Development of cane toad (*Chaunus marinus*) tadpoles in the presence of intraspecific and interspecific competition under resource limiting conditions

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ABSTRACT

Increased competition between and within species of amphibians, resulting from reduced volume breeding pools, represents one facet of climate change's impact on ecosystem dynamics that remains relatively unstudied. This study examines the effect of competition on the development of larval cane toads (*Chaunus marinus*) at varying levels of intraspecific and interspecific competition using *Lithobates forreri* as the competitor. While intraspecific competition was shown to be a significant factor affecting *C. marinus* growth, the effect was not significant for interspecific competition. However, survivorship was shown to be significantly lower in the presence of high interspecific competition as compared with low competition. These results demonstrate an antagonistic relationship between *C. marinus* and *L. forreri*, and suggest that high intraspecific competition—potentially resulting from reduced pond volume—can result in a different and potentially detrimental growth pattern in anuran larvae.

RESUMEN

Aumento en la competencia entre la misma y diferentes especies de anfibios, resultando en la reducción del volumen de los charcos de reproducción, representan una faceta del efecto del cambio climático en la dinámica de los ecosistemas relativamente poco estudiado. Este estudio examina el efecto de la competencia en el desarrollo de los renacuajos del sapo común (*Chaunus marinus*) a varios niveles de competencia intra e interespecífica usando *Lithobates forreri* como competidor. Mientras que la competencia intraespecífica muestra ser un factor significativo en el crecimiento de *C. marinus*, el efecto no fue significativo para la competencia interespecífica. Sin embargo, la sobrevivencia es significativamente menor cuando hay gran competencia comparada con baja competencia. Este resultado demuestra una relación antagonista entre *C. marinus* y *L. forreri*, y sugiere que una alta competencia intraespecífica, resultando potencialmente en una disminución en el volumen del charco, puede resultar en un diferente y potencialmente deterioro del patrón de crecimiento de las larvas de anuros.

INTRODUCTION

Reports of widespread amphibian declines and extinctions suggest that amphibians are suffering from the effects of climate change at an unprecedented rate (Bennett 1999). Thousands of amphibian species have declining populations, and many are critically endangered or have disappeared entirely (Blaustein 1994). While a number of proximate causes of amphibian declines have been proposed, the primary ultimate mechanism of global amphibian declines is generally considered to be climate change (Pounds *et al.* 2006). In the Monteverde area, climatic

conditions have been heavily impacted by climate change corresponding with a marked decrease in the regional misting cloud cover (Graham 1995) and increased minimum daily ambient air temperatures (Pounds *et al.* 2006). Reduced cloud cover during the day and the corresponding increased solar radiation could result in smaller amphibian breeding pools (Pounds and Crump 1994).

One suggested result of reduced breeding pool volume is increased intraspecific competition (Bennett 1999). Tadpoles in smaller volumes of water should experience higher levels of conspecific competition due to increased confinement and competition over limited food resources (Wells 2007). Likewise, several experiments have tested interspecific competition, a factor that would be expected to increase in accordance with decreased water volume. However, compared with the controlled laboratory studies examining intraspecific competition, relatively few studies have examined interspecific competition, and remarkably few field studies have examined interspecific competition between sympatric species (McDiarmid and Altig 1999).

Chaunus marinus (Bufonidae), a highly fecund species that is responsible for extensive damage to local ecosystems in areas of its introduction, is nevertheless subject to factors inhibiting its reproductive success such as competition (Shine 2010; Zug and Zug 1979). The majority of studies focusing on breeding sites and development of *C. marinus* have focused on its introduced ranges, and comparatively few have been conducted in its native range—Central and South America (Evans *et al.* 1996).

