

**Effects of food concentration in the competition between larvae of the
introduced cane toad (*Rhinella marina*) and the native white-lipped frog
(*Leptodactylus albilabris*)**

By

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ABSTRACT

Invasive species are a major focus within conservation biology. The widely introduced cane toad, *Rhinella marina*, has gained particular interest in studies of effects of invasive species on native communities. In this study I examined the effects of invasive cane toad larvae on the growth, development and survival of a Puerto Rican native species, the white-lipped frog, *Leptodactylus albilabris*, when tadpoles were reared with different food levels. I conducted two experiments: (1) a food calibration experiment to determine low and high food concentrations adequate for larvae growth and survival, and (2) a competition experiment with varying species rations and food availability. In the food calibration experiment, lower food levels reduced survival and increased larval period for both species. Contrary to the expected results in the competition experiment, the presence of *R. marina* had no negative effects on *L. albilabris* development. Rather, the opposite outcome was observed; larvae of *R. marina* experienced severe reduction in growth and survival when in the presence of *L. albilabris*, particularly in the high food level treatments. Significant effects of the presence of *L. albilabris* on *R. marina* development were observed as early as the first two weeks, and continued on to metamorphosis. Adaptations to life on ephemeral habitats such as larger body size, higher activity levels and reduced larval period, combined with high food levels, may have provided *L. albilabris* with a competitive advantage over *R. marina*. Further studies are needed to determine if these interactions produce similar results under natural conditions.

RESUMEN

Las especies invasivas son el enfoque de la biología de la conservación. El sapo de la caña de azúcar, *Rhinella marina*, ha ganado particular interés en estudios para demostrar los efectos de especies invasivas en comunidades de especies nativas. En este estudio se examinaron los efectos de las larvas del sapo de la caña en el crecimiento, desarrollo y sobrevivencia de una especie nativa puertorriqueña, la ranita de labio blanco, *Leptodactylus albilabris*, bajo diferentes niveles de alimento. Se utilizaron dos procesos experimentales: (1) experimento de calibración para determinar niveles adecuados (bajo y alto) de comida para crecimiento y sobrevivencia de los renacuajos y (2) experimento de competencia entre ambas especies bajo dos niveles de comida (alto y bajo). Los bajos niveles de comida redujeron la sobrevivencia y aumentaron el periodo larval de ambas especies. Contrario a los resultados esperados para el experimento de competencia, la presencia de *R. marina* no tuvo efectos negativos en el desarrollo de *L. albilabris*. Se observó la situación inversa, donde las larvas de *R. marina* experimentaron reducciones severas en crecimiento y sobrevivencia cuando se encontraban juntas con *L. albilabris*, particularmente con niveles altos de comida. Se observaron efectos significativos de la presencia de *L. albilabris* en el desarrollo de *R. marina* desde las primeras dos semanas de desarrollo, los cuales continuaron hasta la metamorfosis. Las adaptaciones que presenta *L. albilabris* para vivir en ambientes temporeros como mayor tamaño, alto nivel de actividad y periodos larvales reducidos, combinado con altos niveles de alimento, pudieron haber dado la ventaja competitiva a esta especie sobre *R. marina*. Se necesitan estudios futuros para ver si estas interacciones producen resultados similares bajo condiciones naturales.

DEDICATION

Al único que estuvo conmigo en cada etapa de este trabajo. No sería nadie si no me hubieses salvado. Gracias.

El único con el poder para salvarte es Dios.

El único con el poder para ayudarte es Dios.

Él es lo único que necesitas.

El día es hoy. El momento es ahora. Dios te ama y quiere librarte de las aflicciones de este mundo. Él es el único. Él puede hacer un milagro en tu vida, pero tú debes dar el primer paso.

Cuando comienzas a aceptar que no puedes solo, las cargas dejan de pesar. Cree en que Él puede obrar. Cree en que te va a restaurar. Cree que Dios te ama y te quiere ayudar. El dolor se irá. Las cargas desaparecerán. El vacío se llenará. Los problemas serán dejados atrás. Los miedos ya no importarán.

Tu vida será nueva.

Una nueva vida en Cristo está tocando tu puerta. Déjala entrar.

El día es hoy.

El momento es ahora.

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“Because no journey is really meant to be made alone.”

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INTRODUCTION

Invasive species are a significant threat to native biodiversity. The introduction of invasive species, either accidental or deliberate, has been mostly caused by human activities. Among invasive species effects there are changes in community structure, nutrient cycling, and plant productivity (Mack *et al.*, 2000). The effects on native communities are exacerbated when accompanied by other effects such as habitat loss, climate change and pollution (Platenberg, 2007). Invasive species also modify inter- and intraspecific interactions among native species by both direct and indirect mechanisms (Crossland, 2000; Crossland *et al.*, 2008; 2009). Direct mechanisms include competition, predation, parasitism, reproductive interference, hybridization and toxic effects (Crossland, 2000; Pizzatto and Shine, 2009). For these and many other reasons, the biology of invasive species has become a focus of conservation biology (Smith, 2005).

Although invasive species are known for their devastating effects on native fauna (Lampo and DeLeo, 1998) and have caused major changes in populations, communities and ecosystems (Mack *et al.*, 2000), uncertainty remains regarding the effects of these invasive species on native ecosystems (Crossland *et al.*, 2008; Crossland and Shine, 2010). Many detrimental effects believed to be caused by invasive species have been based on circumstantial evidence; therefore, caution must be exercised when attributing observed changes in a native ecosystem to introduced species (Crossland, 2000; Mack *et al.*, 2000). Little direct evidence exists on the mechanisms of impact of invasive species (Smith, 2005; Crossland *et al.*, 2008; Crossland and Shine, 2010), and because of this, detailed scientific understanding of the nature of these impacts is fundamental (Crossland and Shine, 2010). At present, great effort is being devoted to understanding ecological changes on native ecosystems following the introduction of a new species and which

factors increase success probability of invasive species (Lampo and DeLeo, 1998). However, improvement is still needed to better understand invasive species' mechanisms of impact and their influence on native species to improve conservation efforts and predictions of threat (Crossland *et al.*, 2009; Pizzatto and Shine, 2009). Presently, predation and habitat alteration are seen as the most important mechanisms of impact, but competitive impacts may be underestimated (Lodge, 1993).

Invasive species may impact native species by means of interspecific competition if their niches overlap. This phenomenon can be seen in amphibian species that undergo metamorphosis, since they appear to be limited in their use of breeding sites by abiotic and biotic constraints on their larvae (Dayton and Fitzgerald, 2001). According to Smith (2005), tadpole communities are frequently characterized by high population densities in temporally and spatially restricted aquatic habitats, making them vulnerable to competition with invasive species. Thus, tadpole communities are ideal for the study of competition between native and invasive species. Indeed, tadpole communities have been widely used as experimental models to test intra- and interspecific competition, predation, and environmental stochasticity as structuring forces in community ecology (see Bardsley and Beebee, 1998). Nevertheless, there has been little research on the impact of nonindigenous tadpoles on native tadpoles via competition *per se*, and this interaction is difficult to demonstrate in invaded communities (Smith, 2005). Competition occurs by means of exploitation, interference, or both. Among tadpoles, this interaction has been observed as intra- and intercohort competition within species, interspecific competition within the tadpole guild, competition with other vertebrates, and competition with members of other phyla (Alford, 1999). Among these, there is special concern in understanding competition interactions between native and invasive tadpole species.

One invasive species that has gained interest among herpetologists has been the cane toad, *Rhinella marina* [*Bufo marinus*] (Anura: Bufonidae). *R. marina* is the most widespread and common American amphibian (Zug and Zug, 1979), and it is one of the most harmful invasive species worldwide. Several traits have made *R. marina* a successful invader, including prolific breeding (females can lay up to 35,000 eggs in long “rosary” strings that can hatch after approximately 36 hours (Rivero, 1998), long distance dispersal capability, exceptional adaptability (Zug and Zug, 1979), and toxicity (Crossland, 2000; Smith, 2005; Pizzatto and Shine, 2009). Although this species can occur at high densities, it seems to do so only where it is invasive (Zug and Zug, 1979). *R. marina* was introduced into many countries during the 1900’s, primarily as a biological control agent for agricultural pests (Esteal, 1981). The species was introduced to Puerto Rico from Jamaica and Barbados in 1920, 1924 and 1926. Initial reports from Puerto Rico indicated that the introduction was a success, but *R. marina* is now considered a pest in the island (Rivero, 1998).

R. marina larvae are small (approximately 10-25 mm in total length), round-bodied, black or dark brown tadpoles (Zug and Zug, 1979) that typically occur in high densities relative to native species (Williamson, 1999). They can grow and metamorphose in one to two months (Zug and Zug, 1979). It has been suggested that they are aggressive feeders, a trait that may provide a competitive advantage (Alford, 1999). Invasion by this species can affect invertebrates, not only by direct predation, but also by competition in the larval phase (Pizzatto and Shine, 2009). Additionally, both the eggs of this species and its larvae produce toxins that can affect invertebrates and vertebrates that consume them (Crossland *et al.*, 2008; 2009; Crossland and Shine, 2010). Furthermore, bufonid larvae have been shown to negatively affect growth and development of other anuran species (Wilbur and Alford, 1985 in Williamson, 1999). Several

characteristics of larval *R. marina* suggest that this species may impact native tadpole communities (Smith, 2005). Among these characteristics are potential competition with native species (Smith, 2005), toxicity of eggs (Crossland *et al.*, 2008; Crossland and Shine, 2010), and toxicity of larvae (Crossland, 2000). Although this species is potentially harmful, there is scarce information concerning its impacts on native herpetofauna in Puerto Rico. Platenberg (2007) mentions that tadpoles probably compete for limited freshwater resources with the white-lipped frog, *Leptodactylus albilabris* (Anura: Leptodactylidae), but no studies have been conducted to confirm this hypothesis.

Leptodactylus albilabris is native to Puerto Rico and the U.S. and British Virgin Islands (Henderson and Powell, 2009). Tadpoles of this species hatch in approximately four days from eggs laid in terrestrial foam nests, and they develop in temporary ponds or channels after being washed away by water runoff during heavy rains (Rivero, 1998). Metamorphosis can occur in 35 days, but nutritional stress can extend this time to 73 days (Dent, 1956). Although *L. albilabris* is one of the most abundant anurans in coastal wetlands in Puerto Rico, little is known about its life history (Ríos-López, 2008) and larval biology (Lebrón *et al.*, 1995). Most of the studies on this species have concentrated on adult ecology (Joglar, 2005), so there is still much to discover about this native species and its life history. In Puerto Rico, this is the only native anuran species that it is not endangered and presents a larval stage, making it ideal to observe larval impacts of the introduced *R. marina* on native anuran larvae.

