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Increasing conspecific density weakens the ability of intermediate predators to develop induced morphological defences to top predators

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SUMMARY

1. Intraguild predation is common in nature, but it is unclear how species that both compete and eat each other can persist together. One possibility is that intermediate predators possess inducible morphological defences that protect them from top predators while not compromising their ability to compete with top predators.
2. The ability of intermediate predators to develop morphological defences may be compromised in environments with a high density of conspecifics because of reduced resource availability and predation risk due to the saturating functional response of top predators. Furthermore, since morphological defences take time to develop, the type and extent of morphological defences may vary during development.
3. We conducted an experiment to measure the phenotypic responses of an intermediate predator (larvae of the salamander *Ambystoma opacum*) to the presence of a caged top predator (larvae of the dragonfly *Anax* spp.) throughout ontological development in environments that differed in the density of conspecifics present. We also assessed how intermediate predators, reared in the different environments, differed in their vulnerability to top predators and ability to deplete their food resources.
4. We found that *Anax* induced morphological defences in *A. opacum*, but the extent of morphological change declined with the density of conspecifics. Moreover, some morphological traits disappeared, while others appeared just prior to *A. opacum* metamorphosis. The change in *A. opacum* phenotype in response to *Anax* made *A. opacum* less vulnerable to predation by *Anax* but had no significant effect on the foraging ability of *A. opacum*.
5. Our study demonstrates that top predators can induce phenotypes in intermediate predators that reduce their vulnerability to top predators while not compromising their ability to feed on a common prey. An increase in intermediate predator density, however, could diminish the ability of intermediate predators to develop the full suite of morphological defences. The inability to develop the full suite of morphological defences may reduce the probability of persistence with top predators.

Keywords: amphibian, induced defences, intraguild predation, larval development, phenotypic plasticity

Introduction

Intraguild predation is common in nature despite the consumptive and competitive pressure that intermediate predators face from top predators (Polis, Myers & Holt, 1989; Arim & Marquet, 2004). Among the mechanisms hypothesised to explain how intermediate predators persist with top predators is the induction of anti-predator defences in intermediate predators. An inducible defence is a response in phenotypically plastic prey that reduces the prey's vulnerability to predation but often comes at the expense of other fitness components

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(West-Eberhard, 1989; DeWitt, Sih & Wilson, 1998). For example, predators induce *Daphnia* to develop protective helmets and neck teeth that reduce both *Daphnia*'s vulnerability to predation and fecundity (Tollrian & Dodson, 1999). Theory (Bolker *et al*., 2003; Werner & Peacor, 2003; Mougi & Kishida, 2009) predicts inducible defences in prey will increase population and community stability because, although they reduce prey fecundity when the defence is expressed, they also weaken predator–prey interactions when predators are present. Recent empirical work (Boeing & Ramcharan, 2010) supports this prediction. While there is ample evidence demonstrating predator-induced change in the behaviour and morphology of many different taxa of herbivorous prey (see Tollrian & Harvell, 1999 and Benard, 2004 for reviews), most research on inducible defences with intermediate predators has focused on behavioural responses (Huang & Sih, 1990; Eklöv & Werner, 2000) rather than the morphological responses to top predators that are likely to be more costly (Van Buskirk & Schmidt, 2000; Kishida, Trussell & Nishimura, 2009; Hammill & Beckerman, 2010; Stoks, Swillen & De Block, 2012). It is unclear whether intermediate predators have sufficient phenotypic plasticity to allow them to develop effective morphological defences in response to the presence of predators in the way that herbivorous prey do.

Top predators may not induce intermediate predators to alter their morphology in the same way as herbivores for at least three reasons. First, intermediate predators compete with their predators, while herbivores do not (Yurewicz, 2004; Banerji & Morin, 2009). Theory suggests that intermediate predators are more likely to persist with top predators if intermediate predators are stronger competitors for shared food resources (Holt & Polis, 1997). Thus, any change in the phenotype of intermediate predators should (i) reduce vulnerability to predators while not sacrificing competitive ability, (ii) enhance competitive ability while not making them more vulnerable to predation or (iii) reduce vulnerability and enhance competitive ability. None of these expectations involve a trade-off between competitive ability and vulnerability in intermediate predators, unlike herbivorous prey (DeWitt, 1998; Relyea, 2002; Dzialowski *et al*., 2003), but costs associated with an induced phenotype may manifest in other ways (e.g. reduced fecundity or survival at a subsequent life stage). Second, intermediate predators and herbivores differ in their intraspecific trait correlations (e.g. various aspects of head shape and tail shape), which could impose different trade-offs for the two groups (Van Buskirk & Schmidt, 2000). Third, similar induced trait responses by intermediate predators and herbivores to predators may not have the same ecological consequences. For example, Yurewicz (2004) noted that predatory salamanders from her study and herbivorous tadpoles from another study (Relyea, 2001) altered tail morphology in a similar way when exposed to predators, but this morphological change had different consequences for the salamanders and tadpoles. Specifically, the vulnerability of salamanders to predation was enhanced, while the vulnerability of tadpoles to predators was reduced.