This study examines two factors influencing rate of tadpole development in *C. marinus*: intraspecific competition and interspecific competition. It is predicted that competition will have a more pronounced impact on tadpole development than water volume due to the increased competition over food resources. However, selection for temporary pools may be an adaptation promoting the rapid development of tadpoles because tadpoles in lower volume pools should have a perceived need to complete metamorphosis before the pond dries up (Evans *et al.* 1996). Therefore, volume is predicted to have a significant impact on tadpole development. Higher interspecific competition with *Lithobates forreri* (Ranidae) is predicted to result in reduced rates of tadpole development in *C. marinus* as both species belong to the same herbivorous or semi-omnivorous feeding guild (Wells 2007).

MATERIALS AND METHODS

Chaunus marinus tadpoles were collected during the month of October, 2010 from a flooded pasture in Cañitas, Guanacaste, Costa Rica. Although also observed in the flooded pasture in Cañitas, *L. forreri* tadpoles were collected from an artificial pond nearby. Both ponds were located in lower montane wet forest according to the Holdridge Life zone classification system.

Intraspecific competition and volume

To test the effect of water volume and conspecific tadpole competition on the development of *Chaunus marinus*, four treatments were selected: low volume (250 ml) and low competition (5 tadpoles), low volume and high competition (10 tadpoles), high volume (500 ml) and low competition, and high volume and high competition (Fig. 1). Each treatment was replicated ten times for a total of 40 plastic Glad containers (10 cm x 10 cm x 6 cm) and 300 tadpoles. A "natural" treatment was not performed because random sampling of individuals of *C. marinus*

ranged from 2 to >200, suggesting that accurately simulating natural densities would be fallacious.

Interspecific competition

To test the effect of interspecific competition on the growth of *C. marinus* in the presence of *L. forreri*, two additional treatments were selected: low competition and high competition. The low competition treatment consisted of five *Chaunus marinus* tadpoles and one *L. forreri* tadpole, while the high competition consisted of 5 *C. marinus* tadpoles and three *L. forreri* tadpoles (Fig. 2). All interspecific competition treatments were conducted in 1000 ml water to accommodate the large size of *L. forreri*. Each treatment was replicated five times for a total of ten containers (20 cm x 10 cm x 9 cm). Data collection for interspecific competition ceased when *L. forreri* achieved Gosner stage 40-41 where resorption of the tail begins (McDiarmid and Altig 1999).

Measurements and husbandry

The mass (g) of the tadpoles in all treatments was recorded every two days. One container of tadpoles, replicated either 5 or 10 times depending on the treatment, represented the unit of measurement. Tadpoles in each container were weighed together and an average mass for each treatment was calculated. The two different species of tadpole in the interspecific competition treatments were measured separately. Average mass of a tadpole per container for each day was calculated by dividing by the number of containers per condition, and by the number of tadpoles per container. The water was changed every two days. In addition to mass, survivorship information was recorded for interspecific experiments. Food was prepared by mixing 1.5 g fish food with 30 ml water and dispensing 1/6 ml into each treatment every two days. After the experiment, all tadpoles and froglets were returned to their respective ponds.

