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Environment-dependent trade-offs and phenotypic plasticity in metamorphic timing

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ABSTRACT

Background: Fitness trade-offs of plastic traits between alternative environments are a prerequisite for the evolution of phenotypic plasticity; however, the costs associated with plastic traits have yet to be determined. Most empirical studies have assessed the costs of plastic traits by investigating just two environments (to elicit plasticity), and only one or two environments to evaluate the consequences of plasticity. In contrast, in nature, organisms are constantly subjected to multiple environments, and the expression and magnitude of the costs of plastic traits are occasionally context-dependent.

Objective: Analyse the costs of plastic traits across multiple environments.

Methods: We determined the benefits and costs of two plastic responses (predator- and prey-induced morphologies) of larvae of the salamander *Hynobius retardatus* to larval survival, time to metamorphosis, and body size at metamorphosis in three different environments [using tadpoles of an anuran frog as prey, larvae of a predatory dragonfly, or no change agent (conspecific larvae only)].

Results: The benefits of the alternative phenotypes were evident in the two inducing environments, but the costs were greater or more easily detected in crossover environments. The trade-offs appeared in combinations in the crossover environments, and thus were context-dependent.

Conclusions: The cross-environmental costs of plastic traits are necessary for the evolution of phenotypic plasticity. Our findings highlight the importance of measuring the costs and benefits of plastic traits across multiple environments.

Keywords: competing risk, Cox proportional hazards model, metamorphosis, survival analysis.

INTRODUCTION

In nature, organisms have to maximize fitness under temporally and spatially heterogeneous conditions (Roff, 2002). Phenotypic plasticity allows organisms to modify their phenotype in response to changes in the environment, and this appears to be the adaptive strategy for survival in variable environments. Since phenotypic plasticity is not ubiquitous, its

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evolution, including the factors that drive and constrain this process, has attracted much attention (Auld *et al.*, 2010). Fitness-trade-offs between alternative environments is a prerequisite for the evolution of phenotypic plasticity (Moran, 1992; Sultan and Spencer, 2002).

The potential costs of plastic traits include those that are incurred by individuals when plastic traits are expressed in environments different from those to which they are adapted. Mismatches between a trait and the environment can lower fitness, with selection operating on suites of traits (Sultan and Spencer, 2002). However, empirical studies on such costs have largely been restricted to investigations of just two environments [e.g. the presence or absence of a predator (Susana *et al.*, 2008)]. Environments are rarely this simple. Nonetheless, it is an important first step in determining whether a particular response is costly, hence research conducted with a small number of environmental variables. In nature, since most organisms are frequently subjected to multiple environmental factors (e.g. biotic environmental factors such as predators, competitors, and prey items), and the expression and magnitude of the costs of plastic traits are occasionally context-dependent (Callahan *et al.*, 2008; Hoverman and Relyea, 2009), the costs of plastic traits ought to be assessed across multiple environments.

Some organisms that encounter variable environments show phenotypic plasticity in life-history traits (Dorken and Barrett, 2004; Jensen *et al.*, 2008; Lardies and Bozinovic, 2008; Michimae and Emura, 2012). Amphibian larvae, in particular, have been shown to be an excellent model system for exploring trade-offs between the benefits afforded by plasticity in one trait and the consequences of that plasticity for other traits that affect fitness. Many anuran larvae generally develop relatively large tails and small bodies, and show more sustained behavioural inhibition in the presence of predators such as predatory invertebrates (Van Buskirk, 2002; Relyea and Auld, 2005). Several studies have shown that organisms with predator-induced responses are generally less vulnerable to predation than those in which phenotypes are produced in the absence of predators (Benard, 2006). However, these predator-induced responses are typically associated with fitness costs such as reduced growth investment (Relyea and Auld, 2005; Middlemis *et al.*, 2013). Besides such fitness trade-offs between survival and growth during the larval period, survival trade-offs also occur. Some amphibian larvae show consistent plastic responses to variation in prey items (Crump, 1992). In particular, some salamander larvae develop relatively short tails, a long snout–vent length (SVL), and wide heads and mouths; furthermore, they exhibit more aggressive behaviour in environments crowded with prey items, such as conspecific or heterospecific anuran larvae (Michimae and Wakahara, 2002). Therefore, predators and prey typically induce opposite behaviour and morphology in salamander larvae (Murray *et al.*, 2004; Michimae and Hangui, 2008), implying that plastic responses to the change-inducing environment might have a negative effect on survival in other environments.

