Hierarchical competitive ability and phenotypic investments in prey: inferior competitors compete and defend

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Short title : Phenotypic investments in asymmetrically competing prey

Abstract

It is commonly assumed that prey must make growth-defense trade-offs when in the presence of predators under resource-limited conditions. Thus, it is predicted that prey will allocate limited resources either to competitive phenotypes to grow or to defensive phenotypes to defend, not both. Evidence from plants, however, has suggested that under strong selection prey might evolve phenotypes that allow them to simultaneously compete and defend. In a controlled laboratory experiment, we investigated phenotypic investment in asymmetrically competing tadpoles of *Rhacophorus schlegelli* (RS) and *Pelophylax nigromaculatus* (PN) in the presence and absence of predatory dragonfly larvae. We predicted that, because of differences in resource acquisition abilities, tadpoles would invest in competitive and defensive phenotypes differently, depending on their relative competitive ability in the presence of predators. RS was the superior competitor and depressed the growth of PN whether predators were present or absent. As expected, the inferior competitor (PN) responded to competition by elongating its gut in predator-free environments. In contrast, the superior competitor (RS) did not. In the presence of predators, both tadpoles invested in larger tails, a common defensive trait, while the presence of competitors did not influence tail size. When reared alone in the presence of predators, PN grew faster and tended to have a longer gut. In contrast to theoretical predictions, however, in the presence of both predators and competitors the inferior competitor (PN) still maintained the competitive (long gut) and defensive (large tail) phenotypes despite being depressed in growth. Optimal digestion theory suggests that gut elongation should enhance assimilation efficiency and energy gain. In this view, when a competitive phenotype also serves to benefit prey in predator environments, prey may be able to both compete and defend.

Keywords: alimentary tract, asymmetric competition, growth-defense models, optimal digestion theory, phenotypic plasticity

Introduction

Competition and predation often act as selective pressures that drive prey adaptation (Gurevitch, Morrison & Hedges 2000). Animals and plants respond to competition and predation with adaptive plastic phenotypes, which increases fitness in the presence but not in the absence of a threat (Schlichting & Pigliucci, 1998). But because of the often conflicting demands of competition and predation on prey phenotypes, their independent and simultaneous impacts can strongly differ.

Under competition, organisms generally reduce investment in structures that are costly to maintain, investing instead in phenotypes that enhance competitive ability and growth. For example, competition-induced plastic changes in plants often involve increased root density and stem length, leading to better nutrient and light acquisition (Dudley & Schmitt, 1996). In animals, responses to competition for food involve increased foraging activity (Anholt & Werner, 1995), aggressive behavior (King, 1973), and elongation of the alimentary tract in order to improve nutrient assimilation (Sibly, 1981; Relyea & Auld, 2004). Conversely, in the presence of predators prey phenotypes that increase survival are favored, but they generally come at the cost of slower growth and development. In plants, responses to herbivory involve the production of defense chemicals (Karban & Baldwin, 2007) at the cost of reduced root length and density, making plants less able to compete for nutrients and water (Karban & Strauss, 1993; Ohgushi, 1997; Armitage & Fourqurean, 2006; but see Whittaker, 2003). Animal prey species typically reduce foraging activity (Lima & Dill, 1990) and/or build defense structures, at the cost of diverting energy from growth in order to maintain these structures (Tollrian & Harvell, 1999). Recent evidence shows that one way in which prey can compensate for investment in defense structures is through the shortening of the gut (Kehr & Gómez, 2009; Venesky *et al.*, 2013), reducing resource assimilation efficiency and thus reducing growth (Relyea and Auld, 2004; but see Steiner, 2007).