Due to the negative impacts of *R. marina* reported on many native species, I believed that *R. marina* larvae would have a negative impact on growth, development and survival of native *L. albilabris*. Studies of competition between *L. albilabris* and *R. marina* larvae could aid us to better understand the mechanisms of impact of an introduced species to a native species. This

information can also be useful in conservation management of both the invasive species and endangered species such as the Puerto Rican crested toad, *Peltophryne lemur*. Additionally, studying the impacts of *R. marina* on *L. albilabris* tadpoles could give us insight into the larval biology of our native species, contributing to the depauperate knowledge base that presently exists for this species.

OBJECTIVES

General:

- Assess the impact of *R. marina* larvae on the growth, development and survival of native *L. albilabris* under aquarium conditions.

Specific:

- (1) Determine the minimal and maximal amount of food required for *R. marina* and *L. albilabris* larval development under aquarium conditions.
- (2) Determine if there are competitive interactions between both species when reared with different food levels and how this competition affects development, growth and survival of *R. marina* and *L. albilabris* larvae.

LITERATURE REVIEW

Competitive interactions in tadpoles

The larval stage is characterized by being non-reproductive and highly specialized within the complex life cycle of amphibians (Viertel and Richter, 1999). It is also the stage of energy gathering for metamorphosis (Hoff *et al.*, 1999), during which larval development and adult anatomy can be affected by population density, temperature, pH, dissolved oxygen and diet (McDiarmid and Altig, 1999). For these reasons, a short larval period reduces the amphibian's exposure to potential stresses (Vitt and Caldwell, 2009). Selection is predicted to favor larvae capable of growing to the largest size in the shortest period, enhancing their probability of completing metamorphosis and reaching the reproductive stage (Hoff *et al.*, 1999). For example, Kupferberg (1997a) observed that tadpoles forage selectively on the algal foods that promoted most rapid growth and development. Nevertheless, growth rates may be influenced by competition when there is resource overlap (Alford, 1999).

Space, food, and time resources comprise three critical ecological axes that define tadpole niches. In larval amphibian communities, an almost complete overlap in the use of feeding resources is frequently found (Díaz-Paniagua, 1985 in Pavignano, 1990), and niche overlap may be greater among generalist feeders (Rossa-Feres *et al.*, 2004). Niche overlap can lead to exploitation competition, in which the intensity of the competition depends on the degree of resource overlap between species (Alford, 1999). For instance, Alford and Crump (1982) observed that larger tadpoles of *Rana utricularia* occupied areas where more food was available, thus indirectly reducing foraging efficiency for smaller tadpoles. Also, Kupferberg (1997b) observed that competition between tadpoles of *Rana catesbeiana* and *Rana boylei* appeared to be

mediated by algal resources. On average, large tadpoles tend to monopolize more resources than smaller tadpoles (Richter-Boix *et al.*, 2007).

These interactions between tadpoles depend not only on their feeding preferences, size and activity levels, but also on adult reproductive behavior. According to Alford (1999), the phenology and reproductive site preferences of adult frogs control the exposure of tadpoles to competitors and predators. Tadpoles are thus likely to encounter a variety of predators and both intra- and interspecific competitors. Interspecific predation among anurans, particularly predation by tadpoles on the eggs and hatchlings of other species, may be very common. Predators will gain nutrition while reducing or eliminating potential competition or predation. Thus, interspecific interactions among anuran larvae are complex, and can vary among species.

Rhinella marina

Many researchers have focused in studying the effects of *R. marina* on native amphibian species. Williamson (1999) studied the effects of larval *R. marina* on native anurans from southern Queensland. He used both artificial ponds and natural enclosures to conduct competition trials. In artificial ponds, *Rhinella* reduced the survival of many of the native larvae species. A survey of the natural breeding sites, however, showed that there was little pool overlap between *Rhinella* and native species. He concluded that although *R. marina* may negatively affect growth and survival of native anurans under some circumstances, the potential impact of *R. marina* may be minimal if there are always many breeding sites where native anurans can breed in the absence of *R. marina*.

Smith (2005) also studied the competitive effects of the larvae of introduced *R. marina* and *Osteopilus septentrionalis* on the development of two native species in Florida, *Bufo*

terrestris and *Hyla cinerea*. He used artificial ponds with four experimental treatments: native control (36 native larvae), *R. marina* treatment (18 native larvae with 18 *R. marina* larvae), *O. septentrionalis* treatment (18 native larvae with 18 *O. septentrionalis* larvae), and *R. marina* + *O. septentrionalis* treatment (12 of each larvae). Although he found no significant effects of *R. marina* on native tadpoles, he observed a trend to reduced survival for *H. cinerea*. He concluded that fitness and survival of adult native anurans could be affected by the presence of nonindigenous species even if larval survival *per se* was not affected.

Other researchers have focused on the effects of *R. marina*'s toxicity, both of the eggs and the larvae, on native species. In one of such studies, Crossland (2000) used artificial ponds to investigate the impact of eggs and hatchlings of *R. marina* on two native anuran larvae in Australia. He observed that one native species, *Limnodynastes ornatus*, experienced reduced survival when exposed to toxic *Rhinella* eggs and hatchlings. In contrast, *Litoria rubella* experienced an increased survival when exposed to both *Rhinella* and *L. ornatus* tadpoles. This happened because the negative impact of *Rhinella* on *L. ornatus* tadpoles reduced the intensity of predation of *L. ornatus* on *L. rubella* eggs and hatchlings. He concluded that *R. marina* plays an important role in structuring native larval anuran communities via direct and indirect mechanisms, and that *Rhinella* may have both negative and positive effects on populations of native anuran larvae.

In another study, Crossland *et al.* (2008) showed that the most numerous vertebrate victims of *R. marina* invasion in Australia are native tadpoles that die when they consume toad eggs. They documented 11 episodes of mass mortality of tadpoles of 10 species within a single wet season shortly after *Rhinella* invaded. However, despite the high mortality rates found, they

concluded that toad invasion does not appear to threaten the viability of anuran populations because native frogs often breed in ponds not used by *Rhinella*.

Additionally, Crossland *et al.* (2009) used a mesocosm experiment to quantify the impact of eggs and larvae of *Rhinella* on fitness-related traits of native Australian *Opisthodon ornatus*. Their results showed that growth and survival of *O. ornatus* tadpoles were enhanced when they preceeded *R. marina* tadpoles, but were reduced instead when they followed *Rhinella* tadpoles into ponds. However, they also observed that even when exposure to *Rhinella* eggs reduced the number of *O. ornatus* metamorphs, it increased their body size. The authors determined that the increase in size compensated for the reduced survival, concluding that minor interspecific differences in the seasonal timing of oviposition have the potential to massively alter the impact of invasive cane toads on native anurans.

A study by Crossland and Shine (2010) was designed to clarify the determinants of native tadpole vulnerability to *Rhinella* eggs. They exposed tadpoles of 15 species to *Rhinella* eggs with or without alternative food. Their data showed that *Rhinella* eggs were highly toxic to native tadpoles, but tadpole mortality by egg encountering depends upon a complex interaction between the native species, its body size, and the presence of alternative food. Their results demonstrate the complexity of vulnerability determinants, and reveal the importance of identifying ecological factors such as tadpole size and presence of alternative food as the primary determinants of cane toad impact on native tadpoles.

Even when toxicity of larvae and eggs of *R. marina* are detrimental to some species, there are others that can prey on their larvae without ill effects. For example, Heyer *et al.* (1975) found a predator-prey system in which tadpoles of *Leptodactylus pentadactylus* were carnivores on other tadpoles. They conducted several experiments, one of which was to study the relative

ability of predators to capture prey and their preference for certain prey species, where tadpoles of *Physalaemus pustulosus* and *Rhinella marina* were used as prey. In this experiment, both *R. marina* and *P. pustulosus* were offered individually and in combination with *L. pentadactylus* tadpoles. Their results indicated that significantly more *R. marina* tadpoles were eaten compared to *P. pustulosus* on both experimental designs. They concluded that it was possible that these statistical differences could be due to *R. marina* and *P. pustulosus*'s differential success at avoiding predation rather than predator choice, or it could have been a combination of both. Also, they hypothesized that *R. marina*'s vulnerability was due to the relatively slow swimming of these highly visible tadpoles that are normally in aggregations, making them easy prey for predators that are not deterred by their unpalatability.

Not only can some species consume larvae or eggs of *R. marina*, but *R. marina* tadpoles are active cannibals on eggs. Crossland *et al.* (2011) tested three hypotheses as to the benefits of conspecific egg consumption: (1) transfer of toxins from eggs to tadpoles, (2) nutritional input, and (3) reduction of future competition. Their results yielded no evidence for the first hypothesis. In contrast, they found that there was sufficient nutritional value on the eggs for tadpole development, and that this action provided the advantage of reduced competition. Also, they believed that cannibals are unlikely to consume relatives, so this behavior may be favored, rather than opposed, by kin selection.

Leptodactylus albilabris

There are few studies concerning *Leptodactylus albilabris*' larval phase, so this section will focus on what is known to date of this species larval biology. Stejneger (1902) was one of the first to describe its natural history and tadpole morphology. He measured the tadpoles' body

length, inter-orbital and inter-narinal distance, spiracle position, tail appearance, beak structure, and lateral line system, among other features. He also provided illustrations of the lateral, ventral and dorsal view of the tadpole, along with its mouthparts.

In 1956, Dent also described observations on the life history and development of *L. albilabris*. His observations revolved around the earlier development of the species (before larvae hatched from the eggs). Additionally, he provided descriptions of the earlier larval stages and mentioned that metamorphic stages follow the pattern observed for *Rana pipiens* by Taylor and Kollros (1946, in Dent, 1956).

Time to metamorphosis has also been recorded by several researchers. Dent (1956) mentioned tadpoles metamorphosed within 73 days with food deprivation, and estimated that metamorphosis was likely to happen within 35 days under natural conditions. Also, Joglar (2005) collected three wild clutches and raised them under captivity, observing that tadpoles completed metamorphosis in 21-29 days.

The diet of *L. albilabris* during its larval stage has not been recorded. Lebrón *et al.* (1995) reported observations on tadpoles seen feeding on a dead earthworm and an anole carcass. They suggested then that larvae of *L. albilabris* are facultative carnivores, a phenomenon that has been seen in other members of the genus *Leptodactylus*. Also, they suggested that this behavior highlights the dietary plasticity and opportunistic feeding that generalized tadpoles are capable of.

Studies of factors affecting larval development for *L. albilabris* are scarce. Candelas *et al.* (1961, in Joglar, 2005) studied aspects of larval metabolism, finding that tadpoles of *L. albilabris* are capable of reducing their metabolic respiration rate. This allowed them to survive, with

nutritional stress, up to forty days in damp cotton. Interestingly, tadpoles were able to restore their normal metabolic rates when returned to water.