The ability to develop effective anti-predator defences may vary depending on other aspects of the environment (Schlichting & Pigliucci, 1998). The density of conspecifics could influence the development of anti-predator defences through three mechanisms. First, if organisms modify morphology in a way that forces a trade-off between vulnerability to predation and competitive ability, an increase in the density of conspecifics could weaken the response of prey to predators if the cost is too great (e.g. reduced competitive ability in environments where competition is likely to be strong and important; Peacor, 2003; McCoy, 2007). McCoy (2007) observed that herbivorous prey exhibited weaker morphological responses to predators when conspecific density was high than when conspecific density was low. This mechanism may be less important for intermediate predators involved in intraguild predation if changes in intermediate predator morphology are not associated with a trade-off between competitive ability and vulnerability to predation. Consequently, we would expect predator-induced changes in intermediate predator morphology to be similar in environments varying in conspecific density. Second, stronger competition in environments with a high density of conspecifics could reduce the amount of resources available to an individual that could be used to develop a different phenotype in the presence of predators (Sih, Englund & Wooster, 1998; Relyea & Auld, 2004). This mechanism predicts that intermediate predators would have weaker responses to their predators in environments with a high density of conspecifics. Third, the saturating functional response of predators can result in prey (including intermediate predators) experiencing a lower predation risk when conspecifics are available to the predator (Abrams, Hill & Elmgren, 1990; Peacor, 2003; Van Buskirk *et al*., 2011). Consequently, predators may be less likely to induce defensive phenotypes in prey when a high density of prey is present because prey vulnerability to predation is lower when in a crowd.

We investigated the extent to which larval dragonflies (top predators) induce larval salamanders (intermediate predators) to alter their phenotype in ponds varying in the density of intermediate predators present. Although others have documented the occurrence of predator-induced morphological plasticity in intermediate predators (Van Buskirk & Schmidt, 2000; Kishida *et al*., 2009; Hammill & Beckerman, 2010; Stoks *et al*., 2012), our study enhances knowledge of predator-induced plastic responses in intermediate predators by (i) evaluating how conspecific density affects the ability of intermediate predators to develop anti-predator defences in response to top predators, (ii) assessing whether the development of plastic responses by intermediate predators is restricted to a particular developmental stage and (iii) evaluating whether the plastic responses of intermediate predators to top predators and conspecific environment affects the relative performance of intermediate predators (e.g. survival and foraging efficiency).

Methods

Study system

Dragonfly larvae, *Anax* spp, were the top predators, and larvae of the marbled salamander, *Ambystoma opacum*, were the intermediate predators in this study. Larval *Anax* are voracious predators of larval amphibians in temporary pond communities (Van Buskirk, 1988; Davenport & Chalcraft, 2012). *Ambystoma opacum* are common in temporary pond communities of the eastern U.S.A. and can function as a keystone predator of larval anurans (Morin, 1995; Chalcraft & Resetarits, 2003). Larval *A. opacum* are susceptible to *Anax* predation through the entire larval period (Davenport & Chalcraft, 2012). *Anax* and *A. opacum* consume shared prey resources consisting of macroinvertebrates and small larval anurans (e.g. *Bufo* spp., *Pseudacris* spp., and *Rana* spp.; Van Buskirk, 1988; Morin, 1995; Chalcraft & Resetarits, 2003).

In the first of three experiments, we measured the behavioural and morphological response of larval *A. opacum* to the presence of a caged larval *Anax* in environments that differ in the density of newly hatched *A. opacum*. It is difficult to experimentally isolate the effects of competition and predation between a top predator and an intermediate predator. However, by placing *Anax* in cages, we prevented them from directly consuming and competing with *A. opacum*. Therefore, we assessed the response of intermediate predators to the presence of a top predator that both eats and competes with intermediate predators rather than the response that directly results from the top predator either consuming or competing with the intermediate predator. We measured phenotypic responses during the early, middle and late stages of larval salamander development. This experiment will hereafter be referred to as the 'plasticity experiment'. For the second and third experiments, we assessed the performance of different *A. opacum* phenotypes in terms of their ability to (i) forage for food resources (a measure of competitive ability) and (ii) escape predation. We hereafter refer to these two experiments as 'performance trials'.

Plasticity experiment

This experiment was conducted in 1100 L mesocosms (1.9 m^2 surface area) designed to mimic generic features of natural ponds. Researchers have demonstrated that processes found to be important in mesocosms are also important in natural settings (Resetarits & Fauth, 1998; Van Buskirk & McCollum, 1999; Rubbo *et al*., 2006; Van Buskirk, 2009). The experimental design included two levels of predator manipulation (larval *Anax* absent or caged larval *Anax* present; head width range 5.92–8.11 mm; mass range 0.392–0.689 g) crossed with three levels of larval *A. opacum* density (10, 20 or 40 hatchlings per mesocosm) to produce six treatments. Densities of larval salamanders and larval *Anax* in this experiment are comparable to those observed in nature (Smith, 1988; Petranka, 1989; Wilbur & Fauth, 1990).

Each of the six treatments was randomly assigned to one mesocosm within each of four spatial blocks for a total of 24 mesocosms, located at the West Research Campus of East Carolina University. Each mesocosm was equipped with a standpipe to control water levels and fibreglass mesh lids to prevent non-study animal colonisation and escape of study animals. Mesocosms were filled with well water and received one kilogram of leaf litter on 16–18 November 2007. Aliquots of concentrated plankton from local ponds were added to each mesocosm on 27 November 2007 to provide a source of primary producers to support the pond food web and zooplankton and other small invertebrates to provide a source of food for *A. opacum* and *Anax*. We collected 28 *A. opacum* egg clutches and larval *Anax* from the Croatan National Forest on 4–7 November 2007 and 29 November 2007, respectively. Eggs were induced to hatch on 29 November 2007 by inundation in filtered pond water. Study animals were randomly assigned to treatments and placed into mesocosms on 6 December 2007. Water depth in mesocosms was initially 50 cm, but this decreased throughout the study as the result of an

experimentally imposed drying regime of 178 days (following the procedure of Wilbur, 1987). All mesocosms were dried at the same rate, and this drying regime was representative of the hydroperiod in North Carolina ponds where *A. opacum* can be found.