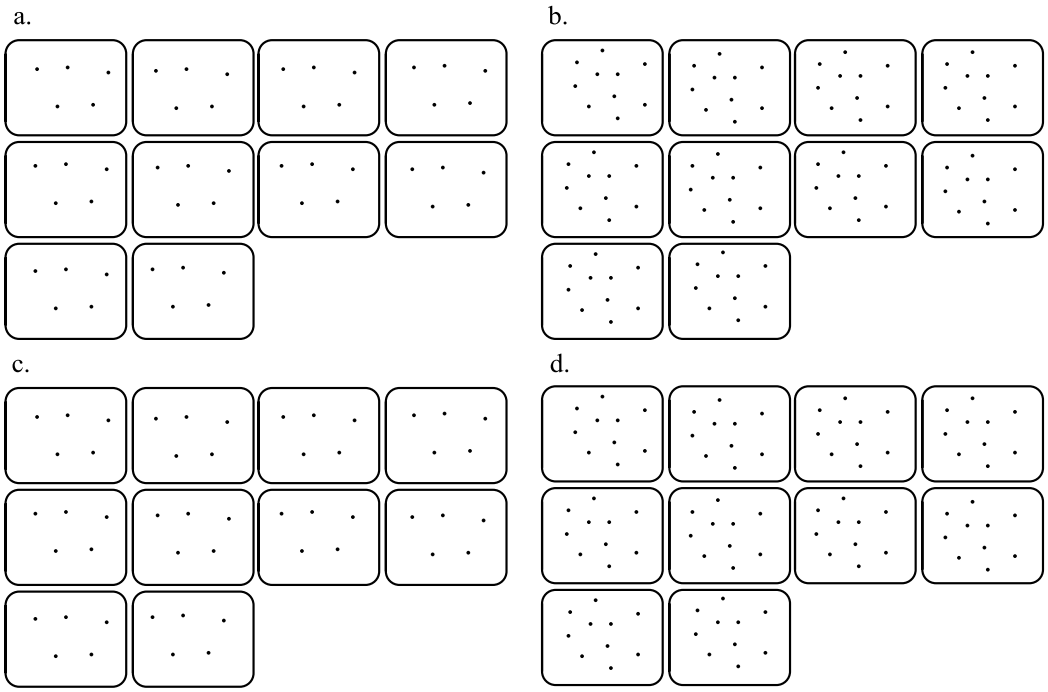

Figure 1. Experimental setup for intraspecific competition experiments involving *Chaunus marinus*. Treatments **a** and **b** have 250 ml water while treatments **c** and **d** have 500 ml. Likewise, **a** and **c** have low density (5 tadpoles), while **b** and **d** have high density (10 tadpoles). Each dot corresponds to 1 tadpole. Each container was 10cm x 10cm x 6cm.

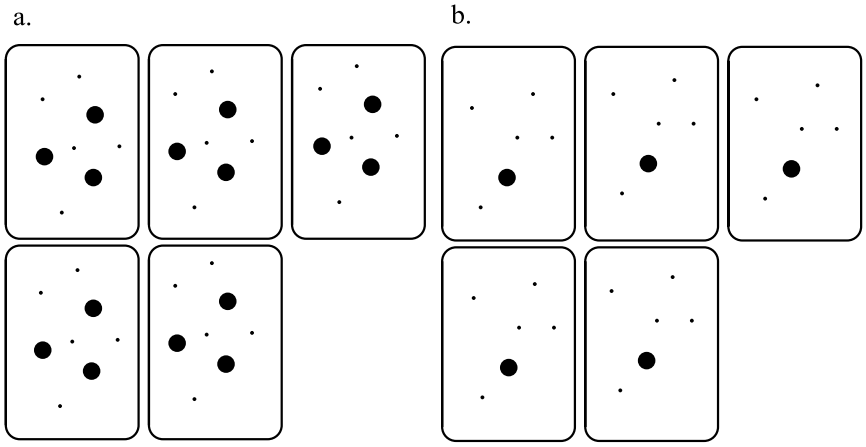

Figure 2. Experimental setup for interspecific competition experiment involving both *Lithobates forreri* (large dots) and *Chaunus marinus* (small dots). **a**, setup for high competition. **b**, setup for low competition. Each dot corresponds to 1 tadpole. All containers (20cm x 10cm x 9cm) were filled with 1000 ml tap water.

RESULTS

Intraspecific competition

On the first day of measurement (day 2) there was no significant difference in mass per tadpole between treatments (Table 1, Fig.3). The initial average between all treatments was 0.066 g. By day 10, tadpole mass in treatments with low competition was higher than in high competition (Table 1, Fig. 3). Low competition treatments reached a peak joint average tadpole mass of 0.11 g while high competition reached a peak average weight of 0.077 g, constituting a 30% difference in mass. Mortalities occurred in all growth conditions, including full mortalities in container 7 of the treatment with low volume and high competition. Both number of containers and numbers of individuals per container were accounted for when calculating the average mass per tadpole for each treatment. Treatments with low competition continued to be significantly different from those with high competition through day 17 (Table 1, Fig. 3). However, tadpole mass was not significantly different by day 19, and through day 21 (Table 1, Fig. 3). Between the first and last day of measurement, there was no significant difference in tadpole mass between high and low levels of competition despite the significant difference for days 10 through 17. However, there was an upward trend in overall change in mass (Fig. 3). Averaged between all treatments, tadpole mass at day 21 was 0.071 g, constituting a 7.6% increase in mass from the first day of measurement. There was no significant difference in mass between low volume and high volume treatments for all days between equivalent levels of competition (Table 1, Fig. 3).

