

Osamu Kishida · Kinya Nishimura

Bulgy tadpoles: inducible defense morph

Received: 17 November 2003 / Accepted: 26 April 2004 / Published online: 9 June 2004
© Springer-Verlag 2004

Abstract Predator induced morphological defenses are marked morphological shifts induced directly by cues associated with a predator. Generally, remote cues, i.e., chemical substances emitted from predators or injured conspecifics, are considered to be ideal signals to induce morphological change in aquatic environments rather than close cues, i.e., close chemical or tactile cues, since chemical substances that can propagate over relatively long distances and persist for a long period may allow organisms to keep safe and to deliberately change their morph. In fact, most organisms adopting an inducible morphological defense utilize remote chemical cues to detect predation risk and to produce morphological defenses. In this paper, we report a unique and functionally well designed inducible morphological defense strategy where the induction process requires close cues from a predator. The tadpoles of *Rana pirica* exhibited a bulgy bodied morphology when threatened with predation by larval salamanders, *Hynobius retardatus*, in close proximity. Predation trials and a function experiment showed that the induced bulgy morph is an adaptive defense phenotype against the gape-limited predator larval *H. retardatus*. Furthermore, *R. pirica* tadpoles use two adaptive strategies in terms of cost saving, i.e., adjustment of the extent of bulginess according to predation risk and reversibility by actual shrink of bulgy body after removing the predation threat. In general, *R. pirica* hatch earlier than *H. retardatus*. In natural ponds, during the early developmental stage *R. pirica* tadpoles live in close proximity to young *H. retardatus* larvae. As they grow, the salamanders gradually become serious predators and the predator–prey interaction becomes intimate. After a while, predation, cannibalism and metamorphosis decrease the number of salamanders in the ponds, and the predator–prey interaction weakens. Such a phenology in the predator–prey

interaction allows the evolution of a close-cue detection system and adaptive cost-saving strategies. Our results highlight that the characteristics of the inducible defense depend on the intensity and specificity of the predator–prey system.

Keywords Phenotypic plasticity · Close cue · Remote cue · Predation · Reversibility

Introduction

Organisms demonstrate various defensive systems, such as escape and hide behavior, and possess defensive morphologies against biotic hazards. In general morphological defenses tend to be constitutive compared with behavioral defense systems (West-Eberhard 1989). However, some organisms do not pay a major part of the morphological defense cost in advance until they detect signs of pressing needs. They perceive a cue for a predation risk that will come later, in an uncertain distant future, and construct a defense morph before the actual risk arises (Adler and Harvell 1990; Tollrian and Harvell 1999). Such strategies, inducible morphological defenses, are known in many taxa in aquatic environments (reviewed by Tollrian and Harvell 1999), and are a form of phenotypic plasticity. The benefit of an inducible defense over a constitutive defense is the cost savings of only deploying the defense when being exposed to an intense regime of a selective agent. Organisms that adopt inducible defenses, therefore, can use the cost savings for other needs, if they can spend in the absence of predation hazards (Clark and Harvell 1992).

One of the important prerequisite for inducible defenses is the availability of a reliable cue concerning the existing predation threat (Tollrian and Harvell 1999). Signals indicating a predation risk must have been adopted according to the combination of its reliability, the time lag between occurrences of the cue and the actual risk, and the time required to respond to an impending crisis. Of the available cues, including sonic, visual, chemical, and tactile cues, most organisms use remote chemical cues to

O. Kishida (✉) · K. Nishimura
Graduate School of Fisheries Sciences, Hokkaido University,
Hakodate, 041-8611, Japan
e-mail: kishida@fish.hokudai.ac.jp
Tel.: +81-138-408827
Fax: +81-138-408827

detect predation risk and to produce morphological defenses (Brönmark and Pettersson 1994; Dahl and Peckarsky 2002; Hanazato 1990; Kusch 1993; Laurila et al. 2001; Stemberger and Gilbert 1984; Stenson 1987; Tollrian 1995; Trussell 1996; Van Buskirk and Arioli 2002). In general a remote chemical cue is a good signal for detecting a predator, for aquatic organisms adopting an inducible morphological defense, since chemical substances that can propagate over relatively long distances and persist for a long period may allow organisms to deliberately change their morph (Adler and Harvell 1990; Havel 1987; Tollrian and Harvell 1999). In contrast, if prey organisms require close cues to trigger the morphological change, what ecological properties favor such an induction process?

In terms of cost savings, adjusting the extent and reversibility of inducible defense phenotypes constitutes the conditional fine-tuning adaptation that depends on internal and external conditions. According to the principle of resource allocation, the cost of an inducible defense should result in fewer resources being available for growth, reproduction, or longevity (Dewitt et al. 1998). Therefore, adjusting the degree of a defense and reversibility of the inducible morph according to the degree of predation risk are adaptive strategies leading to cost savings (Clark and Harvell 1992). However, our knowledge of such the adjustment and reversibility of inducible defensive morphs are limited (Brönmark and Pettersson 1994; Hanazato 1990; Relyea 2003; Van Buskirk and Arioli 2002).

