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The presence of *Polypedates cruciger* tadpoles reduces the growth of *Duttaphrynus melanostictus* tadpoles under laboratory conditions

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Abstract. Competition is one of the main types of interactions in ecological communities. Interspecific competition of many organisms including anuran tadpoles has been demonstrated in numerous previous studies. However, a few studies have also shown that interspecific competition is absent or insignificant in certain tadpole communities. In this study, we conducted experiments to assess whether the presence of one species of tadpole affects the growth of another species. We selected tadpoles of two commonly co-occurring species of anurans in Sri Lanka: *Duttaphrynus melanostictus* and *Polypedates cruciger*. The length and width of tadpoles were used as a measure of growth. The laboratory experiments revealed that the presence of *P. cruciger* tadpoles negatively impacted the growth of *D. melanostictus* tadpoles. Tadpoles of *D. melanostictus* that were raised with *P. cruciger* tadpoles (treatment) were significantly smaller than those raised with conspecifics (control). *P. cruciger* tadpoles that were raised with *D. melanostictus* tadpoles were significantly bigger than those raised with conspecifics. We believe that these effects are due to the superiority of *P. cruciger* tadpoles to *D. melanostictus* tadpoles in terms of competition.

Keywords. Length, width, growth, competition, experiment, tadpoles, *Polypedates cruciger*, *Duttaphrynus melanostictus*, Anura, larvae, asymmetric competition

Introduction

Many examples of larval competition among amphibians have been demonstrated in artificial systems (Wilbur, 1972; Wilbur, 1982; Morin, 1983a). Subsequent field studies have documented that tadpoles can have considerable impact on food density in natural ponds (primarily algae) suggesting that competition may also occur under natural conditions (DeBenedictis, 1974; Seale, 1980). One of the obvious indicators of competition stress among animals is the reduction of growth rates (Alford, 1999). Growth reduction has been used to infer interspecific and intraspecific competition among tadpoles (Smith, 2005). Competition could be of several types: intra and inter-cohort competition within species and interspecific within the "guild" (Root, 1967). The mechanism of competition could be exploitation or interference. Interference competition may be behavioral or allelopathic, which could be caused by chemicals, parasites, or pathogens. However, there are reports that show that interspecific competition among tadpoles in natural environments is nonexistent or insignificant (Skelly, 1995; 1997). It is reported that in certain tadpole communities, predator pressure is a more significant factor than interspecific competition between tadpoles (Skelly, 1995).

In this communication we report the results of an experiment designed to determine effects on growth when tadpoles of two species of anurans found in Sri Lanka are raised together under laboratory conditions. The two selected species were the endemic species of tree frog, *Polypedates cruciger* Blyth, 1858 (Rhacophoridae) and non endemic toad, *Duttaphrynus melanostictus* Schneider, 1799 (Bufonidae). These two species were selected for the study because they are sympatric species which commonly co-occur and breed in semi-natural habitats such as ponds in home gardens. Our objective was to determine whether the presence of one species of tadpole affects the growth of another species. The length and width of tadpoles were used to determine whether there is growth retardation in tadpoles under laboratory conditions. We hope the results of our study will make an important contribution to the knowledge of community ecology of Sri Lankan amphibian larvae and stimulate further research in this discipline.

Materials and Methods

The tadpoles were identified by the descriptions given by Kirtisinghe (1957). Tadpoles of the two selected species were obtained from egg clutches collected from Peradeniya University garden

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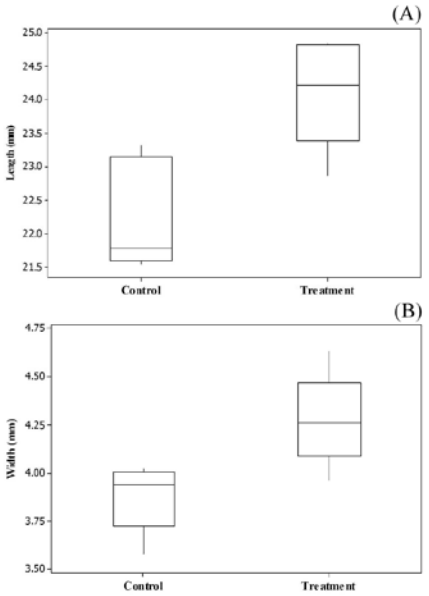

Figure 1. Box plots showing (A) length and (B) width of *Polypedates cruciger* tadpoles in the competition test: kept with conspecifics (control) or with *Duttaphrynus melanostictus* tadpoles (treatment).