In another study, Heatwole *et al.* (1968) studied heat tolerances on tadpoles of *L. albilabris* and *R. marina*. Although they found that *L. albilabris* larvae have less heat tolerance than those of *R. marina*, they found that *L. albilabris* tadpoles have other adaptations to survive in ephemeral ponds. These adaptations, as cited by Joglar (2005) are: (1) eggs are laid in foam nests where early development occurs using vitellum as energy source; (2) larvae are released to the water body in periods when there are high water levels rather than low, because when nests flood, the foam dissolves; (3) larval developmental time (in a water body) is reduced by development occurring in foam nests; and (4) tadpoles can survive under rocks or mud and reduce their metabolic rate if water levels lower.

More recently, Ríos-López (2008) studied the effects of salinity on tadpole growth and survival to metamorphosis as a means to provide insight into the potential impacts of sea-level rise on anuran assemblages in coastal wetlands. He conducted laboratory experiments with larvae of *L. albilabris* and *R. marina*, and found that survival to metamorphosis was reduced for both species in 22-25% seawater, which is within the salinity levels found in their natural distribution. In this salinity range, *L. albilabris* showed ~100% metamorphosis failure while *R. marina* showed ~60% metamorphosis failure. The author concluded that tadpoles may be living near their physiological limit for salinity.

MATERIALS AND METHODS

Study species

Rhinella marina (Fig. 1) was introduced to Puerto Rico from Jamaica and Barbados in 1920, 1924 and 1926. Females can lay up to 35,000 eggs in long “rosary” strings that can hatch after approximately 36 hours (Rivero, 1998). Larvae of this species are small (approximately 10-25 mm in total length), round-bodied, black or dark brown tadpoles (Zug and Zug, 1979) that typically occur in high densities relative to native species (Williamson, 1999). They can metamorphose from 16 days (Crossland *et al.*, 2009) to one or two months after hatching (Zug and Zug, 1979). It has been suggested that they are aggressive feeders, a trait that may provide a competitive advantage (Alford, 1999). *Leptodactylus albilabris* (Fig. 1) is native to Puerto Rico and the U.S. and British Virgin Islands (Henderson and Powell, 2009). Tadpoles of this species hatch in approximately four days from eggs laid in terrestrial foam nests, and they develop in temporary ponds or channels after being washed away by water runoff during heavy rains (Rivero, 1998). Metamorphosis can occur from 21 to 35 days (Dent, 1956; Joglar, 2005). There is scarce information concerning its larval phase. In Puerto Rico, both species breed throughout the year, with peaks during the wet season (Rivero, 1998).

Tadpole collection and aquarium design

I collected tadpoles of both species from wild populations using a dip net at early developmental stages (Gosner stage 25; Gosner, 1960). Developmental staging allows for recognition of morphological landmarks that are useful in comparing the sequence of events in a

developmental continuum (McDiarmid and Altig, 1999). Gosner stage 25 marks the disappearance of external gills and the transition to an active, feeding tadpole (Gosner, 1960).

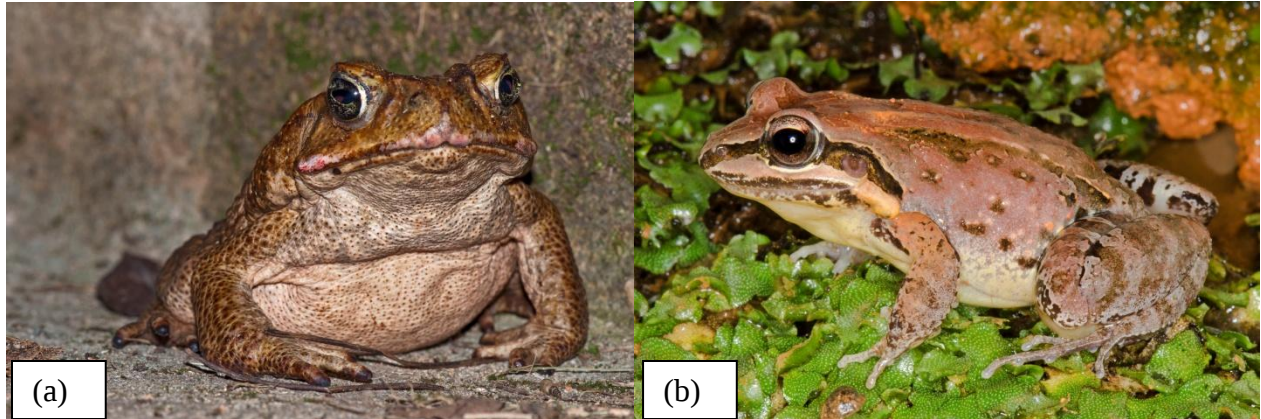


Figure 1. Adults of (a) *Rhinella marina* and (b) *Leptodactylus albilabris*. Photos were taken by José Almodóvar, University of Puerto Rico, Mayagüez Campus.

Multiple tadpoles from each species were collected in different areas to reduce genetic effects on competitive ability (Travis, 1980 in Smith, 2005). *R. marina* tadpoles were collected from an artificial pond located in the Botanical Garden of Caguas, Puerto Rico (N 18° 14' 36.6", W 66° 03' 49.0"); while *L. albilabris* tadpoles were collected from ephemeral ponds in Hormigueros, Mayagüez and Añasco, Puerto Rico (N 18° 08' 13.6", W 67° 07' 14.3"; N 18° 16' 08.8", W 67° 09' 33.2"; N 18° 16' 59.7", W 67° 09' 53.8", respectively).

Tadpoles were kept in aquaria with aged tap water prior to use. Aquaria used for the experiment consisted of Sterilite[®] plastic containers (13.5" x 8" x 5"; 5.7 L) filled with 2 L of aged tap water. Each aquarium was considered an experimental unit. Holes with a diameter of 5 mm were cut in the topmost part of the container to prevent water from overflowing during rainfall. The center of the lid was removed and covered with metallic screening. Each aquarium was cleaned every three days to prevent accumulation of metabolic waste and colonization of potential food resources (i.e. algae, etc.). Throughout the experiment, aquaria were maintained

with air pumps and randomly placed in a covered terrace, where they were exposed to ambient light cycles and temperature.

Experimental procedures and statistical analyses

I used two experimental procedures to assess the impact of *R. marina* larvae on the growth, development and survival of native *L. albilabris* under aquarium conditions: 1) Food calibration experiment (conducted from May-August, 2011) and 2) Competition experiment (September 2011-January 2012).

Food calibration experiment

I conducted this experiment to determine the relationship between food and tadpole development under the conditions to be used in the competition experiment. With the results from the food calibration experiment, a low food level and a high food level were determined to be used in the competition experiment. These levels allowed for comparisons in competitive interactions between both species when tadpoles were reared with scarce food resources and abundant food resources. The low and high food levels to be used in the competition experiment were mainly determined based on survival to metamorphosis.

The food calibration experiment was conducted separately for each of the two species. Four treatments consisting of different amounts of tadpole chow (3:1 ground rabbit pellets (Alimentos Agronutre, Mayagüez, Puerto Rico) and commercial fish flakes (TetraFin[®], Tetra Holding (US), Inc.) were used following Smith (2005). Treatments were as follows: 0.12 g, 0.23 g, 0.45 g, and 0.90 g per aquarium. Tadpole chow was applied every three days to all treatments.

Tadpoles were assigned to treatments at Gosner stage 26-28 (Gosner, 1960). Each aquarium (experimental unit) consisted of twelve tadpoles of *L. albilabris*, which were randomly assigned to one of the four experimental treatments. These treatments were repeated for *R. marina* tadpoles. Each treatment consisted of three replicates for a total of 24 aquaria (12 for *L. albilabris* and 12 for *R. marina*).

Gosner stage, mass (g) and body length (mm) were determined for each tadpole at the beginning of the experiment (Table 1). For body length measurements, tadpoles were placed in a Petri dish with water over a ruler and photographed. These photos were then observed to determine the following measurements for body length: total length (TL; from the tip of the snout to the tail tip) and body length (BL; from the tip of the snout to the junction of the posterior body wall). After the beginning of the experiment, Gosner stage, body length and mass were determined for each tadpole once a week. Tadpoles were monitored daily for survival and metamorphosis. Days to metamorphosis (larval period) and total matured were recorded. Metamorphosis was defined as the emergence of both forelimbs (Gosner stage 42). Metamorphosed tadpoles were removed from the experiment. Dead tadpoles were also removed from the experiment and their date of death, body length and developmental stage were recorded.

Table 1. Mean Gosner stage, mass and body length of tadpoles at beginning of food calibration experiment (± 1 S. D.)

Species	Gosner stage	Mass (g)	Total length (mm)	Body length (mm)
<i>Leptodactylus albilabris</i>	27.19 $\pm$ 0.68	0.052 $\pm$ 0.002	16.48 $\pm$ 0.17	6.22 $\pm$ 0.06
<i>Rhinella marina</i>	26.56 $\pm$ 0.66	0.019 $\pm$ 0.002	11.12 $\pm$ 0.09	4.75 $\pm$ 0.04

Linear regressions were conducted to determine the effects of food treatments on mass, total length, and survival to metamorphosis as well as larval period. Data at metamorphosis was

analyzed because timing and size at metamorphosis are the best indicators of performance during anuran larval phase (Williamson, 1999). The low food level to be used in the competition experiment was decided by a 45%-50% survival for the larvae. The high food level was decided by a > 75% survival. Univariate analysis of variance were conducted to determine if species responded differently to different food concentrations in mass at metamorphosis, total length at metamorphosis, survival to metamorphosis and larval period.

Competition experiment

Following the results from the food calibration experiment, a high food level and a low food level required for *R. marina* and *L. albilabris* development were used in the second experimental procedure to determine if the two species compete for food. Tadpoles were randomly assigned to one of eight experimental treatments following a 4 x 2 fully factorial design. The two factors were food and species composition. *Food* had two levels, low (0.23 g every three days) and high (0.68 g every three days). *Species composition* had four levels, 12 L: 0 R (12 *Leptodactylus* larvae and 0 *Rhinella* larvae), 0 L: 12 R (0 *Leptodactylus* larvae and 12 *Rhinella* larvae), 6 L: 6 R (6 *Leptodactylus* larvae with 6 *Rhinella* larvae) and 4 L: 8 R (4 *Leptodactylus* larvae with 8 *Rhinella* larvae). *Species composition* treatments were repeated with the low food level and the high food level. Each experimental treatment was applied to six replicates for a total of 48 aquaria (12 tadpoles per aquarium, 576 tadpoles in total). Data collection was the same as in the food calibration experiment (Table 2). Data from the first two weeks of development (“early growth”) was also analyzed due to high mortality of *R. marina* after these weeks.

Statistical analyses were run for each species independently. Larval period for *L. albilabris* was reciprocally transformed and early developmental rate (at two weeks) for *R. marina* was squared transformed to conform to the assumptions of normality. Other variables were sufficiently normal for parametric analysis. Pearson's correlation was applied on all

Table 2. Mean Gosner stage, mass and body length of tadpoles at beginning of competition experiment (± 1 S.D.)