Anuran tadpoles have been used as model organisms in studies of inducible defense, which show common features of the inducible morphological response (Lardner 1998; Laurila et al. 2001; McCollum and Leimberger 1997; Relyea 2001; Van Buskirk 2002). First, the tadpoles commonly increase their tail fin depth and reduce their body in the presence of various predator species, although there are some modifications of the morphology in response to different predator species and different ecological situations (e.g., Relyea 2001; Van Buskirk 2001). Although the mechanical functions of these inducible traits are unclear (Van Buskirk and McCollum 2000a, b), such inducible phenotypes have greater survival rates when predators are present (McCollum and Van Buskirk 1996; Van Buskirk and McCollum 1999). Second, the induction is triggered by remote chemical cues from predators or injured conspecifics (Laurila et al. 2001; McCollum and Leimberger 1997; Relyea 2001; Van Buskirk 2002; Van Buskirk and Arioli 2002). A few studies using anuran tadpoles addressed the fine-tuning adaptations of inducible defenses, i.e., adjusting the extent (Van Buskirk and Arioli 2002) and reversibility of defense phenotypes (Relyea 2003).

We report a unique and functionally well designed inducible morphological defense strategy of *Rana pirica* tadpoles under the intimate predator–prey interaction with the larval salamander *Hynobius retardatus*. We demonstrate the following issues experimentally: (1) Tadpoles change their body to bulgy phenotype responding to the

presence of larval salamander. (2) Induction of the bulgy phenotype needs a close cue from the salamander larvae. (3) The mechanical function of the inducible morph is definitely clarified. The induced bulgy bodied morphology prevented tadpoles from being swallowed by larvae of *H. retardatus*, which is a gape-limited predator. (4) The degree of induction decreases with own size, in parallel to a reduced predation risk. (5) The induced bulgy morph actually shrinks and reverses to the typical non-induced morph when the predator is removed. We discuss the implications of these results in terms of the specific phenological characteristic of the predator–prey interaction.

Materials and methods

Tadpoles and predator salamanders

Rana pirica and *H. retardatus*, which are forest creatures, usually spawn in small transient ponds formed by melting snow in early spring, in Hokkaido, Japan. The larvae of both species frequently form loose aggregations within parts of a pond (Sato 1990). *H. retardatus* larvae prey on animals, including *R. pirica* tadpoles and conspecifics in the mixed school. In early spring, other predators have not yet emerged, and the two species interact closely (Michimae and Wakahara 2002). Predation success depends on gape size and prey body size, because the salamander larva swallows its prey whole (Michimae and Wakahara 2002; Ohdachi 1994). Generally, *R. pirica* hatches earlier than *H. retardatus*, although the egg-laying periods overlap (Sato 1990). Tadpoles and larval salamanders of various sizes coexist in a pond due to the random heterogeneity of the environment. Small tadpoles incur a size disadvantage in terms of predation threat.

R. pirica and *H. retardatus* eggs were collected from a pond in Akaigawa, Hokkaido, Japan from late May to early July in 2001. We conducted an induction experiment, a predation trial, and a reversion experiment in a laboratory at 16°C and a LD regime of 14:10 h throughout the experiments. A function experiment was conducted in another laboratory at 25°C with a natural light–dark regime.

Induction of the defensive morph

We designed an experiment to determine whether *R. pirica* tadpoles show an induced response to remote or close cues in the presence of *H. retardatus* larva.

We used *R. pirica* eggs from a single egg mass. The *H. retardatus* eggs were from a single clutch. The both eggs were kept in separate 18-l aquaria containing aged tap water. The *H. retardatus* larvae hatched on 26 May and the *R. pirica* tadpoles hatched on 9 June. We fed the tadpoles fish food and the salamander larvae live freshwater oligochaetes (Tubifex) twice a day. We changed the rearing water in order to remove any residual food late every afternoon.

The experiment was started on 20 June 2001. The experimental units were four 18-l (40.4 cm × 24 cm × 14 cm) predator aquaria and one no-predator aquarium. Each was filled with 16 l of aged tap water, and contained two saucer-shaped colanders made of stainless steel, which were 18 and 15 cm in diameter at the top and bottom, respectively. First, we randomly assigned 15 similarly sized, 11-day-old tadpoles (mean ± SD, body length = 6.39 ± 0.40 mm) to each colander. In the four predator aquaria, each colander was designated a “remote-cue” or a “close-cue” treatment in the following manner. We introduced one larval salamander from a holding tank, in which they were fed sufficient *R. pirica* tadpoles, into one colander in each aquarium (close-cue treatment). At the start of experiment, snout-vent length of the salamander larvae was 15.17 ± 0.74 mm

(mean \pm SD). Actual predation was minimized by replacing the larval salamander with others that were kept in the holding tank. In the replacement, larval salamanders were randomly chosen from the holding tank. The other colander did not contain a larva (remote-cue treatment).

We defined close cues as tactile or close-chemical cues, and remote cues as chemical cues. Visual cues do not induce the defensive morph (O. Kishida, unpublished data). In the close-cue colander, the tadpoles received close and remote cues. In the remote-cue colander, the tadpoles received remote cues that diffused from the adjacent close-cue colander. The no-predator aquarium contained two colanders without a salamander; we placed 15 tadpoles in each colander and called it the “no-cue” treatment.

