Research

Interactions between the information content of different chemical cues affect induced defences in tadpoles

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Animals often alter their behaviour, morphology and physiology in the presence of predators. These induced defences can be fine-tuned by a variety of environmental factors such as predator species, acute predation risk or food availability. It has, however, remained unclear what cues influence the extent and quality of induced defences and how the information content of these cues interact to determine the development of antipredator defences. We performed an experiment to study the significance of direct chemical cues, originating from the predators themselves, and indirect cues, released by attacked or consumed prey, for phenotypic responses in *Rana dalmatina* tadpoles. We reared tadpoles in the presence of caged predators (*Triturus vulgaris*, *Aeshna cyanea*) fed either one or three tadpoles every other day outside the tadpole-rearing tanks. Fifteen hours after food provisioning, predators were put back into the tanks containing focal tadpoles either after washing (direct + digestion-released cues) or with the water containing remnants of the prey (direct + all types of indirect cues). Our results suggest that direct cues together with digestion-released cues can be sufficient to induce strong antipredator responses. Induced defences depended on both direct cues, affecting predator-specific responses, and the quantity of indirect cues, resulting in graded responses to differences in predation threat. Moreover, direct and indirect cues interacted in behaviour, resulting in predator-specific graded responses. We also observed a decrease in the extent of predator-induced responses in large tadpoles as compared to small ones. Our results, thus, suggest that prey integrate multiple cues about predators to optimize induced defences and that this process changes during ontogeny.

Life-history theory suggests that predator-induced defences need to be adjusted carefully, since expression of these plastic defences should be costly, and plastic responses that are accurately adjusted should deliver the largest net benefits (Werner 1986, Houston et al. 1993, Werner and Anholt 1993, Lima and Bednekoff 1999, Urban 2007a). Empirical work suggests that fine-tuned antipredator-responses are taxonomically widespread (e.g. protists: Kusch 1995, fishes: Kusch et al. 2004, arthropods: Laforsch et al. 2004, molluscs: Freeman et al. 2009). For example, tadpoles have been shown to adjust defences according to the predator species (Relyea 2001), the type and quantity of prey eaten by predators (Laurila et al. 1998, Schoeppner and Relyea 2005), or to predator density (Van Buskirk and Arioli 2002). Responses to predators may on the other hand be constrained by environmental factors (e.g. presence of competitors: Relyea 2004, pH-level: Teplitsky et al. 2007), and depend on the intrinsic state of the individual (e.g. body size: Fraker 2008).

Despite a growing body of research concerned with predator-induced defences, relatively little is known about the cues that are proximately used by prey species to adjust their phenotypic responses. While tactile and visual cues may sometimes deliver important information (Moore et al. 2004, Rot-Nikcevic

et al. 2005), predator-induced defences are usually triggered by chemosensory information in aquatic environments (Tollrian and Harvell 1999). For example, prey may respond to (1) chemical cues passively released from injured prey tissue ('damage-released cues'; Chivers and Smith 1998), (2) general prey metabolites excreted at an increased rate into the environment during stress-response to the predator ('no-cost disturbance signals'; Kiesecker et al. 1999), (3) special disturbance cues that are costly to synthesize and are released actively by prey upon attack by a predator ('alarm pheromones'; Fraker et al. 2009), (4) constituents of prey tissue released through digestion by predators ('digestion-released cues'; LaFiandra and Babbitt 2004), and (5) cues originating directly from the predator unrelated to its recent feeding history ('kairomones'; Petranka and Hayes 1998).

Kairomones deliver information about the predator directly, so these are often referred to as a type of direct cues, whereas cues originating from disturbed, attacked or digested prey indirectly deliver information about the presence of a predator, so these are often referred to as indirect cues. Direct cues may play an important role in the development of fine-tuned responses, as they may allow identifying the predator species, however, the quantity and type of indirect cues may

also deliver important information to prey, especially on the acute predation risk. While direct or indirect cues alone can induce defensive behavioural responses in tadpoles (Petranka and Hayes 1998, Fraker et al. 2009), the presence of all types of chemical cues may be required to develop the full suite and magnitude of morphological defences (Schoeppner and Relyea 2005, 2009). One explanation for this may be that the information content of different types of cues can interact in several ways. Interactions between the information content of different types of cues may be synergistic, complementary or conflicting. For example, kairomones indicating a dangerous predator and the simultaneous presence of large quantities of indirect cues may enhance responses. Also, the same quantity of damage-released cues may indicate very different predation risks in the presence of a chewing or a swallowing predator. Finally, large quantities of indirect cues normally induce lowered activity that can lead to lowered ingestion rates, but in the presence of a gape limited predator, it may pay off to increase activity to enhance growth (Urban 2007a, 2007b). Nonetheless, we currently know very little about the significance of the interactions between the information delivered by the different types of chemical cues.