Species	Gosner stage	Mass (g)	Total length (mm)	Body length (mm)
<i>Leptodactylus albilabris</i>	27.30 $\pm$ 0.47	0.061 $\pm$ 0.026	17.16 $\pm$ 1.87	6.61 $\pm$ 0.68
<i>Rhinella marina</i>	27.41 $\pm$ 0.36	0.029 $\pm$ 0.007	11.94 $\pm$ 0.88	5.48 $\pm$ 0.40

dependant variables for early development (at first two weeks) and at metamorphosis. Principal Components Analysis (PCA) was used to study the structure of the response variables and to reduce their number. Response variables used were: early mass, early BL, early TL, early developmental rate, early survival, larval period, mass at metamorphosis, BL at metamorphosis, TL at metamorphosis and survival to metamorphosis. Varimax Rotation was used to maximize the sum of the variances and associate each variable to at most one factor. Principal components scores were used as dependant variables in 2 x 3 fully factorial General Linear Models (GLMs). Univariate analyses of variance were used to determine the effects of food availability, species ratio and the interaction between the two for early survival and survival at metamorphosis. These univariate analyses were conducted to understand how survival was affected in the main results from the PCA. Larval period was also analyzed separately to compare time needed to reach metamorphosis with late development and survival. All statistical analyses were conducted in SPSS (ver. 17.0).

RESULTS

Food calibration experiment

Food treatment had a significant effect for *L. albilabris* on mass at metamorphosis, survival to metamorphosis and larval period (linear regression, Table 3). There was a positive association between food levels and mass at metamorphosis (Fig. 2) and between food levels and survival to metamorphosis (Fig. 3). Higher food levels resulted in a reduction of larval period (Fig. 4). There was no significant effect of food on total length (TL) at metamorphosis. Tadpoles reached metamorphosis at similar lengths regardless of food availability (Fig. 5).

Mass at metamorphosis and survival to metamorphosis were also positively affected by food levels in *R. marina* (Figs. 2 and 3). Similar to *L. albilabris*, there was no significant effect of food level on total length (TL) at metamorphosis for *R. marina* (Fig. 5). Larval period, however, was not significantly affected by food levels, but there was a trend to reduced larval period with higher food treatments (Fig. 4; Table 3).

Table 3. Linear regression results for the four variables measured in the food calibration experiment for *Leptodactylus albilabris* and *Rhinella marina*

Species	Survival to metamorphosis	Larval period	Mass at metamorphosis	Total length at metamorphosis
	$r^2 = 0.49$	$r^2 = 0.66$	$r^2 = 0.64$	$r^2 = 0.01$
<i>Leptodactylus albilabris</i>	$F_{1,10} = 9.52$	$F_{1,10} = 19.54$	$F_{1,10} = 18.09$	$F_{1,10} = 0.06$
	$p = 0.012$	$p = 0.001$	$p = 0.002$	$p = 0.804$
	$r^2 = 0.43$	$r^2 = 0.29$	$r^2 = 0.40$	$r^2 = 0.13$
<i>Rhinella marina</i>	$F_{1,10} = 7.61$	$F_{1,10} = 4.10$	$F_{1,10} = 6.66$	$F_{1,10} = 1.44$
	$p = 0.020$	$p = 0.070$	$p = 0.027$	$p = 0.257$

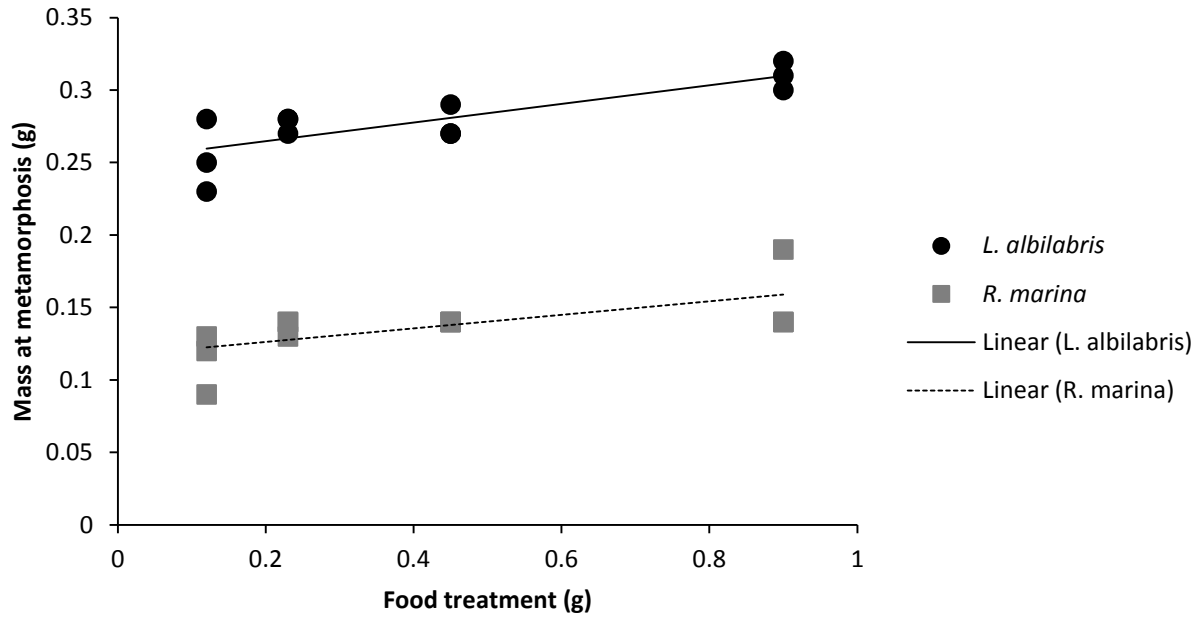


Figure 2. Food treatment effects on mass at metamorphosis for *Leptodactylus albilabris* and *Rhinella marina* in the food calibration experiment. Figure shows linear regression for mass at metamorphosis. Food treatments: 0.12 g, 0.23 g, 0.45 g and 0.90 g.

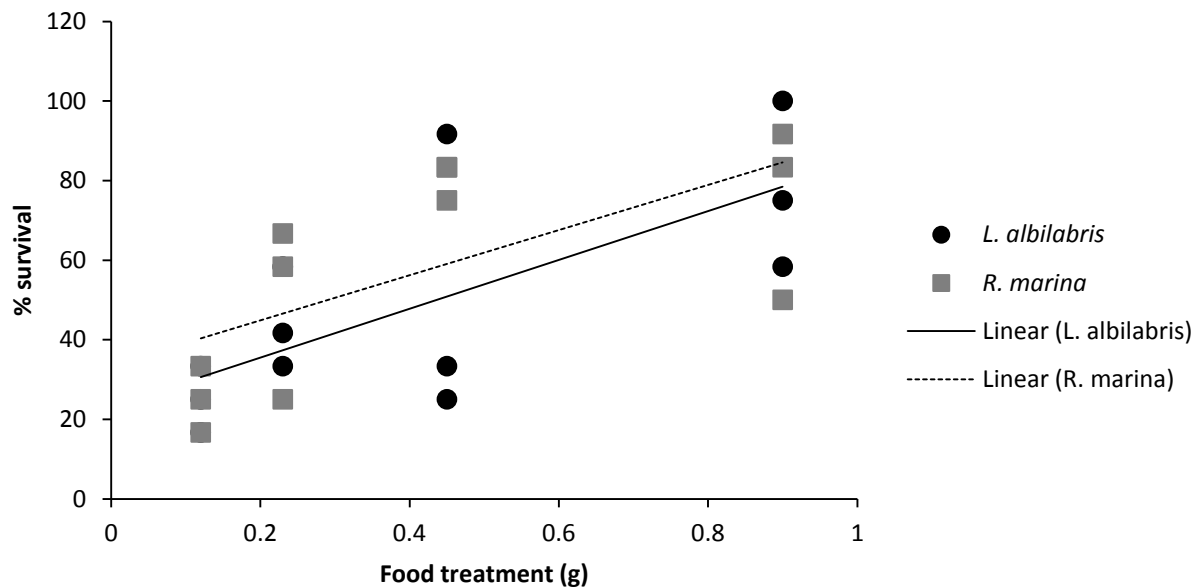


Figure 3. Food treatment effects on survival to metamorphosis for *Leptodactylus albilabris* and *Rhinella marina* in the food calibration experiment. Figure shows linear regression for survival to metamorphosis. Food treatments: 0.12 g, 0.23 g, 0.45 g and 0.90 g.

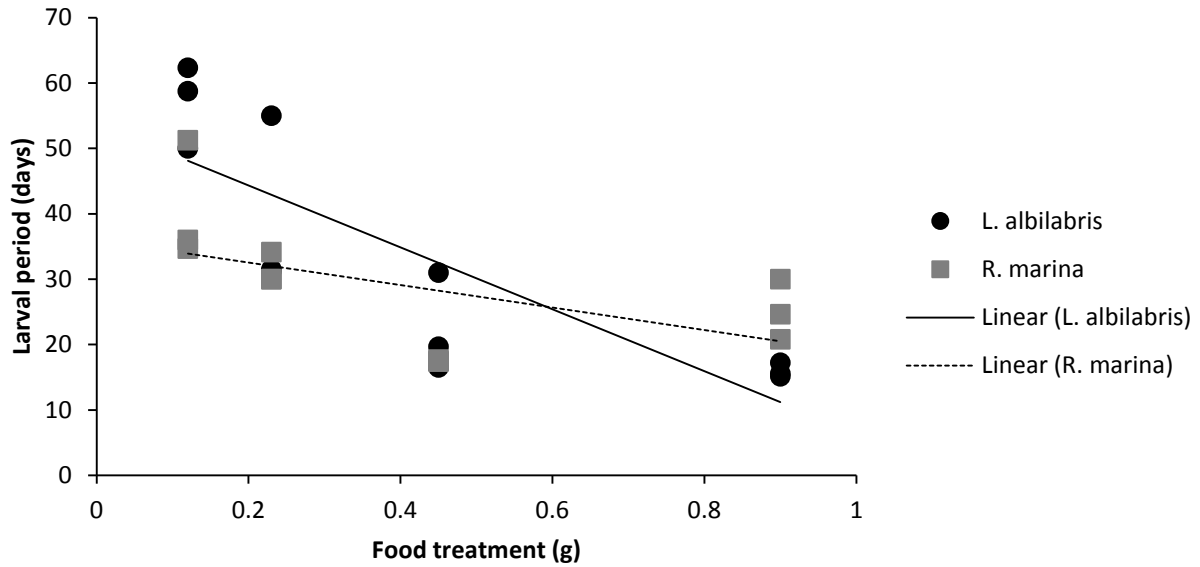


Figure 4. Food treatment effects on larval period for *Leptodactylus albilabris* and *Rhinella marina* in the food calibration experiment. Figure shows linear regression for larval period. Food treatments: 0.12 g, 0.23 g, 0.45 g and 0.90 g.

