

# Determinants and co-expression of anti-predator responses in amphibian tadpoles: a meta-analysis

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A wide range of taxa respond to perceived predation risk (PPR) through inducible defenses, and many prey are capable of responding both behaviorally and morphologically to the same risk event. In cases where multiple defenses confer protection by independent means (i.e. they are mechanistically independent) responses will either be co-expressed, or the expression of one defense will limit the capacity (or need) to respond along another axis. Our ability to generate a broad understanding of these patterns has been limited, in part, by difficulties in comparing results across studies that employ distinct experimental protocols. Using the extensive literature on tadpole responses to PPR, we conducted a meta-analysis to identify the ecological and experimental determinants of inducible defence expression. We then assessed whether the magnitude of response to PPR along behavioural versus morphological response axes was positively, or negatively, correlated. The most commonly quantified responses to perceived risk in tadpoles included reductions in movement and swimming behaviour, and altered tail morphology. Our analyses reveal that tadpole behavioural responses are strongly influenced by prey family, predator taxon, evolutionary history with the predator (native versus non-native), amount of prey consumed by the predator, and how perceived risk was manipulated (e.g. presence versus absence of alarm cues). Tail morphology was similarly influenced by these factors, but also whether the target prey was palatable to predators. Thus, our results identify ecological and experimental features that critically influence the observed effect size in tadpole responses to PPR. A positive correlation between behavioural and morphological responses in studies where both were measured indicates that trait co-specialization is the predominant pattern of defense deployment in larval amphibians. This positive relationship suggests that survival tends to be maximized in tadpoles through equivalent co-activation of multiple independent axes of protection, opposed to maximal expression along any single axis.

Synthesis

Our understanding of plastic responses to perceived predation risk (PPR) has benefited substantially from the vast amount of experimental work examining inducible defences in anuran tadpoles. Indeed this research has illustrated the wide variety of ways that prey animals can respond to the same risk event. We conducted a meta-analysis to identify the key ecological and experimental determinants of inducible defence expression. We then show that, in most cases, behavioural and morphological responses to PPR tend to be co-expressed suggesting that responding along one axis (moving behaviour) does not limit their ability to respond along another distinct axis (tail morphology).

Prey animals routinely encounter spatial and temporal variability in predation risk and are under strong pressure to detect and avoid predators (Lima and Dill 1990, Lima 1998, Benard 2004). A broad range of taxa use visual and chemical cues to detect and respond to variation in predation risk (Lima 1998, Tollrian and Harvell 1999), and many prey respond to perceived risk by expressing one or more inducible defense (Chivers and Smith 1998). Broadly speaking, inducible defenses fall under behavioural or morphological axes and include: reduced activity (Relyea 2001), increased refuge use (DeWitt et al. 1999, Amo et al. 2004), habitat shifting (Werner et al. 1983), development of spines or other protective structures (Repka and Pihlajamaa 1996, DeWitt et al. 2000), and changes in body shape (Chivers

et al. 2008). While these changes are understood to be adaptive in that they increase survival following exposure to predators (DeWitt et al. 1999, Tollrian and Harvell 1999), we currently have only a modest understanding of how multiple inducible defenses are integrated (Relyea 2004). Indeed, the majority of published studies assessing antipredator responses restrict their evaluation to a limited number of metrics along a single response axis (Relyea 2004), leaving a fragmented picture of how species generally respond to PPR. To solidify our understanding of the ecological and evolutionary processes that generate anti-predator responses or influence their efficacy we must therefore clarify how multiple response axes are related (Mikolajewski and Johansson 2004, Sih 2004).

Defenses can be integrated in several ways, and DeWitt et al. (1999) proposed a framework that identifies four functional relationships between morphological and behavioural defenses: codependence, complementation, co-specialization and compensation. Codependence represents cases where the defense axes are mechanistically linked and the efficacy of one defense depends on the expression or presence of another (e.g. escape behavior in fish depends on a tail morphology that facilitates burst swimming). In complementation, the response axes are mechanistically independent, but the appropriate strategy along one axis depends on the strategy expressed in another (e.g. cryptic colouration is enhanced by quiescence, active escape is facilitated by stripes). Defense axes are also mechanistically independent in co-specialization, but here the strategy along one axis does not influence protection gained by responding along the other axis. For example, snails reared with crayfish respond both by crawling out of the water which reduces predator detection and encounter, and by developing shells with a narrow aperture which makes it difficult for crayfish to kill and consume them (DeWitt et al. 1999). Critically, co-specialization is characterized by a positive correlation in the expression of defenses that confer protection through distinct means. Finally, compensation represents cases where weakly expressed defenses along one axis are counterbalanced by strong responses along another axis, and is evidenced by negatively correlated expression of these defenses (DeWitt et al. 1999).

While other functional relationships may theoretically exist (e.g. mechanistically independent defense axes where expression is not correlated), we can distinguish between co-specialization and compensation by examining directionality in the correlation between behavioral and morphological responses to a common risk event. Unfortunately, studies relating defense types typically involve induced behaviour and fixed morphology (Rundle and Brönmark 2001, Cotton et al. 2004, Mikolajewski and Johansson 2004), revealing little about the functional relationship between multiple inducible defense axes. Moreover, even when the responses along multiple defense axes are quantified, general patterns remain obscured by important differences in the prey or predator species involved, and how PPR was manipulated. Accordingly, we lack a synthetic understanding of how prey mount multiple inducible defenses when faced with PPR.

Larval anurans exhibit impressive plasticity along both behavioural and morphological axes in response to PPR, and are perhaps the most extensively studied group in the context of inducible defenses (Van Buskirk et al. 1997, Werner et al. 1983, Relyea 2001, Benard 2004). Notably, recent work has also revealed that the protection conferred by behavioural and morphological defence axes is mechanistically independent in tadpoles (Dijk et al. 2015, but see Van Buskirk and McCollum 2000). This vast body of work therefore provides a unique opportunity to assess the functional relationship between inducible behavioural and morphological defenses. Yet our ability to detect a positive or negative correlation in the expression of behavioural versus morphological responses could be obscured if experimental variables differently affect the responses along these axes. To accurately determine the functional relationship between response axes we must also identify the key sources of substantial variability in the expression of plastic anti-predator responses reported in the

tadpole literature (Van Buskirk 2002, Relyea 2003, Teplitsky et al. 2004), and assess whether these sources are common across defense axes.