In the present study, we aimed at disentangling the importance of direct and indirect cues for phenotypic responses in agile frog *Rana dalmatina* tadpoles. More importantly, we studied the effects of interactions between the information content of direct and indirect cues on antipredator responses. We used a gape-limited, actively foraging predator, which swallows its prey whole (the newt *Triturus vulgaris*), and a chewing, sit-and-wait predator (larvae of the dragonfly *Aeshna cyanea*). Due to the differences in feeding mechanism and foraging mode of these predators, we predicted a larger decrease in activity and smaller body sizes in the presence of dragonfly larvae as compared to the presence of newts, arising due to a conflicting interaction between the information delivered by direct and indirect cues. By raising tadpoles in the absence of predators and in the presence of predators receiving low or high food levels, we tested the prediction that the presence of direct cues and increasing quantities of indirect cues induce graded defensive responses, resulting from synergistic interactions between the information content of direct and indirect cues. By including or excluding damage-released cues, no-cost disturbance signals and alarm pheromones, we tested the prediction that direct cues have to be complemented by indirect cues in the presence of the gape-limited predator to induce continually high levels of plastic responses. Finally, by sampling behaviour and morphology of tadpoles repeatedly, we also tested the prediction that, in a stable environment, induced defences remain qualitatively similar during ontogeny.

Methods

Tadpoles of *Rana dalmatina* can be found in most of southern and middle continental Europe (Gasc et al. 1997) in a wide array of temporary and semi-permanent ponds (Nöllert and Nöllert 1992). They co-occur with a variety of invertebrate and vertebrate predators and respond to their presence both morphologically and behaviourally (Lardner 2000, Teplitsky et al. 2004, 2005a, 2005b).

We applied a $2 \times 2 \times 2$ factorial randomized block design with predator type (dragonfly larva or newt), predator feeding rate (low or high) and types of cues present (all cues or cue-restricted) as factors. We also had a control treatment receiving no predators. Treatments were replicated once (the control twice) in each of ten spatial blocks with the position of treatments randomized within blocks. We used 100 plastic tanks ($42 \times 25 \times 25$ cm) covered with mosquito nets as experimental containers, placed out in an open field belonging to the Plant Protection Inst. of the Hungarian Academy of Sciences located on the outskirts of Budapest. Tanks were filled one week before the start of the experiment with 16 litres of aged tap-water and were inoculated with 1 litre of pond water. We also added 2 g of rabbit pellets and 5 g of dried beech (*Fagus sylvatica*) leaves to each tank to enhance algal growth and provide nutrients and cover for tadpoles. Tanks further received a 0.75 litre transparent cup with bottom and cover made of mosquito net as predator cages. Transparency and the net covers allowed visual and chemical contact between tadpoles and predators while cages prevented predators from capturing the focal tadpoles.

In late march 2008 we collected eight freshly laid clutches of *R. dalmatina* from a pond in the Pilis Mountains, Hungary (47°42'N, 19°02'E). This pond is a semi-permanent water body completely desiccating approximately every third year, but usually after *R. dalmatina* metamorphs have left the water. It also supports permanent populations of invertebrate (*Aeshna* sp. larvae, *Notonecta* sp., *Nepa* sp., *Dytiscus* imagos and larvae) and vertebrate (*T. vulgaris*) predators. We brought egg-clutches to the field station of the Plant Protection Inst. and reared embryos in shallow dishes containing 5 litres of aged tap-water until hatching. Families of frog embryos were reared separately at this stage. We also collected 40 *T. vulgaris* males and 40 larvae of *A. cyanea* from nearby water bodies, kept them individually in 0.75 litre plastic cups and fed them two *R. dalmatina* tadpoles every other day until assigning them to experimental containers. *T. vulgaris* males and *A. cyanea* larvae are similarly voracious predators of small *R. dalmatina* tadpoles, whereas for large tadpoles dragonfly larvae appear to be more dangerous (Hettyey et al. unpubl.). Hunting efficiency of newts may decrease with increasing tadpole size due to gape-limitation, as during attempts of swallowing large prey individuals the latter can escape relatively easily, whereas dragonfly larvae are more capable of holding also large prey firmly with their labia while ingesting them piecemeal with their mandibles.

