

Predator-specific inducible morphological changes in two Japanese anuran tadpoles

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Abstract. We evaluated inducible morphological changes in tadpoles of two anuran species, the Japanese tree frog (*Hyla japonica*) and the wrinkled frog *Glandirana (Rana) rugosa*, against two predator types, which were represented by a predatory fish (*Carassius auratus*) and the nymph of a dragonfly (*Anax parthenope julius*). In the presence of fish cues, *H. japonica* developed shallower tail fins, whereas *G. rugosa* did not exhibit such morphological change. Both tadpole species developed deeper tail fins in the presence of dragonfly nymph cues. The predator specific responses for the species' tadpoles are likely to optimize avoidance of lethal attacks by predators. The interspecific differences in inducible morphological defence traits might be related to the balance with the effects of other defensive traits and explained by the frequency and time of their encounters with predators.

Keywords. chemical defence, defensive behaviour, fish predator, inducible defence, tail morphology.

Introduction

Many organisms exhibit changes in morphology as a response to predation risk (e.g. development of shells in snails, longer spines in dragonfly nymphs, and deeper bodies in fishes, Brönmark and Miner, 1992; Johansson and Samuelsson, 1994; Hoverman, Auld and Relyea, 2005). These inducible morphological changes have been suggested to represent an adaptive response in order to reduce prey vulnerability. Anuran tadpoles also exhibit inducible morphological defence characters, which are sometimes specific to the types of their predators (Teplitsky, Plénet and Joly, 2004; Kishida and Nishimura, 2005; Wilson, Kraft and Van Damme, 2005; Touchon and Warkentin, 2008).

Morphological responses of tadpoles are mainly induced by predatory fish and large insects. For example, tadpoles raised together with fish possess shallower tails and deeper tail muscles than tadpoles raised in a non-predator environment (Wilson, Kraft and Van Damme,

2005; Touchon and Warkentin, 2008). Morphological adaptation in this sense can have a survival benefit for tadpoles due to an increase in swimming speed during predation events. On the contrary, tadpoles faced by large predatory insects (e.g. dragonfly nymphs) often develop deeper tail heights and shallower tail muscles than tadpoles raised in a non-predator environment (Wilson, Kraft and Van Damme, 2005; Touchon and Warkentin, 2008). Dragonfly nymphs, which are ambush foragers, are often the dominant predators in fishless ponds (Wellborn, Skelly and Werner, 1996). Such changes in tail morphology can attract strikes by these nymphs to the more expendable tail region and away from the more vulnerable head and body (Van Buskirk et al., 2003).

Characteristics of morphological adaptation in tadpoles are thought to be associated with the balance among other defence strategies. For example, a tadpole species characterized by chemical defences appears not to exhibit defensive behaviour against fish predators (Kats, Petranka and Sih, 1988). Here, we hypothesize that the characteristics of morphological adaptation as a response to predators differ among tadpole species with different defensive traits.

In Japan, the Japanese tree frog (*Hyla japonica*) and the wrinkled frog *Glandirana (Rana) rugosa* have been observed together in both temporary and permanent water bodies inhabited by both fish and dragonfly nymph predators. *Glandirana rugosa* is known to employ chemical defences against fish predators, as chemicals on the skin improve survival during and after attacks