Two types of models have typically been used to predict the interactive effects of competition and predation on prey phenotypes (Teplitsky & Laurila, 2007). The simple allocation model predicts low investment in defense under strong competition, when limited available resources should be invested in growth (Harvell, 1990), but prioritization of defense under weak competition, when increased resource availability would allow such investment (Relyea, 2004). The second, the growth-defense model (Herms & Mattson, 1992), predicts the converse: that prey should invest less in defense and prioritize growth in high resource environments (e.g., van Velzen & Etienne, 2015), but should increase investment in defense when there is little opportunity for growth and they will have to spend more time remaining in risky environments (e.g., Parejko & Dodson, 1991; Pauwels, Stoks & De Meester, 2010).

These two models both assume that prey can invest in either a competitive or a defensive phenotype, but not in both (Lind *et al*., 2013). Evidence from plants, however, has suggested that prey do not always make such a trade-off. This may occur in the presence of strong selection by competition and weaker selection by predation (Uriarte, Canham & Root, 2002), or when a single phenotype is beneficial under both competition and predation (Siemens et al., 2002). Evidence of such a situation is still scarce in animals, but a recent study reports that prey facing increasing predation risk can prioritize investment in growth and in morphological defenses even when resource availability is reduced (Costa & Kishida, 2015), suggesting that prey may also compete and defend.

In this study we examine the phenotypic investment of asymmetrically competing prey species in both the presence and absence of predators. Because of disproportionality in resource acquisition, we expected superior and inferior competitors to invest differently in competitive and defense phenotypes. From a prey perspective, the presence of interspecific competitors is potentially more challenging than the presence of conspecifics because of potential interspecific differences in competitive ability and resistance to predators (Cipollini, 2004). Thus, maladaptive phenotypic investment in the presence of interspecific competitors may increase selection against the less defended or the less competitive prey. This situation might induce phenotypic investment in prey to not follow theoretical models. Asymmetric competition is pervasive in nature (Schoener, 1983), and is common among anuran larvae (Morin & Johnson, 1988; Werner, 1992; Parris & Semlitsch, 1998; Smith, Dingfelder & Vaala, 2004), which are often model systems in studies of phenotypic plasticity. Tadpoles typically respond to competition by reducing their tail size (Relyea, 2004) and elongating the gut (Relyea & Auld, 2004). Conversely, in the presence of predators, tadpoles grow larger tails and shorten their gut, likely to compensate for building this structural defense (Relyea & Auld, 2004; Kehr & Gómez, 2009). Here, we used gut length and tail fin size as proxy estimates of investment in competitive and defensive phenotypes, respectively.

Following the predictions of theoretical models, we expected 1) that in predator-free environments, inferior competitors would invest less in costly structures (i.e., smaller tail fin size) and more in phenotypes that foster growth (i.e., longer gut) in response to lower resource gain; 2) that in asymmetric competition in the presence of predators, inferior competitors would develop either a long gut to compete or a large tail to defend, but not both, and that which phenotype developed would depend on the relative effects of predation and competition on growth; and 3) that due to higher resource gain, superior competitors would not develop an elongated gut and would maintain a larger tail in the presence of competitors and predators.

Materials and methods

Rhacophorus schlegelli (hereafter RS) and *Pelophylax nigromaculatus* (hereafter PN) are two coexisting anuran species occurring on the main island of Japan, breeding in small ponds and paddy fields. The two species differ in breeding mode: *Pelophylax nigromaculatus* produces more offspring (500-600 eggs per clutch) than *Rhacophorus schlegelli* (200-300 eggs per clutch) (Uchiyama *et al.*, 2002). Under limited resources, RS and PN are known to compete asymmetrically (Ramamonjisoa & Natuhara, unpublished data) and represent a good system for our experiment. We collected four and two egg masses of *Rhacophorus schlegelli* and *Pelophylax nigromaculatus,* respectively. Eggs were collected in early June 2013 from two rice paddy fields in Toyota city, Japan. Eggs were hatched in the laboratory and tadpoles were kept in plastic containers at 15 individuals.L^{-1}. Tadpoles were fed rabbit chow every other day until the start of the experiment.