Caging predators is an effective way to assess nonconsumptive effects of predators on prey (Benard, 2004). We placed two cages (10 cm × 10 cm) made of PVC pipe and window screening on the bottom of each mesocosm. Mesocosms assigned to a caged predator treatment had one larval *Anax* placed into each cage, while mesocosms assigned to no-predator treatments had empty cages. Caged *Anax* were each fed one salamander (matched for size among all tanks) every 3 days for the duration of the experiment. Empty cages were lifted from the mesocosm bottom on feeding days to simulate the disturbance required to feed *Anax*.

Eight morphological measurements (head length, head depth, head width, torso length, tail length, tail fin depth, tail muscle depth and tail muscle width) were measured during the early (30 January–2 February 2008), middle (26–28 March 2008) and end (12–13 May 2008) of the larval period of *A. opacum*. During each sampling period, we captured 40% of the larvae in each mesocosm with a dip net, weighed and photographed the lateral and ventral sides of each salamander and subsequently used imaging software (ImageJ: Rasband, 2012) to measure morphological traits from digital images (Van Buskirk & Schmidt, 2000). To facilitate photography, we anaesthetised salamander larvae in an Orajel® solution (Cecala, Price & Dorcas, 2007) before placing individual animals in a photo chamber (described in Van Buskirk & Schmidt, 2000). Salamanders recovered in a container filled with pond water for 4–7 min before being returned to experimental tanks. No mortality was experienced during the photographing sessions. Mean trait values from each tank were calculated from measured individuals and used as response variables in analyses.

Behavioural observations were made during the early larval period of *A. opacum* but not later because the water in mesocosms became too murky (after 21 March) to make accurate observations. Behaviour was assessed by recording the number of active and inactive larvae observed using a scan sampling technique (Altmann, 1974). Each tank was observed for 10–15 s every 6 h over a 24-h period. We averaged the number of active larvae and the number of observed larvae across the four observations periods over the 24-h period and estimated larval activity as the mean number of active larvae divided by the mean number of observed larvae.

Data were analysed using PROC MIXED (SAS, 2010). We performed a factorial ANOVA on mortality rates (ln-transformed proportion of individuals surviving to end of experiment) of *A. opacum* with the three following main factors and their two-way interactions: (i) block, (ii) *A. opacum* density and (iii) presence of caged *Anax* cues in the larval environment. Block was treated as a random effect, while the other two factors were treated as fixed effects. The log of *A. opacum* mass was analysed with a repeated-measures factorial ANOVA. The same factorial design was used for the analysis of mortality data, but time (early, middle or late in development) and the two-way and three-way interactions involving *Anax* presence, *A. opacum* density, block and time were also included in the analysis. We considered 4 possible covariance structures for these analyses (unstructured covariance structure, variance components and compound symmetry with homogeneous variances and compound symmetry with heterogeneous variances) with and without the assumption of homogeneity of variances among treatment groups. The model with an unstructured covariance structure and homogeneous variances was used for the repeated-measures ANOVA on mass data, while the model with the variance components covariance structure with homogeneous variances was used for the factorial ANOVA on mortality because models with these assumptions fit the data better according to various information theoretic indices (e.g. AIC).

We also employed PROC MIXED (SAS, 2010) to evaluate differences in *A. opacum* phenotype among treatments. We used the same factorial model as described for the analysis of *A. opacum* mortality above to compare arc-sin transformed larval activity. The model with the variance components covariance structure and homogeneous variances best described larval activity data. We compared each of the mean morphological traits among treatment groups with planned contrasts associated with repeated-measures ANCOVA. The ANCOVA model included treatment (six levels represented by the different combinations of *Anax* present or absent crossed with low, intermediate or high density of *A. opacum*) and block as the independent factors, time at which the morphological trait was measured as the repeated factor, and the mean mass of *A. opacum* (log transformed) as the covariate because larger individuals are expected to have larger trait values. The model included the two-way and three-way interactions involving treatment, time and mass to account for potential treatment effects that varied with time and for the potential for the allometric relationship between mass

and the trait measurement to vary among treatments, across time and among treatments and across time. We included two-way and three-way interactions involving the covariate in the model because a visual inspection of graphs suggested that the slope of allometric relationships varied among treatments, across time or the effect of treatment on the allometric relationship appeared to vary with time. Furthermore, tests of the statistical significance of the two-way and three-way interactions involving the covariate generally resulted in relatively low (<0.15) *P*-values for all of the traits examined (Table S1). Models with an unstructured covariance matrix and homogeneous variances were used for the analysis of morphological data because models with these assumptions fit the data better. We employed an ANCOVA approach to remove the effects of size rather than a principal components approach because recent simulations (Berner, 2011) indicate that a principal components approach is inappropriate for size correction.

For each morphological trait, we conducted nine planned contrasts associated with the repeated-measures ANCOVA model performed on that trait. Each contrast compared the effect of *Anax* on the least square mean estimates of the trait values derived from the repeated-measures ANCOVA model. A separate contrast evaluated the effect of predators for each environment that varied in *A. opacum* density and for each time (three environments differing in *A. opacum* density x three times results in nine contrasts). We did not compare morphological traits among environments that differ in the density of *A. opacum* because body mass (the covariate in the ANCOVA) varied among environments differing in *A. opacum* density, but not in environments differing in the presence of *Anax* (see results), so *A. opacum* density treatments are confounded with mass. Given that (i) body size varied among environments differing in *A. opacum* density, (ii) salamanders became larger through time as they grew, (iii) larger salamanders tend to have larger trait values, and (iv) there was sufficient evidence to suggest that allometric relationships differed between treatments and/or through time, we constructed each contrast to compare the least square estimate of the trait value between the *Anax* treatments that corresponds with the average-sized individual that would be found in the particular environment (i.e. *A. opacum* density) and time of development that the contrast applies too. Given that we tested the same hypothesis (predator effects) on eight different morphological traits at a particular time, we derived adjusted *P*-values for each contrast to control the false discovery rate (FDR; Verhoeven, Simonsen & McIntyre, 2005).