We therefore conducted a meta-analysis on the expression of inducible defenses in tadpoles to determine key factors that influence response magnitude along behavioural and morphological axes. The existing literature provides a strong basis for understanding cues which trigger anti-predator trait expression in tadpoles (Kiesecker et al. 1996, Peacor 2006, Schoeppner and Relyea 2009b), however, numerous other factors reportedly influence these responses. Specifically, responses appear to be stronger in prey with increased vulnerability to predation, due to high palatability (Jara and Perotti 2009) or small relative body size (Feminella and Hawkins 1994, Jara and Perotti 2009). Weaker responses are reported when prey are exposed to non-native compared to native predators (Polo-Cavia et al. 2010, Pujol-Buxó et al. 2012), or when prey are exposed to predators fed heterospecific rather than conspecific prey (Schoeppner and Relyea 2009a). Where predators are housed (e.g. within versus outside of experimental arena), and how PPR treatments are administered (e.g. the presence versus absence of alarm cues) could also substantially influence the magnitude of response observed. Critically, while the primary goal of our meta-analysis was to facilitate our examination of the functional relationship between response axes, it also provided a timely assessment of the key factors that affect the observed magnitude of anti-predator response in tadpoles.

We predicted that the expression of anti-predator responses along both response axes (i.e. behavioural and morphological) would be positively correlated and increase with greater risk (trait co-specialization). Further, if protection is conferred proportionally by both response axes, then the change in one axis should be comparable to change in the other (i.e. slope of the relationship between morphological and behavioural responses should overlap with 1.0). However, if responses along one axis limit deployment of defensive traits along the other axis, or if prey mount strong responses in one axis to mitigate inadequate responses along the other axis, we should observe a negative relationship indicating trait compensation. We submit that at this juncture the abundant anuran tadpole literature provides the best available dataset to assess whether trait co-specialization versus compensation is typical of induced defenses, and our analysis may serve as a model for anti-predator trait expression across a broad range of prey taxa.

## Material and methods

### Data collection

An extensive body of published literature has examined tadpole responses to PPR, where the risk treatment is induced via exposure to caged predators or to the chemical cues released by predators (i.e. kairomones), and/or the cues released by killed or injured prey (i.e. alarm cues). These studies involve a range of amphibian and predator species and are conducted using a variety of experimental approaches and protocols, depending on the question of interest. We used Web of Science and Google Scholar to search for

studies evaluating the effect of predation risk on induced behavioural and morphological responses in larval amphibians. Specifically, we queried various search terms including 'anti-predator', 'morpholog\*', 'behavior\*', 'activity', 'defense', 'response', 'amphibian', 'anuran' and 'tadpole'. Following this initial scan of citations, we searched reference lists in relevant papers for additional candidates. Approximately 9000 search hits were initially obtained, of these, roughly 2200 met the initial retention criteria (appropriate topic, taxon, experimental design) and were further considered for inclusion in the analysis. After reviewing titles and abstracts, 320 papers were selected for full text review based on suitability of the topic and study design (i.e. met the data retention criteria outlined below). This preliminary review indicated that the most consistently reported behavioural response to PPR was activity level (moving or swimming), whereas the prevalent morphological response was tail depth measurement. These three metrics served as our primary measures of anti-predator response, while studies which examined other response metrics were dropped. All articles used in our analyses were published prior to 2014, and generally employed ad libitum or high food-resource conditions.

We established data retention criteria that included studies having: 1) untreated controls that were not exposed to predation risk; 2) extractable means, variances, and sample sizes for both control and treatment groups; and 3) basic information on experimental design that allowed us to develop a comprehensive set of predictor variables. Complementary web-related and literature searches on the predators, prey and location of each study allowed us to populate our dataset with all the necessary predictor variables. Ultimately, our list included 152 papers published from 1990 through 2013 (Supplementary material Appendix 1).

Publications were scored for factors associated with prey, predators and basic experimental design conditions. For scoring purposes, predator and prey attributes not described in each study (e.g. prey palatability, primary habitat type of prey, prey consumption behaviour, predator hunting mode) were gathered from Merritt and Cummins (1996), Lannoo (2005), Preisser et al. (2007), and the IUCN Red List Data (IUCN 2014). Prey-related factors considered as predictor variables were: taxonomic family; palatability to predators; primary habitat type in the wild (bromeliad, ephemeral, permanent, both); and mean body mass. Palatability was assigned based on specific predator-prey pairs. Tadpole body mass corresponded to the reported mass of the animals while the experiment was conducted. Predator-related predictors included: taxonomic group (e.g. crayfish, Aeshnidae larvae, fish etc.); evolutionary origin (native, non-native); prey consumption behaviour (chewing, piercing, engulfing); hunting mode (active, sit-and-wait); predator diet in experiment (conspecific, congeneric, confamilial, other amphibian, invertebrate, starved predator); and the number of predators.

Because anti-predator responses can vary depending on study design, we also recorded factors that could influence expression of anti-predator traits, including: study duration (Van Buskirk 2001); number of tadpoles consumed by the predator (i.e. an additional indicator of risk intensity); total prey mass (mg) consumed by the predator over

the duration of experimental exposure to PPR (i.e. a similar indicator of risk intensity, Van Buskirk and Arioli 2002); presence of refuge habitat (Semlitsch and Reyer 1992); and tadpole density (i.e. the number of tadpoles per litre of water). Because experimental venue can affect predation risk responses (Skelly 2002, Winkler and Van Buskirk 2012), we differentiated between studies conducted in mesocosms (venues containing mini-ecosystems, e.g. leaf litter, phyto- and zoo-plankton additions) or in microcosms (simplified laboratory environments). Prey naïveté to predation risk also may influence the intensity of risk responses (Polo-Cavia et al. 2010), so we assessed prey source (captive bred, wild collected as eggs or wild collected as tadpoles), as an indicator of experience. Studies using hatchlings that were still attached to the egg mass were classified as eggs rather than tadpoles. The means by which empiricists manipulated PPR was differentiated based on PPR point of origin (i.e. caged predators housed and fed in main arena, caged predators housed but not fed in main arena, predators housed and fed outside of main arena with predator-treated water added to main arena, flow-through system with predator-treated water), delivery timing of alarm or dietary cues relative to a predation event ('immediate': caged predators fed within arena, 'delayed': water additions or caged predators fed outside of arena, 'absent': starved predators), and PPR delivery duration ('continuous', 'intermittent'). This level of differentiation in our description of PPR manipulation was important because if these differences in methodology influence the prey's perception of risk it would complicate direct comparisons of experimental results across studies. In general, variable and intermittent predation risk should be associated with stronger behavioural responses (Lima and Bednekoff 1999). All of our predictor variables are described more fully in the supplementary material (Supplementary material Appendix 2 Table A1).

