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Investment in defense and cost of predator-induced defense along a resource gradient

Abstract

An organism's investment in different traits to reduce predation is determined by the Fitness benefit of the defense relative to the Fitness costs associated with the allocation of time and resources to the defense. Inherent tradeoffs in time and resource allocation should result in differential investment in defense along a resource gradient, but competing models predict different patterns of investment. There are currently insufficient empirical data on changes in investment in defensive traits or their costs along resource gradients to differentiate between the competing allocation models. In this study, I exposed tadpoles to caged predators along a resource gradient in order to estimate investment in defense and costs of defense by assessing predator-induced plasticity. Induced defenses included increased tail depth, reduced feeding, and reduced swimming activity; costs associated with these defenses were reduced developmental rate, reduced growth, and reduced survival. At low resource availability, these costs predominately resulted in reduced survival, while at high resource availability the costs yielded a reduced developmental rate. Defensive traits responded strongly to predation risk, but did not respond to resource availability (with the exception of feeding activity), whereas traits construed as costs of defenses showed the opposite pattern. Therefore, defensive traits were highly sensitive to predation risk, while traits construed as costs of defense were highly sensitive to resource allocation tradeoffs. This difference in sensitivity between the two groups of traits may explain why the correlation between the expression of defensive traits and the expression of the associated defense costs was weak. Furthermore, my results indicate that genetic linkages and mechanistic integration of multiple defensive traits and their associated costs may constrain time and resource allocation in ways that are not addressed in existing models.

Investment in defense and cost of predator-induced defense along a resource gradient

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An organism's investment in different traits to reduce predation is determined by the fitness benefit of the defense relative to the fitness costs associated with the allocation of time and resources to the defense. Trade-offs inherent in time and resource allocation should result in differential investment in defense along a resource gradient, but competing models predict different patterns of investment. Currently, there is insufficient empirical data on changes in investment in defensive traits or their costs along resource gradients to differentiate among the competing allocation models. In this study, I exposed tadpoles to caged predators along a resource gradient, to estimate investment in defense and costs of defense by assessing predator-induced plasticity. Induced defenses included increased tail depth, reduced feeding, and reduced swimming activity; associated costs of these defenses were reduced developmental rate, reduced growth, and reduced survival. At low resource availability, costs were predominately paid by reduced survival, while at high resource availability costs were paid by reduced developmental rate. Defensive traits responded strongly to predation risk, but did not respond to resource availability (with the exception of feeding activity), whereas traits construed as costs of defenses showed the opposite pattern. Therefore, defensive traits were highly sensitive to predation risk while traits construed as costs of defense were highly sensitive to resource allocation trade-offs. This difference in sensitivity between the two groups of traits may explain why the correlation between the expression of defensive traits and the expression of the associated costs of defense was weak. Furthermore my results indicate that genetic linkages and mechanistic integration of multiple defensive traits and their associated costs may constrain time and resource allocation in ways that are not addressed in existing models.

Keywords: adaptive plasticity, phenotypic plasticity, *Rana temporaria*, time allocation trade-off, trait integration

INTRODUCTION

Susceptibility to predation is a major determinant of fitness; therefore selection acts on traits that reduce predation. However, the optimal expression of defensive traits must also take into account any fitness costs associated with the investment in defense (Stearns 1992). There are competing conceptual models that predict a shift in the optimal allocation of time and resources to defense along a resource gradient, but the nature and direction of the shift is fundamentally different among models.

The simple allocation model predicts increasing investment in defense with increasing resource availability (Harvell 1990; Tuomi et al. 1991; Werner and Anholt 1993). At low resource availability, all investment is made to maintain basic life functions and no investment in defense can be afforded. At high resource availability the maintenance of basic life functions is easily achieved and investment in defense is possible and beneficial.

Contrary to the simple allocation model, the defense-growth model predicts decreasing investment in defense with increasing resource availability (Myers and Bazely 1991). At low resource availability growth is reduced resulting in small individuals that are very vulnerable to predators and which spend more time in vulnerable stages (Arendt 1997). At high resource availability individuals can grow quickly, allowing individuals to escape predation risk by either early metamorphosis, or rapid attainment of a size beyond the reach of gape-limited predators (Kishida and Nishimura 2004). Therefore, defensive mechanisms are more important to slow growing individuals and investment in defense should be higher at low resource availability.

The growth-differentiation model (Tuomi et al. 1991; Gomulkiewicz and Kirkpatrick 1992; Herms and Mattson 1992; Steiner and Pfeiffer in press), combines the previous two models. At low resource availability, this model follows the logic of the simple allocation model,

whereas at high resource availability, the model follows the logic of the defense-growth model. Therefore, investment in defense peaks at intermediate resources.

While allocations to defense are difficult to measure directly, the expression of defensive traits is clearly a function of underlying time and resource allocations and the predator-induced plasticity of these traits are reasonable approximations for investment (Van Buskirk 2000; Teplitsky et al. 2005). Predator-induced defenses have proved to be particularly useful in this regard (Tollrian and Harvell 1992). By exposing individuals to non-lethal predators, the expression of the full defensive response is exhibited without actually suffering predation. The difference in expression of defensive traits between induced and non-induced individuals, or the predator-induced plasticity in defensive traits (Tollrian and Harvell 1992), can be taken as a measure of investment in defense. Similarly, the predator-induced plasticity of non-adaptive traits (Agrawal et al. 2002; McPeck 2004) is a measure of the costs of defense. In this context, non-adaptive traits are traits that are related to fitness and respond to predation risk, but do not decrease predation. As an example, an individual exposed to predators, in addition to expressing defensive traits, might reduce its growth rate (i.e. a non-adaptive trait). The induced reduction in growth rate can be viewed as the cost of allocating resources to defensive mechanisms. Note that non-adaptive responses are not maladaptive when seen from a predatory-defense perspective, because the fitness benefit of the defense necessarily outweighs the costs.