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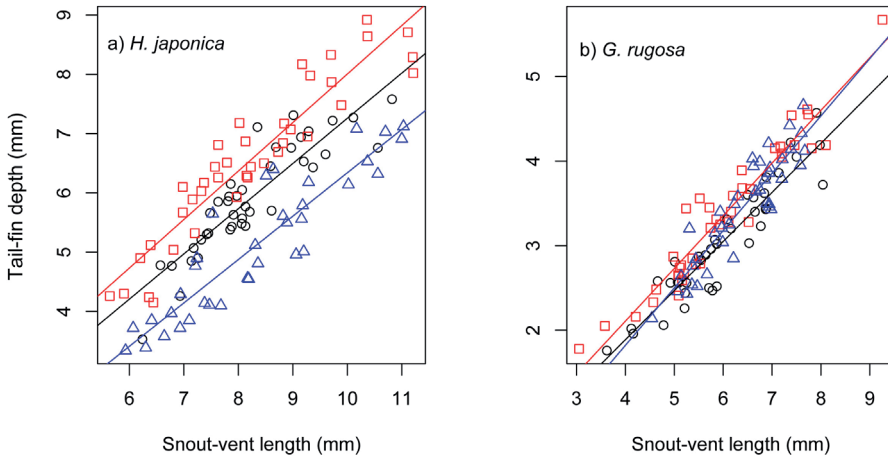


Figure 1. Relationships between snout-vent length and maximum tail-fin depth of tadpole species; *Hyla japonica* (a) and *Glandirana rugosa* (b) incubated with control (black circle), fish cue (blue triangle) or dragonfly cue (red square) on day 12. Each line on the plots shows the significant regression line by GLMMs.

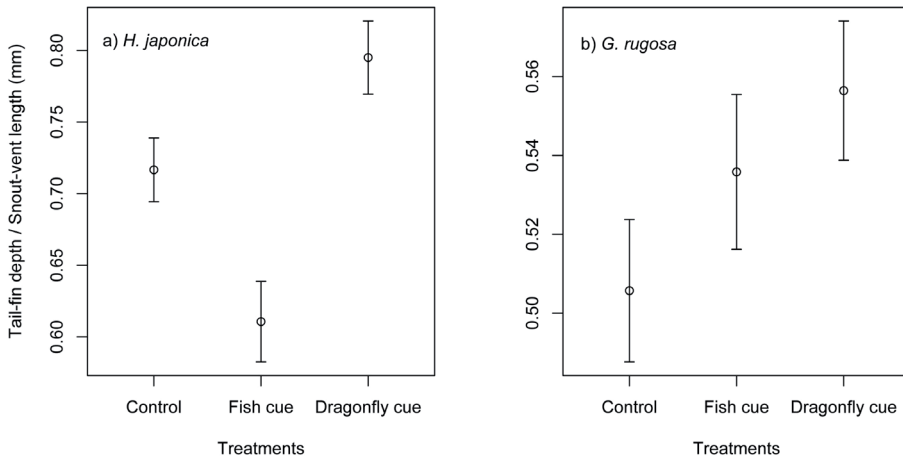


Figure 2. The ratio of maximum tail-fin depth/snout-vent length for inducible morphological changes in *Hyla japonica* (a) and *Glandirana rugosa* (b) in the three treatments (control, fish cue or dragonfly cue). Error bars show $\pm 99\%$ confidence intervals. For *H. japonica*, the differences among the three treatments were significant, but *G. rugosa* only differed between the dragonfly cue and the control significantly (see Results).

by a fish predator (Takahara *et al.*, 2011). *Hyla japonica* does not employ chemical defences but behaviourally decreased activity to avoid predation by fish (Takahara *et al.*, 2006, 2011). This response can be regarded as an effective defensive behaviour, as it reduces the probability of detection by a predator (Takahara *et al.*, 2008). Conversely, both species exhibited common defence behaviour in the presence of dragonfly nymph cues (Takahara, Kohmatsu and Yamaoka, 2008). To

clarify the inducible morphological changes for two tadpole species as a response to fish and dragonfly predators, we evaluated how tail morphology of the tadpoles changes in response to cues of these predators.