After 10 days, all the surviving tadpoles were killed by immersion in 5% formalin. We measured the following seven morphological traits with digital calipers (± 0.01 mm): total length, body length (the tip of snout to the most posterior point of the body excluding the cloacal tailpiece), maximum body depth, maximum body width, maximum tail depth, maximum tail muscle width, and maximum tail muscle depth. In order to reduce the number of dimensions needed to describe the variation in shape, these morphometric data were subject to principal-component analysis (PCA) using the correlation matrix of all measurements.

We conducted an analysis of covariance with size (PC1, see Results) as the covariate for differences in shape (PC2, see Results) between the close- and remote-cue treatments. We regarded each aquarium as a block and treated it as a random effect; the treatment \times block effect was the denominator for the *F*-test of predation main effects. We do not consider the individuals' morphometrics in each tank as a case of pseudoreplications.

Although we attempted to inhibit larval salamanders from attacking tadpoles, some tadpoles were eaten or a part of the tail was chewed. We excluded these animals from the statistical analyses. For each treatment colander, the number of data points ranged from 9 to 15 [close-cue treatment (9–13), remote-cue treatment (14–15), and no-cue treatment (15)].

Effect, function, and reversion of the induced morph

Predation trials

A requirement of an inducible defense is that the defense has a beneficial effect on predation avoidance. We conducted a susceptibility experiment to determine how the induced morph really works to prevent predation by the larval salamander.

We selected tadpoles from three egg masses, mixed the hatchlings in a tank, and fed them fish food ad libitum. After 7 days, we randomly placed tadpoles into two plastic tanks (30 cm \times 25 cm \times 17.5 cm): one was the induction tank and the other was no-induction tank. We placed three 7-day-old larval salamanders in the induction tank to induce the morphological change. We did not introduce any larval salamanders to the no-induction tank; the tadpoles retained the typical morph. Fifteen days later we conducted predation trials.

A number of studies have revealed that tadpoles reduce their activity in the presence of predators so as to decrease the encounter rate with predators (Relyea 2001; Skelly and Werner 1990; Van Buskirk and McCollum 2000a). This predator-induced behavioral change masks the effect of the induced morph. Since *R. pirica* tadpoles reduce their activity when exposed to predators (personal observation), to eliminate possible differences in the activity of induced and typical tadpoles, we conducted the experiment in a small container in which the movements of both induced and typical tadpoles were uniformly restricted. We placed one tadpole of each phenotype in a small container (7 cm \times 5 cm \times 4.5 cm) filled two-thirds full with aged water. The tadpoles were of similar size; the difference in total length between the two phenotypes was within 1 mm. The body widths of tadpoles with the induced and typical morphs were 8.47 ± 0.85 and 6.49 ± 0.64 mm (mean \pm SD), respectively. Then, we randomly added a starved larval salamander (head

width: 8.67 ± 1.98 mm), which had hatched around the same time as the *R. pirica* tadpoles and had not been fed for at least 2 days, to the container. We terminated each trial when the salamander took the first victim, and recorded its phenotype. The predation trial was replicated 40 times.

Function experiment

We examined the gape-size limitation on predation by the larval *H. retardatus* in order to clarify how the relative sizes of tadpoles and salamanders influence predation by the latter. Various sizes of *R. pirica* tadpoles (non-induced phenotypes) and *H. retardatus* larvae from stock tanks were used in this experiment. We measured tadpole body width and salamander head width. We grouped tadpoles in terms of body width at 0.5 mm intervals. We placed larval salamanders, which had not been fed for at least 1 day, in a small container and then added one tadpole that had a body width 1 mm larger than the head width of the salamander. After 15 min, if the tadpole had not been preyed on, we replaced the tadpole with one that was 0.5 mm smaller. This procedure was repeated until predation occurred. We conducted a simple linear regression of the body width of the prey tadpoles on the head width of the predator salamanders.

Reversion experiment

To investigate whether the induced morph is reversible, we conducted the following experiment. The two phenotypes (induced and non-induced morphs) were created in the four aquaria using the same protocol used for the predator aquarium in the induction experiment. After 14 days for induction, we placed each tadpole in a separate 250-ml cup filled with aged tap water to eliminate the predation risk. We fed them fish food ad libitum and exchanged the water every day. The body width and length of each tadpole 0, 3, 6, and 9 days after isolation were measured with digital calipers (± 0.01 mm).

The analysis used the data for tadpoles at Gosner (1960) stage 35 or earlier because the induced incrassate epithelial tissue (see Results) is resolved gradually after this stage (O. Kishida, personal observation). We compared the body shape of the morph types (induced vs typical) 0 and 9 days after eliminating the predator cues. We traced the morphometric changes in the course of growth and reversion at census points at days 0, 3, 6, and 9. The analysis used data for 27 induced and 34 typical phenotypes.

Results

Induction of the defensive morph

The two major axes (PC1 and PC2) of the PCA of *R. pirica* tadpole morphology account for 87.4% of the total variance of all morphological measurements (Table 1). According to the signs and magnitudes of the elements of the eigenvectors, we interpret PC1 as a size axis. A large PC1 value implies a large size. Since the elements along the body axis and the elements of the axes on the plane orthogonal to the body axis have opposite signs, we interpret PC2 as a shape axis that describes variation in body bulginess and the depth of the tail. A low PC2 value implies increased depth and width of the body, and depth of the tail.