## Effect size

Effect sizes were calculated for each study as standardized mean differences (SMD), using the *escalc* function from the *metafor* package (Viechtbauer 2010) in R ver. 2.14.1 (<[www.r-project.org](http://www.r-project.org)>). In both behavioural analyses, positive effect sizes indicate reduced activity. In contrast, tail depth is quantified using a range of distinct approaches that generate results that are often not directly comparable. For example, in Relyea (2003) a deeper tail is indicated by a larger residual tail depth score, whereas a more strongly negative PC1 score indicates a deeper tail in Bennett et al. (2013). Moreover, specific predator-prey pairs also induce tail shape changes in opposite directions (Touchon and Warkentin 2008). Yet our metric of interest is the magnitude of morphological deviation relative to their non-exposed counterparts (i.e. opposed to contraction versus expansion of tail depth per se), and this measurement should not depend upon directionality of the response. We therefore calculated the magnitude of the morphological response for each study by calculating the absolute value of effect size. In doing so we generated a common metric which represents the magnitude of response to predation risk (relative to controls) across experiments with distinct methods, thereby facilitating a more comprehensive analyses

using data from numerous studies. We acknowledge that an implicit, but reasonable, assumption made here is that tail shape responses to PPR are adaptive irrespective of the directionality of their response. Preliminary analysis indicated that ‘moving’ and ‘swimming’ behaviours were not affected similarly by the factors we examined, so we analyzed these responses in separate meta-analyses. These two behavioral responses differ in scale and goal, with ‘moving’ including all small-scale movements involving foraging or tail flicks, and ‘swimming’ including only larger-scale movements such as burst swimming or relocation events. Studies that defined activity as ‘any movement’ were included in the ‘moving’ analysis, rendering our distinction between moving and swimming behaviours more conservative.

A common experimental approach in amphibian predator-prey research is to conduct multiple smaller studies on risk response within a single publication (e.g. multiple predator treatments, variable predator or prey densities, alternate prey population sources). We addressed this by considering individual studies within a publication as separate data points. In addition, some publications provided data at multiple times for a single study, thereby creating the potential for non-independence of these multiple measures. For studies involving multiple measurements on the same animals, the last reported measurement was used exclusively to guard against non-independence but still allow sufficient time for morphological responses to develop. When studies addressed responses to variables not considered in the current paper (e.g. multiple prey populations of a prey species), the mean effect size was calculated (e.g. an average value for the species). For studies involving multiple predator or prey densities and predator consumption levels, separate effect sizes were retained if our preliminary single-variable analysis indicated a significant ( $p < 0.05$ ) effect of that predictor. Otherwise, effect sizes within a paper were averaged as above to account for any possible lack of independence. For example, preliminary analysis indicated no effect of prey density, so for subsequent analyses, effect size was averaged within a given publication so that variation is considered across, but not within, studies. For publications that examined multiple factors that varied among treatment levels or among experiments conducted within a single paper (e.g. predator or prey species, PPR point of origin, predator diet or refuge presence), all effect sizes were retained for our multiple-variable analyses.

## Data analysis

Given the large set of candidate predictor variables, we first conducted separate single-predictor meta-regression tests (‘single-variable analyses’) to identify the relationships between predictor variables and the response metrics (i.e. moving, swimming and tail depth responses) that were worthy of subsequent consideration. For each response metric we then performed a preliminary model selection exercise to determine which study design variables (e.g. experimental duration, prey mass consumed) influenced the observed effect size. This was necessary to ensure comparability among studies which employed a variety of procedures that added an unknown degree of variability. Study design variables in the top model for this preliminary analysis were considered

to have an important influence on effect size, and were retained in subsequent analyses. Having now identified a set of predictors worthy of consideration, and the key study design variables, we generated a series of mixed effects meta-regression models for each response metric (‘multiple-variable analyses’). We then employed model selection and multi-model inferencing to determine the combination of variables that best explained the observed variation in effect size (Burnham and Anderson 2002). Because several predictor variables were categorical and had small sample sizes within individual classes, we excluded classes with  $n < 5$  from the analysis (e.g. we excluded ‘leech’ as a class of predator taxa, and ‘bromeliad’ as a habitat class). Multi-collinearity between predictors was examined using the *vif* function (Lin et al. 2011) and if appropriate, correlated variables were excluded. For example, the three different levels for assessing risk delivery (i.e. PPR point of origin, PPR timing of exposure, PPR duration of exposure) were correlated and not included in the same candidate model, but were instead included in separate models that were compared using multi-model inference. Importantly, while the results from our single-variable meta-regressions provide preliminary insight, our subsequent analyses which directly compare multiple competing models provides a more reliable means to identifying key sources for variation in effect size. Our analyses used prey family as a predictor to examine phylogenetic effects on the observed effect size. We recognized that this may not fully account for within family phylogenetic correlations and to assess the influence of phylogeny more fully, we quantified phylogenetic signal in each of the response variables. These details are summarized in the Supplementary material Appendix 3.

Meta-regressions were conducted using the *rma* function in *metaphor* (Viechtbauer 2010), and model evaluation was corrected for small sample size ( $AIC_c$ , Burnham and Anderson 2002). Support for alternate models was determined by calculating  $AIC_c$  differences ( $\Delta_i$ ) and  $AIC_c$  weights ( $w_i$ ); we considered that models within 2  $AIC_c$  values from the best fit model were not statistically distinguishable (Burnham and Anderson 2002). Two-way interactions in multiple variable models were also tested, but none were detected.

## Publication bias

We tested for publication bias using funnel plots of observed effect sizes against standard errors, via the *funnel* function in *metafor*. The *regtest* function revealed funnel plot asymmetry using Egger’s regression method (Egger et al. 1997); publication bias is inferred if the regression slope is different from zero. If asymmetry was detected, *trimfill* was used to determine the number of studies needed to obtain symmetry; the trim and fill method then augmented observed data by ‘filling in’ the missing studies to assess sensitivity of the results to publication bias (Duval and Tweedie 2000).

## Trait co-specialization versus compensation

Once we had determined the primary factors affecting anti-predator responses in tadpoles, we investigated whether prey responded to PPR through co-specialization or compensation. Specifically, we examined the relationship between



moving and tail depth responses because our multi-model inference (outlined above) indicated that these response axes were influenced by a similar set of predictor variables. Only studies reporting responses in both moving behaviour and morphology (tail depth) were included in this analysis, and studies had to provide both behavioural and morphological data from a single experiment (i.e. same group of tadpoles) under the same experimental conditions. As such, those variables that influence the response magnitude (effect size) should similarly affect both axes despite variation in experimental protocol among experiments – an assumption that we assessed qualitatively by examining the model-averaged coefficients from our best fit models (Supplementary material Appendix 2 Table A4). Regression was performed on behavioural and morphological effect sizes on the full data set ( $n = 80$  data points), and a second regression then focused exclusively on wood frogs *Lithobates sylvaticus* because this was the best-represented prey species in our dataset. Supplemental analyses on effect size averaged at the species-level indicated that accounting for phylogeny was not necessary in our regression that included data from multiple species (Supplementary material Appendix 3). Herein we examine only linear models because we were primarily focused on whether the relationship in trait expression was positive (co-specialization) or negative (compensation), as opposed to determining the functional shape of the relationship.