Materials and Methods

Adults of *H. japonica* and *G. rugosa* were caught using hand nets in the paddy fields in Kyoto city (35°04' N, 135°44' E) during

May–July in 2003 and 2004, respectively. Adults of each species were kept as single male/female pairs in plastic cages (30 × 20 × 17 cm) which contained aerated water (depth: 5 cm). Multiple clutches of eggs were obtained the next day. After hatching, tadpoles of each species were kept separate in 50-L plastic containers until the beginning of the experiments. Tadpoles were fed flake food (TetraFin®) daily. Predatory fish (*Carassius auratus*) were obtained from a local fish farmer, kept in 50-L plastic containers, and were fed a flake food twice a week. Dragonfly nymphs (*A. parthenope julius*) were caught using hand nets at a pond in Otsu city (34°58'N, 135°57'E). All nymphs were individually kept in perforated plastic cups (diameter: 8.5 cm; height: 8.5 cm) that were placed in plastic containers which contained 5-L aerated water, and were fed two tadpoles, which were consistent with the tadpole species being evaluated in each experiment, twice a week. All animals were maintained on a 16-h/8-h light/dark photoperiod at 25 ± 2°C and the water, which was continuously filtered in all containers, was changed weekly.

We evaluated inducible morphological change (i.e. change of tail morphology) by exposing each tadpole species to cues from caged predators for 12 days. For the experiments 24 plastic tanks (30 × 20 × 17 cm) filled with aerated water (6 L) and gravel substrate (depth: 5 mm) were prepared. Twelve tanks were assigned for each anuran species, and held 10 tadpoles that were randomly selected from a mixture of three clutches (i.e. 10 tadpoles tank⁻¹). The snout-vent length of *H. japonica* tadpoles was 5.3 ± 0.4 mm (mean ± SD, n = 12) and that of *G. rugosa* tadpoles was 5.2 ± 0.3 mm (n = 12) at the beginning of the experiments. In each tank, one fish (total length: 56.1 ± 0.7 mm, n = 8) or one dragonfly nymph (body length: 35.6 ± 1.8 mm, n = 8) was placed in an opaque perforated plastic cup (diameter: 10 cm; height: 7 cm; holes: < 500 µm), which was suspended in the upper part of the tank for exposure of the predator presence (hereafter, predator cues). Tanks for treatment without predators (i.e., non-predator cues, hereafter, control) were used with an empty cup. Air was forced through an air stone into the cup in all tanks. Experiments for each species consisted of three treatments (fish cue, dragonfly nymph cue and control) and four replicates for each treatment.

Tadpoles were given per capita rations (6% of the weekly mean wet weight of the tadpoles in each tank) of powdered food three times a week. Dragonfly nymphs and fish were fed with two tadpoles and flake food, respectively, twice a week after removal from the cups, after which they were returned to the experimental tanks. The tadpole species fed to the dragonfly nymphs was consistent with the tadpole species being evaluated in each experiment. The water in the tanks was completely changed after one week.

The relative tail-fin depth of tadpoles was recorded on day 12 by means of digital calipers to the nearest 0.01 mm. Snout-vent length and maximum tail-fin depth were measured. Because the maximum tail-fin depth depends, in part, on tadpole body size, relative tail-fin depth was compared among the three treatments using analysis of covariance (ANCOVA test by GLMM) with snout-vent length as covariate. A generalized linear mixed model [GLMM; (Venables and Ripley, 2002)] was used to estimate differences among the three treatments (α = 0.05). We treated the experimental tank as the random factors in the GLMM. We tested the significance of the differences between two ANCOVA mo-

dels of GLMMs; (relative tail-fin depth) = b (snout-vent length) + treatment, and (relative tail-fin depth) = b (snout-vent length). We compared the models by Akaike Information Criteria (AIC) and likelihood test by L ratio (α = 0.05).

To quantify the effects of predator cues, we calculated the relative tail-fin depth against snout-vent length [$(X_{\text{maximum tail-fin depth}}/X_{\text{snout-vent length}})$]. The statistical significance difference in the ratio of maximum tail-fin depth/snout-vent length among the three treatments was determined by the 99% confidence interval of the ratio. We performed all statistical analyses and graphics using R ver. 2.13.0 software (R Development Core Team, 2011) and “nlme” package for GLMM (see, R code in the Appendix 1).