Based on time and resource allocation arguments high investment in defense is associated with high cost of defense. Therefore, investment in defense and costs of defense should be correlated, i.e. predator-induced plasticity of defensive traits should be correlated to predator-induced plasticity in non-adaptive traits.

Predator-induced defenses can be found in various morphological, behavioral, life-historical and physiological traits (Herms and Mattson 1992; Lima 1998). However, most studies of predator-induced defenses did not investigate different resource conditions, or used only two resource levels and cannot detect non-linear effects (Angilletta et al. 2003). Also, previous studies did not investigate investment in multiple defensive traits and their fitness costs in an integrated way (Van Buskirk 2000; Teplitsky et al. 2005).

The goal of this study was to investigate changes of investment and costs with various defensive traits and non-adaptive traits along a resource gradient. I used *Rana temporaria* tadpoles and one of their most common predators (the sit-and-wait predatory dragonfly larva *Aeshna cyanea*) as a model system. Tadpoles express multiple (adaptive) defenses, such as reduced swimming and feeding activity and an increase in tail depth (Skelly and Werner 1990; Van Buskirk and McCollum 2000). Costs of defenses are expressed in non-adaptive responses, such as reduced growth, reduced development and reduced survival not caused by predation (Skelly 1992; Anholt and Werner 1995; McCollum and VanBuskirk 1996; Anholt et al. 2000; Van Buskirk 2000; Van Buskirk 2002; LaFiandra and Babbitt 2004). The defenses are known to reduce predation risk and the non-adaptive responses relate to reduced fitness but do not decrease predation (Van Buskirk and McCollum 2000; Altwegg and Reyer 2003).

I conducted an experiment in which I exposed tadpoles to a predator environment (with non-lethal caged predators) and a no-predator environment. I assessed the predator-induced responses along a resource gradient for three (adaptive) defensive traits (swimming, feeding and tail depth) and three non-adaptive traits (body size, time to metamorphosis and survival). I selected those traits because they largely respond independent from each other (Van Buskirk and McCollum 2000; Relyea 2002; Steiner 2005).

I did not have specific predictions for responses in the different defensive traits, beside the general prediction that investment in defenses and cost of defenses should be correlated and investment of defense in each trait should follow one of the above outlined models. To start with specific predictions would require a better understanding of integration of multiple defensive traits and associated costs. This study improves our understanding of investment and correlated costs of defense and provides a basis for making predictions about genetic linkage and mechanistic integration of multiple traits.

MATERIALS AND METHODS

For the experiment I used *R. temporaria* tadpoles hatched from clutches collected from a population at 1159 m elevation near Wildhaus, Switzerland. I reared tadpoles in 0.28 m² outdoor plastic pools filled with 80 liters at the University of Zürich, Switzerland. At the outset on 4 May 2002 (day 1), I stocked 100 pools with 20 tadpoles (71 tadpoles/m²) consisting of two individuals each from ten clutches. Stocked tadpoles were 4-5 day old (15 mg, stage 24-25, Gosner 1960). I covered the pools with shade cloth to prevent invasion of predators and escape of metamorphs. I moved tadpoles simultaneously to new pools when the water in any of the pools started to get cloudy. This was done on day 19, 32 and 46. Water quality degraded only at the highest food level, the treatment with the highest survival. I terminated the experiment on day 54 (26 June 2002) when most tadpoles reached metamorphosis.

Treatments

The experiment had two temperature treatments (warm and cold), two predator treatments (non-lethal predator and no-predator) and five food levels (resource availability), replicated in five complete randomized blocks (100 pools in total). The differences between the

two temperature treatments (1.64 ± 0.05 C°) were not enough to manipulate growth rates as initially intended. I mention the temperature manipulation for integrity reasons and will not discuss the (lack of) temperature effect in detail. Details about the initial reasoning for manipulating temperature are available upon request.

Each pool contained one floating cage (~1 L volume), which contained either one final instar dragonfly larva (*A. cyanea*) or was left empty. I fed the dragonfly larvae 300 mg of *R. temporaria* tadpoles three times a week. This feeding schedule of the caged predators is known to induce a full defensive response in tadpoles exposed to the kairomone (chemical cue released by the predator) (Van Buskirk and Arioli 2002). I rotated the dragonflies within the non-lethal predator treatment among pools each time I fed them to equalize for possible differences among individual *Aeshna*. I rotated the cages among the no-predator pools to control for effects of disturbance.

The five resource availability levels were 1%, 2%, 4%, 8% and 16% (16% equals *ad libitum* food) of tadpole body mass fed per day. Before each feeding event I calculated the amount of food by weighing two extra sets of twenty tadpoles for each resource availability level. After day 18 I weekly measured the average wet weight of the experimental tadpoles in each treatment and based the amount of food on these averages, which allowed me to base the amount of dry food on the actual average wet weight of the experimental tadpoles in each treatment combination. I fed the tadpoles twice a week with a mixture of ground rabbit chow and fish food. I adjusted the amount of food in each pool to changes in tadpole densities, which were caused by metamorphosing individuals and mortality.

Sampling morphology, behavior, life-history and survival data

I measured morphology on each sampling day (18, 26, 33, 40 and 49) by randomly selecting five tadpoles from each pool and photographing them in lateral and ventral view. I present only results from day 18 here. Results from day 26 were similar, but the data from later samples were unusable because tadpoles in high-resource treatments began entering metamorphic climax. I weighed the tadpoles (mass at day 18 presented) and promptly returned them to the pool. I used image analysis software (Optimas 6.5, Media Cybernetics 1999) to measure tail depth and estimate body size as the centroid size calculated from 26 landmarks positioned in three-dimensional space (Bookstein 1991, Electronic Supplementary Material S1). I obtained size-corrected tail depth by regressing the tail depth for all measured tadpoles against body size and the square of body size. Using size corrected residuals can bias results (Darlington and Smulders 2001; Garcia-Berthou 2001; Freckleton 2002). Using an ANCOVA with body size and the square of body size did not alter the results (ANCOVA results are not reported). For ease of graphical display (Fig. 1), I used size corrected residuals in all analysis on tail depth. I could not measure morphology and wet weight in two and one pool respectively, due to technical problems.