On 25 April, when tadpoles reached a free-swimming state (developmental stage 25–26; Gosner 1960), we started the experiment by randomly distributing predators into the cages hung into the rearing tanks of tadpoles and assigning 16 tadpoles, two per sib-group, to each experimental container. Resulting initial densities (one individual per litre) lie within the range that can be found under natural conditions (Hettyey unpubl.). We fed predators every other day in the following manner: We (1) removed 0.2 l of water from each rearing tank and placed 0.75 l transparent feeding cups filled with 0.2 l of aged tap water on the ground next to the rearing tanks, (2) removed the predators from their cages and placed them into the feeding cups, and (3) added *R. dalmatina* tadpoles to the feeding cups. Next morning (ca 15 h after 1, 2 and 3), we (4) removed tadpoles that were

still alive in the feeding cups and (5) poured predators back into the predator cages either together with the predation water containing remains of food items (all-cues treatment) or put back predators after rinsing and added 0.2 l of aged tap water to rearing tanks (cue-restricted treatment). In the control treatment, we poured 0.2 l of aged tap water into the rearing tanks after each feeding round. Half of the predators received two, the other half six similarly sized tadpoles at a time. After ten days, when tadpoles used as predator food grew bigger, we reduced this amount to one and three tadpoles, respectively.

Ten and 27 days after start of the experiment (5 May and 22 May), we monitored activity of tadpoles three times a day, at 12:00, 14:00 and 16:00 h. For each rearing tank we noted how many tadpoles were swimming in the water column or feeding on the walls of tanks, added these counts together and used the number of tadpoles visible as an estimate of tadpole activity. We calculated the ratio of tadpoles visible by dividing the number of tadpoles visible with the total number of tadpoles in the container. Wherever it was necessary, we corrected for spontaneous mortality in the rearing tanks by means of linear interpolation (mortality averaged 3.5% and was nowhere higher than 19%). The two dates of sampling were selected to obtain measurements for both small and large tadpoles.

On day 11 and 28 (6 May and 23 May), we haphazardly took a sample of ten tadpoles from each tank and anaesthetized them by placing them into 0.02 m/m % MS-222 (tricaine) until they became immobile. We then rinsed tadpoles with aged tap water, photographed them with a digital camera to obtain pictures on their lateral view and, after recovery, put them back into the rearing tanks they were taken from. Mortality during this procedure was very low ($< 1\%$). From the photographs, we later measured four parameters that are known to show plasticity in response to the presence of predators (Laurila et al. 2004, Teplitsky et al. 2004): body length, tail length, maximum tail muscle depth and maximum tail fin depth. Body shape measures were defined following Van Buskirk and McCollum (2000). For digital measurements, we used ImageTool 3.0. After the second sampling occasion, we terminated the experiment, transported tadpoles and predators to the Pilis Mountains and released the animals at their sites of collection.

Statistical analyses

To obtain an overall measure of body size and to be able to control for body size when analyzing body shape, we first performed a principal components analysis (PCA) for each sampling occasion separately. Bivariate correlations between body length, tail length, maximum tail muscle depth and maximum tail fin depth all showed strong positive relationships at both sampling occasions (Pearson correlation; all $r > 0.75$, all $p < 0.001$). The first component explained a large proportion of the variance (first sampling occasion: 84.7%; second sampling occasion: 82.9%) and original variables loaded strongly and positively on PC1 (all $r > 0.88$ at both sampling occasions). We used PC1 scores as measures of body size in subsequent analyses.

When analysing potential effects on body size, body shape and activity, we first performed an analysis involving all

treatments and testing for the effect of predator presence/absence. In a second step, we tested for effects of predator type, feeding rate and types of cues present. In these analyses, we had to exclude the control treatment, as feeding rate and types of cues present could not be replicated in the control.

To analyze variation in body size, we used linear mixed effect models (LMM) by entering body size as the dependent variable, rearing tank as a random factor and presence/absence of predators as a fixed factor. We further performed LMM analyses with body size as the dependent variable, predator type, feeding rate and types of cues present as fixed factors and rearing tank as a random factor.

We analyzed variation in body shape of tadpoles using multivariate general linear models (GLM) with body length, tail length, maximum tail muscle depth and maximum tail fin depth entered as dependent variables, predator presence/absence as a fixed factor and body size as a covariate. We further built GLMs with body length, tail length, maximum tail muscle depth and maximum tail fin depth entered as dependent variables, predator type, feeding rate and types of cues present as fixed factors and body size as a covariate. Analyses on body shape were based on measures averaged over individuals within rearing tanks to avoid pseudo-replication.