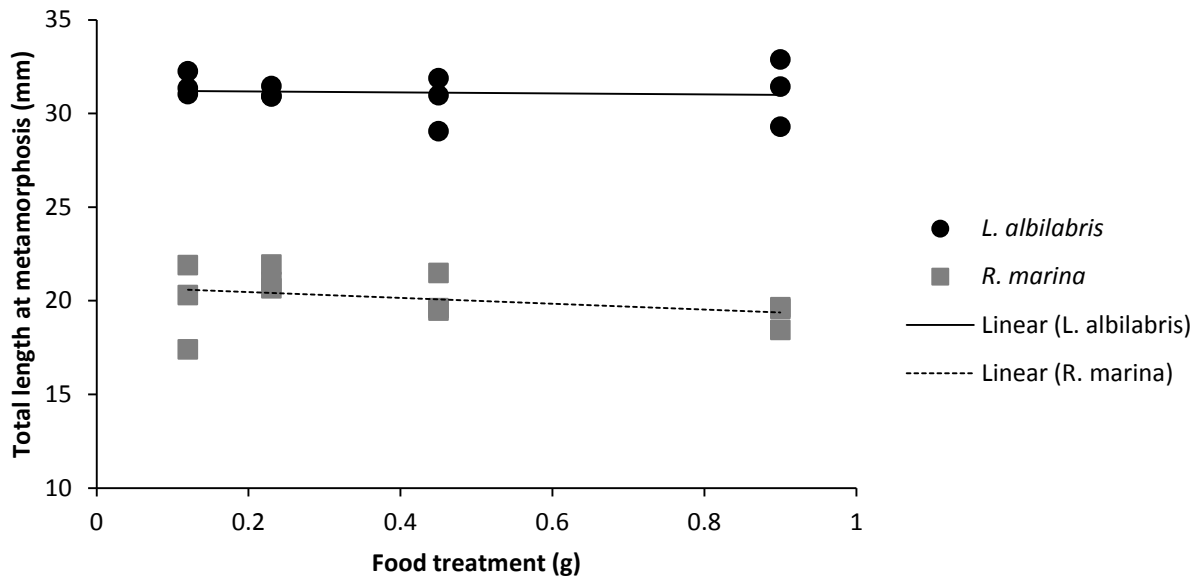


Figure 5. Food treatment effects on total length at metamorphosis for *Leptodactylus albilabris* and *Rhinella marina* in the food calibration experiment. Figure shows linear regression for total length at metamorphosis. Food treatments: 0.12 g, 0.23 g, 0.45 g and 0.90 g.

To determine the low food level for the competition experiment, a 45% to 50% survival to metamorphosis was established. In the 0.12 g treatment, survival for *L. albilabris* and *R. marina* was 25%, which was lower than the survival rate established for the food calibration experiment. In treatment 0.23 g, however, *L. albilabris* larvae experienced a 44% survival and *R. marina* a 50% survival. These survival percentages were in accordance to the values established, so the 0.23 g treatment was selected as the low food level to be used in the competition experiment. A percentage of survival to metamorphosis > 75% was established to determine the high food treatment to be used in the competition experiment. Treatment 0.45 g allowed for a 50% survival to metamorphosis for *L. albilabris* and an 80% in *R. marina*. Treatment, 0.90 g allowed for a 77% survival for *L. albilabris* and a 75% for *R. marina*. Because of differences in survival to metamorphosis between treatments 0.45 g and 0.90 g, I believed that tadpoles of *R. marina* could suffer disproportionately from water fouling in the 0.90 g treatment. For this reason, I decided to use an intermediate quantity (0.68 g) as the high food level in the competition experiment.

L. albilabris tadpoles were, on average, 0.033 g heavier and 5.36 mm longer than *R. marina* tadpoles (Table 1). There was a significant difference in mass at metamorphosis ($F_{1,20} = 131.77$, $p < 0.001$) and total length at metamorphosis ($F_{1,20} = 139.55$, $p < 0.001$) between species, but these differences were not attributable to different food concentrations (mass at metamorphosis $F_{1,20} = 0.32$, $p = 0.574$; TL at metamorphosis $F_{1,20} = 0.57$, $p = 0.459$). Differences in size between the two species likely contributed to differences in larval period and size needed to reach metamorphosis. There was a significant difference in larval period between species ($F_{1,20} = 6.29$, $p = 0.021$), and species responded differently in larval period depending on the food concentrations available ($F_{1,20} = 4.87$, $p = 0.039$). The shortest larval period in the 0.12 g

treatment was 44 days for *L. albilabris* and 24 days for *R. marina*. *L. albilabris* tadpoles needed more time during the larval period to reach metamorphosis with scarce resources than those of *R. marina* (Table 4). Even with these differences between species, there was a trend for both species regarding larval period and mass at metamorphosis. The first tadpoles to reach metamorphosis in each treatment did so at a lower mass than those that stayed longer (Table 4). Survival to metamorphosis did not differ significantly between species ($F_{1,20} = 0.48$, $p = 0.497$), and species did not respond differently to different food concentrations ($F_{1,20} = 0.03$, $p = 0.874$) in survival to metamorphosis.

Table 4. Variations in larval period and mass at metamorphosis due to food treatment effects in *Leptodactylus albilabris* and *Rhinella marina*

Species	Food Treatments (g)	Larval period (days)		Mass at metamorphosis (g)	
		Minimum	Maximum	Minimum larval period	Maximum larval period
<i>Leptodactylus albilabris</i>	0.12	44	80	0.223	0.273
	0.23	18	85	0.204	0.337
	0.45	12	60	0.254	0.626
	0.90	12	25	0.303	0.306
<i>Rhinella marina</i>	0.12	24	63	0.071	0.198
	0.23	18	55	0.158	0.152
	0.45	15	34	0.100	0.209
	0.90	18	51	0.105	0.229

Competition experiment

Pearson's correlation showed that the measured variables were highly correlated for *L. albilabris* (Table 5) and *R. marina* (Table 6), validating the use of Principal Components Analysis (PCA) to reduce the number of variables.

Due to high mortality of *R. marina* larvae in the 6 L: 6 R treatment, data for *R. marina* in this treatment were excluded from the Principal Components Analysis (PCA). PCA was

conducted for the two species separately. Response variables used for the PCA's were: early mass, early BL, early TL, early developmental rate, early survival (at first two weeks), larval period, mass at metamorphosis, BL at metamorphosis, TL at metamorphosis and survival to metamorphosis. Larval period was reciprocally transformed for *L. albilabris* and early developmental rate was squared transformed for *R. marina*. Three principal components (PC) with Eigenvalues > 1 were extracted after each PCA, explaining 86% of the variation in the data for *L. albilabris* and 88% for *R. marina*. Extracted PC's were named based on variables with high weightings. Variables related to the first two weeks of development (early mass, early total length, early body length and early developmental rate) weighted strongly for PC1 "Early

Table 5. Pearson's correlation results for variables of *Leptodactylus albilabris* measured in the competition experiment*

Variables	Early mass	Mass at metamorphosis	TL at metamorphosis	BL at metamorphosis	Early TL	Early BL	Early developmental rate	Reciprocal larval period	Early survival	Survival at metamorphosis
Early mass	1	0.284	0.182	0.377	0.955	0.912	0.865	0.585	-0.259	0.177
Mass meta	0.049	1	0.837	0.763	0.166	0.309	0.041	-0.091	-0.335	-0.225
TL meta	0.147	<0.001	1	0.632	0.135	0.156	-0.110	0.017	-0.129	-0.043
BL meta	0.013	<0.001	<0.001	1	0.217	0.373	0.091	-0.068	-0.103	-0.119
Early TL	<0.001	0.171	0.220	0.105	1	0.905	0.874	0.619	-0.218	0.270
Early BL	<0.001	0.036	0.185	0.014	<0.001	1	0.796	0.431	-0.391	0.025
Early developmental rate	<0.001	0.407	0.265	0.302	<0.001	<0.001	1	0.754	-0.262	0.283
Reciprocal larval period	<0.001	0.302	0.462	0.348	<0.001	0.005	<0.001	1	-0.132	0.348
Early survival	0.066	0.025	0.230	0.278	0.105	0.010	0.064	0.225	1	0.646
Survival meta	0.154	0.097	0.402	0.248	0.058	0.443	0.050	0.020	<0.001	1

*r values are shown above the main diagonal. P values are shown below the main diagonal. Meta (metamorphosis), TL (total length), BL (body length).

development”. Variables related to later larval growth (mass at metamorphosis, total length at metamorphosis and body length at metamorphosis) weighted strongly for PC2 “Late development”. Lastly, early survival and survival at metamorphosis weighted strongly for PC3 “Survival” (Tables 7 and 8). Variables in PC3 (early survival and survival to metamorphosis) were analyzed separately for each species with an univariate analysis of variance to understand how early survival was affected with PC1 “Early development” and how survival to metamorphosis was affected with PC2 “Late development”. An univariate analysis was also conducted for larval period to evaluate it with PC2.

Table 6. Pearson’s correlation results for variables of *Rhinella marina* measured in the competition experiment*

Variables	Early mass	Mass at metamorphosis	TL at metamorphosis	BL at metamorphosis	Early TL	Early BL	Squared early developmental rate	Larval period	Early survival	Survival at metamorphosis
Early mass	1	0.126	0.235	0.084	0.876	0.901	0.844	-0.467	0.247	0.571
Mass meta	0.617	1	0.949	0.917	0.196	0.226	-0.083	0.334	0.055	0.438
TL meta	0.349	<0.001	1	0.871	0.260	0.264	0.023	0.198	0.095	0.448
BL meta	0.741	<0.001	<0.001	1	0.197	0.214	-0.061	0.443	0.076	0.443
Early TL	<0.001	0.435	0.297	0.433	1	0.969	0.888	-0.482	0.333	0.605
Early BL	<0.001	0.368	0.289	0.395	<0.001	1	0.888	-0.431	0.295	0.656
Squared early developmental rate	<0.001	0.744	0.928	0.811	<0.001	<0.001	1	-0.681	0.221	0.514
Larval period	0.051	0.176	0.432	0.066	0.043	0.074	0.002	1	-0.067	0.102
Early survival	0.244	0.828	0.708	0.764	0.112	0.162	0.300	0.790	1	0.659
Survival meta	0.004	0.069	0.062	0.066	0.002	<0.001	0.010	0.686	<0.001	1

*r values are shown above the main diagonal. P values are shown below the main diagonal. Meta (metamorphosis), TL (total length), BL (body length).