To test our predictions, we conducted a laboratory factorial design in which the presence or absence of a caged predator dragonfly larva (*Orthetrum* sp) was crossed with the absence or presence of competitors (8 RS, 8 PN, 4 RS + 4 PN). *Orthetrum* sp. larvae are important tadpole predators in paddy fields in Japan. Densities were manipulated to fall within the range of their density in nature (Nakanishi *et al.*, 2009). For each species, tadpoles from different egg masses were pooled and then randomly allocated to the containers. This allowed us to homogenize the composition of each experimental unit while introducing genetic variability. Tadpoles were at Gosner 26-27 (Gosner, 1960) at the start of the experiment and initial body masses did not differ at the start of the experiment (t-test, n=15, t_{28}=1.488, p=0.148).

We conducted the experiment in rectangular plastic tubs (15 x 24 x 12 cm) filled with 2.5 L of aged tap water, following the same experimental design as Smith, Dingfelder & Vaala (2004) in studying asymmetric competition in tadpoles. Each treatment was replicated six times. We used pellets (described below) to simulate clumped resources and to stimulate species interaction (Kiesecker, Blaustein & Miller, 2001). Under such conditions, PN and RS tadpoles compete asymmetrically and the strength of interspecific interaction typically increases with decreasing resource levels (Ramamonjisoa and Natuhara, unpublished data). This laboratory setting allowed us to control for the amount of resources and the environmental conditions the tadpoles are exposed to, facilitating the inference of causal factors of observed effects.

The food materials consisted of a mixture of rabbit chow (Marukan, Ltd protein: 15%, lipid=5%) and commercial algae (Sun Nutrition, protein = 57%, lipid = 7%) in equal weights. Food protein content (~36%) is within the range of dietary protein content that pond dwelling tadpoles meet in the field (Schiesari, Werner & Kling, 2009). Foods were pulverized into fine powder and bound into a 0.5% (in mass) agar solution. The solution was poured into petridishes (85 mm x 15 mm) and allowed to harden at 7°C. To create pellets, we bored in the agar-bound food material using a 6 mm diameter cylinder (pellet dimension 6 mm x 15 mm). As applied in previous studies (e.g., Bennett, Pereira & Murray, 2013), we provided food weighing about 4-5% of the total mass of the tadpoles per aquarium every day to simulate environments with moderate food restriction.

Dragonfly larvae were collected from an experimental paddy field inside Nagoya University. Each aquarium contained one small cylinder covered with a net that was either empty or held a dragonfly larva. This method allowed simulating the threat of predation while avoiding actual predation, prevented tadpoles from feeding on predator's egestion but allowed predator waterborne chemical cues to diffuse in the water. Dead predators were immediately replaced with living ones. We fed predators with two tadpoles every other day. Predators in single-species tadpole treatments were only fed tadpoles of that species. The mixed tadpole-predator treatments were fed individuals of each tadpole species (Costa & Vonesh, 2013). We provided approximately the same prey biomass to reduce variation among treatments. To maintain the chemical cues in containers, half of the water volume was siphoned and replaced every 3-4 days; feces were removed with a pipette. Containers were randomly reorganized in the experimental room every time water was changed. There were no food leftovers in the containers prior to feeding. The experiment was conducted in a room at 24 °C with natural daylight regime. We terminated the experiment after 35 days when the tadpoles were at Gosner 34-36.

At the end of the experiment, the tadpoles were removed from their containers, blotted dry and weighed. Feeding ended 30 hours before the end of the experiment, which allowed tadpoles to "clear" their gut. As applied in previous studies (e.g., Bennett, Pereira & Murray, 2013), mean individual species growth rates were computed by the differences in body mass (final body mass – initial body mass) over the number of experimental days (35 days) and the number of tadpoles of the respective species in each container. Mortalities occurred in the first days of the experiments (until day 3), during which dead individuals were immediately replaced by new alive ones.