We compared the differences in tadpole shape (PC2) between close-cue and remote-cue treatments. Analyzing

Table 1 Coefficients and percentage of variance of the major principal component axis (PC1: size axis and PC2: shape axis) of the morphometrics of *R. pirica* in the induction experiment

Morphometric variable	PC1	PC2
Total length	0.365	0.490
Body length	0.400	0.005
Body depth	0.376	-0.530
Body width	0.395	-0.397
Tail depth	0.404	-0.201
Tail muscle width	0.341	0.441
Tail muscle depth	0.361	0.293
Percent variance	77.04	10.38

tadpole shape (PC2) using an ANCOVA correcting for size (PC1), there was a difference between the close- and remote-cue treatments (Table 2). That is, when *R. pirica* tadpoles were reared for 2 weeks with larval *H. retardatus* in the predation aquaria, the close-cue treatment induced a bulgy morphology resulting from incrassate epithelial tissue (Fig. 1) in the tadpoles, while the remote-cue treatment did not. Furthermore, we conducted an ad hoc a posteriori comparison of the values for the remote-cue and no-cue treatments; there was no difference between the two treatments (Table 3). These results show that a bulgy morph was induced when the predator was in close proximity, but not when the predator was at a distance (Fig. 2).

Since PCA scores are not intuitively appealing and we realized that marked morphological changes had occurred in the body, further analyses were conducted using two actual measurements of body shape: body width and body length. In the analyses individual tadpoles in different blocks (aquaria) were pooled because block effects were not significant in the preliminary analyses. The body widths of tadpoles subjected to the close-cue and remote-cue treatments differed statistically (body widths were adjusted using the covariate body length, $F_{1,99}=138.07$, $P<0.001$, ANCOVA). Note that the regression line for body width on the body length of tadpoles with the typical morph runs through the origin ($t_{57}=0.810$, $P=0.422$), while the intercept of the regression line for tadpoles with the

Table 2 Results of ANCOVA testing the effect of cue types (close-cue or remote-cue) and blocks (aquaria) on variation in shape (PC2). In the preliminary analysis for the full model, the interaction terms of covariate were not significant (i.e., PC1 \times cue-type ($F_{1,82}=0.522$, $P=0.47$); PC1 \times aquarium ($F_{3,82}=0.452$, $P=0.72$); PC1 \times cue-type \times aquarium ($F_{3,82}=1.374$, $P=0.26$)). Therefore, the variance components of the interaction terms were pooled and included in the error term

	SS	df	F	P
Cue-type	34.385	1	40.514	0.008
Aquarium (block)	1.095	3	1.247	0.298
PC1 (covariate)	7.099	1	24.258	<0.001
Cue-type \times aquarium	2.546	3	2.900	0.039
Error	26.044	89		

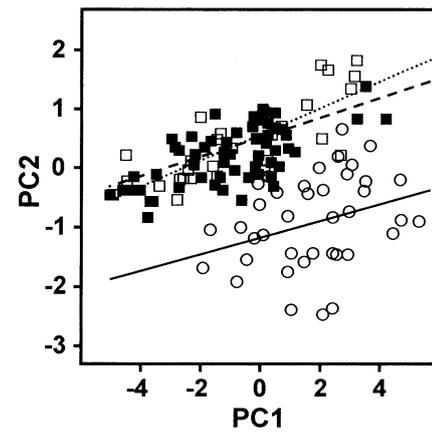


Fig. 1 Morphometric characteristics of tadpoles in three treatments (close cue, open circles; remote cue, solid squares; and no cue, open squares). A large PC1 value indicates large size, and a low PC2 value indicates bulginess. Regression lines of PC2 (Y) on PC1 (X), close cue: $Y=-1.10+0.13X$, $r^2=0.09$; remote cue: $Y=0.48+0.16X$, $r^2=0.4$; no cue: $Y=0.59+0.22X$, $r^2=0.63$

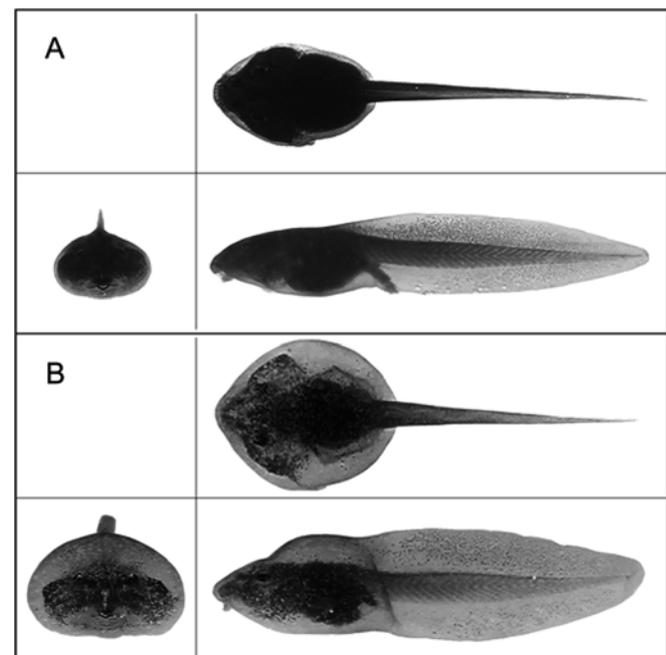


Fig. 2a, b Tadpoles with typical and bulgy morphs. **a** Typical morph without the predator or with only the remote cue of the predator. **b** Bulgy morph induced in the close-cue treatment in the induction experiment