## Data deposition

Data available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.t8g60>> (Hossie et al. 2016).

## Results

Of the 152 publications retained for our analyses, 71 provided 314 observations of moving behaviour, 45 provided 154 separate observations on swimming behaviour in response to predation risk, and 48 provided 179 observations of tail depth. Most studies involved anuran tadpoles from the Ranidae (moving: 54%, swimming: 66%, and tail depth: 68%) as prey. Aeshnidae dragonfly larvae were the most common predator in the moving and morphology datasets (60% and 51% of cases, respectively), whereas fish were the most common predators in the swimming dataset (37% of cases). Heterogeneity tests revealed that PPR affected moving ( $Q_{313} = 979.72$ ,  $p < 0.0001$ ;  $1.43 \pm 0.06$  (SMD  $\pm$  SE)), swimming ( $Q_{153} = 564.76$ ,  $p < 0.0001$ ;  $1.07 \pm 0.07$ ), and morphology ( $Q_{178} = 830.66$ ,  $p < 0.0001$ ;  $2.32 \pm 0.15$ ).

## Publication bias

Regression analysis revealed significant funnel plot asymmetry for all datasets (all  $Z \geq 12.21$ , all  $p < 0.001$ ). For moving behaviour, we estimated that 83 cases with negative effect sizes were needed to obtain symmetry, which represents a 26% increase over our existing sample size. With inclusion of the missing studies, there remained a significant effect of predation risk on prey moving behaviour ( $Q_{396} = 1639.45$ ,

$p < 0.001$ , SMD =  $0.96 \pm 0.09$ ). The regression test for swimming behaviour also indicated positive bias and an estimated 25 cases are needed to obtain symmetry, representing a 29% increase. Including the missing cases for swimming behaviour also yields a significant effect of predation risk ( $Q_{178} = 803.99$ ,  $p < 0.001$ , SMD =  $0.84 \pm 0.10$ ). For the morphology dataset, 51 studies with negative effect sizes were needed to obtain symmetry, representing a 16% increase. Note that because we used the absolute value of effect size in our analysis, a bias toward positive effect sizes is expected in the case of morphology. However, with the missing cases filled in, there remained a significant effect of predation risk on prey morphology ( $Q_{229} = 1324.62$ ,  $p < 0.001$ , SMD =  $1.36 \pm 0.21$ ).

## Single-variable analysis

Mean effect sizes (Supplementary material Appendix 2 Table A2) and statistical test results (Supplementary material Appendix 2 Table A3) from single-variable analyses of predictor variables are reported for the three anti-predator response metrics. In terms of study design, experiment duration was important for each of the three response metrics, but effect sizes were highly variable (Supplementary material Appendix 2 Table A2). Across all response metrics effect size was greater when experiments were conducted in mesocosms compared to microcosms (Supplementary material Appendix 2 Table A2–A3). Overall, response magnitudes in tadpole movement, swimming, and morphology were influenced by many similar factors, and of the 20 predictors under consideration only tadpole density failed to influence any of the three response metrics. In contrast, predator taxon, experiment duration, tadpole mass consumed, and PPR timing of exposure, each affected the three response metrics. Interestingly, refuge presence was related to changes in both tadpole behaviour and morphology, and likewise PPR point of origin, PPR duration of exposure, and PPR timing of exposure each influenced both response axes (Supplementary material Appendix 2 Table A2–A3). Notably, while both immediate and delayed exposure to PPR elicited strong behavioural and morphological changes, those associated with immediate risk were especially influential on tail morphology (Supplementary material Appendix 2 Table A2–A3).

In general, Ranidae (true frogs) had stronger induced responses whereas Bufonidae (toads) had weaker responses to perceived risk, and responses to predators including beetle larvae, Aeshnidae dragonfly larvae, and fish, were especially pronounced. Interestingly, palatability of the tadpoles to their predator only appeared to influence effect size in the tail depth response, with unpalatable species having significantly weaker responses (Supplementary material Appendix 2 Table A2–A3). Predator hunting mode appeared to influence the effect size of the swimming response, with tadpoles expressing a more pronounced response when exposed to sit-and-wait predators. Tadpole movement was least responsive to piercing predators, and swimming responses were more pronounced with exposure to piercers; tail morphology was more strongly influenced by chewing predators (Supplementary material Appendix 2 Table A2–A3).

Table 1. Multiple-variable models from meta-regression of anuran tadpole movement responses to perceived predation risk.

| Model  | k | n   | AICc   | ΔAICc | w <sub>i</sub> |
|--|---|-----|--------|-------|----------------|
| Prey family + Predator group + Prey mass consumed + PPR timing of exposure                   | 5 | 233 | 692.96 | 0     | 0.51           |
| Prey family + Predator group + Predator origin + Prey mass consumed + PPR timing of exposure | 6 | 233 | 693.12 | 0.27  | 0.46           |
| Predator taxon + Prey mass consumed + PPR timing of exposure                                 | 4 | 233 | 699.14 | 6.1   | 0.024          |
| Predator taxon + Predator origin + Prey mass consumed + PPR timing of exposure               | 5 | 233 | 699.27 | 6.31  | 0.022          |

## Multiple-variable analysis

Model-averaged parameter estimates and unconditional standard errors were calculated for predictor variables contained in the high-ranking ( $w_i > 0.01$ ) multiple variable models assessing movement (four models: Table 1), swimming (six models: Table 2), and tail morphology (six models: Table 3) responses, and are provided in the Supplementary material Appendix 2 Table A4. Several important factors underlying tadpole responses to predation risk were common to the three response metrics, including tadpole taxonomic family (Fig. 1), predator taxonomic group (Fig. 1), and amount of prey consumed. Number of predators and PPR point of origin were important factors influencing swimming responses (Fig. 2), whereas PPR timing of exposure influenced moving and morphological responses (Fig. 1, 3; Supplementary material Appendix 2 Table A4). Evolutionary origin of predators (i.e. native versus non-native) was moderately important for all three response types (movement:  $\sum w = 0.47$ ; swimming and morphology:  $\sum w = 0.57$ ) and prey palatability was moderately important to morphological variation ( $\sum w = 0.57$ ). Finally, the habitat where the tadpoles are found was weakly important in relation to tadpole swimming behaviour ( $\sum w = 0.20$ ). Overall, our meta-analyses revealed that the factors which influenced the movement and tail depth responses were highly congruent (Table 1, 3; Supplementary material Appendix 2 Table A4), justifying our approach of comparing these responses directly in the co-specialization versus compensation analyses described below.