Morphological responses

At the end of the experiment, we randomly sampled a subset of three tadpoles of each species from each container and took digital images of the tadpoles. We measured body length, tail length, body height, tail height, tail depth, muscle depth and tail fin area following Warkentin (1999) (on some occasions, morphometrics were conducted on only two individuals). When needed, a piece of glass was placed under the tail to avoid distortion during measurement. Morphometric measures were performed with the software ImageJ (http://imagej.nih.gov/ij/). Upon completion of morphological measurements, the tadpoles (not necessarily the ones used for morphometrics) were euthanized by an overdose of MS-222, weighed to the nearest 0.1mg and dissected or frozen (-20°C) for later measurement. We uncoiled the entire gut system without stretching and measured length to the nearest 0.1mm with a digital caliper. Measurements were repeated two times by the same person and averaged for one individual tadpole.

Data analyses

We evaluated the effects of “competition” and “predation” (factors) on tadpole “growth rates”, “gut length”, and “tail fin area” (response variables) with general linear models (GLM). Upon significance, we conducted post-hoc TukeyHSD tests to compare tadpole growth rates among treatments. Partial etas squared are reported as estimate of effect sizes. All statistical analyses were conducted on tank means (N = 6) for all response variables. Data were log-transformed when necessary to improve assumptions of normality and homogeneity of variances.

Gut length was standardized using the Scaled Mass Index, a robust technique to correct metric size to body mass (Peig & Green, 2009). Tail fin area was corrected following Berner (2011): we first entered a subset of the metric traits (body length, tail length, body height, tail height and muscle depth), log-transformed when necessary to increase linearity, into a Principal Component Analysis to estimate a latent size PC1. We then size-corrected the tail fin area (square-rooted) using that PC1 as covariate in a GLM. This method has been suggested to be more reliable than common techniques based on residual analysis (Berner, 2011). PC1 scores were averaged and centered prior to analysis (Gabriela, 2016). We used Sigmaplot (ver. 13, SYSTAT Software Inc.) and SPSS (ver. 17, IBM) to perform the analyses and SPSS (ver. 17, IBM) to make the figures.

Results

Growth rate

Competition and predation influenced tadpole growth rates but competition exerted a larger effect (Table 1). The two species competed asymmetrically: RS was the superior competitor whether in the presence or absence of predator (Fig. 1). Compared to when reared alone, in mixed rearing, RS grew faster at the expenses of PN (Table 2). Intraspecific competition was more detrimental to RS than interspecific competition while the opposite pattern was observed in PN. The presence of predators did not reverse the outcome of interaction: RS still grew faster at the detriment of PN (in comparison to when reared alone, Table 2). PN grew faster when reared alone in the presence of predators but RS did not (Table 2). Competition and predation significantly interacted on PN growth: PN decreased growth under competition but grew faster in the presence of predators.

Morphology

PN and RS exhibited larger tails in the presence of predators (Fig. 2, Table 2). In contrast to expectations, investment in tail defense was not affected by the presence of competitors (Table 2). The inferior competitor PN elongated its gut in the presence of competitors and predators (Fig. 2, Table 2). Gut length in the superior RS did not change in any treatments.

Discussion

We investigated how the relative competitive abilities of two tadpole species affected competitor and predator induced phenotypes. PN and RS tadpoles competed asymmetrically in which RS was the superior competitor in both the presence and absence of predators. This may be due to the fact that the relative impacts of competition on tadpole growth exceeded the positive impacts of predation. Such a pattern has been previously reported in other systems in which predation can unidirectionally benefit one competitor (Chase *et al.*, 2002). We used food pellets and this may have increased the strength of interaction between the two species by simulating clumped resources (Kiesecker, Blaustein & Miller, 2001).