To investigate tadpole activity, we used a LMM with the arcsine square-root transformed ratio of tadpoles visible entered as the dependent variable, predator presence/absence, and date of sampling occasion as fixed factors and rearing tank as a random factor. Finally, we performed another LMM with activity of tadpoles entered as the dependent variable, predator type, feeding rate, types of cues present and date of sampling occasion as fixed factors and rearing tank as a random factor.

We included all possible interactions into initial models and performed backward removal of terms with $p > 0.1$ to avoid problems potentially arising due to the inclusion of non-significant terms (Engqvist 2005). We re-entered removed variables one by one to the final model to obtain relevant statistics. All tests were two tailed. Statistics were calculated using SPSS 15.0 for Windows.

Results

Size

We did not detect differences in body size between the control treatment and treatments that contained a predator either 11 (LMM; $F_{1,95.5} = 0.02$, $p = 0.89$) or 28 days ($F_{1,97.9} = 0.01$, $p = 0.91$) after start of the experiment (Fig. 1), but observed considerable variation among rearing boxes on both sampling occasions (11 d: Wald $Z = 5.58$, $p < 0.001$; 28 d: Wald $Z = 6.57$, $p < 0.001$).

When analyzing treatments containing a predator, we observed no effect of feeding rate (LMM; 11 d: $F_{1,77} = 0.04$, $p = 0.84$; 28 d: $F_{1,78} = 0.25$, $p = 0.62$) or types of cues present (day 11: $F_{1,77.2} = 0.17$, $p = 0.69$; 28 d: $F_{1,78} = 0.15$, $p = 0.7$). There was a non-significant tendency for predator type to affect tadpole size on day 11 with tadpoles being bigger in the presence of newts than in the presence of dragonfly larvae ($F_{1,77.3} = 3.39$, $p = 0.07$; Fig. 1), this tendency, however, completely diminished by the second sampling occasion
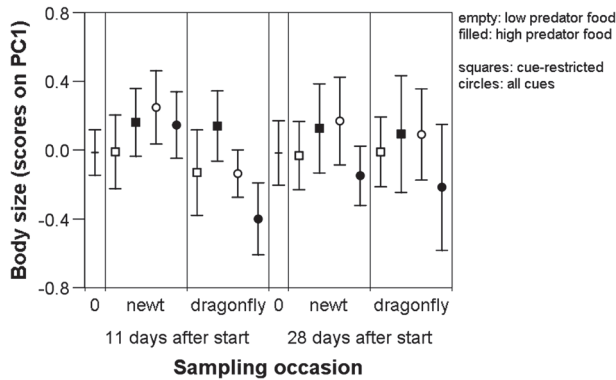
Figure 1. Body size of tadpoles (means ± SE) in the nine treatments at the two sampling occasions. Empty symbols represent treatments where predators received little food, filled symbols represent treatments where predators received much food. Squares represent cue-restricted treatments, whereas circles represent all-cue treatments. The figure is based on rearing tank-averages of factor scores on PC 1 from PCAs on body length, tail length, maximum tail muscle depth and maximum tail fin depth performed for the two sampling occasions separately. Larger values indicate larger body sizes.

($F_{1,78} = 0.05$, p = 0.83; Fig. 1). Effects of all two-way and the three-way interactions were non-significant at both sampling occasions (all p > 0.17).

Shape

Relative measures of tadpole shape differed in the presence of predators from that found in the control treatment at both sampling occasions (Table 1, Fig. 2, Appendix 1). Univariate tests revealed that in the presence of predators, the body of tadpoles was relatively shorter and tail fins were deeper on both sampling occasions, whereas tail length was not affected and the tail muscle only tended to be narrower at the first sampling occasion (Table 1, Fig. 2, Appendix 1).

The analysis of treatments containing a predator revealed that predator type affected relative body shape measures on both sampling occasions, whereas feeding rate did not (Table 1, Fig. 2). There was a marginally non-significant tendency for types of cues present influencing shape at the first sampling occasion, this tendency, however, disappeared by the second sampling (Table 1, Fig. 2, Appendix 1). The interaction between predator type and feeding rate tended to affect body shape of tadpoles on day 28, all other two-way interactions were non-significant (Table 1, Appendix 1). Univariate tests suggested that, at both sampling occasions, tadpoles had a shorter tail and a deeper tail fin in the presence of dragonfly larvae than when they were reared together with newts, whereas predator type had no effect on body length or tail muscle depth at either sampling (Table 1, Fig. 2, Appendix 1). Furthermore, at the first sampling occasion, tail fins were deeper and tail muscles tended to be shallower in the cue-restricted treatments as compared to when all cues were present, but types of cues present had no effect on body length or tail length (Table 1, Fig. 2, Appendix 1). At the second sampling occasion, the interaction between predator type and feeding rate had a significant effect on tail fin depth and a marginally

non-significant effect on body length with tadpoles in the low food - newt treatments having more control-like phenotypes than in the other predator treatments, but there was no effect on tail length or tail muscle depth (Table 1, Fig. 2, Appendix 1).