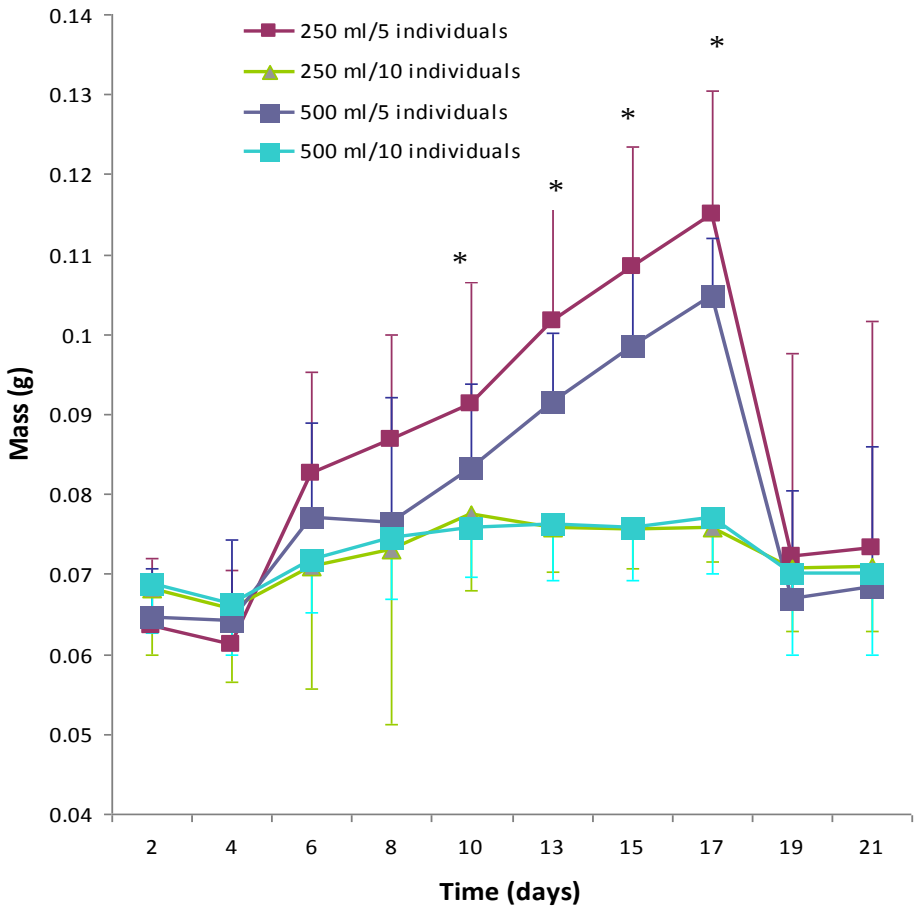

Figure 3. Average (±SD) mass (g) for *Chaunus marinus* tadpoles exposed to four different growth conditions varying in water volume and level of competition over a three week period (N = 40). Each condition was replicated 10 times. Asterisks indicate the days on which there were significant levels of difference between treatments with 5 individuals and treatments with 10 individuals.

Table 1. One-way analysis of variance showing degrees of significance between treatments on each day of measurement for high and low levels of intraspecific competition and high and low levels of volume. All intraspecific experiments tested the growth of *Chaunus marinus* under resource limiting conditions during October, 2010. Tadpoles were collected from a flooded pasture in Cañitas, Guanacaste, Costa Rica.