Larvae of the salamander *Hynobius retardatus* have a well-documented ability to exhibit prey-induced plastic morphology, namely, the broad-headed morph (Michimae and Wakahara, 2002). Under conditions of crowding with heterospecific anuran (*Rana pirica*) tadpoles, *H. retardatus* larvae become behaviourally more aggressive and frequently develop wider heads and larger mouths than conspecifics reared under conditions of lower heterospecific larval density (Michimae and Wakahara, 2002); this phenotype helps them to swallow their amphibian prey (Takatsu and Kishida, 2013). Furthermore, *H. retardatus* larvae develop relatively large tails and small bodies in the presence of dragonfly (*Aeshna nigroflava*) larvae (Michimae and Hangui, 2008). This predator-induced plastic morphology is qualitatively similar to that found previously in anuran tadpoles and salamander larvae in response to chemical cues from predatory dragonfly larvae (Van Buskirk, 2002; Kishida and Nishimura, 2005; Relyea and Auld, 2005;

Middlemis *et al.*, 2013). Although the morphological responses of *H. retardatus* larvae to heterospecific anuran tadpoles and predatory dragonfly larvae are clear, our knowledge of the costs and benefits of the two responses is limited. Therefore, it is unclear whether the expression and magnitude of the costs of plastic responses are dependent on the type of environment. When *H. retardatus* larvae exhibit the predator-induced morphology, the plastic response may increase resistance to predation in the predator-induced environment; however, these *H. retardatus* larvae may incur fitness-related costs such as delayed time to metamorphosis or reduced size at metamorphosis in the same or other environments. Moreover, this may lead to lower survival during the larval period. When *H. retardatus* larvae develop a prey-induced offensive morphology, the plastic response may raise the efficiency of predation in environments containing prey such as frog tadpoles (Takatsu and Kishida, 2013), improve their growth, and lead to higher survival. However, the offensive morphology could increase their vulnerability to predation. Given that *H. retardatus* larvae co-exist with a range of functionally diverse predator and prey species in natural communities (Michimae, 2011), assessing whether ecological phenotypes (morphologies) induced by multiple factors lead to fitness trade-offs within or across multiple ecologically relevant environmental conditions is critical for predicting the evolution of phenotypic plasticity in natural populations.

In this study, we generated three different morphologies (i.e. non-, predator-, and prey-induced morphologies) and established three different environments: prey tadpoles of the anuran frog *Rana pirica* (tadpole environment), larvae of the predatory dragonfly *Aeshna nigroflava* (dragonfly environment), and non-induced (conspecific larvae only; conspecific environment). The primary objectives of the present study were as follows: (1) identify the benefits and costs of the two phenotypically plastic responses (predator- or prey-induced morphology) of salamander larvae in the three different environments by assessing survival during the larval period and life-history traits such as timing of and size at metamorphosis; (2) determine the trade-offs between the benefits afforded by a plastic morphology and the consequences of that plastic morphology for other traits that affect fitness in or across multiple environments; and (3) explore whether fitness-related trade-offs are dependent on the type of environment.