Behaviour

Tadpoles were more active in the absence of predators than in their presence and 27 days after the start of the experiment than after 10 days (Table 2, Fig. 3). However, the interaction between predator presence and the date of the sampling occasion was also significant with a smaller decrease in activity during the later sampling occasion (Table 2, Fig. 3).

The analysis of treatments containing a predator indicated that, on average, tadpole activity was lower in the treatments where predators received more food, and repeated a previous result that tadpole activity increased between the first and the second sampling occasion (Table 2, Fig. 3). Tadpoles tended to be more active when all types of cues were present than in the cue-restricted treatments, whereas the main effect of predator type did not have a significant effect on activity (Table 2, Fig. 3). The interactions between predator type and date of sampling occasion and types of cues present and date of sampling occasion were significant (Table 2). During the first sampling occasion, tadpoles were more active in the presence of newts than in the presence of dragonflies, and more active when all types of cues were present than in the cue-restricted treatments, whereas during the second sampling occasion there were no such differences (Table 2, Fig. 3). The interaction between predator type and feeding rate showed a non-significant tendency, whereas all other interactions were non-significant (Table 2).

To further dissect the effects of predator type, types of cues present and feeding rate, we performed two more analyses, one for each sampling occasion. At the first sampling occasion, tadpoles were more active in the presence of newts (LMM; $F_{1,76} = 4.51$, p = 0.037), when all types of cues were present ($F_{1,76} = 5.4$, p = 0.023) and when the predators were fed less ($F_{1,76} = 5.18$, p = 0.026, Fig. 3). The interactions were non-significant (all p > 0.23). At the second sampling occasion, none of the main effects seemed to influence tadpole activity (all p > 0.17), but the interaction between predator type and feeding rate was significant ($F_{1,76} = 5.39$, p = 0.023) with tadpoles being more active in the presence of newts than in the presence of dragonfly larvae at low but not at high predator feeding rate (Fig. 3). All other interactions were non-significant (all p > 0.4).

Discussion

The observed lack of induced changes in tadpole body size, the present morphological responses and the decrease in activity all agree well with what has previously been observed in other tadpole species (Relyea 2001, Van Buskirk 2002), and in *R. dalmatina* tadpoles specifically (Lardner 2000, Teplitsky et al. 2004, 2005a, 2005b). Apart from delivering further support for these well-documented phenotypic changes, our experimental design also allowed us to draw some conclusions on how interactions between the information content

Table 1. Results of multivariate general linear models on body length (body L), tail length (tail L), tail muscle depth (tail MD) and tail fin depth (tail FD). For the univariate tests, F-values are provided in the cases where the multivariate tests yielded significant results.

Effect	Multivariate tests				Univariate tests				
	DF	Wilk's λ	F	p	DF	body L	tail L	tail MD	tail FD
day 11									
Control incl.									
Body size	4,94	0.00	$4.61×10^9$	<0.001	1,97	1910.56****	1193.20****	533.51****	1142.99****
P/A of predators	4,94	0.46	27.79	<0.001	1,97	44.63****	0.63	3.19*	76.28****
Control excl.									
Body size	4,73	0.00	$347×10^9$	<0.001	1,76	1512.47****	990.96****	458.32****	1196.67****
Predator type	4,73	0.74	6.43	<0.001	1,76	0.59	5.72**	1.24	23.13****
Feeding rate	4,72	0.97	0.57	0.687					
Types of cues present	4,73	0.88	2.41	0.057	1,76	0.60	0.33	3.41*	8.22****
Predator type × Feeding rate	4,71	0.98	0.36	0.835					
Predator type × Types of cues present	4,72	0.95	0.88	0.478					
Feeding rate × Types of cues present	4,71	0.94	1.09	0.366					
Predator type × Feeding rate × Types of cues present	4, 68	0.96	0.70	0.593					
day 28									
Control incl.									
Body size	4,94	0.00	$1.48×10^{10}$	<0.001	1,97	4287.04****	5827.98****	4020.32****	3309.98****
P/A of predators	4,94	0.84	4.39	0.003	1,97	6.48**	2.25	0.00	13.77****
Control excl.									
Body size	4,72	0.00	$1.12×10^{10}$	<0.001	1,75	3604.46****	5255.84****	2844.35****	3053.49****
Predator type	4,72	0.81	4.33	0.003	1,75	2.39	6.60**	0.02	14.37****
Feeding rate	4,72	0.93	1.44	0.230					
Types of cues present	4,71	0.92	1.52	0.206					
Predator type × Feeding rate	4,72	0.89	2.18	0.079	1,75	3.86*	0.00	0.02	4.64**
Predator type × Types of cues present	4,70	0.98	0.44	0.781					
Feeding rate × Types of cues present	4,70	0.93	1.27	0.292					
Predator type × Feeding rate × Types of cues present	4, 68	0.92	1.46	0.225					