Performance trials

We set up 32 mesocosms on 17 November 2009 to induce phenotypes observed in four of the six larval environments considered in the plasticity experiment. Salamanders from these mesocosms were used to assess whether individuals with different phenotypes differed in their performance. We focused on four of the six larval environments considered for the plasticity experiment due to constraints on the number of mesocosms available, choosing the more extreme larval environments from the plasticity experiment. These were (i) 10 *A. opacum*, no caged *Anax;* (ii) 10 *A. opacum*, caged *Anax;* (iii) 40 *A. opacum*, no caged *Anax;* and (iv) 40 *A. opacum*, caged *Anax*. We utilised 24 mesocosms for raising larval *A. opacum* in low densities (10 individuals: 12 with caged *Anax*, 12 without) and eight mesocosms in high densities (40 individuals: four with caged *Anax*, four without). Our prior research suggested this should produce enough larval salamanders (assuming low survival of about 50%) to compare differences in predator vulnerability (i.e. mortality rates due to predation) and foraging efficiency of the different phenotypes with at least eight replicates.

Mesocosms were established on 20–22 November 2009 and maintained in the same manner as in the plasticity experiment. Twenty-seven *Ambystoma opacum* egg clutches were collected on 3–10 November 2009, and larval *Anax* were collected on 23 November 2009. All organisms were collected from the same ponds that the organisms for the plasticity experiment were collected from. Eggs were induced to hatch on 19 November 2009 by inundation in filtered pond water. The experiment began on 1 December 2009 after all mesocosms had been randomly assigned treatments and study organisms. We waited to conduct performance trials until the larval salamanders achieved a size comparable to that observed during week 17 in the plasticity experiment. The comparable size was reached at week 22 (mean mass (g) for low-density treatments $\pm$ 1 SE: 0.543 $\pm$ 0.007, mean mass (g) for high-density treatments $\pm$ 1 SE: 0.345 $\pm$ 0.007), and we observed that the phenotype in each environment was the same as that observed for the same environment in the plasticity experiment.

We assessed the vulnerability of *A. opacum* phenotypes from each of the four larval environments to a free-swimming, lethal *Anax* in 31 L experimental tubs (52.1 cm × 36.1 cm × 30.7 cm) with 30 g of leaf litter. Tubs were located outdoors at the West Research Campus of East Carolina University. We measured vulnerability for each *A. opacum* phenotype in 12 replicate

blocks (11 replicates for the 10 *A. opacum* with caged *Anax* phenotype due to a limited supply of individuals). To measure vulnerability, five *A. opacum* of a particular phenotype were placed in a tub with a single *Anax*. Vulnerability was measured as the absolute value of the instantaneous per capita death rate due to predation. Instantaneous death rates were measured as the ln-transformed proportion of surviving *A. opacum* of each phenotype over a 24-h period (Lieberman *et al.*, 1985; Sheil, Burslem & Alder, 1995; Rogers & Chalcraft, 2008; Davenport & Chalcraft, 2013). Given that all deaths were due to predation, the absolute value of the instantaneous per capita death rates provides a measure of vulnerability to predation. We used PROC MIXED (SAS, 2010) and the same factorial model (independent and interactive effects of *Anax* presence, *A. opacum* density and block) used for the analysis of survival in the plasticity experiment to assess differences in vulnerability measured in the performance trials. The model with the variance components covariance structure and homogeneous variances was used for the analysis because this fit the data better than models with other assumptions.

We measured foraging efficiency of 10 individuals from each of the four larval environments. Thus, this experimental design comprised 4 treatments (phenotypes from the 4 larval environments) that were replicated in 10 spatial blocks. To measure larval foraging efficiency, we placed one salamander in a 31-L tub of filtered pond water. We placed one individual of each phenotype in the foraging trials to ensure any differences between treatments were due to differences between phenotypes rather than density dependence. Each tub contained 20 g of leaf litter and 40 *Daphnia* spp. individuals as prey. After 24 h, we removed the larval salamander and rinsed the leaf litter to remove all *Daphnia*. We filtered water in each tub (including the wash water) through a series of sieves (500 and 250 μm) to retrieve any remaining *Daphnia*. Ten sets of tubs without larvae were established to measure our efficiency at extracting *Daphnia*. Larval foraging efficiency was defined as the difference in the number of *Daphnia* removed from tubs without larvae versus the number of *Daphnia* removed from tubs with larvae. We used PROC MIXED (SAS, 2010) and used the same factorial model (independent and interactive effects of *Anax* presence, *A. opacum* density and block) used for the analysis of survival in the plasticity experiment to assess differences in foraging efficiency measured in the performance trials. The model with the variance components covariance structure and homogeneous variances was used for the analysis because it better described variation in foraging efficiency data.

Results

Plasticity experiment

On average, 73% of salamanders in mesocosms survived to the end of the experiment. Mortality of *A. opacum* was not affected by conspecific density ($F_{2,\ 6} = 1.08$, $P = 0.399$), the presence of caged *Anax* ($F_{1,\ 6} = 2.11$, $P = 0.242$) or the interaction between conspecific density and caged *Anax* ($F_{2,\ 6} = 0.21$, $P = 0.816$). Salamanders grew larger during the experiment ($F_{2,36} = 161.01$, $P < 0.001$), and an increase in the density of *A. opacum* caused salamanders to be smaller ($F_{2,18} = 31.80$, $P < 0.001$) although the presence of caged *Anax* had no effect on *A. opacum* mass ($F_{1,18} = 2.08$, $P = 0.166$). None of the interactions involving the effects of *Anax* presence, *A. opacum* density or time accounted for a significant amount of variation in *A. opacum* mass (all $P \geq 0.075$). During early stages of development, *Anax* reduced larval activity ($F_{1,\ 3} = 17.15$, $P = 0.026$), but the effect of *Anax* decreased as larval density increased ($F_{2,\ 6} = 5.57$, $P = 0.043$; Fig. 1).