I recorded behavior data by instantaneously sampling the activity of the visible tadpoles as swimming, feeding or resting. Feeding behavior consists of scraping algae (mouth movement) at the pool walls and bottom, often accompanied by a characteristic tail waggle. Swimming was all other movement. I recorded the data by visiting each pool four times over a three and a half hour period on day 22 one day after I fed the dragonflies and the same day I fed the tadpoles. Most tadpoles were visible: only 4.6% were hiding.

Given that all tadpoles entered the experiment at the same age and date, I used time to metamorphosis as a measure of developmental rate. I removed tadpoles that reached the four-

emerged-limbs stage (Gosner stage 42) from the pools and kept them in tilted boxes with little water until the tail was absorbed (stage 46). I noted the date when metamorphosis was completed (Gosner stage 46). I checked the pools and boxes for metamorphs at least every second day. I noted tadpoles (17.7%) that did not reach the four-emerged-limbs stage by the termination of the experiment (day 54) as metamorphosed on day 55. This is a conservative way of analysis, because those 17.7% of the tadpoles were noted as metamorphosed on (theoretically) the earliest possible date.

I noted tadpoles that reached the four-emerged-limbs stage (Gosner stage 42) by day 54 as survivors. Non-surviving tadpoles were tadpoles that disappeared or died before day 54, and those 17.7% that did not reach the four-emerged-limbs stage by day 54. Tadpoles that have not metamorphosed that late in the season have low survival probabilities (Altwegg and Reyer 2003). To ascertain that my definition of survival for the tadpoles that did not reach the four-emerged-limbs stage had no substantial effect on the results, I reanalyzed the survival data excluding these tadpoles. Results changed only slightly (mixed model as described in statistical analyses, with survival as response variable: logarithm of food $F_{1,88} = 32.55$, $p < 0.0001$; predation $F_{1,88} = 11.38$, $p = 0.0011$; logarithm of food by predation interaction $F_{1,88} = 14.25$, $p = 0.0003$).

Statistical analyses

I performed two tests for each trait. First, I tested the overall effects of predators, temperature and food and possible interactions on the six traits with a mixed model (proc mixed, type III SS, SAS 9.1, SAS Institute 2002) including block as random factor, predator and temperature as categorical fixed factors, and the logarithm of food levels as continuous fixed factor. I defined each pool as independent unit. For all traits I used the pool means in the

analysis. I arcsine-square-root transformed survival and behavioral ratios for all analysis (allowing values >1 , Fig. 1f). I accounted for multiple testing (six traits) with Bonferroni-corrections. I used the logarithm of food availability in all analyses, because I believe that a doubling of the amount of food is biologically more meaningful to an individual than increasing the amount of food by some units (mg). I describe only the main effects of food and predators for this first test, because interactions between food and predators are the object of the second test. For temperature, I only describe significant main effects and significant interactions between temperature and food. All interactions between temperature and predators, and temperatures, predators and food were non-significant (see Table 1).

The second test describes the shape of the predator-induced plasticity along the resource gradient. Predator-induced plasticity was the absolute difference between the no-predator and non-lethal predator treatment for the different traits (calculated for each treatment combination within each block). I used absolute plasticity values to have a direct scale unit with the exception of tail depth, which was corrected for body size. No trait response curve was fundamentally altered nor does the interpretation of the results change when I analyze relative values. To describe the shape of the predator-induced plasticity along the resource availability gradient, I used model selection, based on Akaike's Information Criterion of small samples (AICc), on three candidate models. I assessed the support for each model for each trait separately. The three candidate models included a) an intercept-only model, which describes no change in predator-induced plasticity along the resource gradient (control model), b) a linear term for the resource availability effect (simple allocation model and defense-growth model), and c) a linear and quadratic term for the resource availability treatment (growth-differentiation model). I calculated the Akaike weight and evidence ratio to determine how much better the best fitting model was

supported in comparison to the other models (Burnham and Anderson 2002). I used model selection and not a second-order model, because I was interested in the overall support for the model and did not want to test the significance of each single parameter in the model.

RESULTS

Predator-exposed tadpoles had 11.6 % deeper tails than predator-naïve tadpoles (Fig. 1a, Table 1). Predator-induced plasticity in tail depth, the difference between predator-exposed and predator-naïve tadpoles, which I use to measure investment in defense, did not change with increasing resource availability (Fig. 1g, Table 2).

Behavior responded strongly to predators (Table 1), but only swimming activity changed with resource availability disregarding predation risk (Fig. 1b & c, Table 1). Feeding activity decreased in response to predator-exposure by 34%. Swimming activity decreased in response to predators by 72%, and with increasing resource availability it increased by 39%. The predator-induced plasticity in feeding activity was greatest at intermediate food levels and was therefore best explained by a curvilinear relationship (Fig. 1h, Table 2), while the predator-induced plasticity in swimming activity did not change with increasing resource availability and was best explained by an intercept-only model (Fig. 1i, Table 2).

Time to metamorphosis was affected by resource availability and predation risk (Fig. 1d, Table 1). At high resource availability tadpoles metamorphosed about 8 days earlier than at low resource availability and predator-naïve tadpoles metamorphosed about two days earlier than predator-exposed tadpoles. Temperature affected the time to metamorphosis only at high resource availability but not at low resource availability (Fig. 1d, Table 1). Predator-induced

plasticity in time to metamorphosis increased along the resource gradient and was best described by a linear relationship (Fig. 1j, note negative values; Table 2).