MATERIALS AND METHODS

Study species, study area, and sampling

In late May 2012, we collected 20 clutches of salamander eggs (*H. retardatus*), 30 masses of frog eggs (*R. pirica*), and several dragonfly larvae (*A. nigroflava*) from a number of ponds in the Teshio experimental forest, Horonobe-chou, Hokkaido, Japan. Clutches of salamander eggs were placed in stock tanks (37.0 × 25.0 × 13.5 cm) filled with 10 litres aged tap water until hatching. Frog eggs were arbitrarily divided (200–300 individuals per tank) and placed in stock tanks (33.4 × 20.0 × 10.0 cm) filled with 2 litres aged tap water. After the frog eggs had hatched, two pieces of rabbit chow (dry weight, 0.40 g) were added to each tank as food every two days. The dragonfly larvae were placed separately in small plastic pots (8.4 × 5.7 × 4.4 cm) filled with 100 mL aged tap water. One small salamander larva (200 mg) was placed in each pot as food every two days. All animals were housed in an experimental room, which was maintained at 16°C with a natural light/dark regime. Water in the rearing tanks was changed every 2 days.

Induction treatments

After the salamander eggs had hatched (SVL, 12.04 ± 0.93 mm; gape size, 3.74 ± 0.64 mm; mean ± standard deviation, $N = 20$), five randomly selected salamander hatchlings were placed in each of 90 tanks (33.4 × 20.0 × 10.0 cm) filled with 2 litres aged tap water (i.e. a total of 450 salamander hatchlings). Three induction treatments were conducted on the salamander larvae to generate 'non-induced', 'prey-induced', and 'predator-induced' morphologies. The prey-induced morphology was generated by placing 100 *R. pirica* tadpoles (SVL, 5.73 ± 0.50 mm; body width, 3.48 ± 0.29 mm; $N = 20$) in each of 30 tanks as induction agents. We generated the predator-induced morphology by placing a cage containing one third-, fourth- or fifth-instar dragonfly larva in each of 30 tanks as the induction agent. We reduced the effect of individual variability of the dragonfly larvae by replacing the dragonfly larvae every 2 days, while maintaining the others in stock tanks. For each replacement, one small salamander larva was placed in each cage as food. Salamander larvae with a non-induced morphology were obtained by adding no induction agents to the remaining 30 tanks. A week after the treatments began, salamander larvae with the prey-induced morphology were clearly larger than those in other treatments, possibly because they had consumed *R. pirica* tadpoles. To maintain similar salamander larvae body size among treatments, larvae used in the prey-induced treatment were refrigerated (4–8°C) for a few days. An additional exposure to water at a low temperature (4°C) for a period of time (e.g. 30–90 days at intervals of 30 days) delayed metamorphosis of salamander larvae (corresponding to 30–90 days of delay), but did not affect the size at metamorphosis (Iwasaki and Wakahara, 1999). However, in the present study, this additional treatment for such a short period would have had little effect on the timing or size at metamorphosis of the prey-induced morphology.

There were no significant differences in the body size [breast–vent length (BVL)] of salamander larvae among the three treatments 17 days after beginning the induction treatments (prey-induced morphology, 11.00 ± 0.43 mm; predator-induced morphology, 10.92 ± 0.55 mm; non-induced morphology, 10.95 ± 0.44 mm; $N = 15$). During the induction treatments, water from the rearing tank was exchanged three times each week; salamander larvae were fed *Tubifex* and brine shrimp, and tadpoles were fed rabbit chow three times each week.

Field experiments

We conducted three field experiments in an outdoor artificial pond (area = 10 m × 15 m) in the Teshio experimental forest of Hokkaido University (45°01′77.65′N, 142°01′47.71″E). This pond had no tree canopy, a depth of 38 cm, and a bottom comprising soil and small rocks. In this pond, adult salamanders and frogs typically begin to spawn in late April, and larvae of both amphibians co-exist and interact from spring to late summer. Invertebrate predators, including larvae of the dragonfly *A. nigroflava*, are also naturally distributed in this pond. Twenty-seven square enclosures (60 cm × 60 cm × 60 cm) with a polyvinyl chloride (PVC) frame and 1-mm nylon mesh on all sides were placed in the pond. Small water insects such as chironomids (Chironomidae spp.), water boatmen (Corixidae spp.), and ephemerids (Baetidae spp.) passed through the mesh to colonize the enclosures. These animals are potential prey for salamander larvae. Three sets of nine enclosures were used for each of the following three experiments.