Results

The relative tail-fin depths of both tadpole species were significantly different among the three treatments (ANCOVA; *H. japonica*, L ratio = 26.7, $P < 0.001$; *G. rugosa*, L ratio = 10.8, $P < 0.01$; Fig. 1). The ratio of maximum tail-fin depth/snout-vent length of *H. japonica* in the fish cue treatment was significantly lower than that in the control, and the ratio in the dragonfly cue treatment was significantly higher (Fig. 2a). Meanwhile, the ratio of *G. rugosa* in the fish cue treatment did not significantly differ from that in the control, and the ratio in the dragonfly cue treatment was significantly higher (Fig. 2b).

Discussion

The relative tail-fin depth of *H. japonica* exposed to fish cues was shallower on day 12 of the experiment in comparison to that of tadpoles in the control group, wherein that of *G. rugosa* was not. Confirm to our observations on *H. japonica* tadpoles, also *Pseudacris regilla* developed shallower tail fins when exposed to chemical cues from fish (Benard, 2006). As a shallower tail-fin depth is associated with increased tadpole survival following predatory encounters with fish species (Benard, 2006), we suggest that also *H. japonica* shows an inducible morphological defence in response to fish cues.

Under predation risk by fish, *G. rugosa* would depend primarily on chemical defence as this species did not exhibit inducible morphological change in this study. A lack of defensive behaviour in tadpoles of this species has additionally been presented (Takahara et al., 2011). The chemical defence of *G. rugosa* is however highly beneficial to avoid predation risks by fish, as the tadpoles survived at a high rate (80%) after being attacked by fish predators (Takahara et al., 2011).

Both tadpole species increased relative tail-fin depth in response to dragonfly nymph cues. Such morphological response is known as general anti-predator adaptation against large aquatic insects, such as dragonfly nymphs and diving beetles (Van Buskirk and McCollum, 2000; Relyea, 2001; Kishida and Nishimura, 2005; Benard, 2006). Tadpoles with relatively larger tails likely reduce capture by predators as a result of lure effects or faster swimming capability (McCollum and Leimberger, 1997; Van Buskirk *et al.*, 2003).

Hyla japonica and *G. rugosa* have often been observed in the same ponds and likely encounter similar predator species in their habitats. Nevertheless, their morphological responses differed with respect to predator species. The interspecific differences in inducible morphological defences might be caused by the combination and balance of effects of other defensive traits, which differ per anuran species and can evolve as a response to encounter frequency with predators. Anuran tadpoles are likely to favour predator-avoidance strategies that are effective against predators that are encountered most frequently (Teplitsky, Plénet and Joly, 2003). Future studies should therefore focus on how the combination of tadpole defensive traits and the resulting predator-avoidance strategies have evolved under predation risks.

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Appendix 1: R code for ANCOVA analysis in this study

```
### Load R packages for GLMM
library(nlme)
library(MASS)

### ANCOVA test for tail-fin depth with snout-vent length by GLMM
###For Hyla japonica
summary(TDHy<-lme(TD~SVL+Treatment, random=~1|Tank, data=Hy12,
  method="ML"))
summary(TDHy2<-lme(TD~SVL, random=~1|Tank, data=Hy12, method="ML"))
summary(TDHy3<-lme(TD~SVL*Treatment, random=~1|Tank, data=Hy12,
  method="ML"))
anova(TDHy, TDHy2, TDHy3)
###For Glandirana rugosa
summary(TDGI<-lme(TD~SVL+Treatment, random=~1|Tank, data=G112,
  method="ML"))
summary(TDGI2<-lme(TD~SVL, random=~1|Tank, data=G112, method="ML"))
summary(TDGI3<-lme(TD~SVL*Treatment, random=~1|Tank, data=G112,
  method="ML"))
anova(TDGI, TDGI2, TDGI3)
```