Table 7. Principal components extracted and loadings of variables measured for *Leptodactylus albilabris* in the Principal Components Analysis for the competition experiment

Variables	PC1 Early development	PC2 Late development	PC3 Survival
	Eigenvalue = 4.45	Eigenvalue = 2.62	Eigenvalue = 1.53
Early body length	0.875	0.249	-0.214
Early total length	0.956	0.138	0.028
Early mass	0.936	0.251	-0.036
Early developmental	0.965	-0.076	-0.003
Reciprocal larval period	0.759	-0.144	0.162
Body length at metamorphosis	0.140	0.872	-0.046
Total length at metamorphosis	-0.005	0.903	0.025
Mass at metamorphosis	0.071	0.928	-0.223
Early survival	-0.269	-0.093	0.893
Survival to metamorphosis	0.279	-0.085	0.903

*High loadings for each PC are shown in bold.

Table 8. Principal components extracted and loadings of variables measured for *Rhinella marina* in the Principal Components Analysis for the competition experiment

Variables	PC1 Early development	PC2 Late development	PC3 Survival
	Eigenvalue = 4.22	Eigenvalue = 3.20	Eigenvalue = 1.47
Early body length	0.926	0.206	0.157
Early total length	0.933	0.169	0.207
Early mass	0.898	0.127	-0.008
Early developmental	0.944	-0.098	-0.068
Reciprocal larval period	-0.674	0.463	-0.001
Body length at metamorphosis	0.002	0.959	0.104
Total length at metamorphosis	0.132	0.930	0.105
Mass at metamorphosis	0.032	0.970	0.084
Early survival	-0.027	-0.043	0.968
Survival to metamorphosis	0.216	0.393	0.844

*High loadings for each PC are shown in bold.

Early development and survival

Presence of larvae of the introduced *R. marina* seemed to have no significant effect on early development (PC1) and early survival for larvae of *L. albilabris*. PC1 for *L. albilabris* was significantly affected by species composition ($F_{2,29} = 35.53$, $p < 0.001$) and amount of food ($F_{1,29} = 24.13$, $p < 0.001$). The high food level allowed for greater growth, as did a reduced number of conspecifics (Fig. 6a). The interaction between these two factors (species composition and food) was not significant ($F_{2,29} = 0.70$, $p = 0.504$).

There was an interaction effect of species composition and food on early survival of *L. albilabris* larvae ($F_{2,30} = 11.17$, $p < 0.001$). Specifically, high food combined with only 6 larvae of the same species present (6 L: 6 R treatment) allowed for a greater survival when compared to other treatments (Fig. 6b). Similar to PC1, early survival of *L. albilabris* was also mostly affected by species composition ($F_{2,30} = 12.79$, $p < 0.001$) and food ($F_{1,30} = 7.23$, $p = 0.012$).

There was a significant interaction effect of species composition and food ($F_{1,14} = 6.32$, $p = 0.025$) in the early development (PC1) of *R. marina*. Presence of *L. albilabris*, combined with a high food level, had a detrimental effect for early development of *R. marina*. Tadpoles of *R. marina* on these treatments suffered from lower growth than those reared in monospecific aquaria. The effect was particularly pronounced in the 6 L: 6 R treatment (Fig. 7a). There were no significant main effects on early development of *R. marina* by species composition ($F_{1,14} = 4.27$, $p = 0.058$) or food level ($F_{1,14} = 1.07$, $p = 0.318$).

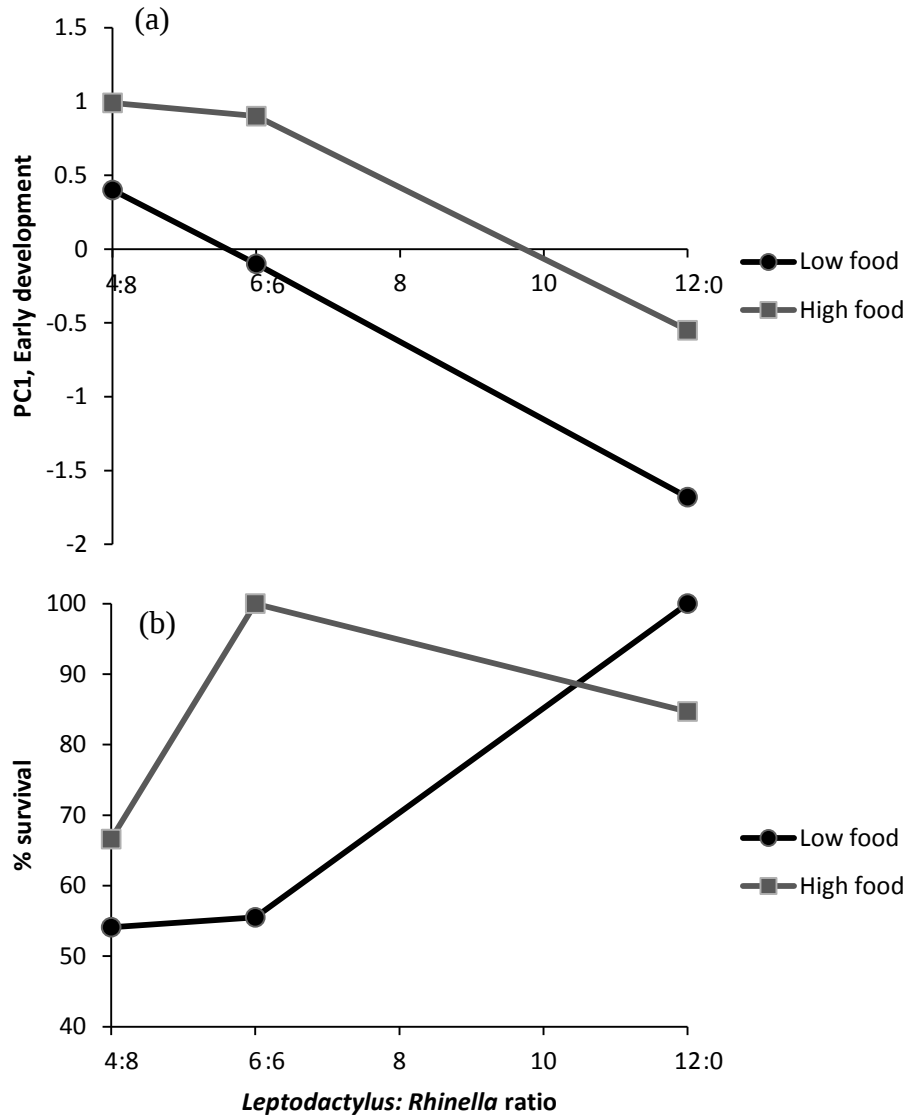


Figure 6. Relationship between food availability and species composition in early development of *Leptodactylus albilabris*. Figure shows (a) estimated marginal means for principal component 1 “Early development” (early mass, early total length, early body length, early developmental rate; 2 x 3 fully factorial GLM) and (b) survival (univariate analysis of variance) during the first two weeks of development. Food treatments: low food (0.23 g), high food (0.68 g). Species composition treatments: 12 L: 0 R (12 *L. albilabris* and 0 *R. marina*), 6 L: 6 R (6 *L. albilabris* and 6 *R. marina*) and 4 L: 8 R (4 *L. albilabris* and 8 *R. marina*).

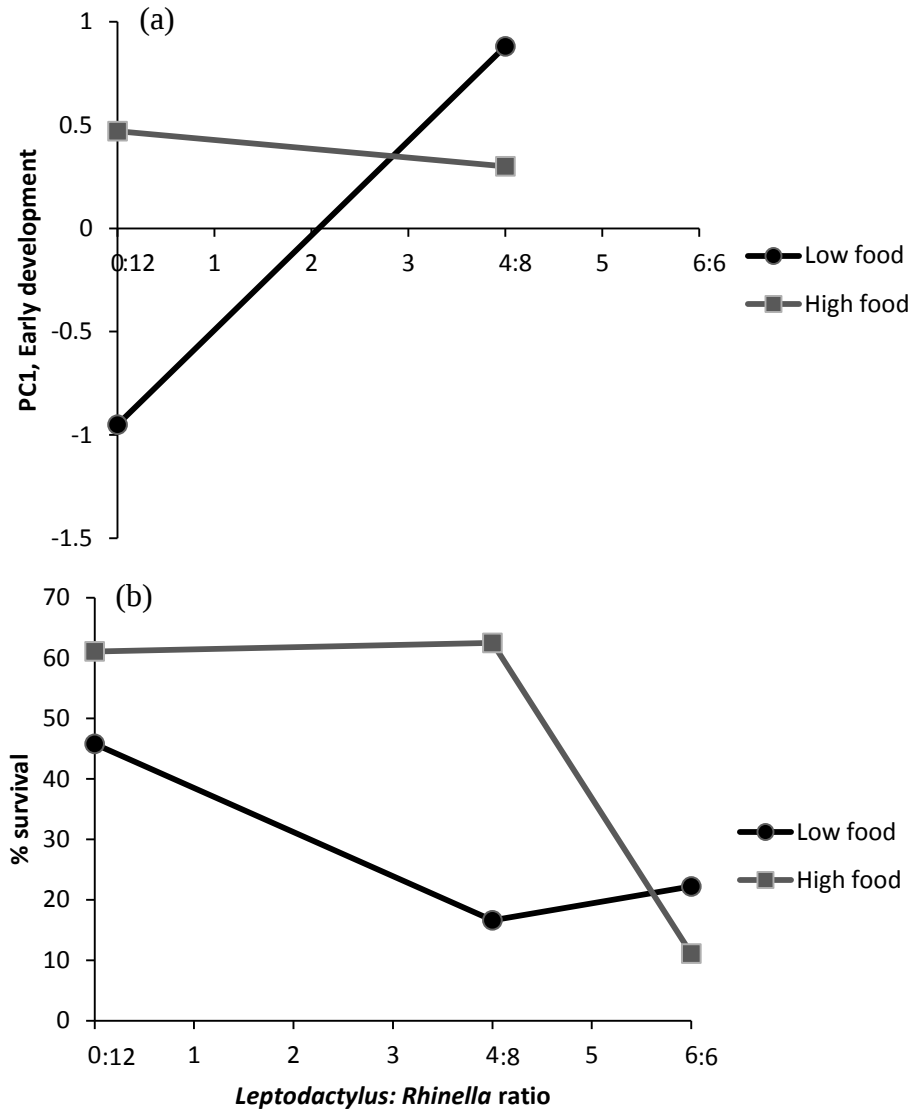


Figure 7. Relationship between food availability and species composition in early development of *Rhinella marina*. Figure shows (a) estimated marginal means for principal component 1 “Early development” (early mass, early total length, early body length, early developmental rate; 2 x 3 fully factorial GLM) and (b) survival (univariate analysis of variance) during the first two weeks of development. Food treatments: low food (0.23 g), high food (0.68 g). Species composition treatments: 0 L : 12 R (0 *L. albilabris* and 12 *R. marina*), 6 L : 6 R (6 *L. albilabris* and 6 *R. marina*; excluded from (a) due to high mortality of *R. marina*) and 4 L : 8 R (4 *L. albilabris* and 8 *R. marina*).