Larger and older salamanders had larger morphological trait measurements than smaller and younger salamanders, but the slope of the relationship between mass and morphological trait measurements generally varied with the developmental stage of the salamander and treatment (Table S1). The presence of *Anax* did not produce any variation in *A. opacum* morphology among the environments differing in *A. opacum* density during the early development of *A. opacum* (FDR adjusted *P*-values for all contrasts ≥0.083). During the middle of larval *A. opacum* development, however, the presence of *Anax* caused *A. opacum* to develop shorter torsos (FDR

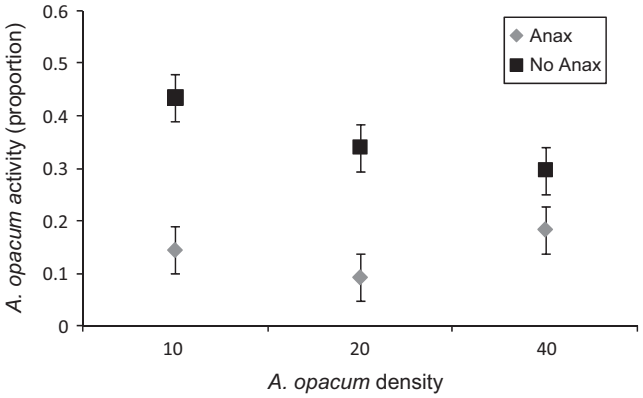

Fig. 1 Least square mean (± 1 SE) activity levels (arc-sine transformed proportion of visible individuals that were active) of *A. opacum* in environments differing in the density of conspecifics and the presence of caged *Anax*.

adjusted *P*-values <0.001; Fig. 2a) and heads (FDR adjusted *P*-values <0.027; Fig. 2b) in environments with a low density of *A. opacum*, but not in environments with an intermediate or high density of *A. opacum* (FDR adjusted *P*-values >0.445). Furthermore, the presence of *Anax* caused *A. opacum* to develop taller tail fins by the middle of larval *A. opacum* development, regardless of the density of *A. opacum* (FDR adjusted *P*-values<0.007; Fig. 2c). Predators did not alter the head depth, head width, tail length, tail muscle depth or tail muscle width of *A. opacum* during the middle of larval development in any of the environments differing in *A. opacum* density (FDR adjusted *P*-values >0.198). By the end of larval development, there were significant differences in head depth (FDR adjusted *P*-values <0.001; Fig 3a) and tail muscle depth (FDR adjusted *P*-values =0.017; Fig. 3b) of *A. opacum* as a result of the presence of *A. opacum*, but these differences only appeared in environments with a low density of *A. opacum*, but not in environments with an intermediate or high density of *A. opacum* (FDR adjusted *P*-values >0.178). When differences appeared late in larval development, *Anax* caused *A. opacum* to develop taller tail muscles and taller heads. The occurrence of *Anax* did not cause differences in any other morphological trait that we measured during the later stages of larval development in any of the environments differing in the density of *A. opacum* (FDR adjusted *P*-values >0.250).

Performance trials

The size of *A. opacum* used for performance trials was not different from that measured during the middle of larval development in the plasticity experiment for either the low-density (t_{14} = 0.2994, P = 0.769) or high-density treatment (t_{14} = 0.1679, P = 0.869). Predator-induced phenotypes of *A. opacum* were less vulnerable to *Anax* than non-predator-induced phenotypes ($F_{1,\ 10}$ = 5.26, P = 0.045; Fig. 4a). Salamanders raised in environments with a high density of conspecifics were also more vulnerable to *Anax* than phenotypes raised in environments with few conspecifics ($F_{1,\ 11}$ = 8.07, P = 0.016; Fig. 4a). This is probably due to the fact the salamanders raised in environments with a high density of conspecifics were smaller than those with a low density of conspecifics (t_6 = 20.001, P < 0.001). Predators and conspecifics appear to generate trait variation that have opposing effects on *A. opacum* vulnerability, but the effects are additive ($F_{1,\ 10}$ = 0.14, P = 0.713; Fig. 4a). Consequently, *A. opacum* from environments with a low density of conspecifics and *Anax* experienced the lowest mortality during the vulnerability trials, while *A. opacum* from environments with a high density of conspecifics and no *Anax* were most vulnerable.