Table 3 Results of ANCOVA testing the effect of cue-types (remote-cue or no-cue) on variation in shape (PC2). The slopes of both treatments were homogeneity, i.e., cue-type \times PC1, ($F_{1,85}=2.299$, $p=0.133$). In the analysis, individual tadpoles in different colanders were pooled, because the colander effects were not significant in the preliminary analyses

	SS	df	F	P
Cue-type	0.073	1	0.461	0.499
PC1 (covariate)	13.522	1	85.537	<0.001
Error	13.595	86		

bulgy morph was positive and significantly different from zero ($t_{37} 2.460, P=0.019$) (Fig. 3). These results show that the body width to body length ratios revealed isometry in typical morph tadpoles, whereas the ratio revealed allometry in the bulgy morph. That is, small tadpoles had bulgier morphologies than larger tadpoles when exposed to the predator.

In conclusion, (1) the tadpoles develop a bulgy morph in response to the close cue from larval salamanders; and (2) the bulginess was adjusted according to the size of the tadpole relative to the predator.

Effect, function, and reversion of the bulgy morph

Predation trials

The binomial test revealed that bulgy morph tadpoles had higher survival than the typical morph (29:11, $P=0.003$). It is clear that the induced bulgy morph is an effective defense against predatory salamander larvae, at least for the sizes studied.

Function experiment

The simple linear regression of the body width of preyed tadpoles on the head width of preying salamanders revealed that the maximum body width of tadpoles captured is positively correlated with the head width of larval salamanders (slope= 0.842 ± 0.039 (mean \pm SE), $F_{1,64}=459.276, P<0.001$, Fig. 4). As a previous study revealed (Ohdachi 1994), since head width is strongly correlated with gape size, *H. retardatus* larvae are gape limited, which can consume tadpoles with the same head (body) width as that of the larvae themselves, but which cannot eat much larger tadpoles.

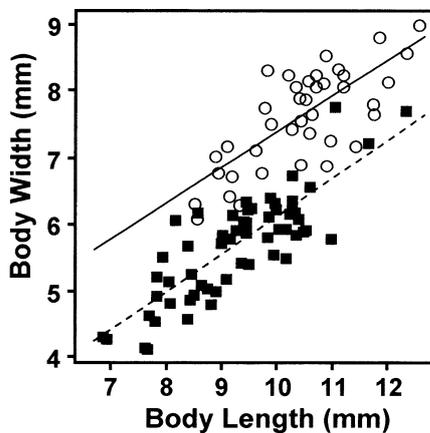


Fig. 3 Body length and body width of tadpoles with bulgy (open circles) and unprotected morphs (solid squares). Regression lines of body length (Y) on body width (X), typical morph: $Y=0.38+0.58X, r^2=0.7$; bulgy morph: $Y=2.02+0.53X, r^2=0.56$

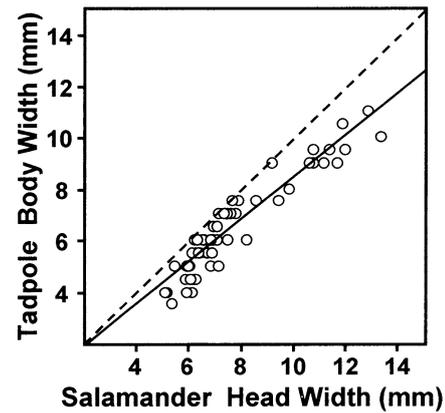


Fig. 4 Linear regression of the maximum body width of preyed tadpoles on the head width of preying salamanders, solid line. The broken line is the isoline

Reversion experiment

The body shapes (body width–length ratio) of the two phenotypes differed significantly at day 0 ($t_{59}=10.997, P<0.001$), but there was no difference at day 9 ($t_{59}=0.032, P=0.975$), and the shape of the typical morph did not change for 9 days (paired t -test: $t_{33}=1.609, P=0.117$) (Fig. 5a). Figure 5b illustrates the morphometric changes in the course of growth and reversion at census days 0, 3, 6, and 9. The body of the bulgy individuals actually shrunk as soon as the risk was removed (days 0–3), and then steadily approached the typical phenotype (days 3–9) with growth and reversion. Indeed, tadpoles with the bulgy morph reverted to the typical morph by actually shrinking their body within 9 days of removing the predation risk.

Discussion

The *R. pirica* tadpoles exhibited an inducible defensive morphology in the presence of the predation threat of larval *H. retardatus*.

The bulgy morph prevented tadpoles from being swallowed by *H. retardatus* larvae, which are gape-limited predators. The predation trials and function experiment showed that the inducible bulgy morphology is an adaptive phenotype against the gape-limited predator larval *H. retardatus*. The need for the induction of a defense morph has not been described for the inducible morphological defenses of non-colony organisms. Most of the aquatic organisms that adopt inducible morphological defenses utilize remote chemical cues (Brönmark and Pettersson 1994; Dahl and Peckarsky 2002; Hanazato 1990; Kusch 1993; Laurila et al. 2001; Stemberger and Gilbert 1984; Stenson 1987; Tollrian 1995; Trussell 1996; Van Buskirk and Arioli 2002). Furthermore, *R. pirica* uses two adaptive cost-saving strategies. Tadpoles adjusted the bulginess according to their own size, and the induced morph reverted to the typical morph after removal of the predation threat.