## Trait co-specialization versus compensation

Twenty-two publications included measures of both movement and tail depth responses to predation risk, and were therefore used to assess the functional relationship between anti-predator response axes. Of the 80 observations available for analysis, most involved one of three frog species

(*Lithobates sylvaticus*: 40%; *Pelophylax lessonae*: 12.5%; *Rana temporaria*: 6.3%). Behavioural and morphological effect sizes were positively correlated across the full data set ( $F_{1,78} = 40.78$ ,  $p < 0.001$ ,  $R^2 = 0.34$ ; Fig. 4a); both the intercept ( $0.91 \pm 0.32$ ) and slope ( $0.42 \pm 0.07$ ) were significantly different from zero ( $p = 0.005$  and  $p < 0.001$ , respectively). For the analysis consisting exclusively of *L. sylvaticus*, effect sizes were positively correlated ( $F_{1,30} = 61.31$ ,  $p < 0.0001$ ,  $R^2 = 0.67$ ; Fig. 4b), with only the slope ( $0.72 \pm 0.09$ ,  $p < 0.001$ ), and not the intercept ( $-0.70 \pm 0.56$ ,  $p = 0.22$ ) being significantly different from zero. Given the positive slope in both regressions involving anti-predator behavior and morphology, we surmise that for larval amphibians, trait co-specialization rather than trait compensation generally describes the pattern of defense deployment.

## Discussion

Our meta-analysis revealed that many of the important factors influencing the magnitude of anti-predator responses in larval anurans affect both behavioural and morphological responses. We found that the observed effect size of all three commonly quantified tadpole anti-predator response metrics is critically influenced by how empiricists choose to manipulate PPR. The strength of each response also differs among anuran families, as well as with exposure to different predators, yet in general anti-predator responses across all axes increase with the total amount of prey consumed and when predators are native to the prey species. Importantly, the expression of inducible behavioural and morphological defenses in tadpoles was positively correlated both across a wide range of anuran families and within the most extensively studied species *Lithobates sylvaticus*. Thus, larval anurans exhibit trait co-specialization, not compensation, across behavioural and morphological response axes. This suggests that tadpole survival is generally maximized though

Table 2. Multiple-variable models from meta-regression of anuran tadpole responses in swimming to perceived predation risk.

| Model   | k | n   | AICc     | ΔAICc | w <sub>i</sub> |
|---|---|-----|----------|-------|----------------|
| Prey family + Predator group + Predator origin + Predator number + Prey number consumed + PPR point of origin   | 7 | 142 | 401.6987 | 0     | 0.5377         |
| Prey family + Predator group + Predator number + Prey number consumed + PPR point of origin                     | 6 | 142 | 403.5474 | 1.85  | 0.21335        |
| Prey family + Prey habitat type + Predator group + Predator number + Prey number consumed + PPR point of origin | 7 | 142 | 403.7906 | 2.09  | 0.1889         |
| Prey family + Predator group + Predator origin + Prey number consumed + PPR point of origin                     | 6 | 142 | 407.1107 | 5.41  | 0.03592        |
| Prey family + Predator group + Prey number consumed + PPR point of origin                                       | 5 | 142 | 409.2256 | 7.53  | 0.01248        |
| Prey family + Prey habitat type + Predator group + Prey number consumed + PPR point of origin                   | 6 | 142 | 409.5869 | 7.89  | 0.0104         |

Table 3. Multiple-variable models from meta-regression of anuran tadpole responses in tail morphology to perceived predation risk.

| Model  | <i>k</i> | <i>n</i> | AICc   | ΔAICc | <i>w<sub>i</sub></i> |
|--|----------|----------|--------|-------|----------------------|
| Prey family + Prey palatability + Predator group + Predator origin + Prey mass consumed + PPR timing of exposure | 7        | 125      | 466.31 | 0     | 0.419                |
| Prey family + Prey palatability + Predator group + Prey mass consumed + PPR timing of exposure                   | 6        | 125      | 467.06 | 0.76  | 0.287                |
| Prey family + Predator group + Predator origin + Prey mass consumed + PPR timing of exposure                     | 6        | 125      | 468.5  | 2.19  | 0.14                 |
| Prey family + Predator group + Prey mass consumed + PPR timing of exposure                                       | 5        | 125      | 469.26 | 2.96  | 0.095                |
| Prey family + Prey palatability + Predator group + Prey mass consumed + PPR timing of exposure                   | 5        | 125      | 470.49 | 4.69  | 0.04                 |
| Predator group + Predator origin + Prey mass consumed + PPR timing of exposure                                   | 5        | 125      | 473.63 | 7.33  | 0.011                |

the concurrent deployment of multiple largely independent defenses, as opposed to maximal expression along any single defence axis. The absence of compensation between these defenses further indicates that the protection conferred by one modality, say morphology, is not equivalent to that conferred by the other, and cannot be replaced simply by responding more strongly along the behavioural axis.

### Determinants of response magnitude

Our meta-analysis clearly illustrates that the means through which PPR is experimentally manipulated influences the observed effect size across each of the response metrics we examined (Fig. 1–3). Stronger movement and tail mor-

phology responses are observed when prey are exposed to caged predators fed within the experimental tank (i.e. where alarm cues immediately paired with predator kairomones), compared to when alarm cues are absent (i.e. experiments using starved predators), when caged predators are fed outside of an experimental tank, or when PPR is manipulated through water addition (Fig. 1, 3). This highlights that tadpoles generally respond more strongly to cues associated with predation events than to cues signalling predator-presence in isolation from other cues. That said, delaying exposure to alarm and/or diet cues (e.g. by employing water additions or using caged predators fed outside of experimental tank) seems to differently affect movement and tail shape responses (Supplementary material Appendix 2 Table A4). Specifically, movement responses were strong except in cases where starved predators were employed, yet tail morphology responses were notably diminished when exposure to alarm or diet cues was delayed. Interestingly, this suggests that while the PPR cues required to elicit strong movement