Experiment 1

In each of the nine enclosures, a clump of emergent plants (*Poaceae* sp.) was used to mimic a typical vegetation structure in ponds with abundant predators. Next, 10 randomly selected salamander larvae with the non-, predator- or prey-induced morphology were assigned to three enclosures each. After the salamander larvae had been allowed to acclimate for one day, two fourth- or fifth-instar dragonfly larvae were placed in each enclosure. The surviving salamander larvae were counted twice daily, in the morning and evening. The count was continued until the last salamander larva expired, and the number of days from placing the dragonfly larvae in the enclosures to the death of each salamander larva was recorded. During each count, all dragonfly larvae were collected and randomly reassigned to enclosures to minimize individual variability among dragonfly larvae with respect to predation efficiency. Because final-instar dragonfly larvae eat less, they were replaced with third-, fourth- or fifth-instar larvae during the experiment.

Experiment 2

In each of the nine enclosures, two artificial floating islands made of polystyrene foam plates (10 × 15 cm) were introduced for metamorphosing salamanders and tadpoles. Next, 200 *R. pirica* tadpoles (SVL, 9.693 ± 1.070 mm; body width, 6.117 ± 0.685 mm; $N = 20$) were added. Subsequently, 10 randomly selected salamander larvae with the non-, predator- or prey-induced morphology were assigned to three enclosures each. The presence of metamorphosed salamanders was checked once a week, until the first metamorphosed individual was observed. Subsequently, twice-weekly checks were conducted for the presence of metamorphosed salamanders, the remaining individuals were counted, and the time to event (days from placing the salamander larvae in the enclosures to the completion of metamorphosis or larval death) was recorded for each larva. Metamorphosed salamanders and *R. pirica* tadpoles were collected from the enclosures during each check. The ventral surface of the metamorphosed salamanders was scanned, and their body size calculated from the scanned images using ImageJ 1.45s (Schneider *et al.*, 2012).

Experiment 3

In each of the nine enclosures, we placed two artificial floating islands and 10 randomly selected salamander larvae with one of the three morphologies. The presence of metamorphosed salamanders was checked, the time to event was recorded, and the individuals were weighed and measured using the same methods as described for Experiment 2.

Statistical analyses

Each environmental group (predatory dragonfly larvae, prey tadpoles, and conspecific salamander larvae) contained 90 individuals in total, including 30 larvae (10 larvae × 3 enclosures) with each of the three morphologies (non-, predator-, and prey-induced morphology).

Time to event analysis

When analysing time to event data, competing risks arise when the individuals being studied experience only one of the multiple events (death or metamorphosis). For each individual, the time to event and the type of event were recorded. For instance, time to metamorphosis was not recorded if death occurred before metamorphosis. The standard way of analysing time to event data with competing risks is to use proportional hazard regression and cumulative incidence analysis (Bakoyannis and Touloumi, 2012).

Whether larval morphologies had differing effects on the time to death (survival) or the time to metamorphosis (metamorphic timing) during the larval period was determined in the dragonfly environment. All salamander larvae in this environment died before metamorphosis with the exception of one larva; thus data on metamorphic timing and body size at metamorphosis were not available. In the predatory dragonfly larvae environment, a Cox proportional hazards model was used to calculate the effects of morphologies on the time to death (Cox, 1972). Identity of the enclosure was added to the Cox proportional hazards model as a random effect term to take into account the morphology-specific interactions among larvae in their enclosures. The results of this analysis are reported as hazard ratios with 95% confidence intervals (CI). Survival over time was also characterized using the cumulative incidence function (Bakoyannis and Touloumi, 2012).

Whether larval morphologies had different effects on the time to death and time to metamorphosis during the larval period was determined in the prey tadpole environment and the conspecific environment. Survival during the larval period and timing of metamorphosis were analysed using a cause-specific Cox proportional hazards model to assess the effects of the morphologies and enclosure as a random effect term on both the time to death and the time to completion of metamorphosis. The results of this analysis are reported as hazard ratios with 95% confidence intervals. Survival over time was also characterized using the cumulative incidence function (i.e. the overall probability of death in the presence of metamorphosis), as was completion of metamorphosis over time (i.e. the overall probability of metamorphosis in the presence of death) (Bakoyannis and Touloumi, 2012).