Both tadpole species grew larger tails in the presence of dragonfly larvae predators, consistent with previous reports (e.g., Van Buskirk, 2000; Van Burskirk *et al.*, 2003). A larger tail functions as a lure and is thought to increase survival in predator environments (Van Burskirk *et al.*, 2003). Typically the expression of an inducible defense, such as larger tails in tadpoles, is thought to result in a growth/predation risk trade-off, in which vulnerability to predators decreases at the cost of decreased growth and development (Tollrian & Harvell, 1999). However, none of the tadpoles in this experiment exhibited reduced growth when reared alone in the presence of dragonfly larvae. To the contrary, PN grew even faster in the presence of predators. Growth acceleration in response to predators is not unusual, and has been seen in many taxa, including tadpoles with induced morphological defenses (Relyea, 2002; Bennett, Pereira & Murray, 2013, reviewed in Costa & Kishida, 2015). Adopting a high growth strategy is thought to be adaptive: faster growth constitutes a quicker means of reaching a size refuge against predators, and a quicker path to metamorphosis and escape from the hostile environment (Urban, 2007). In tadpoles, vulnerability to predators typically decreases with increasing body size (Brodie Jr & Formanowicz Jr, 1983; Urban, 2007; Costa & Kishida, 2015), and in our system, larger tadpoles are less susceptible to predation from dragonflies (Ramamonjisoa, unpublished data). Furthermore, gape-limited newts are also top predators in our system, suggesting that larger, faster growing tadpoles will have increased fitness in the presence of these predators.

We predicted smaller tails in the inferior competitor species (PN) because of lower resource gain (e.g., Relyea, 2004; Teplitsky & Laurila, 2007), but instead we found that PN elongated its gut while also enlarging its tail. A prediction of the optimal digestion theory is that an organism can modify gut length in response to the quantity and the quality of its diet (Sibly, 1981) as a longer gut increases food assimilation efficiency and energy gain (Sibly, 1981), and is considered an adaptive competitive trait to foster growth in tadpoles (Relyea & Auld, 2004). Although a previous experiment indicated RS can elongate its gut on a low quality diet (N. Ramamonjisoa, unpublished data), only PN did in this experiment, likely as a consequence of it being the inferior competitor. |

The fact that the inferior competitor PN simultaneously grew a competitive phenotype (long gut) and a defensive one (large tail) in the presence of predators goes against the predictions of two classic phenotypic investment models (simple allocation models and growth-defense models; see Introduction). Typically, these models predict a trade-off between competitive and defense traits; that is, we expected tadpoles to invest in either a larger tail for predator defense or a longer gut for competition, but not both (Relyea & Auld, 2004). So why did we find the opposite pattern, in which the inferior competitor invested in both traits, even with decreasing resources? A similar pattern has recently been reported, in which tadpoles facing predation from gape-limited salamanders can prioritize investment in both growth and morphological defenses even when resource availability is reduced (Costa & Kishida, 2015). Although our study was not designed to identify the mechanisms underlying such patterns, several recent studies have shown that prey in the presence of a predators can alter various physiological traits which can reduce metabolic rates, increase assimilation efficiency, and allow for the investment of energy in defense structures (McPeek, 2004; Thaler, McArt & Kaplan, 2012; Thaler, Contreras & Davidowitz, 2014). Assuming that the digestive tract is the key organ mediating digestion efficiency and nutrient assimilation in animals (Sibly, 1981; Clissold *et al.*, 2010), elongating the gut might not be an unusual prey response to predators (but see Steiner, 2007).

Investing in a competitive phenotype while maintaining defense is hypothetically adaptive. For example, Teplitsky & Laurila (2007) has suggested that investing in morphological defense is an important compensation for the commonly induced behavioral risk-taking that prey experience at low resource levels (Lima & Dill, 1990). Because we did not conduct behavioral observations, it is not known whether foraging activity increased in the inferior competitor, but optimal digestion theory (Sibly, 1981) predicts that a longer gut may help tadpoles increase fitness by increasing energy intake to accommodate the nutritional demands in the presence of both competitors and predators. A simple scenario may further illustrate why such a simultaneous investment occurred: lowering investment in competitive phenotypes can be costly and may ultimately exclude the least competitive prey from the local system (Holt, Grover & Tilman, 1994). On the other hand, reducing defense investment in the presence of a better-defended superior competitor would not be adaptive because selection may intensify against the less defended prey species (Cipollini, 2004). Thus, selection for plasticity in both defensive and competitive traits may occur simultaneously, resulting in tadpoles exhibiting larger tails and longer guts with no apparent trade-off between the two traits.