Tadpole mass responded strongly to resource availability (Fig. 1e, Table 1). At high resource availability tadpoles weighed three times more than at low resource availability. Predator exposure affected tadpole mass only at the highest resource availability with predator-naïve tadpoles showing higher masses (Fig. 1e). The predator-induced plasticity in body mass along the resource gradient was best explained by a curvilinear relationship (Table 2).

Survival increased with increasing resource availability and was reduced under predation risk (Fig. 1f, Table 1). At high resource availability survival was almost three times higher than at low resource availability, and 11% more (non-lethal) predator-exposed tadpoles died than predator-naïve ones. The survival advantage of predator-naïve tadpoles (“predator-induced plasticity in survival”) decreased with increasing resource availability (Fig. 1f) and was best explained by a linear relationship (Table 2).

DISCUSSION

The tadpoles in the experiment exhibited the previously described responses to resource availability and predation risk (Skelly and Werner 1990; Skelly 1992; Anholt and Werner 1995; McCollum and VanBuskirk 1996; Werner and Anholt 1996; Laurila et al. 1998; Van Buskirk and Yurewicz 1998; Laurila and Kujasalo 1999; Anholt et al. 2000; Van Buskirk 2000; Peacor 2002; LaFiandra and Babbitt 2004; Relyea 2004). Under limited resource availability, they showed reduced mass, reduced developmental rate and had lower survival. Under predation risk, defenses were expressed by increased tail depth and reduced feeding and swimming activity. Costs of defense were mostly expressed in a reduced developmental rate and reduced survival.

The costs of defense shifted along the resource gradient. At low resource availability, defense costs were not paid by a reduction in development rate or reduced mass, but the consequence for defense was reduced survival. Reduced survival probabilities in response to (non-lethal) predators at low resources, have been found in other studies (Peacor 2002). At high resource availability, the costs of defense were paid by a reduction in the development rate without a reduction in survival, in agreement with previous work (Skelly 1992; LaFiandra and Babbitt 2004). Costs of defense were also expressed in reduced mass in predator-exposed tadpoles, but only at the highest resource level. Most previous studies were not designed to detect shifts in costs along resource gradients, because they either did not manipulate resources or did not investigate costs in multiple traits. The reported shift in costs along the resource gradient indicates that survival and development are mechanically or genetically linked. The shift in costs is likely linked to shifts in the time and resource allocation trade-offs along the resource gradient. Under low resources the time to reach metamorphosis before the end of the growing season is very constrained (Steiner and Pfeiffer in press). Many resources should be devoted to reach a threshold developmental rate and no resources are available to pay for cost of defense. The consequence of costs of defense is then reduced survival. Reduced survival is most likely a cumulative result of numerous effect pathways and reduced allocation to maintenance and should be closely linked to resource allocation tradeoffs. Under high resources, time allocation trade-offs are relaxed, because reaching metamorphosis before the end of the growing season is easily achieved. Therefore, costs of defense are paid by a reduction in development rate and not by a reduction in allocation to maintenance, resulting in similar survival between predator exposed and non-exposed tadpoles.

I expected a strong correlation between investment in defense and cost of defense. At each resource availability defense and costs of defense were expressed. However, in contrast to the expectation investment in defense and cost of defense were not strongly correlated. Predation risk had a more severe effect on defensive traits than resource availability, shown by strong predator-induced plasticity but only weak resource-induced plasticity (responses to resource availability). The opposite pattern was found in non-adaptive traits. The enhanced resource-induced plasticity in the non-adaptive traits indicates that they were under a stricter rule of resource allocation trade-offs, while defensive traits were strictly ruled by the predation risk. This difference in dominance explains why there was no strong correlation between investment in defense and costs of defense.

Defensive traits have evolved in response to predation risk and therefore should act more specific in response to predation risk, as found in my study. Non-adaptive traits, traits that are related to fitness but do not reduce predation, should have evolved in response to many environmental factors including resource availability. Selection should act to reduce cost of defense, i.e. weak responses to predation risk in non-adaptive traits, but strong responses to predation risk in defensive traits are selected for, resulting in the observed pattern of difference in dominance. Defensive traits respond more specific to predation risk compared to non-adaptive traits and show less variability in their response (less interactive effects Table 1 and Table 2). One could conclude that defensive traits show less variability than non-adaptive traits. However, there is a limitation to this conclusion, because most defenses investigated in zoological systems are behavioral or morphological trait responses, whereas traits where costs of defense are expressed are often life-history traits. Hence, we need systems with life-history defensive traits

and morphological and behavioral non-adaptive traits, e.g. systems where development time is reduced under predation risk.

I expected in accordance with the models outlined in the introduction an interaction between resource allocation and predation risk in defensive traits. The lack of this interaction in most defensive traits might be due to the high predation risk level in the experiment. If predation risk dominates over resource availability, individuals should express their maximal defense regardless of the resource availability and the costs of defense. The maximal defense is limited by the maximal phenotypic plasticity, which is genetically determined. I think that defense in tail depth and swimming activity was expressed at their maximum across the resource gradient, which explains why none of the models outlined in the introduction was supported by these traits. Some support for this explanation comes from studies that show that defense is limited and levels off when predation risk continues to increase above a certain threshold (Van Buskirk and Arioli 2002; Relyea 2004; Teplitsky et al. 2005). Support for my findings and disagreement with the theoretical models comes from studies that manipulated resource availability. All of them failed to find a significant interaction between food level and predator effect in defensive traits (Skelly and Werner 1990; Anholt and Werner 1995; Laurila and Kujasalo 1999; Anholt et al. 2000; Peacor 2002; LaFiandra and Babbitt 2004). All of these studies used high levels of predation risk. However, studies which manipulated tadpole densities found an interaction between density and predator effect in defenses as proposed by the simple allocation model (Werner and Anholt 1993; Relyea 2004). The differences in results between the two groups of studies suggest that competition effects cannot be viewed as equivalent to resource manipulation effects, because conspecific densities might change the abundance of cues in the environment,

change the relative predation risk or might affect resource availabilities in non-expected ways (Peacor 2002; Peacor 2003).