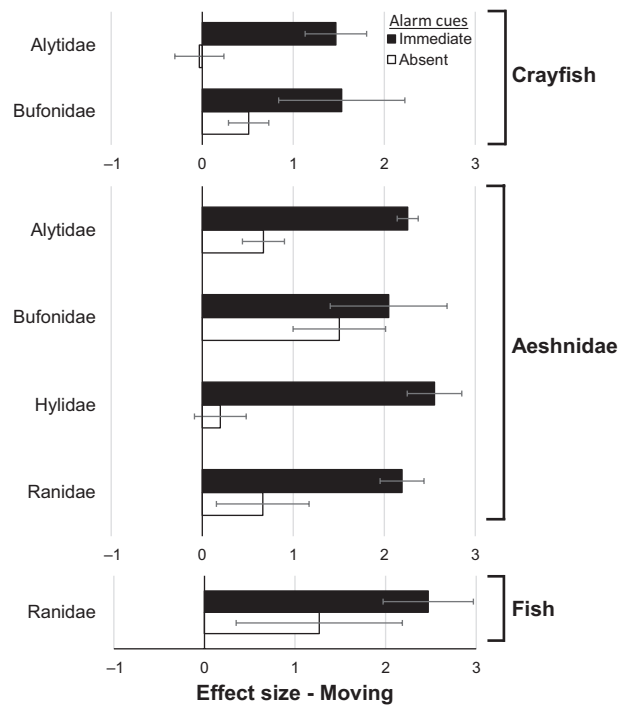


Figure 1. Mean  $\pm$  SE effect sizes for tadpole movement responses upon either immediate exposure to alarm and/or dietary cues (i.e. caged predator housed and fed within the experimental arena) or where alarm and/or dietary cues were absent (i.e. prey exposed to cues from a starved predator). Only data for prey families and predator taxa where an average of at least  $n = 3$  data points could be calculated is displayed.

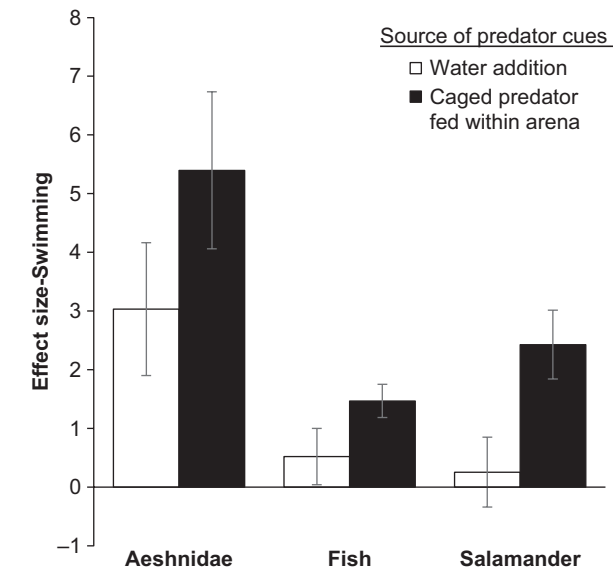


Figure 2. Mean  $\pm$  SE effect sizes for Ranidae tadpole swimming responses upon exposure to perceived predation risk, as manipulated by water additions or by housing and feeding a caged predator within the experimental arena. Only data for the predator taxa where an average of at least  $n = 3$  data points could be calculated is displayed.

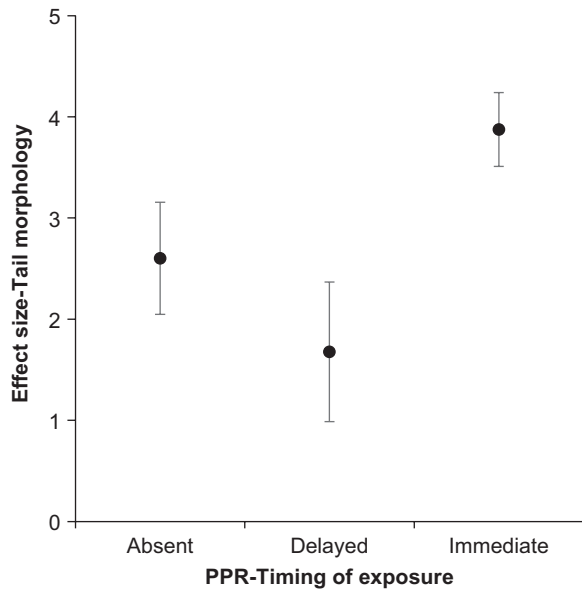


Figure 3. Mean  $\pm$  SE effect sizes for Ranidae tadpole tail morphology responses upon exposure to perceived predation risk (PPR) from Aeshnidae larvae. PPR manipulations categorized based on timing of exposure to alarm and/or dietary cues: 'absent' (cues from starved predators), 'delayed' (cues via addition of predator-conditioned water, or predators housed but not fed within experimental arena), or 'immediate' (cues from predators housed and fed within the experimental arena). Only data where an average of at least  $n = 3$  data points could be calculated is displayed.

responses are present even when exposure to alarm and/or diet cues is delayed, a cue that is important for morphological responses either decomposes quickly or is otherwise functionally absent in treatments where cue exposure is delayed. Peacor (2006) found diminished behavioural responses to 2–4 day old chemical cues associated with a predation event, however, a delay of 5 h yielded similar anti-predator responses as did immediate exposure. Tadpole swimming response was strongly influenced by the source of the PPR cues, with caged predators fed within the experimental arena causing the strongest reduction in swimming (Fig. 2). Caged predators fed outside the main arena elicited intermediate swimming responses, but water additions and flow-through systems both elicited substantially weaker responses. The swimming response may therefore be sensitive to presence of additional predator-related cues (e.g. visual cues). Indeed, visual, acoustic and tactile cues are known to reinforce, or enhance, risk perception in tadpoles, though visual cues in the absence of other predator-related stimuli appear to only weakly influence tadpole behaviour (Stauffer and Semlitsch 1993, Hettyey et al. 2012).

In all cases the total amount of prey consumed influenced the strength of the tadpole's response, yet only swimming behaviour appeared responsive to the number of predators. Schoeppner and Relyea (2008) showed that the strength of wood frog tadpole behavioural and morphological responses are sensitive to the mass of prey consumed, and that the apparent effect of predator (beetle larvae) number can be explained solely by the associated increase in prey consumption (but see Van Buskirk and Arioli 2002). This appears to hold for moving behaviour and tail depth, which are variables

that were also measured by Schoeppner and Relyea (2008), given the absence of 'predator number' in the best fit models (Table 1, 3). In contrast, both the number of prey consumed and predator number were found in the top three best fit models that explain the strength of the swimming response (Table 2), indicating that predator number provided additional explanatory power. Key predators eliciting a strong swimming response included waterbugs (Belostomatidae), crayfish, turtles, and Aeshnidae dragonfly larvae (but not beetle larva, Supplementary material Appendix 2 Table A2, A4), and we suggest that future efforts to disentangle the effects of prey mass consumed versus number of predators should employ a variety of predator taxa and examine a suite of response types.