Although predation and competition typically have opposite effects on prey phenotypes (Relyea & Auld, 2004), the fact that the inferior competitor simultaneously grew a longer gut to compete and a larger tail to defend does not indicate that trade-offs did not exist. Instead, it is possible that trade-offs were not apparent because we looked at a limited set of morphological traits. Clearly, an integrative analysis of physiological, behavioral and morphological traits would provide a more thorough understanding of the phenotypic trade-offs in these tadpoles, although we acknowledge the difficulty of conducting such a study. Furthermore, another drawback of our experiment is the simplified laboratory conditions. Tadpoles typically live in dynamic and complex environments, so the phenotypic responses reported in our simplified scenario might differ from what tadpoles would exhibit in nature. For instance, the tadpoles in this study are typical to small ponds and ephemeral water bodies (paddy fields) in which desiccation could potentially influence prey strategies and phenotypic investments (Richter-Boix, Tejedo & Rezende, 2011).

In conclusion, these prey species differentially invested in gut length, with the inferior competitor (PN) increasing its gut length in response to interspecific competition. Furthermore, this species also increased its gut length in the presence of predators while also maintaining a large tail for defense. This may indicate that when a competitive phenotype (here, the gut) also functions to benefit prey in predator-environments (e.g., increased growth), prey might evolve the ability to simultaneously compete *and* defend. Investment in defense typically induces costs, which arise from diverting energy needed for growth into defense structures. In our experiment, predation did not impair growth, and this apparent lack of defense cost may partially explain our results (e.g., Siemens *et al.*, 2002). While the ability to simultaneously express competitive and defense phenotypes was first thought to be specific to plants (Siemens *et al.*, 2002; Jones *et al.*, 2006), our results indicate that this ability is present in animals too. The costs associated with these investments remain unclear and present interesting avenues for future research.

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Table 1. Results of general linear models summarizing the interactive effects of predation and competition on tadpole growth rate and morphological traits in the superior (*Rhacophorus schlegelli*, RS) and the inferior competitor (*Pelophylax nigromaculatus*, PN). Morphological traits were size-corrected before analysis (see Materials and methods). *P*-values in bold indicate significant effects at alpha = 0.05, n = 6. “Eta” represents partial eta squared. Values in bold indicate significant effects.

		Competition			Predation			Comp. x Pred.		
		F (1, 20)	*P*	*Eta*	*F* (1,20)	*P*	*Eta*	*F* (1,20)	*P*	*Eta*
RS	Growth rate	34.56	**<0.001**	0.64	21.31	**<0.001**	0.51	2.46	0.132	0.11
	Tail fin area*	1.232	0.281	0.06	91.65	**<0.001**	0.82	1.97	0.177	0.09
	Gut length	0.01	0.902	0.01	0.03	0.847	0.01	0.03	0.87	0.01
PN	Growth rate	65.76	**<0.001**	0.77	15.61	**<0.001**	0.43	4.79	**0.04**	0.20
	Tail fin area*	0.04	0.846	0.01	49.3	**<0.001**	0.72	0.69	0.417	0.03
	Gut length	12.49	0.002	0.38	5.78	0.026	0.22	0.87	0.36	0.04

* *F* (1, 19)

Table 2. Tukey post-hoc tests following general linear models testing the effects of predation and competition on tadpole growth rate|, tail fin area and gut length in the superior (*Rhacophorus schlegelli* RS) and the inferior competitor (*Pelophylax nigromaculatus* PN). *P*-values in bold indicate significant effects at alpha = 0.05, n = 6. GR=growth rate, TFA= tail fin area; GL=gut length. Values in bold indicate significant differences.