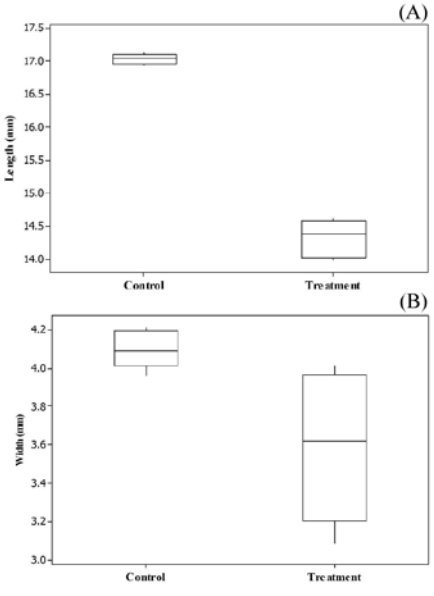

Figure 2. Box plots showing (A) length and (B) width of *Duttaphrynus melanostictus* tadpoles in the competition test: kept with conspecifics (control) or with *Polypedates cruciger* tadpoles (treatment).

(Kandy District, Sri Lanka) and some home gardens in Kandy (Kandy District, Sri Lanka). Complete Randomized design was used to assess the competitive effects on each tadpole species. Five hundred and forty tadpoles from the two species *D. melanostictus* and *P. cruciger* (270 from each species) were used in the experiment. The tadpoles were two weeks old and belonged to "Gosner stage 25" (Gosner, 1960). They were reared in 360 mm diameter and 100 mm high glass aquaria filled with aged tap water (60 mm level). Prior to the experiment tadpoles were measured from the snout to the tip of the tail to make sure that they were similar in size. At the time of introduction both species of tadpoles were nearly similar in size from head to tip of the tail (*D. melanostictus* = 10.97 ± 0.97, *P. cruciger* = 11.02 ± 0.96). A two-sampled t-test showed that there is no significant difference between the lengths of tadpoles of the two species ($T = -0.20$, $P = 0.842$) before introduction. The experimental setting involved 18 tadpoles of *D. melanostictus* and 18 tadpoles of *P. cruciger* reared in the same glass aquarium as the treatment and 36 tadpoles from each species reared in two different glass aquaria of the same size and volume as the control. This was replicated five more times using the same experimental setting. Tadpoles in each glass aquarium were fed daily with 2.0 g of commercial fish food and 2.0 g of filamentous algae. Water was changed once a week. After three weeks the length and width of tadpoles were measured. Measurements were taken using a Mitutoyo Digital vernier caliper up to the nearest 0.01 mm (error ± 0.005 mm).

The data obtained were analyzed using Statistical Analysis Software (SAS) version 9.1. Averages of length and width of tadpoles of each species in each glass aquarium were calculated. One-way ANOVA was conducted using average length and width of the tadpoles in the five replicates to determine whether there is a significant difference ($\alpha < 0.05$) in length and width of tadpoles between treatment and the control. A Fisher's Least significant difference (LSD) test was conducted to separate the means and explore the directionality of the difference between the means of the replicates and the treatments.

Results

During the experiment no mortality was recorded in either of the experimental settings. A One-way ANOVA showed that both length and width of *P. cruciger* tadpoles in the control were significantly different than those in the treatment (length: $F_{1,8} = 13.11$, $P = 0.007$; width: $F_{1,8} = 8.71$, $P = 0.018$; Table 1). One-way ANOVA showed that both length and width of *D. melanostictus* tadpoles in the control were significantly different than those in the treatment (length: $F_{1,8} = 431.82$, $P < 0.0001$; width: $F_{1,8} = 7.95$, $P = 0.022$; Table 1). A Fisher's LSD test further indicated that length and width of *P. cruciger* tadpoles in the control were significantly lower than those in the treatment (Figure 1). Fisher's LSD test also showed that both length and width of *D. melanostictus* tadpoles in the control were significantly greater than those in the treatment (Figure 2). These values show that growth of *D. melanostictus* tadpoles was reduced when they were raised with *P. cruciger* tadpoles. In contrast, growth of

Table 1. Average length (±SD) and width (±SD) of tadpoles in the five replicates in the competition test: kept with conspecifics (control) or heterospecifics (treatment).

Experimental Setting	Length (mm) ± SD	Width (mm) ± SD
Polypedates cruciger control	22.25 ± 0.83	3.88 ± 0.18
Polypedates cruciger treatment	24.13 ± 0.81	4.27 ± 0.24
Duttaphrynus melanostictus control	17.04 ± 0.07	4.10 ± 0.10
Duttaphrynus melanostictus treatment	14.31 ± 0.28	3.59 ± 0.39

P. cruciger tadpoles was greater when they were raised with *D. melanostictus* tadpoles.

Discussion

Competition and predation are two of the most significant types of ecological interactions that structure communities (Morin, 1999). These ecological interactions are equally observed and reported in anuran larval communities and are known to affect the fitness of the competing tadpoles negatively. These negative effects are known to result in mortality and to decrease recruitment of individuals to the breeding population. Competition can be symmetric or asymmetric. Asymmetric competition arises during an encounter between two or more individuals for a limited resource and as a result these resources are divided unequally and the larger individual wins the contest (Clutton-Brock et al., 1979). Asymmetric competition is not rare among different species of tadpoles: several studies have experimentally shown that one species of tadpole can have a big impact on the growth and development of another species (DeBenedictis, 1974; Wilbur, 1982; Morin, 1983b; Werner, 1992; Alford, 1999; Smith, 2005). However, it has also been demonstrated that interspecific competition among tadpoles can be less significant than the effect of predators on the growth and metamorphosis (Skelly, 1995). Further, it has been criticized that experimental venue (i.e., laboratory *vs* natural habitat) has a strong effect on the outcome of the results of experiments in tadpole competition studies (Skelly and Kiesecker, 2001). According to Skelly and Kiesecker (2001) interspecific effects among tadpoles are stronger in artificial environmental settings.