*$p<0.10$, **$p<0.05$, ***$p<0.01$, ****$p<0.001$

of different types of chemical cues may have shaped phenotypic responses.

Young tadpoles were less active and tended to be smaller in the presence of dragonfly larvae than in the presence of newts. This difference may have resulted from a conflicting interaction between the information content of direct and indirect cues in the case of the gape-limited predator, and/or from a synergistic interaction in the case of dragonfly larvae. While the presence of similar amounts of indirect cues should have induced similar responses to both predators, it seems that the direct cues delivering information on the type of predator weakened responses in the case of newts and/or enhanced them in the case of dragonflies. This difference could have evolved because newts are gape-limited and are active foragers, also finding immobile prey. Consequently, it may pay to maintain relatively high activity levels for the returns in growth rate in the presence of newt predators (Kishida and Nishimura 2005, Urban 2007a, 2007b). Larvae of aeshnid dragonflies, however, are not gape-limited and are sit-and-wait predators, so that decreasing activity will lower encounter rates with this predator. Consequently,

decreasing foraging activity may be a viable strategy in the presence of dragonfly larvae, even if this comes at a cost of decreased growth (Relyea 2002). An alternative explanation for the weaker responses to the presence of newts compared to that of dragonfly larvae could be that newts pose a weaker threat on tadpoles than dragonflies (Van Buskirk 2001). Our observations, however, suggest that *T. vulgaris* are not less voracious predators of small *R. dalmatina* tadpoles than *A. cyanea* larvae (Hettyey et al. unpubl.). As the effect of predator type was not large, further studies are needed to assess the robustness and significance of this result.

A proximate, mechanistic explanation for the observation that dragonfly larvae induce stronger responses than newts may be that as the former chew prey tadpoles and the latter swallow prey without chewing, more alarm substances may be released into the water from tadpoles that are consumed by dragonfly larvae than by newts (sensu Ferrari et al. 2007). Under our experimental conditions, however, this is not a likely explanation for the observed between-predator-treatment differences in the strength of responses, as in that case the interaction between predator type and types of cues present should
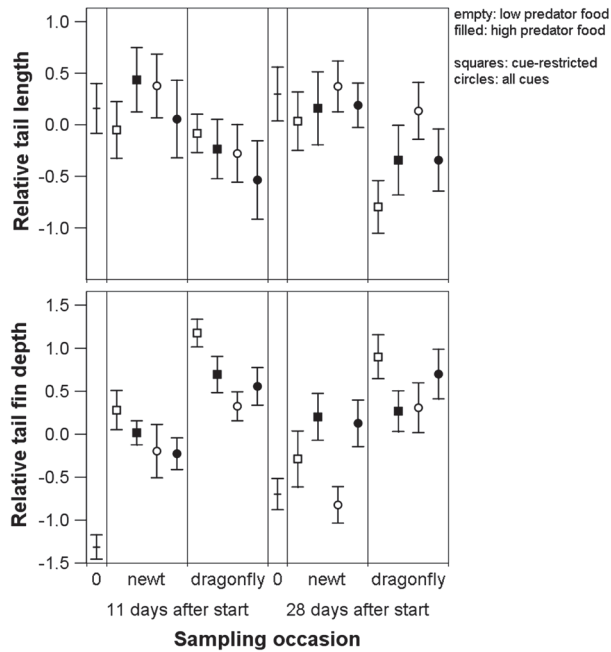
Figure 2. Relative tail length and relative tail fin depth (means ± SE) in the nine treatments at the two sampling occasions. Similar results on relative body length and relative tail muscle depth are presented in an electronic appendix. Empty symbols represent treatments where predators received little food, filled symbols represent treatments where predators received much food. Squares represent cue-restricted treatments, whereas circles represent all-cue treatments. Relative size values are residuals from regressions on body size (the latter calculated as factor scores on PC 1 from PCAs on body length, tail length, maximum tail muscle depth and maximum tail fin depth). The figure is based on rearing tank-averages. Larger values indicate larger sizes relative to overall body size.