However, one defense trait was affected by resource levels, suggesting that the dominance of the predation risk in defensive traits was not absolute. Feeding activity agreed in its investment in defense (predator-induced plasticity) with one of the theoretical models, the growth-differentiation model. At low resource availability the feeding activity was dominated by acquiring the scarce resources and no strong response to predation risk was expressed, which has been described before (Werner and Anholt 1996; Van Buskirk and Yurewicz 1998; Relyea 2004). At high resource availability the expressed strategy of predator induced-tadpoles was to spent as much time feeding as the predator-naïve tadpoles. One explanation could be that at high resource availability tadpoles escaped predation by reaching a size threshold as outlined in the simple allocation model. Though, we would expect a similar reduction in investment in defense for the swimming activity. Predator exposed tadpoles might also be willing to take a higher risk of foraging at high resource availability for unknown reasons. If we accept that defense is expressed in reduced feeding activity and that feeding activity is optimized to maximize fitness, we have to conclude that a reduction in feeding activity under predation risk was only beneficial at intermediate resource availability, i.e. investment in defense was only made at intermediate resource availability. I expected that high investment in defense at intermediate resources would evoke high costs at intermediate resources, which was not the case. This shows that defense and their costs are not closely linked.

Empirical data on interactions between resource allocation and predation risk in defensive traits in other systems are rare. In *Daphnia* head length a peak investment in defense at intermediate resources has been described (Barry 1995). The best empirical data comes from

plant-herbivore systems and induced chemical defenses, but in many of these studies it is difficult to distinguish between defense (adaptive responses) and cost of defense (non-adaptive responses) (Karban and Baldwin 1997; Agrawal et al. 2002). We need studies that investigate effects across various resource levels and various predation risk levels.

The difference between the experienced environment of predator-induced and predator-naïve tadpoles in the experiment likely covers the extremes observed in nature (Van Buskirk and Arioli 2002). We know that predator densities vary substantially in natural ponds (e.g. low predator densities in temporal ponds and high predator densities in permanent ponds). We also know that tadpole survival in natural ponds is low (5-7%) and assume that predation is the major cause (Riis 1991). However, accurately estimating realized predation rates or quantifying chemical cues (kairomones) in nature is difficult (Van Buskirk 2005; Van Buskirk and Arioli 2005). The variation of resource availability used in the experiment is also likely to be found in nature. There are natural ponds with *ad libitum* food. When we consider that some *R. temporaria* lay their clutches with thousands of eggs in temperate ruts, it becomes clear that resource availability in nature can be very low. Conducting experiments at the extremes (high predation risk, *ad libitum* food) might be problematic and can reveal different defense patterns than observed in experiments done under intermediate conditions (Steiner and Pfeiffer in press).

Not all studies exploring costs of defenses in tadpoles and other systems are consistent in their findings. Opposite patterns in growth rates, size at metamorphosis, and survival in response to (non-lethal) predators were found (Werner and Anholt 1996; DeWitt et al. 1999; Van Buskirk 2002; Benard 2004; Hoverman et al. 2005). Some of this variation might be due to different adaptation to various predator types and different defense strategies of prey species (Laurila et al. 1998; Hoverman et al. 2005). Some variation might also be explained by differences in resource

availability. If only one of the non-adaptive traits would have been assessed in this study, the conclusion would have changed. There would be reduced (no) costs of defense at low or high resource availabilities respectively, despite equal amount of defenses across the whole range of the resource gradient. It is important to assess costs and benefits of defense in multiple potentially interacted traits.

The predicted correlation between defense and cost of defense could not be shown in this study. For a better understanding of the origin of the discrepancy between theories and empirical data, we need a better knowledge of effect pathways linking defense and costs of defense. For instance, the widely assumed link between feeding activity, resource acquisition and conversion of acquired food in body mass (growth) has been shown to be unclear in tadpoles and a number of damselfly larvae (McPeck 2004; Steiner 2005). Improved knowledge of effect pathways will allow us to develop allocation models in close proximity to empirical data, which include intrinsic costs that can explain the linkage of defenses and associated costs (Yearsley et al. 2002). A better understanding about mechanistic integration and genetic linkage of multiple traits is important for predictions about adaptation to various environments which has implications for population dynamics, adaptation to changing environments and community dynamics. My study shows, that non-adaptive traits, where costs of defenses are expressed are mechanistically or genetically linked (shift in cost along the resource gradient), but that defenses and their costs are largely independent of each other. We are lacking a good understanding of the evolution of complex traits and more studies on (predator-) induced plasticity in various systems along environmental gradients such as resource and predation risk, spanning over multiple behavioral, physiological, morphological and life-historical traits, could likely fill this gap.

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REFERENCES

- Agrawal AA, Conner JK, Johnson MTJ, Wallsgrove R (2002) Ecological genetics of an induced plant defense against herbivores: Additive genetic variance and costs of phenotypic plasticity. *Evolution* 56:2206-2213
- Altwegg R, Reyer HU (2003) Patterns of natural selection on size at metamorphosis in water frogs. *Evolution* 57:872-882
- Angilletta MJ, Wilson RS, Navas CA, James RS (2003) Tradeoffs and the evolution of thermal reaction norms. *Trends Ecol Evol* 18:234-240
- Anholt BR, Werner EE (1995) Interaction between food availability and predation mortality mediated by adaptive-behavior. *Ecology* 76:2230-2234
- Anholt BR, Werner E, Skelly DK (2000) Effect of food and predators on the activity of four larval ranid frogs. *Ecology* 81:3509-3521
- Arendt JD (1997) Adaptive intrinsic growth rates: An integration across taxa. *Quart Rev Biol* 72:149-177
- Barry MJ (1995) The role of nutrition in regulation of predator-induced crests of *Daphnia-carinata*. *Freshw Biol* 34:229-239
- Benard MF (2004) Predator-induced phenotypic plasticity in organisms with complex life histories. *Ann Rev Ecol Evol System* 35:651-673
- Bookstein FL (1991) *Morphometric tools for landmark data: geometry and biology*. Cambridge University Press, Cambridge
- Burnham KP, Anderson DR (2002) *Model selection and multimodel inference: a practical information-theoretic approach*. Springer, New York