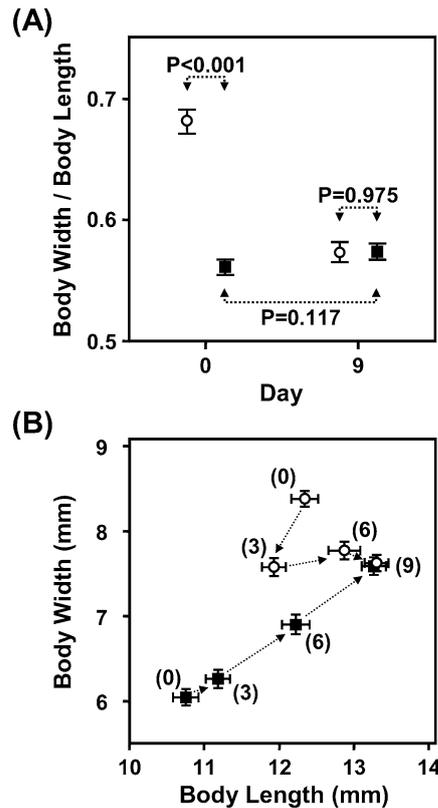


Fig. 5a, b Body shape change of the two phenotypes after the risk was removed. **a** Ratios of body width to body length of tadpoles with bulgy (*open circles*) and typical (*solid squares*) morphs, 0 and 9 days after eliminating the predator cues. Error bars denote one standard error. **b** Trajectory of the change in body width and length of tadpoles with the bulgy (*open circles*) and typical (*solid squares*) morphs. The numbers in parentheses are the days after the predation risk was removed. Error bars denote 1 SE

Defensive morph for size-dependent predation

Rana pirica tadpoles developed a bulgy body which has a mechanical function against gape-limited *H. retardatus* larvae. This differs markedly from morphs reported in previous studies of inducible defense in anuran larvae, in which tadpoles developed higher tail fins and smaller bodies when exposed to the threat of predators (e.g., Van Buskirk 2002). Although past studies have shown that inducible phenotypes have higher survival rates when predators are present (McCollum and Van Buskirk 1996; Van Buskirk and McCollum 1999), the mechanical roles of the inducible traits are unclear (Van Buskirk and McCollum 2000a,b).

Plastic enlargement of body size as a defense against predators is observed in several taxonomic groups (Brönmark and Miner 1992; Stenson 1987). For example, when *Holopedium gibberum*, a plankton species, is exposed to water-soluble factors released by predatory *Chaoborus obscuripes* larvae, it induces a thicker gelatinous capsule that renders it more difficult for predators to capture (Stenson 1987). With such defenses, prey capture success depends on the balance between the size of the capturing organ of the predator and prey size.

Recent studies have demonstrated that *H. retardatus* larvae have two distinct morphs: “normal” and “broad-headed” morphs. The latter is induced by a high density of *R. pirica* tadpoles or conspecifics, and is advantageous for the consumption of larger prey (Michimae and Wakahara 2001,2002). Accordingly, the inducible bulgy morph of *R. pirica* tadpoles might have been selected as the result of an arms race with larval *H. retardatus*, with the intimate predator–prey relationship. This predator–prey system is a noteworthy model of reciprocal phenotypic plasticity, which has been a focus of attention (Agrawal 2001).

Cues for induction

Contrary to the common feature of most existing inducible morphological defenses that are triggered by remote cues, the induction of the defense morph of *R. pirica* tadpoles responding to larval *H. retardatus* was not triggered only by a remote cue, i.e., a close cue must be required for the induction. In general, immediate response cued by remote signals should be thought of as a prerequisite for inducible morphological defenses, since morphological responses required time to respond. A bryozoan, *Membranipora membranacea*, constitutes an exceptional example; it produces spines in response to a close-proximity signal, i.e., localized attack by predatory nudibranches (Harvell 1984). It is a clonal modular sessile organism and induction is initiated after part of the colony is eaten.

Of various signals indicating a predation risk, a prey organism adopts some signal as cues for induction based on its reliability, time lag between occurrences of the cue and the actual risk, and the time required to respond to an impending crisis. Reliability and time lag between the cue and the attack are related not only to the sensory ability and escape ability of the prey but also the hide strategy and attack strategy of the predator. Signal adoption is deeply related to those characteristics, which depend on life history of predator and prey.

Rana pirica and *H. retardatus*, spawn in small transient ponds formed by melting snow in early spring. Generally, *R. pirica* tadpoles hatch earlier than *H. retardatus* larvae (Sato 1990). In time, there are vast number of *R. pirica* tadpoles and *H. retardatus* hatchlings in these ponds [just after hatching of *H. retardatus*, the population density (numbers per meter square) of *R. pirica* tadpole and *H. retardatus* larvae in natural coexisting ponds range from 817 to 0.79 and from 152 to 0.93, respectively (O. Kishida, unpublished data)], at which density most larval *H. retardatus* are not substantial predators. For a certain period, the predation risk remains low and *R. pirica* tadpoles live in close proximity to *H. retardatus* larvae. The salamanders around the tadpoles become serious predators as their size increases and catches up with the size of the tadpoles, without developing a special hide hunting strategy. In the phenological process with unsynchronized size developments, use of a close signal detection system would have been evolutionarily favored rather than utilization of a remote signal detection system.