Analysis of size at metamorphosis

Whether larval morphologies had different effects on SVL at metamorphosis was determined in the tadpole and conspecific environments. In the analysis of SVL at metamorphosis in the two environments, if a larva had died, the associated SVL measurements were deleted before performing the following analysis. In the two environments, a generalized linear mixed model was used to calculate the effects of morphologies on SVL. Identity of the enclosure was added to the generalized linear mixed model as a random effect term to take into account the morphology-specific interactions among larvae in their enclosures. *Post hoc* comparisons between larval morphologies in the two environments were performed using a Tukey-Kramer multiple comparison test.

RESULTS

Survival time

In the dragonfly environment, the estimated hazard ratio of predator-induced to non-induced morphology was 0.561 (95% CI, 0.331–0.948); the hazard ratio and the upper limit of the associated 95% CI were less than 1, indicating that the time to death was increased. The estimated hazard ratio of prey-induced to non-induced morphology was 1.727 (95% CI, 1.014–2.939); the hazard ratio and the lower limit of the associated 95% CI were more than 1, indicating that the time to death was shortened. The cumulative incidence analysis showed that salamanders with the predator-induced morphology had longer survival times and those with the prey-induced morphology had shorter survival times than

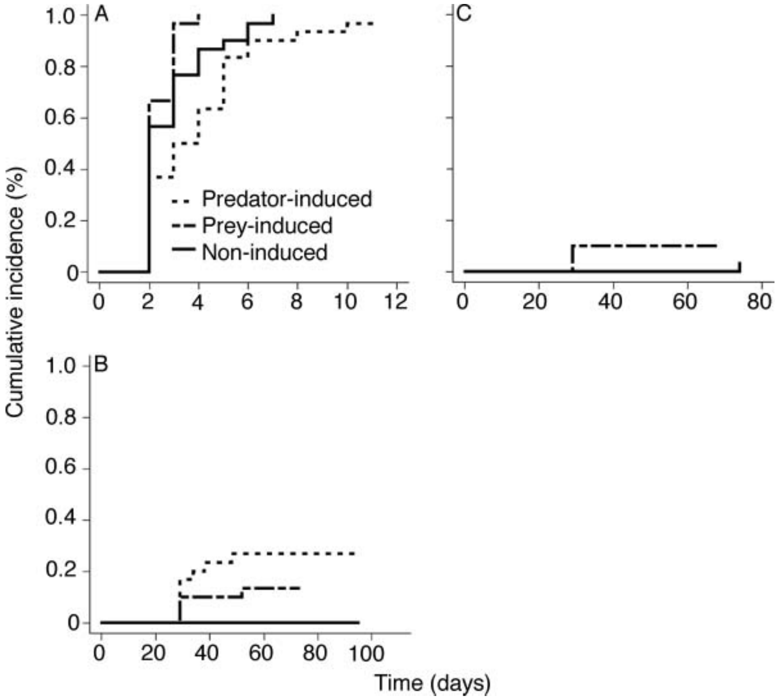

Fig. 1. Cumulative incidence functions (%) for time to death in the environment with predatory dragonfly larvae (A), anuran tadpoles (B) or conspecifics only (C).

those with the non-induced morphology (Fig. 1A). The variance of the random effect for enclosures showed no within-enclosure correlation ($P = 0.93$).

Parameter estimates (regression coefficients) of the Cox model did not converge to finite values in either of the other two environments because of few events (death) experienced by the non-induced morphology. In the tadpole environment, survival time was shorter for the prey-induced and predator-induced than the non-induced morphologies (Fig. 1B). In the conspecific environment, there were no marked morphological differences in the larvae among the non-induced, prey-induced, and predator-induced morphologies (Fig. 1C).