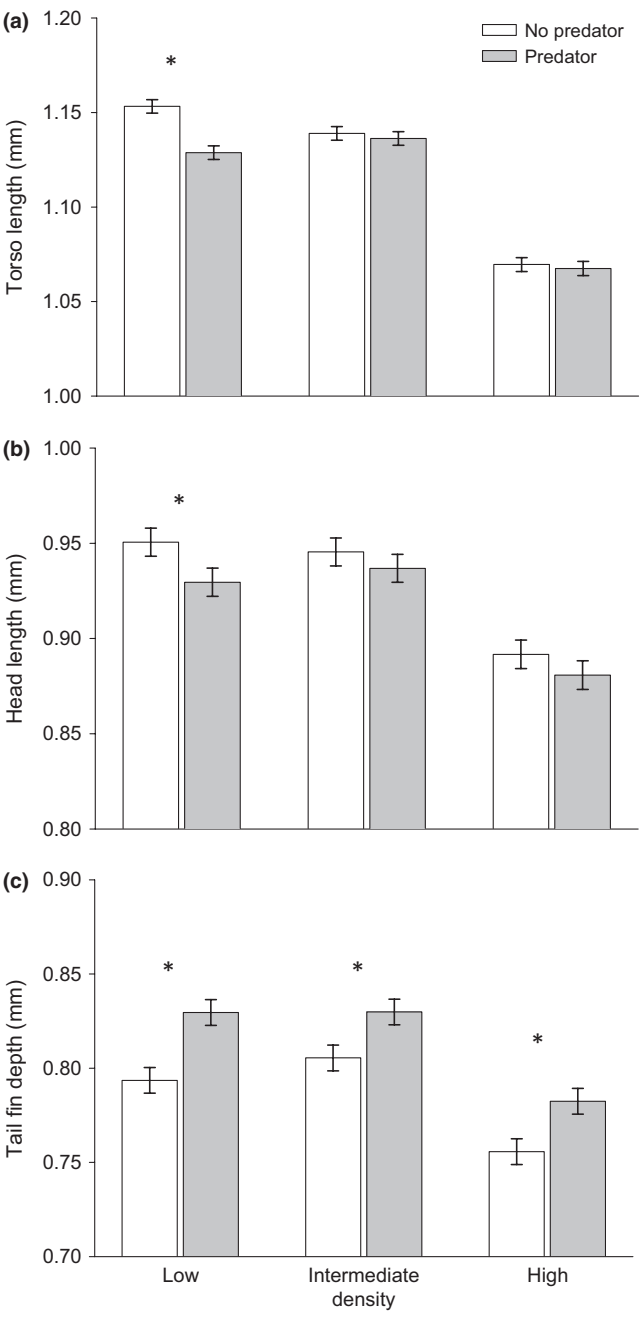

Fig. 2 Least square estimate of mean (± 1 SE) (a) torso length, (b) head length and (c) tail fin depth of *A. opacum* raised in environments differing in the density of conspecifics and the presence/absence of *Anax*. Least square means for each of the six treatments were estimated for the size of salamander that was representative (i.e. the mean) of that environment during the middle stage of larval development. Asterisks above a set of bars representing environments that differ in the densities of *A. opacum* indicate that predator treatments differed statistically in the least square mean value of that trait in that environment.

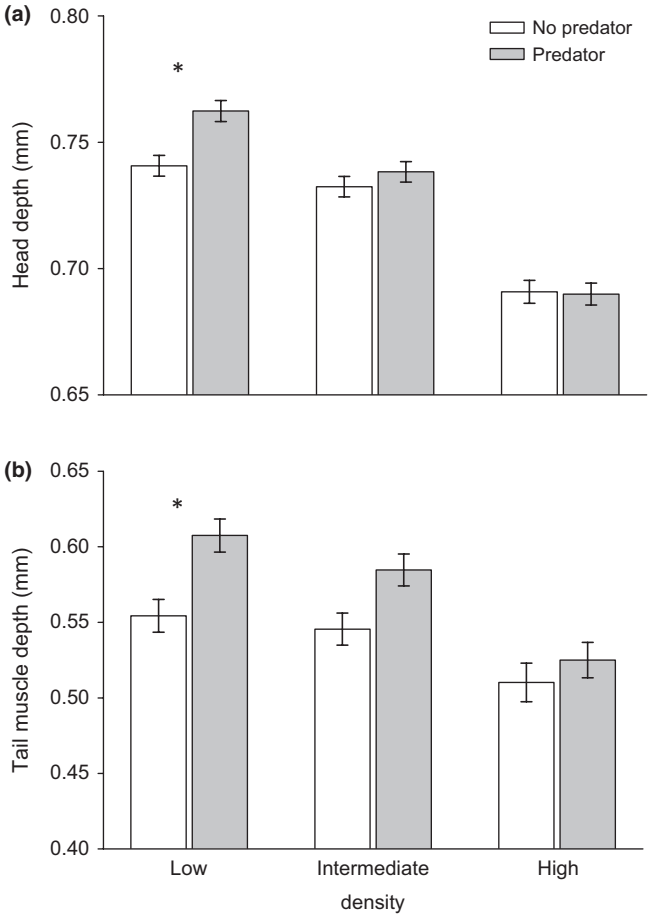

Fig. 3 Least square estimate of mean (± 1 SE) (a) head depth and (b) tail muscle depth of *A. opacum* raised in environments differing in the density of conspecifics and the presence/absence of *Anax*. Least square means for each of the six treatments were estimated for the size of salamander that was representative (i.e. the mean) of that environment during the late stage of larval development. Asterisks above a set of bars representing environments that differ in the densities of *A. opacum* indicate that predator treatments differed statistically in the mean value of that trait in that environment.