have been significant: we should have found similar responses to the two predators in the treatments excluding damage-released cues, no-cost disturbance signals and alarm pheromones, and stronger responses to dragonflies than to newts when all types of alarm cues were present. This was, however, not the case for either body size or body shape or behaviour. Consequently, and as food intake of predators was set to an equalized level by controlled feeding, our data indicate that differences in the kairomone-profile, and/or digestion cues provided by the predators may have determined predator-specific responses in tadpoles. Interestingly, kairomones and digestion cues together appear to be sufficient to mount strong inducible defences in behaviour and morphology in *R. dalmatina* (see also LaFiandra and Babbitt 2004, Richardson 2006, Schoeppner and Relyea 2009).

The amount of prey eaten by predators had a clear effect on tadpole activity: when predators were provided with more food, tadpoles generally responded with a larger decrease in activity. Such a graded response to the perceived predation risk has been observed in some studies before in anuran tadpoles (Van Buskirk and Arioli 2002, Teplitsky et al. 2005a, Schoeppner and Relyea 2008) and other taxa (Tollrian 1993, Wiackowski and Staronska 1999, Ferrari et al. 2006). At the second sampling occasion, tadpoles decreased their activity in the presence of little-fed newts less than in the presence of well-fed newts, whereas there was no such difference in

Table 2. Results of linear mixed effect models on tadpole activity. Significant effects are highlighted in bold.

Effect	DF	F	p	Wald Z	p
Control incl.					
P/A of predators	1,98	84,14	**<0.001**		
Date	1,498	89,89	**<0.001**		
P/A × date	1,498	88,39	**<0.001**		
Tube identity				4,60	**<0.001**
Control excl.					
Predator type	1,75	2,13	0.148		
Feeding rate	1,75	6,00	0.017		
Types of cues present	1,75	2,97	0.089		
Date	1,397	438.61	**<0.001**		
Predator type × Feeding rate	1,75	3,05	0.085		
Date × Predator type	1,397	7,71	**0.006**		
Date × Type of cues present	1,397	8,01	**0.005**		
All other interactions			>0.1		
Tube identity				3,99	**<0.001**

the presence of dragonflies. We observed the same pattern in responses in tadpole shape (tail fin depth and body length). Thus, as opposed to our prediction to find synergistic interactions between the information content of direct and indirect cues, these interactions seem to have been complementary: In the presence of newt kairomones, tadpoles fine-tuned their antipredator responses to the concentration of indirect cues informing them about the level of acute predation risk (Kiesecker et al. 2002), whereas such a fine-tuning seemed redundant in the presence of dragonfly kairomones. A possible explanation for this difference is that, apart from infrequent moults, large *Aeshna* larvae are always effective predators of large *R. dalmatina* tadpoles, whereas adult newts are gape-limited and only become dangerous when fully grown, if alternative prey is scarce and breeding activity is low (Griffiths 1985, Kishida and Nishimura 2005, Urban 2007a, 2007b).
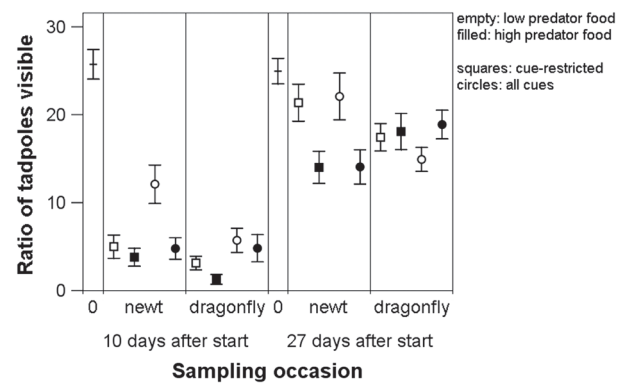

Figure 3. Ratio of tadpoles visible (means ± SE), used as an estimate of tadpole activity, during the first and second sampling occasion. Empty symbols represent treatments where predators received little food, filled symbols represent treatments where predators received much food. Squares represent cue-restricted treatments, whereas circles represent all-cue treatments. For the ease of interpretation, untransformed data are shown.