Time to metamorphosis

In the tadpole environment, the estimated hazard ratio of prey-induced to non-induced morphology was 7.62 (95% CI, 3.812–15.23); the hazard ratio and the lower limit of the associated 95% CI were greater than 1, indicating that the time to metamorphosis was shortened. The estimated hazard ratio of predator-induced to non-induced morphology was 1.17 (95% CI, 0.673–2.05); timing of metamorphosis was shorter for the prey-induced than for the non-induced morphology, but there was no significant difference in timing between the non-induced and predator-induced morphologies (Fig. 2A). The variance of the random effect for enclosures showed no within-enclosure correlation ($P = 0.93$).

In the conspecific environment, there were no differences in larval morphology among the non-induced, prey-induced, and predator-induced morphologies (Fig. 2B).

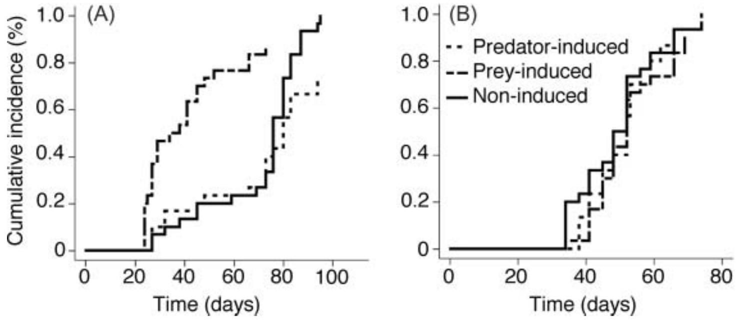

Fig. 2. Cumulative incidence functions (%) for time to metamorphosis in the environment with anuran tadpoles (A) or conspecifics only (B).

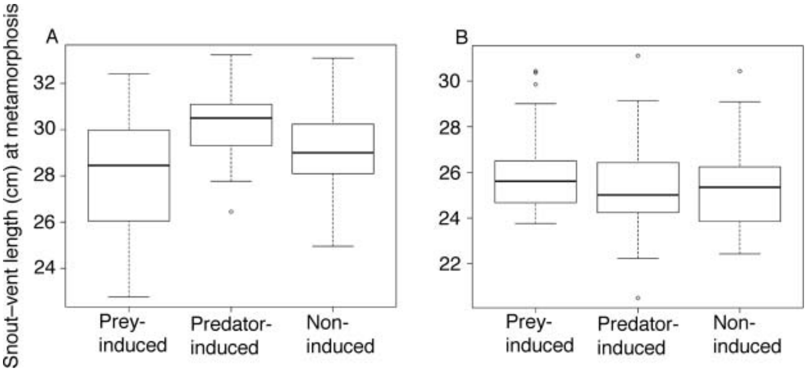

Fig. 3. Body size [snout–vent length (SVL) in centimetres (mean ± standard error, S.E.)] at metamorphosis in the environment with anuran tadpoles (A) or conspecifics only (B).

SVL at metamorphosis

In the tadpole environment, larval morphologies had a significant effect on SVL at metamorphosis ($F_{2,62} = 6.26$, $P < 0.01$), but the enclosures had no effects on SVL at metamorphosis ($z = 0.51$, $P = 0.31$). Snout–vent length was significantly less for the prey-induced morphology than for the non-induced morphology ($t = -2.39$, $P = 0.05$; Fig. 3A) and predator-induced morphology ($t = -3.49$, $P < 0.01$; Fig. 3A), but there was no significant difference in SVL at metamorphosis between the non-induced and predator-induced morphologies ($t = 1.30$, $P = 0.40$; Fig. 3B). In the conspecific environment, there was no evidence of statistically significant morphological ($F_{2,76} = 0.40$, $P = 0.67$) or random effects ($z = 0.80$, $P = 0.21$) on SVL at metamorphosis (Fig. 3B).