Prey family was an important predictor in all three of the anti-predator responses we examined, and Ranidae expressed relatively strong responses along behavioural and morphological axes. Ranidae species are known to vary considerably in their responsiveness to predators along behavioral (Richardson 2001, Relyea 2001, Van Buskirk 2002) and morphological (Relyea 2001, Van Buskirk 2002) axes, and this is reflected in the wide unconditional standard error surrounding the prey taxa coefficients (Supplementary material Appendix 2 Table A4). Examining the specific nature of taxonomic differences in responses to PPR was beyond the scope of our study, but such efforts do help determine whether phylogenetic constraints have influenced larval anuran responses to risk. More generally, low data availability for taxa from families other than Ranidae, Bufonidae and Hylidae highlights the need for PPR research to be conducted more widely across the anuran phylogeny. Prey palatability is at least partly related to prey family (e.g. Bufonidae tadpoles are generally unpalatable), however, our analyses did reveal that tail depth responses were weak when tadpoles were unpalatable irrespective of taxonomic affiliation. Strong tail depth responses may be redundant when prey are unpalatable, given that both of these defenses primarily function to increase survival following detection or attack. Though little is known about the plasticity of unpalatability in tadpoles, some Bufonidae are known to increase investment in chemical defences upon exposure to crushed conspecifics (Hagman et al. 2009, but see Bokony et al. 2016). Nevertheless, unpalatability and tail depth may represent distinct strategies to guard against predation in unpredictable environments. Interestingly, palatability did not strongly influence behavioural traits (Table 1–2, Supplementary material Appendix 2 Table A3–A4). Instead, unpalatable prey retain strong movement and swimming responses, suggesting that even unpalatable tadpoles seek to reduce predator encounter and detection upon exposure to PPR.

Aeshnidae dragonfly larvae are a widely-used predator in studies of tadpole response to PPR, and our meta-analyses confirm previous evidence (Van Buskirk 2002) that darner larvae elicit strong responses in movement, swimming, and tail depth (Fig. 1–3; Supplementary material Appendix 2 Table A4). In addition, strong movement and tail depth responses were generated by beetle larvae and fish (Fig. 1), whereas water bugs and crayfish caused strong responses in swimming (Supplementary material Appendix 2 Table A4). Nyström and Åbjörnsson (2000) showed that *Bufo bufo* tadpoles are highly responsive to crayfish, which readily



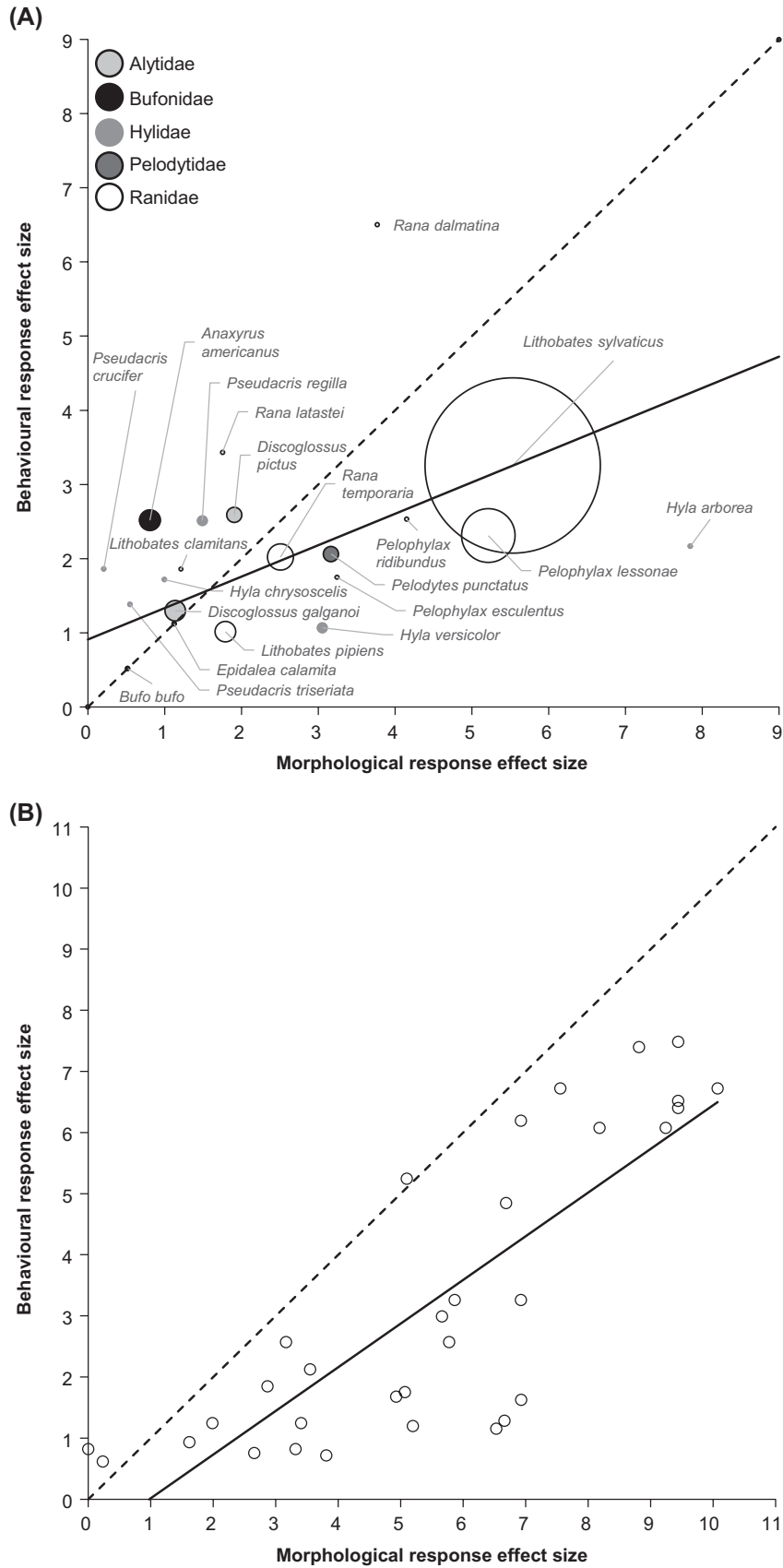


Figure 4. Relationship between expression of behavioural and morphological responses to perceived predation risk in tadpoles for (A) all species, (B) *Lithobates sylvaticus*. The solid line is the line of best fit, and the dashed line represents a 1:1 correlation with a zero intercept. (A) depicts effect sizes as species-level averages to illustrate phylogenetic patterns, and the diameter of each circle reflects the number of data points which came from that species.

consume this prey type despite their unpalatability to other predators. Similarly, Kiesecker et al. (1996) found that *Bufo borealis* tadpoles did not respond behaviourally to newts or trout, which find them unpalatable, but responded strongly to backswimmers and water bugs, which readily consumed them. All species responded more strongly to native predators, irrespective of prey family or predator group, indicating that despite the generality of PPR responses tadpoles possess anti-predator responses that are shaped strongly by their evolutionary history with those predators.