Cost savings modifying the extent and reversibility of bulginess

Defenses are costly in terms of fitness to organisms. This paper addressed the conditional fine-tuning adaptations for costly defense, adjusting the extent and reversibility of the inducible defense phenotype. Here, we describe the cost of the bulgy morph, and then discuss the fine tunings of the bulgy morph in terms of the specific phenological characteristic of the predator–prey interaction.

The induced bulgy tadpoles delay metamorphosis (O. Kishida, unpublished data). The delay in metamorphosis of an induced phenotype should be interpreted as a cost, because it increases susceptibility to pond drying in the larval stage for amphibian species in a temporary pond (Smith 1983). However, the delayed metamorphosis could not be considered as a cost of morphological defense per se, since *R. pirica* tadpoles under the predation risk show behavioral responses as well as a morphological change, i.e., reduction in activity, which leads to low resource acquisition (O. Kishida, personal observation). The reduced resource acquisition of bulgy phenotype is the confounding factor to quantify the cost of morphological defense.

However, we have indirect evidences that indicate the possibility of cost of the morphological defense. The tadpoles developed the bulgy morph using hypertrophic epithelial tissue, which consists of fibroblasts and connective tissue, and which is saturated with lymph fluid (O. Kishida, unpublished data). An estimate from projected photographs indicates that epithelium makes up 45% of the body volume. Furthermore, a preliminary gene subtraction method targeting the activating genes in the epithelial tissue of the bulgy and typical morphs indicated that splitting enzymes, such as fibrinogen, carboxypeptidase B, and elastase, are inhibited and intra- and intercellular substances, such as osteonectin and collagen, are actively synthesized (O. Kishida, unpublished data). Therefore, the bulgy morph requires the production and maintenance of a huge amount of tissue at a substantial metabolic cost.

With respect to cost savings, the defense structures need not be too robust to protect the organisms from hazards. A past study showed that prey organisms can detect and respond to subtle variation in predation risk. *Rana lessonae* tadpoles can adjust the extent of morphological traits according to external conditions, that is the number of predators (Van Buskirk and Arioli 2002).

Here, we demonstrate that the adjustment of defense morph depends on the condition of the tadpoles. *R. pirica* tadpoles modified their degree of bulginess in accordance with their own size. The decreased induction may imply that the adjustment of defense morph is in parallel to a reduced predation risk with size. This, in other words, implies that the tadpoles become less bulgy and save substances for the bulgy morph, with increasing body size. This modification implies that tadpoles exhibit an adaptive modification of bulginess.

Organisms possessing designs for an inducible morph might have the option to withdraw the cost invested in the morph when it is no longer necessary. When the cost of induction of the defense morph is high, selection should favor the organism in which the invested cost is reversed after the predation risk is relaxed, if developmental and life history constraints do not discourage reversion (Gabriel 1999). A few papers have reported the reversibility of inducible morphological defenses after removing cues (Brönmark and Pettersson 1994; Hanazato 1990; Relyea 2003). Relyea (2003) suggested that induced phenotype could revert to the non-induced phenotype by preferentially shunting energy for other growth, which makes the defensive traits relatively smaller.

Interestingly, our study indicates that the body of the induced bulgy morph actually shrunk and almost completely reverted to the typical morph within 9 days of removing the predation risk. Such rapid reversion, as well as the modification of bulginess, is an adaptive cost-saving strategy.

The adaptive significance of the phenotype reversion can be understood from the phenologies of organisms in the pond community. In the early developing season, *R. pirica* tadpoles are the sole prey available to larval *H. retardatus* under the condition of reciprocal size-dependency (Michimae and Wakahara 2002). However, predatory aquatic insects emerge as the water temperature rises, and the feeding of these insects decreases the number of larval *H. retardatus*. Furthermore, cannibalism and metamorphosis of *H. retardatus* may also decrease the number of larval *H. retardatus*. A decline in *H. retardatus* larvae is inevitable in the phenological cycles. After being released from the threat of larval *H. retardatus*, *R. pirica* tadpoles should recoup the cost paid to deter the threat.

The induction of a bulgy morph, the recognition of the presence of the predator using a proximate cue, and the cost-saving strategies of the bulgy morph of *R. pirica* tadpoles could be based on co-evolution in this particular predator–prey interaction, under the phenological regime of their habitat.

Acknowledgements We especially thank to H. Murahana for assistance with some of the experiments. We would like to thank S. Takenaka for helpful comments. We would also like to thank S. Sato for letting us use the laboratories at the Otaru Aquarium.