Early survival for *R. marina* was greatly affected by the interaction between species composition and food ($F_{2,30} = 9.88$, $p = 0.001$). A higher food level allowed for higher early survival, except on the 6 L: 6 R treatment; whereas in the presence of *L. albilabris*, *R. marina*

had a higher early survival when there was a lower food level. Even so, by the first two weeks of the experiment, survival for *R. marina* on this treatment was 22% (low food) and 11% (high food; Fig. 7b). The main effect of food was significant for early survival of *R. marina* ($F_{1,30} = 10.14$, $p = 0.003$). The high food level allowed for higher *R. marina* early survival (Fig. 7b). Species composition also had a significant primary effect on early survival of *R. marina* ($F_{2,30} = 16.82$, $p < 0.001$). *R. marina* experienced higher early survival in the absence of *L. albilabris* larvae (Fig. 7b).

Late development and survival

Late development (PC2) and survival to metamorphosis in *L. albilabris* were not affected by species composition. There were no interaction effects of food and species composition in PC2 for *L. albilabris* ($F_{2,29} = 0.54$, $p = 0.589$). Food levels ($F_{1,29} = 0.52$, $p = 0.478$) and species composition ($F_{2,29} = 0.56$, $p = 0.580$) had no significant primary effects on late development (Fig. 8a). The traits affected during the late development stage were survival to metamorphosis and larval period. Survival to metamorphosis was affected by the interaction between food and species composition ($F_{2,29} = 4.74$, $p = 0.017$), and there was also a primary effect of food availability ($F_{1,29} = 35.45$, $p < 0.001$) on survival to metamorphosis. Low food reduces survival to metamorphosis in *L. albilabris*, whereas high food in the 6 L: 6 R treatment allowed for the highest survival to metamorphosis (Fig. 8b). Larval period for *L. albilabris* was affected by the interaction of both factors studied ($F_{2,29} = 6.92$, $p = 0.003$). There was also a primary effect of food ($F_{1,29} = 45.54$, $p < 0.001$) and species composition ($F_{2,29} = 12.07$, $p < 0.001$) on larval

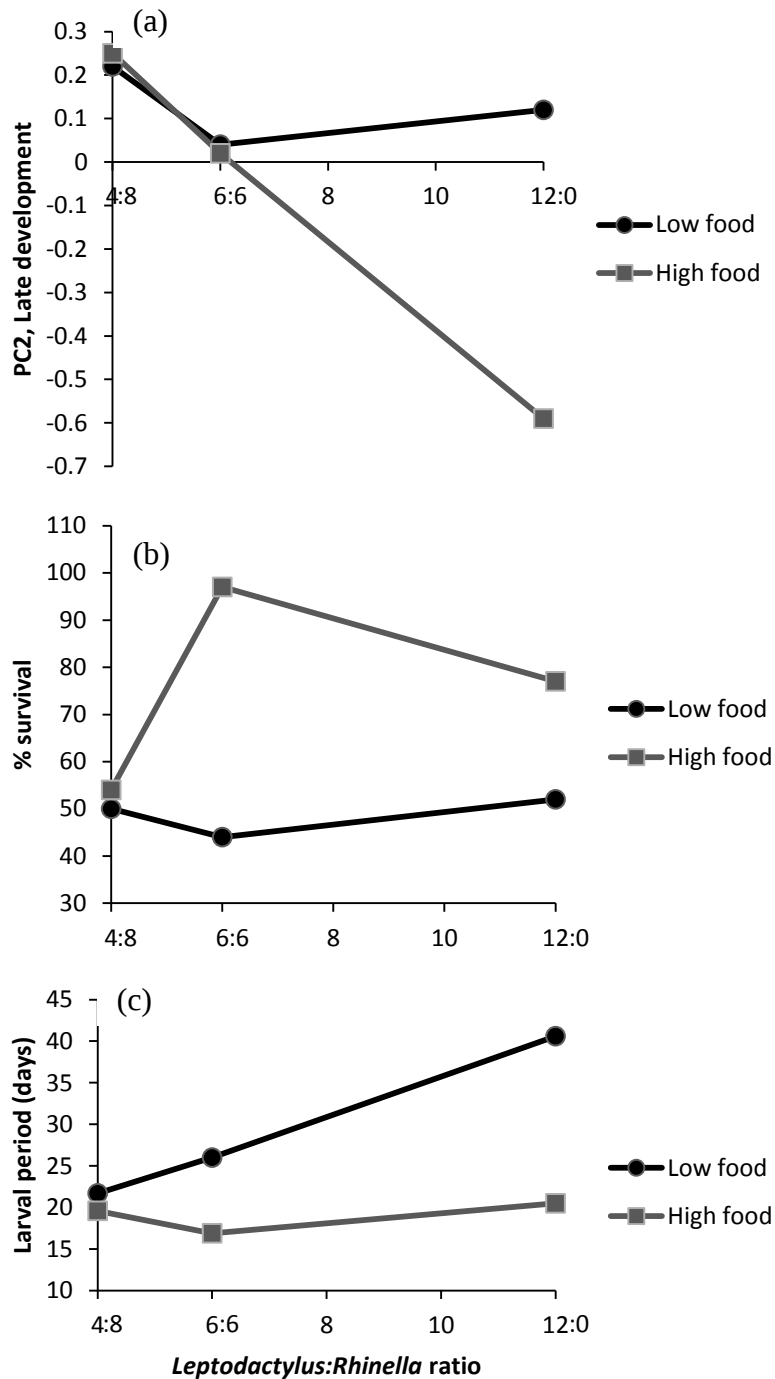


Figure 8. Relationship between food availability and species ratio in late development of *Leptodactylus albilabris*. Figure shows (a) estimated marginal means for principal component 2 “Late development” (mass, total length, body length at metamorphosis; 2 x 3 fully factorial GLM), (b) survival to metamorphosis and (c) larval period (univariate analysis of variance). Food treatments: low food (0.23 g), high food (0.68 g). Species composition treatments: 12 L: 0 R (12 *L. albilabris* and 0 *R. marina*), 6 L: 6 R (6 *L. albilabris* and 6 *R. marina*) and 4 L: 8 R (4 *L. albilabris* and 8 *R. marina*).

period. With a high food level, tadpoles reached metamorphosis faster, with the 6 L: 6 R treatment being the one to produce the shortest larval period in the experiment (Fig. 8c).

Similar to *L. albilabris*, food availability had no significant effect on late development (PC2) of *R. marina* ($F_{1,14} = 0.36$, $p = 0.558$). Tadpoles of *R. marina*, however, reached metamorphosis with decreased late development when *L. albilabris* were present ($F_{1,14} = 17.03$, $p = 0.001$). The interaction between food availability and species composition did not have a significant effect on late development for *R. marina* ($F_{1,14} = 0.94$, $p = 0.348$; Fig. 9a). Survival to metamorphosis for *R. marina*, however, was greatly affected by the interaction between food and species composition ($F = 4.11$, $p = 0.026$). There were also primary effects of food ($F = 4.39$, $p = 0.045$) and species composition ($F = 26.79$, $p < 0.001$) on survival of *R. marina*. When tadpoles of *R. marina* were reared with high food, they experienced relatively high survival to metamorphosis. Survival to metamorphosis was lower, however, in the presence of *L. albilabris* (Fig. 9b). Larval period was only significantly affected by species composition ($F_{2,15} = 5.72$, $p = 0.014$). When *L. albilabris* was present, *R. marina* tadpoles accelerated their metamorphosis when compared to control groups, reaching metamorphosis faster and with reduced growth (Fig. 9c). There were no significant effects of food ($F_{1,15} = 1.20$, $p = 0.290$) or the interaction between both factors ($F_{1,15} = 1.07$, $p = 0.831$) on *R. marina* larval period (Fig. 9c). Change in mass through larval period for both species is shown in Appendix section (Appendix A-D). Growth rates for both species are shown in Appendix section (Appendix E).

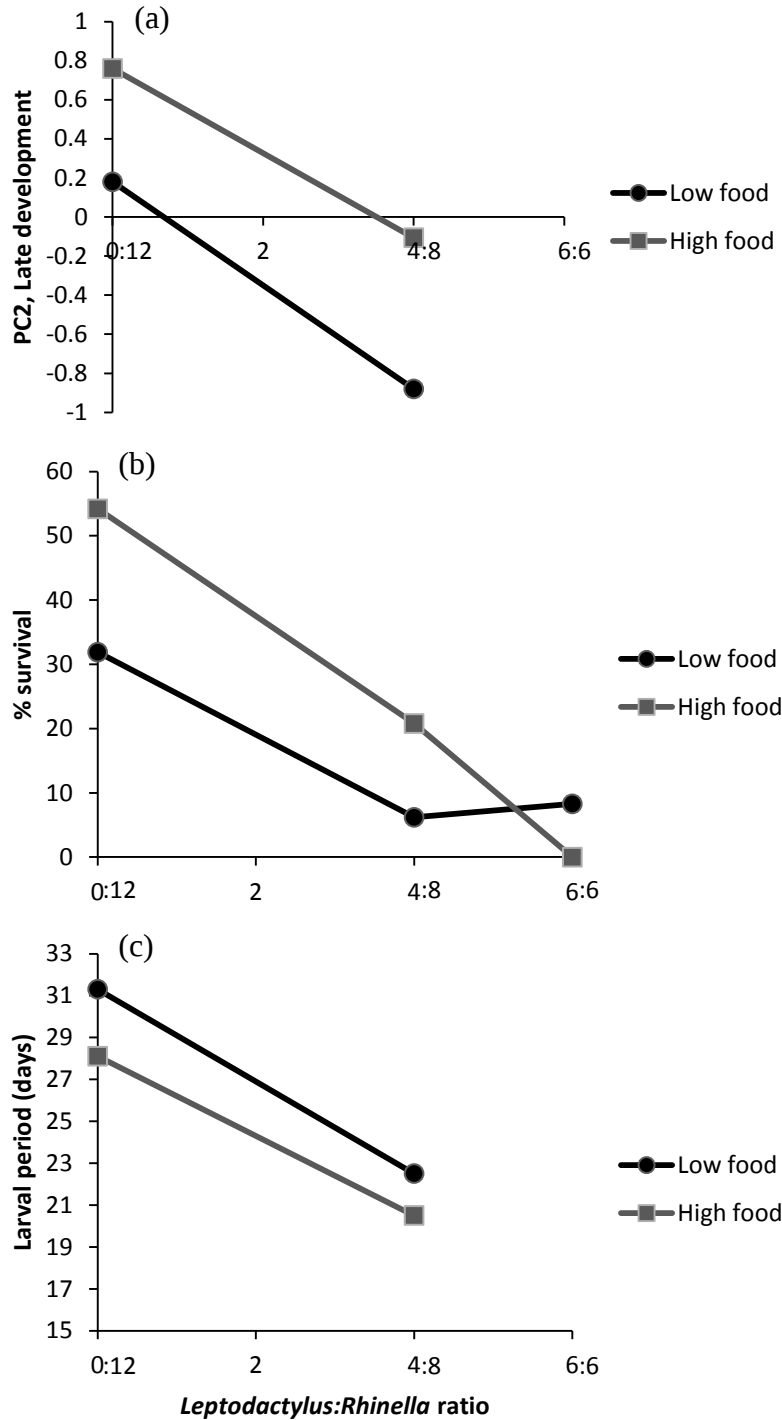


Figure 9. Relationship between food availability and species ratio in late development of *Rhinella marina*. Figure shows (a) estimated marginal means for principal component 2 “Late development” (mass, total length, body length at metamorphosis; 2 x 3 fully factorial GLM), (b) survival to metamorphosis and (c) larval period (univariate analysis of variance). Food treatments: low food (0.23 g), high food (0.68 g). Species composition treatments: 0 L: 12 R (0 *L. albilabris* and 12 *R. marina*), 6 L: 6 R (6 *L. albilabris* and 6 *R. marina*; excluded from (a) and (c) due to high mortality of *R. marina*) and 4 L: 8 R (4 *L. albilabris* and 8 *R. marina*).