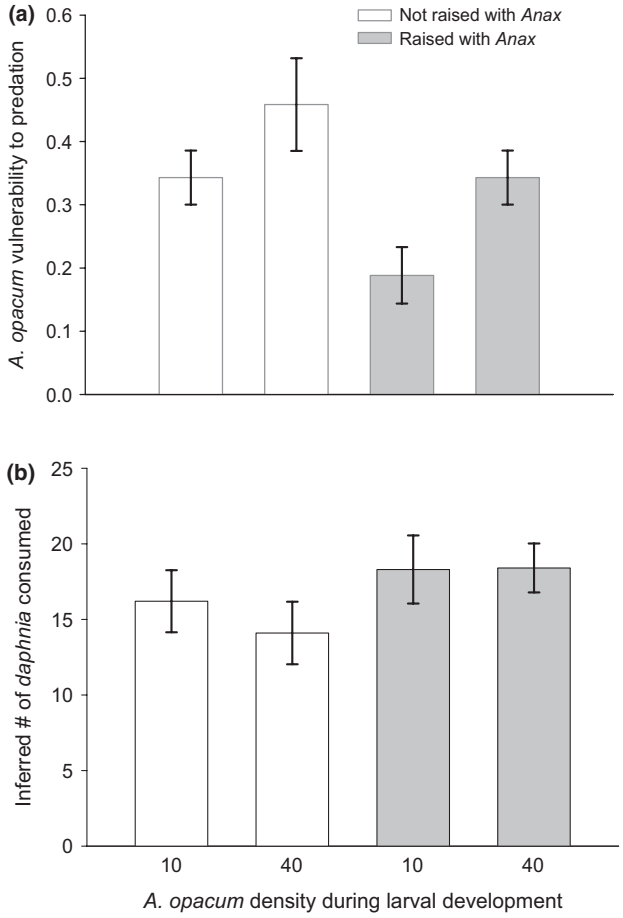

Fig. 4 Least square mean (± 1 SE) (a) vulnerability of each of the four *A. opacum* phenotypes to *Anax* and (b) the inferred (control-treatment) number of *Daphnia* consumed by each of four *A. opacum* phenotypes derived from environments differing in the density of conspecifics and the presence of caged *Anax*. Vulnerability to predation is measured as the absolute value of the instantaneous per capita rate at which *Anax* kill *A. opacum*.

Predator-induced phenotypes did not differ from non-predator-induced phenotypes in foraging efficiency ($F_{1,\ 9}$ = 3.10, P = 0.112; Fig. 4b). Phenotypes produced by differences in the density of conspecifics did not differ in foraging efficiency ($F_{1,9}$ = 0.27, P = 0.618; Fig. 4b). Simultaneous exposure to both predators and high densities of conspecifics did not result in a change in the foraging efficiency of *A. opacum* that might otherwise be expected by the independent influence of predators or higher density of conspecifics ($F_{1,\ 9}$ = 0.37, P = 0.556; Fig. 4b).

Discussion

Prior work on anti-predator defences of intermediate predators has focused primarily on documenting behavioural responses, and there has been less of an emphasis on understanding how environmental conditions alter the extent to which intermediate predators express morphological responses. We found that the top predator, *Anax*, induces anti-predator morphological defences in the intermediate predator, *Ambystoma opacum*, but the magnitude of this response often varied among environments differing in the density of *A. opacum* present. Of the six *A. opacum* traits that were altered by top predators, five were altered when the density of *A. opacum* was low, but not when it was intermediate or high.

The advantages gained by intermediate predators altering their phenotype should make persistence more likely with top predators in environments with a low density of conspecifics than in environments with a high density of conspecifics for at least two reasons. First, microcosm experiments have revealed that intermediate

predators with predator-induced defences persist for longer periods with top predators or cannibalistic intermediate predators than intermediate predators not exposed to top predators or cannibalistic intermediate predators (Banerji & Morin, 2009; Kratina, Hammill & Anholt, 2010). Second, simulation studies (Taylor & Scott, 1997; Taylor, Scott & Gibbons, 2006) reveal that the long-term persistence of *A. opacum* populations is enhanced when larval survival is high. Consequently, *A. opacum* populations are more likely to persist with top predators in environments with a low density of larval conspecifics because *A. opacum* larvae develop phenotypes that enhance larval survival in these environments.

Prior research with herbivores has demonstrated that the development of morphological anti-predator defences can come at the cost of reduced competitive ability (Relyea, 2002). We did not find a similar cost for intermediate predators in our study. Instead, we found that the ability of *A. opacum* to deplete its food resources was not altered by the induction of morphological defences. The absence of such a trade-off for intermediate predators is important because theory suggests that intermediate predators must be competitively superior to top predators in order to coexist with them (Holt & Polis, 1997). Although we cannot state that *A. opacum* are superior competitors, we do know that their ability to capture shared prey was not hampered by the development of morphological defences. Similarly, Hammill & Beckerman (2010) found that phenotypic defences induced by fish in phantom midges (*Chaoborus flavicans*; an intermediate predator) reduced their risk of predation, but the change in phenotype was not associated with a change in the ability of phantom midges to consume their prey. Together, this body of work supports the idea that herbivores do indeed respond to their predators differently than do intermediate predators. More importantly, it demonstrates that when top predators have negative consequences on their prey via multiple mechanisms, the prey do not adjust their morphology in response to one mechanism (e.g. predation) when the negative consequences for other mechanisms (e.g. competition) could be enhanced. Such a trade-off would be especially problematic if the maintenance of competitive superiority is a key component for allowing members of an intraguild food web to persist (Holt & Polis, 1997). Consequently, a trade-off between competitive ability and vulnerability to predation was most likely not the reason that *A. opacum* exhibited more predator-induced morphological changes when present at low density than at high density.

Two explanations for more pronounced changes in intermediate predator phenotype at low than at higher conspecific densities are as follows: (i) intermediate predators are less likely to invest in energetically costly defences when they are competing with conspecifics for resources and (ii) the vulnerability to predation was weaker at higher densities due to a saturating functional response of the predators. The decline in *A. opacum* body size with increasing larval *A. opacum* density (Petranka, 1989; Scott, 1990; this study) supports the hypothesis that intraspecific competition is stronger in environments with a higher density of *A. opacum*. Despite the fact that predator-induced changes in larval *A. opacum* morphology were stronger and involved more traits in environments with a low density of *A. opacum*, predators did cause *A. opacum* to develop taller tail fins across a broad range of *A. opacum* densities. It appears likely that larval salamanders in environments with a high density of conspecifics had access to insufficient resources to develop the full suite of morphological defences, but could develop some defences.