References

- Adler FR, Harvell CD (1990) Inducible defenses, phenotypic variability and biotic environments. *Trends Ecol Evol* 5:407–410
- Agrawal AA (2001) Phenotypic plasticity in the interactions and evolution of species. *Science* 294:321–326
- Brönmark C, Miner JG (1992) Predator-induced phenotypical change in body morphology in crucian carp. *Science* 258:1348–1350
- Brönmark C, Pettersson LB (1994) Chemical cues from piscivores induce a change in morphology in crucian carp. *Oikos* 70:396–402

- Clark CW, Harvell CD (1992) Inducible defenses and the allocation of resources: a minimal model. *Am Nat* 139:521–539
- Dahl J, Peckarsky BL (2002) Induced morphological defenses in the wild: predator effects on a mayfly, *Drunella coloradensis*. *Ecology* 83:1620–1634
- Dewitt TJ, Sih A, Wilson DS (1998) Costs and limits of phenotypic plasticity. *Trends Ecol Evol* 13:77–81
- Gabriel W (1999) Evolution of reversible plastic responses: inducible defenses and environmental tolerance. In: Tollrian R, Harvell CD (eds) *The ecology and evolution of inducible defenses*. Princeton University Press, Princeton, pp 286–305
- Gosner KL (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16:183–190
- Hanazato T (1990) Induction of helmet development by a *Chaoborus* factor in *Daphnia ambigua* during juvenile stages. *J Plankton Res* 12:1287–1294
- Harvell CD (1984) Predator-induced defenses in a marine bryozoan. *Science* 224:1357–1459
- Havel JE (1987) Predator-induced defenses: a review. In: Kerfoot WC, Sih A (eds) *Predation: direct and indirect impacts on aquatic communities*. University Press of New England, Hanover, pp 263–278
- Kusch J (1993) Induction of defensive morphological changes in ciliates. *Oecologia* 94:571–575
- Lardner B (1998) Plasticity or fixed adaptive traits? Strategies for predation avoidance in *Rana arvalis* tadpoles. *Oecologia* 117:119–126
- Laurila A, Crochet PA, Merila J (2001) Predation-induced effects on hatchling morphology in the common frog (*Rana temporaria*). *Can J Zool* 79:926–930
- McCollum SA, Leimberger JD (1997) Predator-induced morphological changes in an amphibian: predation by dragonflies affects tadpole shape and color. *Oecologia* 109:615–621
- McCollum SA, Van Buskirk J (1996) Costs and benefits of a predator-induced polyphenism in the gray treefrog *Hyla chrysoscelis*. *Evolution* 50:583–593
- Michimae H, Wakahara M (2001) Factors which affect the occurrence of cannibalism and the broad-headed “cannibal” morph in larvae of the salamander *Hynobius retardatus*. *Behav Ecol Sociobiol* 50:339–345
- Michimae H, Wakahara M (2002) A tadpole-induced polyphenism in the salamander *Hynobius retardatus*. *Evolution* 56:2029–2038
- Ohdachi S (1994) Growth, metamorphosis, and gape-limited cannibalism and predation on tadpoles in larvae of salamanders *Hynobius retardatus*. *Zool Sci* 11:127–131
- Relyea RA (2001) Morphological and behavioral plasticity of larval anurans in response to different predators. *Ecology* 82:523–540
- Relyea RA (2003) Predators come and predators go: the reversibility of predator-induced traits. *Ecology* 84:1840–1848
- Sato T (1990) Breeding sites of a salamander, *Hynobius retardatus* (in Japanese with English abstract). *Bull Obihiro Centenn City Mus* 8:1–10
- Skelly DK, Werner EE (1990) Behavioral and life-historical responses of larval American toads to an odonate predator. *Ecology* 71:2313–2322
- Smith DC (1983) Factors controlling tadpole populations of the chorus frog (*Pseudacris triseriata*) on Isle Royale, Michigan. *Ecology* 64:501–510
- Stemberger RS, Gilbert JJ (1984) Spine development in the rotifer *Keratella cochlearis*: induction by cyclopoid copepods and *Asplanchna*. *Freshwater Biol* 14:639–647
- Stenson JAE (1987) Variation in capsule size of *Holopedium gibberum* (Zaddach): a response to invertebrate predation. *Ecology* 68:928–934
- Tollrian R (1995) Predator-induced morphological defenses: costs, life history shifts, and maternal effects in *Daphnia pulex*. *Ecology* 76:1691–1705
- Tollrian R, Harvell CD (1999) *The ecology and evolution of inducible defenses*. Princeton University Press, Princeton
- Trussell GC (1996) Phenotypic plasticity in an intertidal snail: the role of a common crab predator. *Evolution* 50:448–454
- Van Buskirk J (2001) Specific induced responses to different predator species in anuran larvae. *J Evol Biol* 14:482–489
- Van Buskirk J (2002) A comparative test of the adaptive plasticity hypothesis: relationship between habitat and phenotype in anuran larvae. *Am Nat* 160:87–102
- Van Buskirk J, Arioli M (2002) Dosage response of an induced defense: how sensitive are tadpoles to predation risk? *Ecology* 83:1580–1585
- Van Buskirk J, McCollum SA (1999) Plasticity and selection explain variation in tadpole phenotype between ponds with different predator composition. *Oikos* 85:31–39
- Van Buskirk J, McCollum SA (2000a) Functional mechanisms of an inducible defence in tadpoles: morphology and behaviour influence mortality risk from predation. *J Evol Biol* 13:336–347
- Van Buskirk J, McCollum SA (2000b) Influence of tail shape on tadpole swimming performance. *J Exp Biol* 203:2149–2158
- West-Eberhard MJ (1989) Phenotypic plasticity and the origins of diversity. *Annu Rev Ecol Syst* 20:249–278