Overall, our meta-analysis reveals that features of the study design (e.g. amount of prey consumed, timing of cue exposure and origin of cue) are dominant factors influencing the strength of response across all response types. Ultimately, this finding underscores the need to carefully consider aspects of study design, both by empiricists and by researchers conducting synthetic analyses such as ours, as variability in design may govern the apparent prevalence and magnitude of anti-predator responses.

### Co-specialization of anti-predator responses

The positive correlation between behavioural and morphological responses across species (Fig. 4A, Supplementary material Appendix 2 Fig. A1) and within wood frog tadpoles (Fig. 4B) supports the trait co-specialization model and indicates that the expression of one defense type does not constrain expression of the other (energetically, physiologically, morphologically or otherwise). Recent work has indicated that behavioural and morphological responses to PPR in amphibian tadpoles are functionally independent (Dijk et al. 2015), however, even if there is not complete independence between these traits, our test offers a reasonable first step in differentiating between trait compensation and co-specialization across a range of anuran species. We note that our focus on inducible defences could influence interpretation of the expression of co-specialization versus compensation. Specifically, consistently high exposure to predators over evolutionary time should prompt evolution of stronger baseline (constitutive) defenses in prey (Tollrian and Harvell 1999) and smaller induced responses to PPR. As such, it may be difficult to detect and classify the functional relationship when comparisons include species that vary in their baseline level of protection or that possess additional constitutive (fixed) defenses. In addition, a large proportion of the data (~40%) used in our multi-species analysis came from a single species (*L. sylvaticus*). The analysis restricted to wood frog tadpoles (Fig. 4B) may represent a more robust test of the functional relationship between defense axes given that both movement and tail depth responses are highly inducible in this particular species, whereas these responses appear to be less plastic in tadpoles of several other anuran species (Relyea and Werner 2000, Relyea 2001; Supplementary material Appendix 2 Fig. A1).

Tadpoles respond to PPR in an remarkably wide variety of ways (e.g. refuge use, Hossie and Murray 2010; tail colour, Touchon and Warkentin 2008; developing bulgy bodies, Kishida and Nishimura 2004), and our analyses focus only on the three most commonly quantified response metrics. The functional relationship between anti-predator

responses not examined here remain to be identified, and we suspect that other functional relationships may be observed within tadpoles as well. For example, Hylidae did not show a strong correlation between movement and tail morphology responses (Supplementary material Appendix 2 Fig. A1), and perhaps other functional relationships are important for this group. Specifically, many hylid tadpoles modify tail colour in conjunction with tail shape responses when exposed to PPR, and both of these traits are known to lure strikes away from their body (Van Buskirk et al. 2003, 2004), thus hylid tadpoles may rely more heavily on these complementary defences. A more important concern, however, is that we still lack a clear framework to understand how various defense axes are integrated within organisms, like tadpoles, that also exhibit plasticity in their life history and physiology.

Both behavioural and morphological responses can be associated with reduced growth and/or development (Skelly 1992, McCollum and Van Buskirk 1996, Steiner 2007), reflecting an energetic cost associated with mounting either response. Consequently, one could expect an inhibition in the expression of one defense (e.g. behaviour) due to allocation of resources to the other (e.g. morphology). The absence of this pattern, however, may be explained by two phenomena. First, trait expression is associated with resource allocation and may involve multiple (and rarely quantified) traits (Agrawal et al. 2010), including changes in gut length (Relyea and Auld 2004), metabolism (Steiner and Van Buskirk 2009), immune function (Rigby and Jokela 2000), food intake rate (Steiner 2007), and energy conversion (Steiner 2007). Therefore, the expression of either behavioural or morphological defenses may not be mutually exclusive due to an offset of energetic costs through another integrated, and not readily discerned, trait (e.g. reduced gut length). Accordingly, in order to fully understand trait interactions associated with anti-predator defenses, we need to better appreciate how multiple traits are integrated and how prey allocate resources among those traits. Second, trait interactions can be masked by variability in resource acquisition (Van Noordwijk and de Jong 1986, Zera and Harshman 2001, Agrawal et al. 2010), due to disparity across species, body size, and resource availability; these differences can influence whether trait compensation or co-specialization are observed. However, this appears not to be the case in our study owing to: 1) the consistent expression of co-specialization when *L. sylvaticus* was examined in isolation; 2) the absence of prey size relevance on anti-predator responses, implying that variation in prey size unlikely masks tradeoffs between behavioural and morphological responses; and 3) our analyses included only studies using high resource or ad libitum conditions. This means that prey likely had sufficient resources to cover the costs of both defenses, and thus are able to co-specialize. In contrast, the relationship between behavioural and morphological defenses could shift from being co-specialized (high resources) to compensatory (low resources) in resource-limited environments, as was observed in a recent experiment manipulating tadpole nutritional status under PPR (Bennett et al. 2013). Thus, while our results indicate that tadpoles generally appear to maximize survival through equivalent co-activation of multiple independent axes of protection, opposed to maximal expression along any single axis, the relationship between function-

ally independent inducible defences may in fact vary with resource availability.

## Conclusion

Our study highlights several areas requiring further research into the expression of behavioural and morphological responses of prey to perceived risk. First, research should address the energetic demands of simultaneously mounting multiple anti-predator defenses. Aside from quantifying effects on immune function or metabolic rates, among the main priorities should be to quantify the expression and costs of multiple defense types (of the > 150 articles surveyed in this study, only 15% could be used in the analysis of trait interactions). Second, conclusions regarding trait compensation or co-specialization are often based on species-, population- or group-level analyses, yet explanations on why these responses occur are mainly based on energetic constraints acting on the individual. While our results suggest that frog tadpoles exhibit trait co-specialization in response to PPR, this is based on group-level analyses averaged across individuals within tanks or treatment types. Logically, a next step is to examine individual tradeoffs and expression of one or more anti-predator responses (DeWitt et al. 1999). Third, amphibian tadpoles are likely the only group currently amenable to such a large a meta-analysis focusing on behavioural and morphological tradeoffs, but the many other taxa demonstrating inducible defenses are candidates for much further empirical work, including those having quantifiable behavioural responses (e.g. snails, daphnia, damselflies and fish). Ultimately, to the fullest extent possible, future work on anti-predator responses should adopt a more holistic view by considering the complexity and interplay among a variety of response types compared to the more restrictive approach that has traditionally dominated research into anti-predator responses.

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*Author contributions* – KL and DLM designed the study, KL and TJH collected data and conducted statistical analysis, TJH, KL and DLM wrote the paper, TJH generated the figures, TJH and DLM addressed reviewer comments.

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Supplementary material (available online as Appendix oik-03305 at <[www.oikosjournal.org/appendix/oik-03305](http://www.oikosjournal.org/appendix/oik-03305)>). Appendix 1–3.