Day	F	df	p
2	1.3	3,36	0.30
4	0.63	3,36	0.60
6	2.0	3,36	0.13
8	1.7	3,36	0.20
10	3.9	3,35	0.017
13	16.4	3,35	<.0001
15	27.6	3,35	<.0001
17	41.6	3,35	<.0001
19	0.21	3,35	0.90
21	0.13	3,35	0.90

Interspecific Competition

On day one, mass of *C. marinus* tadpoles was not significantly different between high and low competition treatments (Table 2, Fig. 4). There was no significant difference in tadpole mass between both treatments for all days (Table 2, Fig. 4). However, there was a visible trend showing gradual loss in mass in the treatment with high interspecific competition as compared with low interspecific competition (Fig. 4). Furthermore, high competition had an initial tadpole mass of 0.071 g (±0.013) and a final mass of 0.058 g (±0.020), whereas low competition had an initial mass of 0.074 g (±0.008) and a final mass of 0.080 g (±0.017), constituting an 18.3% overall decrease in mass for high competition and an 8.1% overall increase in mass for low competition.

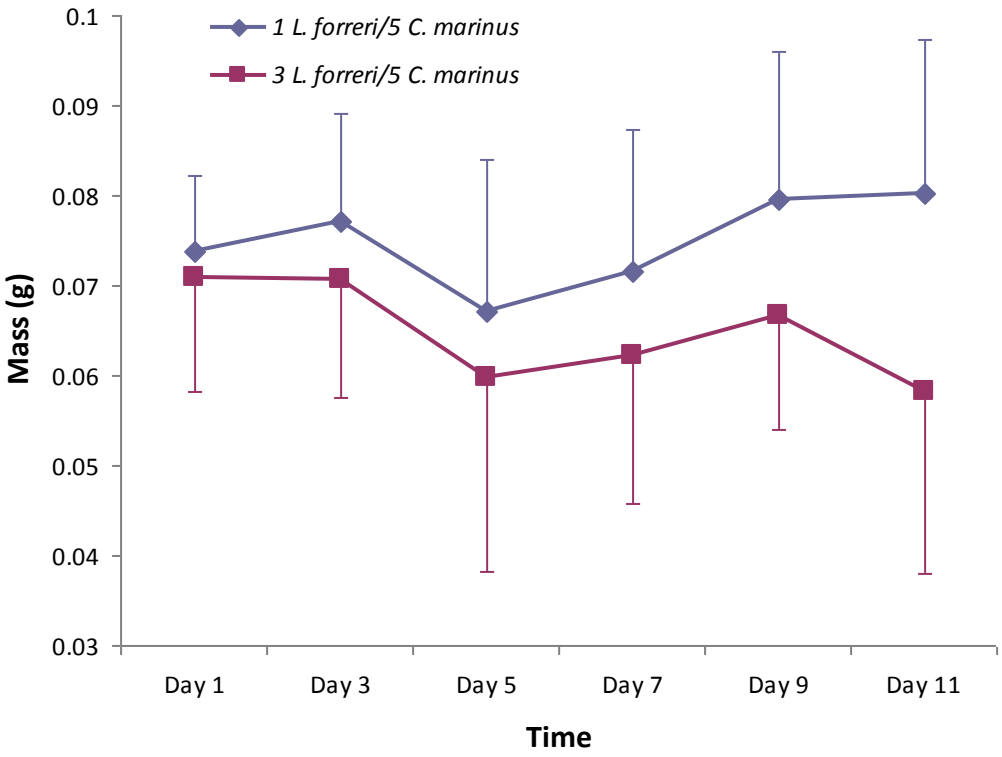

Figure 4. Average (±SD) mass (g) for *C. marinus* tadpoles exposed to high and low interspecific competition by the leopard frog *L. forreri* over an 11 day period (N = 10). Each treatment was replicated 5 times.