DISCUSSION

Survival in the dragonfly environment

Inducing the anti-predator morphology prolonged survival of the salamander *H. retardatus*, thus indicating the survival benefits of this morphology in the dragonfly environment (Fig. 1A). Deeper tails might attract predator strikes to the more expendable tail region and away from the more vulnerable head–body and core muscle regions, allowing escape (Van Buskirk *et al.*, 2003). *Hynobius retardatus* larvae that have previously encountered predators are likely to show more rapid and sustained behavioural inhibition in the presence of predators compared with those that have not, although the behavioural responses of the salamander larvae were not investigated in the present study. Some anuran tadpoles have been reported to show more pronounced predator-avoidance behaviour after witnessing predation events (Murray *et al.*, 2004) or simulation of such (Gonzalo *et al.*, 2007). Predator-avoidance behaviours in response to prior exposure to predators are likely to increase survival in the presence of predators (Alvarez and Nicieza, 2006).

The survival time of salamanders with the broad-headed (or prey-induced) morphology was shorter than that of the morphologically non-induced morphology (Fig. 1B), indicating the survival costs of the broad-headed morphology in the dragonfly environment. This dumpy morphology, with shorter tail length, longer SVL, and wider head and mouth (Michimae and Wakahara, 2002; Michimae and Hangui, 2008), might be a disadvantage in avoiding attack by predators. In amphibians, differences in body shape are known to affect vulnerability to predators during the larval period (Van Buskirk *et al.*, 2003, Benard, 2006). In fact, larvae with the broad-headed morphology show slower burst swimming than those with a non-induced morphology (G. Miyazaki *et al.*, unpublished data).

Survival and metamorphosis traits in the conspecific environment

Unexpectedly, the duration of the larval period and SVL at metamorphosis of *H. retardatus* remained unaffected by larval morphology, indicating no survival costs or developmental or growth costs of predator- or prey-induced morphologies in the conspecific environment (Figs. 1C, 2B, and 3B). In particular, producing and maintaining these plastic morphologies was thought to increase the developmental and growth costs compared with those in non-induced larvae, resulting in delayed metamorphosis and small size at metamorphosis (Van Buskirk and Schmidt, 2000; Relyea and Auld, 2005). However, our results do not support this scenario. *Hynobius retardatus* larvae might be able to consume small aquatic insects such as chironomids (Chironomidae spp.), water boatmen (Corixidae spp.), and ephemerids (Baetidae spp.) in their enclosures to fuel their metamorphosis. Considering that the various morphological types do not differ in resource acquisition, the developmental and growth costs associated with phenotypic plasticity in this environment would not be great (as discussed below).

Survival and metamorphosis traits in the tadpole environment

The somewhat higher mortality (approximately 15%) observed in salamander larvae with the broad-headed morphology may indicate that low-frequency cannibalism occurred in the enclosures (Fig. 1B). However, surprisingly, the mortality of salamander larvae with the anti-predator morphology was approximately 30% (Fig. 1B). Furthermore, the mortality of the anti-predator morphology in the frog tadpole environment occurred within 30 days of the experiment starting (Fig. 1B). *Rana pirica* tadpoles might not compete with *H. retardatus* larvae, but might limit the time that small *H. retardatus* larvae, particularly those with the anti-predator morphology, spend searching for or feeding on prey items such as small water insects. Behavioural responses (i.e. reducing activity) to predatory dragonfly larvae might be widespread in amphibian species (Van Buskirk, 2002; Relyea and Auld, 2005), and our data reveal that they occur in *H. retardatus*. Increased density of *R. pirica* tadpoles may disturb searching or feeding activity in small and easily alarmed *H. retardatus* larvae with prior exposure to predators that are absent in the current environment, which could eventually increase larval mortality.

The metamorphic timing of *H. retardatus* was shortened with the broad-headed morphology (Fig. 2A), indicating the developmental benefits of the broad-headed morphology in the tadpole environment. However, the accelerated metamorphosis ultimately resulted in a smaller body size at metamorphosis (Fig. 3A). These results suggest that the unstable habitat in which amphibian larvae live should be taken into account. Developmental plasticity might allow larval amphibians inhabiting temporary or ephemeral water bodies to complete their metamorphosis rapidly before their habitat dries up. In this case, acceleration of larval development might be an adaptive strategy in drying ponds, because it reduces mortality due to desiccation (Altwegg and Reyer, 2003; Chelgren *et al.*, 2006), even though it is associated with a smaller size at metamorphosis (Fig. 3A), which can adversely affect the survival of juveniles and the breeding success of adults (Altwegg and Reyer, 2003; Chelgren *et al.*, 2006).