Although we found that *Anax* induced more morphological changes in *A. opacum* when *A. opacum* was present at low density than at high density, surprisingly we found that the vulnerability of *A. opacum* to *Anax* was not dependent on a statistical interaction between prior exposure to *Anax* and conspecific density. In other words, the extent to which prior exposure to *Anax* reduced the vulnerability of *A. opacum* was statistically similar in environments that varied in the density of *A. opacum* present. This observation suggests that tail fin depth, the only morphological trait to change as the result of *Anax* presence at all *A. opacum* densities, was mostly responsible for reducing vulnerability to predation. The absence of a statistically significant interactive effect between prior *Anax* exposure and conspecific density on the vulnerability of *A. opacum* does not mean, however, that the other predator-induced morphological changes are not biologically important. The predator-induced phenotype of *A. opacum* experienced a greater reduction in vulnerability to predation compared with the non-predator-induced phenotype that developed when the density of *A. opacum* was low (approximately 12% more survived foraging trials with *Anax* if they had prior exposure to *Anax*) rather than when the density of *A. opacum* was high (7% more survived foraging trials with *Anax* if they had prior exposure to *Anax*). We may have lacked statistical power to detect this small difference (5%) in vulnerability to predation, but small differences in vulnerability to predation could have very important consequences for populations over the long

term (Vance-Chalcraft & Soluk, 2005). For example, Taylor *et al*. (2006) found that even a small change in the survival of larval *A. opacum* can greatly increase the minimal adult survival rate necessary for *A. opacum* populations to persist when larval survival is generally low. Survival of larval amphibians in nature is generally low (Wells, 2007). Nonetheless, it appears that body size and tail fin depth may be the most important traits for reducing predation risk of *A. opacum* to *Anax* over the short term, with size being mediated by *A. opacum* conspecific density. Although our experiment was only performed during the larval stage of *A. opacum*, it is important to note that growth (e.g. size) can have important consequences for long-term dynamics of *A. opacum* populations (Taylor & Scott, 1997; Taylor *et al*., 2006). Specifically, larval growth clearly has significant consequences for overall fitness of individual *A. opacum* and persistence of *A. opacum* populations (Scott, 1994).

We also found that the predator-induced defences employed by *A. opacum* varied through ontological development. The delay in the appearance of morphological responses until intermediate stages of larval development is probably due to a lag in the reallocation of tissues away from overall growth towards growth of morphological defences (Van Buskirk & Schmidt, 2000; Hoverman & Relyea, 2007). This finding supports the hypothesis that morphological defences can take additional time to develop, but organisms can respond immediately to predators with behavioural defences (Relyea, 2003; Hoverman & Relyea, 2007, 2009). Initially, we did find a change in *A. opacum* activity levels so our work accords with this observation.

Some of the *A. opacum* traits that differed among treatments during the middle of larval development were not statistically distinguishable among treatments just prior to metamorphosis. The disappearance of morphological differences between treatments could occur for any of at least three reasons. First, morphological responses during the middle of larval development were found in the larvae's trunk and tail, which may all converge before metamorphosis due to developmental constraints. Unlike tadpoles, salamanders retain their tails after metamorphosis with tail fins becoming absorbed and reduced. This suggests that no matter how deep tail fins are during the larval period, there is a restriction once a salamander metamorphoses. Second, it is possible that there is an optimum body size or minimum trait size (e.g. torso length) that must be achieved before salamanders can initiate metamorphosis. Salamanders with shorter torsos during the middle of their larval development may have enhanced growth of their torso during the latter part of the larval period by reallocating the energy (tissues) from their expressed tall tail fins to torsos. Third, convergence may also coincide with reduced mortality during the later stages of larval development because *A. opacum* approaches a size during this time of larval development that makes it more difficult for *Anax* to capture efficiently. Our findings suggest that the effect of top predators on intermediate predator morphology may not carry across from aquatic to terrestrial stages of intermediate predators although more work is needed to measure fitness components of the different phenotypes during their terrestrial phase. For example, it is possible that the development of induced defences requires resources that affect egg production or metabolism in ways that are independent of size at metamorphosis and which could affect both total egg production and survival during the terrestrial phase of life.

Interestingly, one of the salamander traits that responded differently to predators at middle and late stages of development, head depth, is probably related to the ability of salamanders to capture prey (Nyman, Wilkinson & Hutcherson, 1993). During the middle of development, salamanders did not alter their head depth in response to predators and we found no variation in the ability of salamanders to deplete their prey resources among treatments that varied in the presence of top predators. We did not measure the foraging ability of *A. opacum* late in development, but *A. opacum* did develop deeper heads late in development in response to the presence of *Anax*. Salamanders with deeper heads are less likely to be gape-limited, which would allow them to be more efficient in harvesting prey (Kishida *et al*., 2009). Furthermore, larger heads would allow those individuals to consume larger and more prey items that *A. opacum* and *Anax* compete for (Yurewicz, 2004). Consequently, if anything, the competitive ability of *A. opacum* may be enhanced in the presence of *Anax* during late stages of development and the increased competitive ability could also increase the likelihood of their persistence together.

It is clear that the simultaneous exposure of larvae of intermediate predators to intraspecific competitors and top predators can influence the phenotype of an individual in a way that can lead to differential performance. Consequently, the development of anti-predator strategies in intermediate predators may play a crucial role in allowing them to persist with top predators in nature. Although the development of anti-predator defences in intermediate predators may lower their vulnerability to top predators, our work also demonstrates that the presence of more intermediate predators can limit the

development of the full suite of anti-predator defences in intermediate predators. Ultimately, our work provides some insight into the kinds of environments where intraguild predators are more likely to persist with each other. The influence of competitors and stage of development on morphological traits that develop in response to predators may explain much of the variation in phenotypic traits that is often observed in nature (e.g. Van Buskirk, 2009).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Repeated measures analysis of covariance results for morphological responses of *Ambystoma opacum* to six different larval environments varying in the occurrence of caged *Anax* predators and densities of conspecifics present.

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