Table 2. Mann-Whitney U Test results between high and low levels of interspecific competition showing non-significance for tadpole mass between the two treatments for all days. All interspecific experiments tested the growth of *Chaunus marinus* competing with *Lithobates forreri* under resource limiting conditions during October, 2010. Tadpoles of both species were collected from two ponds in Cañitas, Guanacaste, Costa Rica.

Day	χ^2	df	p
1	0.55	1	0.46
3	0.88	1	0.35
5	0.27	1	0.60
7	0.54	1	0.50
9	1.32	1	0.25
11	3.15	1	0.08

Tadpole Survivorship in the Presence of Interspecific Competition

High competition by *L. forreri* tadpoles resulted in decreased *C. marinus* survivorship over an 11 day period. By day five, three *C. marinus* tadpole had been eaten by *L. forreri* tadpoles in the

presence of high interspecific competition. Comparatively, only 1 tadpole was eaten by day five in the low interspecific competition treatments. No mortality was observed in *L. forreri*. It was shown that there was a significant difference between the two treatments in survivorship for *C. marinus* (Kaplan-Meier, DF = 3, χ^2 = 10.69, P = 0.014, Fig. 5) The Mantel-Haenszel test for posthoc comparison of groups showed that there was a significant difference between tadpole survivorship in the presence of high versus low interspecific competition.

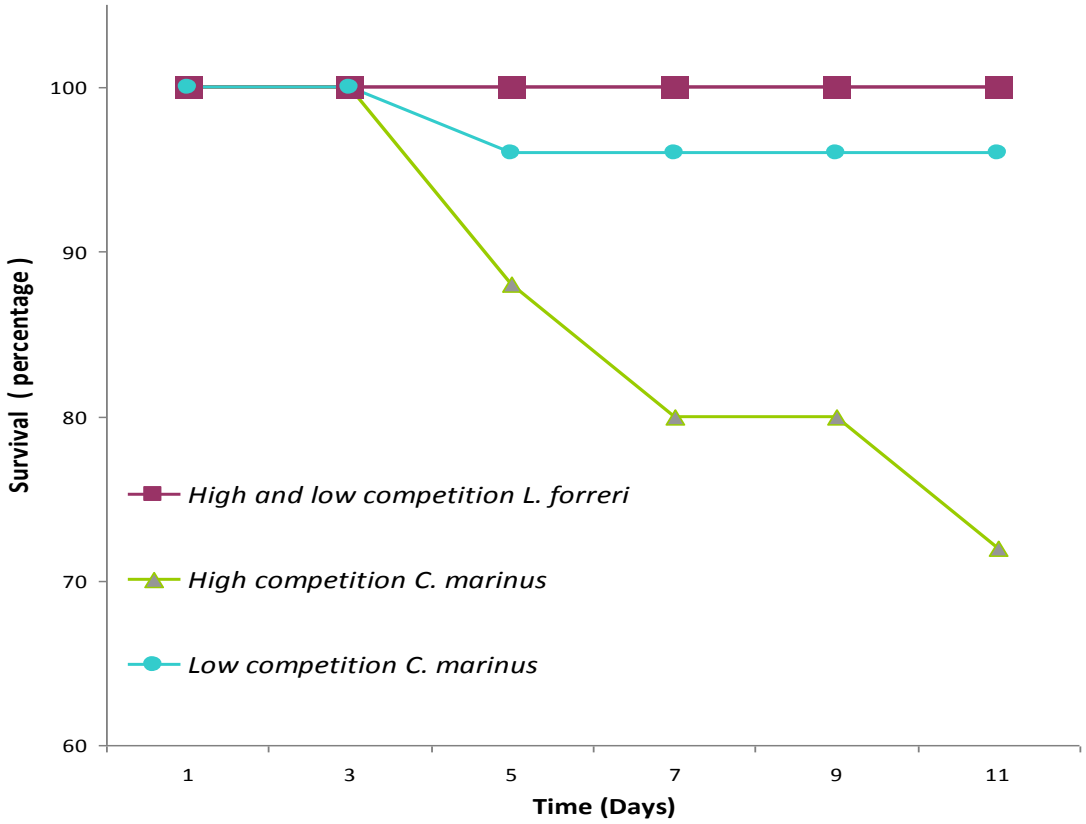

Figure 5. Survival curve showing percentage of *Chaunus marinus* and *Lithobates forreri* survivors in high and low interspecific competition treatments over an 11 day period in containers (20 cm x 10 cm x 9 cm) filled with 1000 ml tap water. Each treatment was replicated 5 times. High competition and low competition trends for *L. forreri* were combined as no mortalities for this species were experienced in either treatment.