We previously investigated the effects of different food items (conspecific larvae, *Tubifex*, and tadpoles) on the body size at metamorphosis of prey-induced and non-induced morphologies (Michimae and Wakahara, 2002). In that experiment, the two morphologies were not exposed to cold temperatures. There were no significant differences between the two morphologies in terms of body size at metamorphosis, but there were significant differences among food items. Salamander larvae fed only tadpoles, regardless of their morphology, were significantly smaller than those fed only *Tubifex* or only conspecific larvae. In the present study, the prey-induced morphology primarily fed tadpoles was significantly smaller than the predator-induced or non-induced morphologies primarily fed small insects. These results are consistent with those of the previous study, suggesting that the addition of the cold environment for the prey-induced morphology did not affect larval growth and development. However, even if the benefits of prey-induced morphologies for development were underestimated, metamorphic timing was still significantly shorter for the prey-induced morphology than for the non-induced or predator-induced morphology.

Environment-dependent trade-offs

Our results suggest that the trade-offs between the benefits and costs afforded by morphologically plastic traits depend on the type of environment in which the plasticity is measured (Figs. 1, 2, and 3). For example, *H. retardatus* with the broad-headed morphology can hasten its metamorphosis in the tadpole environment, which can have survival benefits by decreasing larval mortality due to desiccation when the ephemeral ponds they inhabit dry up (Fig. 2A) (Altwegg and Reyer, 2003; Chelgren *et al.*, 2006). However, this results in costs such as a smaller size at metamorphosis (Fig. 3A) in the same environment and lower survival (Fig. 1A) in an environment with predators. However, the trade-offs disappear when the conspecific and predator environments are compared (Figs. 1C and 3B). Therefore, the trade-offs afforded by plastic traits appear to be dependent on the environment. However, the anti-predator morphology can carry considerable survival costs in the tadpole environment (Fig. 1B) without generating any benefits (Figs. 2A and 3A), as well as producing no benefits or costs in the conspecific environment (Figs. 1A, 2B, and 3B). Instead, survival advantages are observed only in the predatory dragonfly environment (Fig. 1A), suggesting that the trade-offs afforded by phenotypically plastic traits are only seen when comparing combinations of environments. Plasticity trade-offs thus need to be assessed in or across multiple environments.

The costs of plastic traits were found to be higher in stressful environments, i.e. the survival costs of the broad-headed morphology were high in the predatory dragonfly environment and for the anti-predator morphology in the tadpole environment. The costs of plastic traits might be greater or more easily detected in stressful conditions, indicating that ecological costs are increased in the context of competition, predation risk or resource limitation. Environmental stressors (i.e. predatory dragonfly larvae and tadpoles) in our study systems also modified the expression and magnitude of the costs of plastic traits (Figs. 1, 2, and 3). Similar environment-dependent expressions of costs or life-history trade-offs have also been detected in other animals (Messina and Fry, 2003; Luong and Polak, 2007). Our results support the hypothesis that the trade-offs afforded by plastic traits are context-dependent, and that the costs of plastic traits are most likely detected under stressful conditions.

Although the cross-environmental costs of plastic traits are necessary for phenotypic plasticity to evolve (Moran, 1992; Sultan and Spencer, 2002), many studies have failed to detect the costs associated with plastic traits (Relyea, 2007). This may have been because investigations were performed for as few as two environments. The number and range of environments that the organisms encounter and the frequency of encounters with each environment are important determinants of the adaptive value of phenotypic plasticity (Pigliucci, 2001; Sultan and Spencer, 2002), and these effects might be largely attributed to the wider range of environment-dependent costs that organisms have to bear. Our findings highlight the importance of measuring costs and benefits across multiple environments and might stimulate the development of a method for estimating the potential importance of the costs of plastic traits.

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