The presence or absence of alarm cues released during predation-events had relatively weak effects. This result may partly have arisen because some of the tadpole alarm cues have degraded by the time the predation water was poured back together with predators into the tadpole rearing tanks (Turner and Montgomery 2003, Peacor 2006, Ferrari et al. 2008). Nonetheless, young/small tadpoles did respond differentially to the presence or absence of predation-event related indirect cues both morphologically and behaviourally: in their presence, they developed shallower tail fins and deeper tail muscles and lowered activity less than when only digestion-released cues and kairomones were present. These differences in responses disappeared by the second sampling occasion. The diminishing sensitivity of tadpoles to cues on predation threat with increasing size, probably a result of a decrease in tadpole vulnerability to predation (Travis et al. 1985, Semlitsch 1990, Eklöv and Werner 2000), aligns to our other results and to those of previous studies (Van Buskirk 2001, Laurila et al. 2004, Fraker 2008). We expected indirect cues released by the act of predation and ingestion to enhance responses to kairomones and digestion-released cues (also see LaFiandra and Babbitt 2004, Richardson 2006, Schoeppner and Relyea 2009), but observed the opposite. Indeed, our results suggest that the presence of 'old' damage-related and disturbance cues reduce behavioural and morphological defences. However, we can only speculate on the possible reasons for this result and further studies are needed to clarify the causes.

In summary, we observed fine-tuned antipredator responses both in morphology and in behaviour of tadpoles. Induced defences were predator-specific, most likely mediated by kairomones, but a larger amount of indirect cues in the treatments where predators received more food resulted in stronger responses. Our data align to previous studies suggesting that tadpoles use direct cues, and, possibly, also predator-specific digestion-released cues, to adjust the type of responses providing optimal defences against predators using different foraging modes (Kishida and Nishimura 2005, Teplitsky et al. 2005b, Wilson et al. 2005) and indirect cues to adjust the intensity of responses according to the actual predation risk (Van Buskirk and Arioli 2002, Schoeppner and Relyea 2008). Also, large tadpoles reacted only to 'dangerous' newts, probably because these are not always voracious predators of large tadpoles. Information content of direct and indirect cues, thus, seem to have complemented each other. However, small tadpoles did not react to newts as intensively as to dragonfly larvae despite their similar dangerousness, probably because the former is a gape-limited predator. Consequently, the information content of indirect cues indicating the presence of dangerous predators is likely to have interacted antagonistically with the information delivered by kairomones in the case of the newt, and/or synergistically in the case of dragonfly larvae. Finally, we generally observed weaker responses to predators in larger tadpoles, also suggesting that tadpoles optimize their induced defences carefully based on several extrinsic and intrinsic cues, rather than relying on one general cue associated with the act of predation.
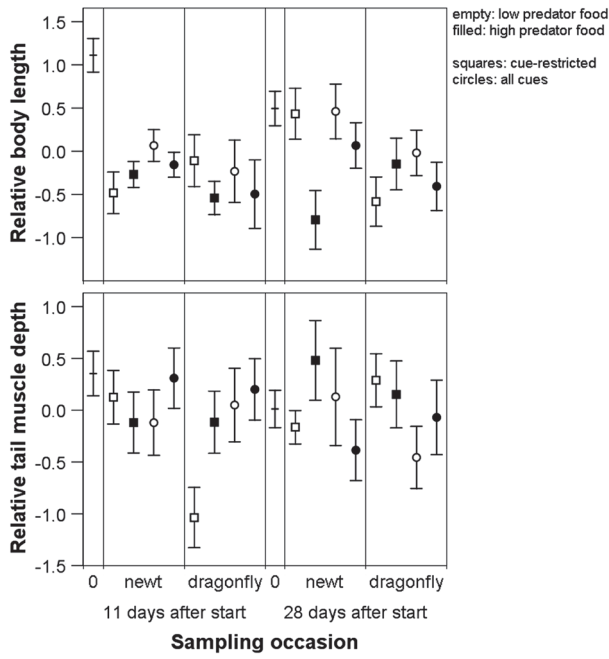
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Appendix 1. Relative body length and relative tail muscle depth (means±SE) in the treatments at the two sampling occasions. Empty symbols represent treatments where predators received little food, filled symbols represent treatments where predators received much food. Squares represent cue-restricted treatments, whereas circles represent all-cue treatments. Relative size values are residuals from regressions on body size (the latter calculated as factor scores on PC 1 from PCAs on body length, tail length, maximum tail muscle depth and maximum tail fin depth). The figure is based on rearing tank-averages. Larger values indicate larger sizes relative to overall body size.