DISCUSSION

In the case of intraspecific competition, results indicate that high competition negatively effects *Chaunus marinus* tadpole development. For both treatments with low competition, a peak average tadpole mass was achieved at day 17, with significant difference from the two high competition treatments starting at day 10 and continuing through day 17. The visible increase in mass from day 2 to 17, followed by a sharp decline in mass from day 19 to 21 likely corresponds to major internal structural and physiological changes known to occur during pre-metamorphosis stages of anuran development including the development of an adult urogenital system and

ossification of skeletal elements (Wells 2007). Although most of these changes would be expected to increase tadpole mass, the sharp decrease in weight for low competition treatments after day 17 likely corresponds to a severe reduction of intestinal elements, which are severely reduced in adult anurans as compared with larval stages (Wells 2007). The growth trend in low competition treatments is consistent with an accelerated version of the tadpole development schematic presented by Brown and Cai (2007) in which *Xenopus laevis* tadpoles achieved a climax stage, and proceeded to experience a 70% loss in mass corresponding to tail and gill resorption, and organ remodeling. The growth trend in the current experiment is accelerated as this climax period occurred for *C. marinus* during the pre-metamorphosis phase whereas it occurred in the experiments testing *X. laevis* immediately after pro-metamorphosis. The absence of a distinct growth trend in high competition treatments suggests that the tadpoles in these treatments were stressed, either because of higher density or lower availability of food. It is predicted that these treatments would show a similar growth trend (a peak in mass followed by a sharp decline in pre-metamorphosis) as seen in the low competition treatments at a later time if the experiment had been allowed to continue. This is supported by the Wilbur-Collins model of tadpole development that predicts a staggered pattern of tadpole growth depending on resource availability (McDiarmid and Altig 1999).

Volume did not have a significant effect on *C. marinus* development. Volume was predicted to have a measurable effect on tadpole development due to the perceived threat of a drying water source (Crump 1991). The lack of significance between high and low volume suggests that it is the number of individuals and not density that influences tadpole development. However, the absence of a distinct trend between high and low volume treatments could result from the relative constancy of volume for the duration of the experiment. This suggests that low versus high volume conditions may not be the most effective method of simulating pond desiccation resulting from climate change. Future work should consider a progressive reduction of water volume over time, simulating the drying conditions resulting from a lifting cloud base and a reduced mist frequency (Bennett 1999). While it is possible that constant volumes of water did not accurately simulate drying conditions in nature, it is also possible that the development *C. marinus* is not as highly impacted by volume. It has been shown that *C. marinus* favors temporary breeding pools (48%) over permanent ones (16%), potentially suggesting that high pond volume is not necessarily an important variable in breeding pool selection for this species (Evans *et al.* 1996).

Regarding interspecific competition, there was no significant difference between high and low competition average tadpole mass for all days. However, a trend was visible insofar as differences in average tadpole weight became progressively more significant from day 5 to 11. One possible explanation for the lack of significance is the high levels of variation within the treatments. The continuation of this experiment was limited by the achievement of the climax phase by *L. forreri*. Interspecific experiments were concluded once this stage was reached since the two species of anurans no longer belonged to the same feeding guild, and were therefore no longer competing. The lack of competition between the two species at this stage was evident as *L. forreri* had achieved an adult frog appearance, and the adult anuran diet is dissimilar from the diet of its larval stage. (McDiarmid and Altig 1999). Nevertheless, interspecific competition was markedly less effectual than intraspecific competition for tadpole growth, a finding that is consistent with a niche-partitioning model of co-existence between species which predicts that sympatric species should limit individuals of their own species more than they limit competitors of other species (Levine and HilleRisLambers 2009).