- Darlington RB, Smulders TV (2001) Problems with residual analysis. *Anim Behav* 62:599-602
- DeWitt TJ, Sih A, Hucko JA (1999) Trait compensation and cospecialization in a freshwater snail: size, shape and antipredator behaviour. *Anim Behav* 58:397-407
- Freckleton R (2002) On the misuse of residuals in ecology: regression of residuals vs. multiple regression. (vol 71, pg 542, 2002). *J Anim Ecol* 71:722-722
- Garcia-Berthou E (2001) On the misuse of residuals in ecology: testing regression residuals vs. the analysis of covariance. *J Anim Ecol* 70:708-711
- Gomulkiewicz R, Kirkpatrick M (1992) Quantitative genetics and the evolution of reaction norms. *Evolution* 46:390-411
- Gosner KL (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16:183-190
- Harvell CD (1990) The ecology and evolution of inducible defenses. *Quart Rev Biol* 65:323-340
- Harms DA, Mattson WJ (1992) The dilemma of plants - to grow or defend. *Quart Rev Biol* 67:283-335
- Hoverman JT, Auld JR, Relyea RA (2005) Putting prey back together again: integrating predator-induced behavior, morphology, and life history. *Oecologia* 144:481-491
- Karban R, Baldwin IT (1997) *Induced responses to herbivory*. University of Chicago Press, Chicago
- Kishida O, Nishimura K (2004) Bulgy tadpoles: inducible defense morph. *Oecologia* 140:414-421
- LaFiandra EM, Babbitt KJ (2004) Predator induced phenotypic plasticity in the pinewoods tree frog, *Hyla femoralis*: necessary cues and the cost of development. *Oecologia* 138:350-359

- Laurila A, Kujasalo J (1999) Habitat duration, predation risk and phenotypic plasticity in common frog (*Rana temporaria*) tadpoles. *J Anim Ecol* 68:1123-1132
- Laurila A, Kujasalo J, Ranta E (1998) Predator-induced changes in life history in two anuran tadpoles: Effects of predator diet. *Oikos* 83:307-317
- Lima SL (1998) Stress and decision making under the risk of predation: Recent developments from behavioral, reproductive, and ecological perspectives. *Adv Stud Behav* 27:215-290
- McCollum SA, VanBuskirk J (1996) Costs and benefits of a predator-induced polyphenism in the gray treefrog *Hyla chrysoscelis*. *Evolution* 50:583-593
- McPeck MA (2004) The growth/predation risk trade-off: So what is the mechanism? *Am Nat* 163:E88-E111
- Myers JH, Bazely D (1991) Thorns, spines, prickles, and hairs: Are they stimulated by herbivory and do they deter herbivores? In: Tallamy DW, Raupp MJ (eds) *Phytochemical induction by herbivores*. John Wiley & Sons, New York, pp 325-344
- Peacor SD (2002) Positive effect of predators on prey growth rate through induced modifications of prey behaviour. *Ecol Lett* 5:77-85
- Peacor SD (2003) Phenotypic modifications to conspecific density arising from predation risk assessment. *Oikos* 100:409-415
- Relyea RA (2002) Costs of phenotypic plasticity. *Am Nat* 159:272-282
- Relyea RA (2004) Fine-tuned phenotypes: Tadpole plasticity under 16 combinations of predators and competitors. *Ecology* 85:172-179
- Riis N (1991) A field study of survival, growth biomass and temperature dependence of *Rana dalmatina* and *Rana temporaria* larvae. *Amphibia-Reptilia* 12:229-243

- Skelly DK (1992) Field evidence for a cost of behavioral antipredator response in a larval amphibian. *Ecology* 73:704-708
- Skelly DK, Werner EE (1990) Behavioral and life-historical responses of larval american toads to an odonate predator. *Ecology* 71:2313-2322
- Stearns SC (1992) *The evolution of life-histories*. Oxford University Press, Oxford
- Steiner UK (2005) Cost of predator-induced plasticity and cost of responding to predators in tadpoles. Mathematisch-naturwissenschaftliche Fakultät, PhD. Universität Zürich, Zürich
- Steiner UK, Pfeiffer T (in press) Optimizing time and resource allocation trade-offs for investment into morphological and behavioral defense. *Am Nat*
- Teplitsky C, Plenet S, Joly P (2005) Costs and limits of dosage response to predation risk: to what extent can tadpoles invest in anti-predator morphology? *Oecologia* 145:364-370
- Tollrian R, Harvell CD (1992) *The ecology and evolution of inducible defenses*. Princeton University Press, Princeton
- Tuomi J, Fagerstrom T, Niemela P (1991) Carbon allocation, phenotypic plasticity, and induced defenses. In: Tallamy DW, Raupp MJ (eds) *Phytochemical induction by herbivores*. John Wiley & Sons, New York, pp 85-104
- Van Buskirk J (2000) The costs of an inducible defense in anuran larvae. *Ecology* 81:2813-2821
- Van Buskirk J (2002) A comparative test of the adaptive plasticity hypothesis: Relationships between habitat and phenotype in anuran larvae. *Am Nat* 160:87-102
- Van Buskirk J (2005) Local and landscape influence on amphibian occurrence and abundance. *Ecology* 86:1936-1947
- Van Buskirk J, Yurewicz KL (1998) Effects of predators on prey growth rate: relative contributions of thinning and reduced activity. *Oikos* 82:20-28