	PN			RS		
Treatments	GR	TFA	GL	GR	TFA	GL
Alone vs. together	**0.002**	0.996	**0.023**	**0.029**	0.998	0.998
Alone vs. alone+pred	**0.001**	<**0.001**	0.117	0.17	<**0.001**	0.994
Alone vs. together+pred	0.037	<**0.001**	**0.002**	<**0.001**	<**0.001**	0.999
Alone+pred vs. together	<**0.001**	**0.002**	0.854	0.808	<**0.001**	0.996
Alone+pred vs. together+pred	<**0.001**	0.975	0.286	<**0.001**	0.257	0.997
Together vs. together+pred	0.606	<**0.001**	0.73	**0.001**	<**0.001**	0.999

Figure 1. Growth rates of the tadpoles of *Rhacophorus schlegelli* (RS) and *Pelophylax nigromaculatus* (PN) when reared alone or together in the presence and absence of non-lethal dragonfly larvae. Data are mean ± SE, n = 6.

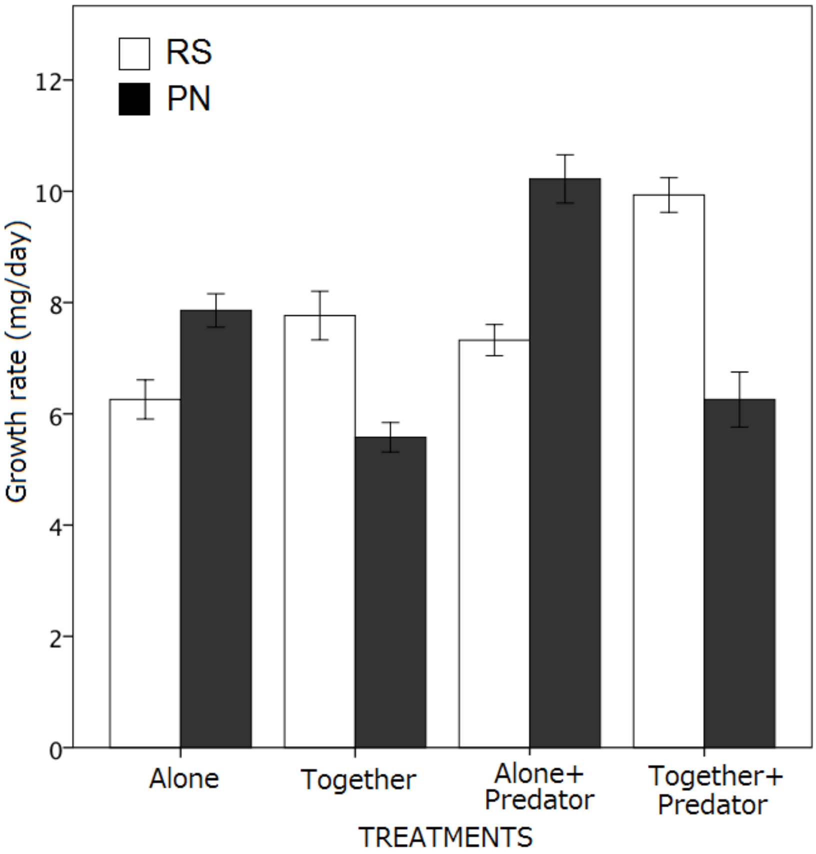

Figure 2. Morphological responses of the tadpoles of *Rhacophorus schlegelli* (superior competitor, RS) and *Pelophylax nigromaculatus* (inferior competitor, PN) when reared alone, together in the presence and absence of non-lethal dragonfly larvae. Tail fin areas and gut length were size-corrected prior to analysis. Tail fin areas were square-rooted and log-transformed. Data are mean ± SE, n = 6.