While interspecific competition did not show a difference in tadpole growth between high and low competition, survivorship between the two conditions for *C. marinus* did show a significant difference. Whereas tadpole survivorship for the high competition treatment approached 70% by day 11, it never fell below 95% for tadpoles in the low competition treatment. By contrast, *L. forreri* experienced no mortalities for both treatments. This suggests that under resource-limiting conditions, *L. forreri* has a selective advantage over *C. marinus*.

The consumption of *C. marinus* tadpoles by *L. forerri* tadpoles in interspecific treatments is a significant characteristic of the study considering the high toxicity of larval *C. marinus* and because this predation suggests that *L. forreri* can become carnivorous in nutrient limiting conditions. Predator release is one factor that may be contributing to the success of *C. marinus* in its invasive ranges. Specifically, it has been shown that consumption of larval *C. marinus* in Australia by sympatric amphibians was always fatal (Shine 2010). Therefore, this study supports the theory that the impact of cane toads in their native ranges is tempered by predation, and that sympatric predators like *L. forreri* may have some resistance to the toxins present in cane toad larvae. Evidence for factors such as predator release is crucial for an understanding of why invasive species are so destructive in their introduced ranges, and can serve as a basis for future control efforts.

Both interspecific and intraspecific competition would be expected to increase as a result of reduced pond volume and resource availability (Bennett 1999). Reduced growth rate could have potentially serious consequences for obligate pond dwellers like tadpoles when their survival depends on completing metamorphosis before ponds dry up, especially in light of evidence for the profound impact of climate change on ecosystem dynamics (Pounds *et al.* 2006). Therefore, the decreased growth rate in tadpoles stressed for available resources due to increased competition, coupled with more rapidly drying breeding pools due to climate change represents a potential contributing factor leading to reduced amphibian reproductive success (Bennett 1999). This suggests that if current trends in decreased mist frequency and warming continue, the reproductive success of tropical amphibians, especially those in restricted climate envelopes, could be at risk.

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LITERATURE CITED

Bennett, Rich 1999. Tropical Ecology and Conservation, p. 233-240. Council on International Educational Exhange, Monteverde.

Blaustein, A. R. 1994. Chicken little or Nero's fiddle? A perspective on declining amphibian populations. Herpetologica 50: 85-97.

Crump, M. L., and Pounds, J. A. Amphibian declines the climate disturbance: The case of the golden toad and the harlequin frog. Conservation Biology Vol. 8, No. 1 (March 1994): 72-85.

Crump, M. 1991. Choice of oviposition site and egg load assessment by a treefrog.

Herpetologica 47: 308-315.
Evans, M., Yáber, C., and Hero, J. 1996. Factors Influencing Choice of Breeding Site by *Bufo marinus* in Its Natural Habitat. *Copeia* 4: 904-912.
Graham, N. E. 1995. Simulation of recent global temperature trends. Science 267: 666-671.
Levine, J. M., and J. HilleRisLambers. 2009. The importance of niches for maintenance of species diversity. Nature 461: 254-258.
McDiarmid, R. W. Altig 1999. Tadpoles: the biology of anuran larvae, p. 247-275. The University of Chicago Press, Chicago: 458.
Pounds, A. *et al.* 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. Nature 439: 161-167.
Shine, R. 2010. The ecological impact of invasive toads (*Bufo marinus*) in Australia. Quarterly review of biology.
Wells, K. D. 2007. The ecology & behavior of amphibians. The University of Chicago Press, Chicago: 1148.
Zug, G. R., and Zug, P. B. 1979. The Marine Toad, *Bufo marinus*: A Natural History Resumé of Native Populations. Smithsonian Contributions to Zoology 284.
Brown, D. D., and Cai, L. 2007. Amphibian Metamorphosis. Developmental Biol. 306: 20-33.