- Van Buskirk J, McCollum SA (2000) Functional mechanisms of an inducible defence in tadpoles: morphology and behaviour influence mortality risk from predation. *J Evol Biol* 13:336-347
- Van Buskirk J, Arioli M (2002) Dosage response of an induced defense: How sensitive are tadpoles to predation risk? *Ecology* 83:1580-1585
- Van Buskirk J, Arioli M (2005) Habitat specialization and adaptive phenotypic divergence of anuran populations. *J Evol Biol* 18:596-608
- Werner EE, Anholt BR (1993) Ecological consequences of the trade-off between growth and mortality-rates mediated by foraging activity. *Am Nat* 142:242-272
- Werner EE, Anholt BR (1996) Predator-induced behavioral indirect effects: Consequences to competitive interactions in anuran larvae. *Ecology* 77:157-169
- Yearsley J, Hastings IM, Gordon IJ, Kyriazakis I, Illius AW (2002) A lifetime perspective on foraging and mortality. *J Theor Biol* 215:385-397

TABLES

Table 1: Relationship between predator exposure and temperature differences along a resource gradient (logarithm of food) for six different traits. Mixed effect models are presented with block as random factor. The interaction between the logarithm of food and predator exposure is investigated in more detail in the results presented in Table 2. Reported degrees of freedom account for all tests except the tests for block effects (random factor).

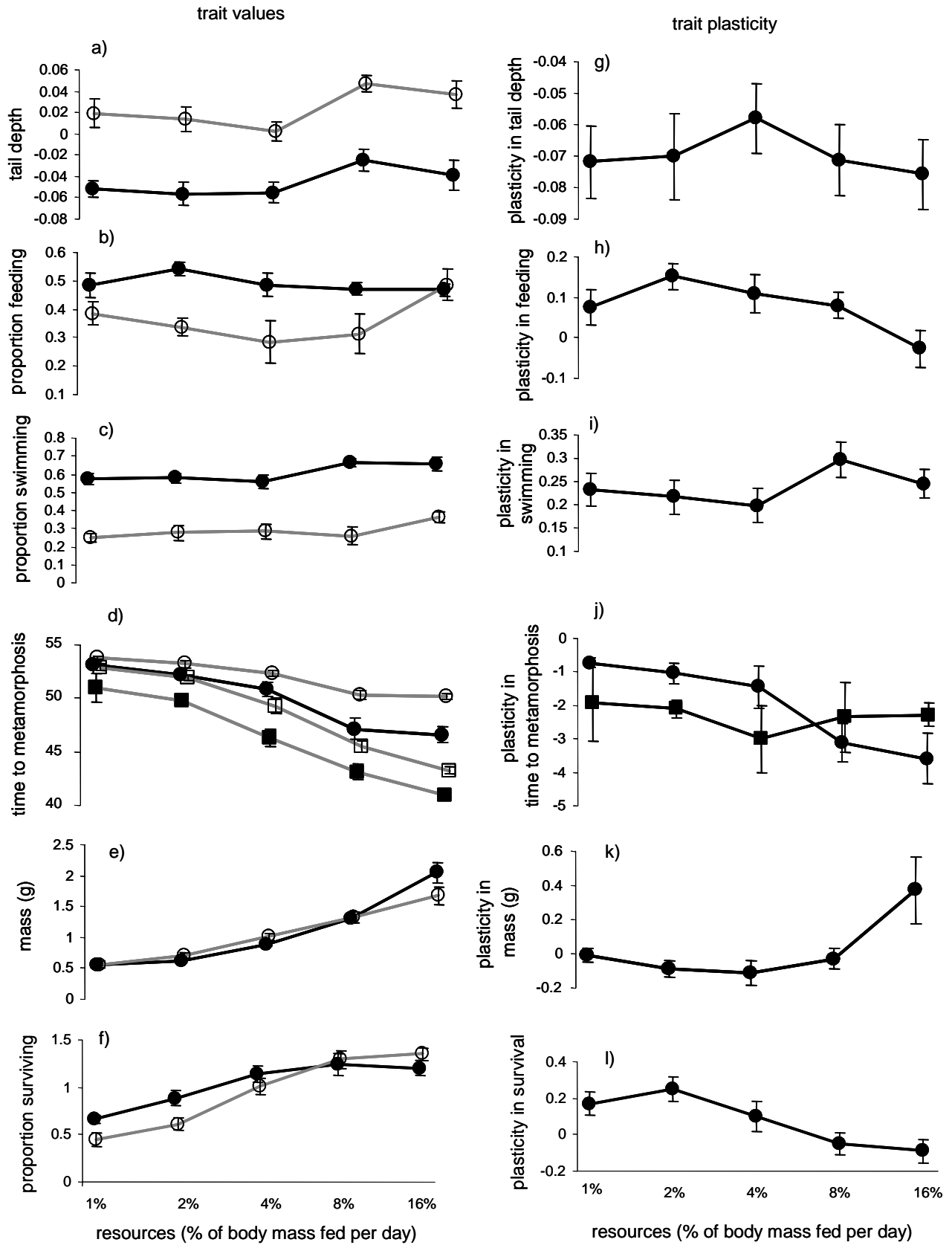
	Tail depth	Feeding activity	Swimming activity	Time to metamorphosis	Mass	Survival
	F _{1,86}	F _{1,88}	F _{1,88}	F _{1,88}	F _{1,87}	F _{1,88}
Block	0	0.49	0	0.79	1.08	0
Predator	33.93*	13.9*	74.5*	8.58*	2.47	11.87*
Temperature	1.3	1.03	0.56	6.81	0.06	1.7
Predator*Temperature	0.3	0.77	0	3.85	0.35	0.03
Logfood	7.26	0.13	9.58*	511.13*	318.3*	121.66*
Logfood*Predator	0.04	1.89	0.08	6.03	5.16	9.22*
Logfood*Temperature	2.3	0.17	0.41	46.67*	3.78	1.81
Logfood*Predator*Temperature	0.18	0.19	0.61	3.68	1.06	0.09