We found that tadpoles of *Polypedates cruciger* have a strong negative effect on the growth of *Duttaphrynus melanostictus* tadpoles under laboratory conditions while the *D. melanostictus* tadpoles have no negative effect on the growth of *P. cruciger* tadpoles. Tadpoles of *P. cruciger* raised with *D. melanostictus* tadpoles gained a larger size than the *P. cruciger* tadpoles that were raised with their conspecifics. In contrast, *D. melanostictus* tadpoles raised with their conspecifics gained a lager size than *D. melanostictus* tadpoles that were grown with their heterospecifics. These observations evidence that there is asymmetric competition between these two species of tadpoles under laboratory conditions, and *P. cruciger* tadpoles are the stronger competitors. We assume that these effects are largely due to exploitative competition caused by the tadpoles of *P. cruciger*.

The type of competition taking place between these two species is actually unknown. Although it was not the scope of this study to evaluate the reasons behind competition, the competition for food is likely to be the main reason. The greater body sizes attained by the *P. cruciger* tadpoles could allow them to out-compete *D. melanostictus* tadpoles when food is a limiting factor. It is reported that members of the competitively superior species obtain a growth rate advantage at the expense of those of the inferior species, whose growth is inhibited and the superior competitor decreases time to metamorphosis while increasing that of the inferior competitor (Morin, 1983b). A chemical secreted by the superior competitor can also reduce the growth rate of tadpoles (chemical interference), which is known for species like *Rana sphenocephala* (Alford, 1999). However, we are unable to confirm that such a chemical is involved in this system, since no experiments were carried out to investigate this possibility.

Previous studies have demonstrated that competition can have strong effects on the development, growth and survival of tadpoles (Alford, 1999). In natural systems such as ponds (DeBenedictis, 1974; Griffiths et al. 1991) and in streams (Kupferberg, 1997; Flecker, 1999) tadpoles are commonly exposed to this factor. Studies conducted on *Hyla gratiosa* and *H. femoralis* tadpoles under laboratory conditions have indicated that one species can have a strong negative effect on the growth of the other species (Wilbur, 1982). Results in Wilburs' study indicated that *H. gratiosa* tadpole induces a negative effect on the rate of metamorphosis and the size at metamorphosis of *H. femoralis* tadpoles (Wilbur, 1982). However, it is demonstrated that *H. femoralis* has no effect on the survival or the size at metamorphosis of *H. gratiosa.* But it lengthens both the minimum and mean larval period. Similar studies conducted on the tadpoles of *Rana pipiens* and *R. sylvatica* has shown strong effects of density and evidence for inter-specific competition (DeBenedictis, 1974).

The main reason for the selection of these two species for this study is because they are sympatric species that co-occur in semi-natural habitats throughout the

southwestern wet zone of Sri Lanka. Moreover they share the same breeding habitats such as artificial ponds found in anthropogenic habitats. However, the adults of the two species occupy two different microhabitats where *D. melanostictus* is terrestrial and *P. cruciger* is arboreal (Dutta and Manamendraarchchi, 1996). Based on the descriptions of Altig and Johnston (1989) the tadpole of *D. melanostictus* can be categorized as a lentic, benthic and littoral or a shallow water inhabiting tadpole, whereas the tadpole of *P. cruciger* can be categorized as a lentic macrophagous nektonic tadpole. However, we note that the experimental conditions in which they were reared were not ideal with respect to the conditions that prevail in their natural breeding habitats. The natural food present in the breeding habitats is likely to be different from the food provided in the experiment. However, this difference was minimized by adding filamentous algae along with commercial fish food. The artificial ratios of the two species used in this study are also not reflective of the natural ratios of tadpoles that are present in ponds. This is because the number of eggs in a *D. melanostictus* clutch is greater than that of *P. cruciger* egg clutch (Ukuwela, personal observation). Therefore, the actual interactions faced by the two species in their natural habitat might differ from the results obtained here.

The primary goal of this experiment was to determine whether the presence of one species of tadpole effects the growth of another species under laboratory conditions. The results revealed that the presence of *P. cruciger* tadpoles negatively impacted the growth of *D. melanostictus* tadpoles, which suggest that tadpoles of *P. cruciger* are superior competitors to the tadpoles of *D. melanostictus*. We believe the results of this short study will add an important dimension to the knowledge of community ecology of the amphibian fauna of Sri Lanka.

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