F values in boldface indicate significance prior to Bonferroni-corrections ($p < 0.05$). *Significance after Bonferroni-corrections for 6 tests ($p < 0.0083$)

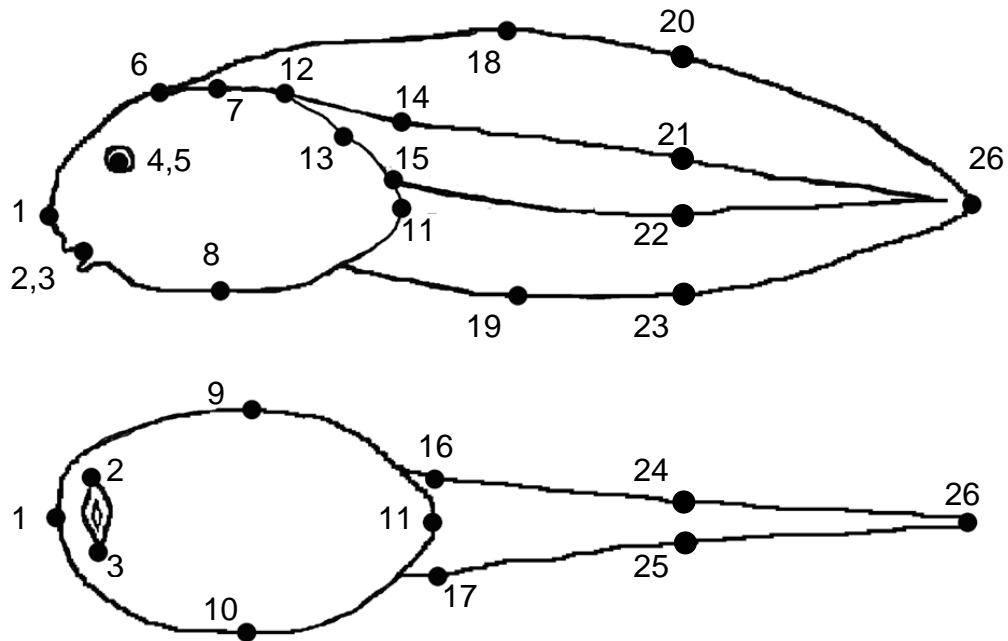
Table 2: Model selection procedure to evaluate support for three candidate models including an intercept-only, a linear (intercept + logfood) or non-linear (intercept + logfood + logfood²) relationship between predator-induced plasticity and resource availability for six different traits. The AICc and Akaike weight of the best supported model is boldfaced. The evidence ratio 1 describes how much better the best fitting model is supported in comparison to the second best model and evidence ratio 2 describes how much better the best fitting model is supported in comparison with the least supported candidate model.

	<u>intercept only</u>		<u>intercept + logfood</u>		<u>intercept + logfood</u>		Evidence ratio 1	Evidence ratio 2
	AICc	Akaike weight	AICc	Akaike weight	AICc	Akaike weight		
Tail depth	-181.7	0.9872	-177.2	0.0110	-175.4	0.0018	90.0	544.6
Feeding	-50.4	0.0259	-50.4	0.0259	-54.0	0.9482	36.6	36.6
Swimming	-71.3	0.9786	-67.4	0.0198	-64.9	0.0016	49.4	601.8
Time to metamorphosis	198.5	0.0003	190.5	0.9238	193.0	0.0758	12.2	2980.9
Mass	45.3	0.0001	42.8	0.0012	36.1	0.9987	812.4	9897.1
Survival	7.2	0.0003	-0.8	0.8453	0.9	0.1544	5.5	2980.9

Fig. 1: a-f) Expression of six traits in predator-naïve (filled symbols) and (non-lethal) predator-exposed (open symbols) *R. temporaria* tadpoles in response to increasing resource availability. For time to metamorphosis (d and j), circles indicate cold temperatures and squares warm temperatures. g-l) Predator-induced plasticity in six traits in response to increasing resource availability. Symbols show means \pm SE of five replicates. Predator-induced plasticity was measured as the difference in the trait expression between predator-naïve and predator-exposed tadpoles (note the negative values on y-axis for g & j). Survival, feeding and swimming activity were arc-sine square root transformed (allowing values >1). Tail depth values were body size corrected. With the exception of time to metamorphosis, for all traits shown in this figure there was no significant effect of temperature on the response variables. I pooled results from the two temperature treatments in the graphs to simplify the graphical illustration of all the traits except for time to metamorphosis.



Appendix A. Location of landmarks for digitizing anurans



The upper image is a view of left side of the tadpole.

The lower image is a view of the bottom of tadpole, reversed by a mirror so that the right side is above and left side below.

Landmark	Description
1	tip of nose
2	right edge of mouth
3	left edge of mouth
4	right eye
5	left eye
6	point at which fin meets dorsal surface of head
7	deepest part of body: top edge
8	deepest part of body: bottom edge
9	widest part of body: right edge
10	widest part of body: left edge
11	back of body
12	point at which top of tail muscle meets body
13	where notochord meets the body
14	top of tail muscle at deepest point
15	bottom of tail muscle at deepest point, (line between 14 and 15 runs perpendicular to the long axis of the tail muscle)
16	right side of tail muscle at widest point

- 17 left side of tail muscle at widest point
- 18 top of tail fin at deepest point
- 19 bottom of tail fin at deepest point
- 20 top of tail fin at half-way point
- 21 top of tail muscle at halfway point
- 22 bottom of tail muscle at halfway point
- 23 bottom of tail fin at halfway point
- 24 right side tail muscle at halfway point
- 25 left side tail muscle at halfway point
- 26 tip of tail

Centroid size was calculated based on 1 to 13, i.e. it excluded the tail measurements

Tail depth = 18 - 19

Tail length = 26 - 13

Tail muscle depth = 14 - 15

Tail muscle width = 16 - 17

Body length = 11 - 1

Body depth = 7 - 8

Body width = 9 - 10