DISCUSSION

Food calibration experiment

Food treatments had a significant effect on mass at metamorphosis, survival to metamorphosis and larval period for *L. albilabris*. For *R. marina*, there were significant effects of food treatments on mass at metamorphosis and survival to metamorphosis. Larval period was not significantly affected by food in *R. marina*, but there was a trend to reduced larval period with higher food levels. There were no significant effects of food on total length at metamorphosis for both species. Timing and size at metamorphosis are the best indicators of performance during the anuran larval phase (Williamson, 1999). The larval period and size at metamorphosis can be determined by food availability (Kupferberg, 1997a). Food availability does not work independently in anuran larval performance, but food deprivation has previously been shown to increase larval period (Dent, 1956; Kupferberg, 1997a; Browne *et al.*, 2003). This effect was demonstrated in this experiment, where there was a positive effect of high nutrient concentration on growth, survival, and larval period of tadpoles (Figs. 2-4). Tadpole size, however, was not significantly affected by food availability. Tadpoles of both species reared with lower food levels reached metamorphosis at similar size to those in higher food levels, but they took longer to do so. This result was similar to other studies where tadpoles exposed to food deprivation or competition metamorphosed at later times but at similar sizes as those with shorter larval periods (Kupferberg, 1997a; Bardsley and Beebe, 1998). Reduced size at metamorphosis may negatively impact fitness by reducing juvenile survivorship and reproductive benefits (Semlitsch *et al.*, 1988; Denver, 1997; Crossland *et al.*, 2009). Size does not seem to be greatly affected by low resource availability for many species, but low resources will force tadpoles to

increase their larval period in order to gain the necessary size needed to initiate metamorphosis (Wilbur and Collins, 1973). A longer larval period may decrease survival by exposing larvae to prolonged habitat deterioration (Crump, 1989; Loman, 1999), predation (Heyer, 1976; Sih *et al.*, 1995; Van Buskirk and Yurewicz, 1998) and intra- and interspecific competition (Alford and Crump, 1982). Thus, tadpoles that reach the largest size in the shortest period will increase their probability of completing metamorphosis and reaching the reproductive stage (Hoff *et al.*, 1999). Resource availability, then, is an important factor during larval development.

Under the conditions studied in this experiment, the lowest food level (0.12 g) severely reduced survival to metamorphosis and increased larval periods for both species (Figs. 3 and 4). Also, the minimal amount of food needed for tadpole development for both species was 0.23 g under the experimental conditions (treatment that allowed a 45% to 50% survival to metamorphosis). Under more natural conditions, however, survival to metamorphosis and larval period could vary depending on species and other biotic and abiotic factors present. Amphibian larval growth is variable and depends on several factors such as temperature, pH, hydroperiod (period of time during which a wetland is covered by water), predation, competition and food quantity and quality (Katzmann *et al.*, 2003). Food quality in particular can play an important role in larvae development. Higher protein levels promote better growth and survival and shorten the larval period (Kupferberg, 1997a; Sretarugsa *et al.*, 1997; Browne *et al.*, 2003). The food mixture used for this experiment (3:1 ratio of rabbit pellets and fish flakes) contained approximately 17% protein in rabbit pellets and approximately 42% protein in fish flakes. According to Schiesari *et al.* (2009), rabbit food contains approximately 16% protein and when mixed with fish flakes at a 3:1 ratio (as used in this experiment) it is approximately 23%, which is considered low when compared to food ingested in nature. In their study with tadpoles of

Lithobates sylvaticus, *L. pipiens*, *L. clamitans* and *L. catesbeianus*, they found that food ingested in nature by these species contained protein percents that ranged from 31% to 44%. The diversity of food found in natural systems may be different to that used in experiments. Because of this, it would be necessary to study in more detail the effects of resource availability on larval development of *L. albilabris*, such as the protein concentrations that larvae consume in nature, and how adding animal matter to their diet can affect growth. These studies could help to better understand life history traits for this native species.

Although this experiment was conducted mainly as a way to determine adequate food levels for the competition experiment between *L. albilabris* and *R. marina*, it allowed me to make several observations that are worthy of inclusion in this manuscript. First, since tadpoles of *L. albilabris* are larger than those of *R. marina*, I expected their larval period to be longer because they would need to grow more than those of *R. marina* to reach metamorphosis. This effect was observed only in the lower food treatment (0.12 g; Table 4). Thus, I conclude that *L. albilabris* can accelerate its growth rate to reach metamorphic size at the same time as a smaller species when resources are available. This trait could give larvae of *L. albilabris* a competitive advantage over other species present at the same breeding site.

Secondly, the Wilbur-Collins' model (1973) on influences on development, growth and survival of tadpoles can be studied for *L. albilabris*. In this model, Wilbur and Collins (1973, as reviewed by Alford, 1999) proposed that tadpoles would slow their developmental rate and metamorphose at a larger body size when there were favorable conditions. Under unfavorable conditions, however, tadpoles would accelerate their developmental rate and metamorphose at a species-specific minimum body size and continue growing in the terrestrial stage. Although the Wilbur-Collins' model (1973) was not studied in this experiment, a trend to follow the pattern

proposed in the model was seen for *L. albilabris* and *R. marina*. In the present experiment, unfavorable conditions were resource limitation and the presence of conspecifics. Resource limitation and density do not act independently on growth rate (Wilbur, 1977b in Alford and Crump, 1982). Although we did not change density levels, we observed that when larvae were in the presence of conspecifics they tended to accelerate development and metamorphose at smaller sizes. In contrast, when tadpoles were released from density stress (all other conspecifics had been removed from the experiment by either mortality or completion of metamorphosis), they tended to remain longer in the aquaria and reached metamorphosis at a bigger size than those who metamorphosed earlier (Table 4). Since time and size at metamorphosis are directly related to fitness, tadpoles should eat more, develop more slowly and grow larger as long as habitat conditions remain favorable (Crump, 1989). Effects of time and size at metamorphosis on fitness would be an interesting aspect of *L. albilabris* larval stage for future studies. Unfavorable conditions can vary, and responses of tadpoles can change accordingly. Candelas *et al.* (1961 in Joglar, 2005) observed that tadpoles of *L. albilabris* reduced their metabolic rate when placed on a damp cotton medium. Tadpoles were able to restore their metabolic rates when returned to water. It would seem then that responses of growth and development of tadpoles of *L. albilabris* also depend on abiotic factors present.

Because of conditions used in the food calibration experiment, a higher food treatment was not possible. In a preliminary run for this experiment I used higher food levels and encountered high mortality of tadpoles due to water fouling. Even so, this experiment was an important step for the competition experiment because it allowed me to use food treatment levels adequate for the aquarium conditions used.

Competition experiment

R. marina is an introduced species and has been documented to have detrimental effects on native biota (Williamson, 1999; Crossland, 2000; Crossland *et al.*, 2008). Contrary to the expected results, *R. marina* seemed to have no negative effects on growth, development and survival of native *L. albilabris* tadpoles. Rather, the opposite outcome was observed; *R. marina* larvae experienced severe reduction in growth and survival when in the presence of *L. albilabris* larvae.

Early development and survival

The larval phase is a period devoted to growth, and many species increase in mass three to four orders of magnitude during this period (Werner, 1986 in Schiesari *et al.*, 2009). According to the normal growth curve presented by Adolph (1931), there is a logarithmic growth that begins at hatching and continues under favorable conditions for about two weeks. It is in this period that body weight can double approximately every two and a half days. Thus, this early stage of tadpole development is important to determine size and time to metamorphosis. It has also been reported that time to metamorphosis is determined by food intake during the first half of the larval growth period (Browne *et al.*, 2003). Resource limitation and competitors will tend to decrease metamorph size and increase larval period (Browne *et al.*, 2003).

Early development (PC1) for *L. albilabris* was negatively affected by species composition and food limitation. This demonstrates that this species suffered more from intraspecific competition rather than interspecific competition during this period. The trend seen in early survival (reduced survival with low food level and higher presence of conspecifics) further evidences this result (Fig. 6). This finding of intraspecific competition effects is

consistent with results reported by Richter-Boix *et al.* (2007), in which a study of intra- and interspecific effects between six anuran species showed that larger species were more affected by intra- rather than by interspecific competition. *L. albilabris* tadpoles in control treatments had a higher early survival when reared with lower food levels, suggesting that intraspecific competition pressure is stronger when there are more resources available (at least up to that point) and more conspecifics present. During this early stage of development, tadpoles of *L. albilabris* will try to gain mass faster, exploiting the resources available and reducing early survival of conspecifics. When conspecific numbers are low, however, higher resources will allow for better growth and higher early survival. Nevertheless, with fewer conspecifics and more of the introduced species, there was a trend toward reduced early survival (Fig. 6b). This finding suggests that if *L. albilabris* breeds where there are high *R. marina* densities, there might be a negative effect of the invasive species on the survival of the native one. This might be the case, since *L. albilabris* can lay an average of 106 eggs per clutch (Joglar, 2005), and *R. marina* can lay up to 35,000 eggs (Rivero, 1998).

Contrary to my expectations, early development (PC1) in *R. marina* was greatly affected by the presence of *L. albilabris* tadpoles. Interaction effects of food and species composition had a negative effect on early growth and development of *R. marina*. PC1 (early development; early mass, early total length, early body length, early developmental rate) for *R. marina* was reduced when *L. albilabris* and high food levels were present. This provides evidence of interspecific competition between both species, where *R. marina* seems to be competitively inferior to *L. albilabris*. This effect on *R. marina* is exacerbated when there is a 50-50 ratio of individuals present and high food levels (Fig. 7). It is possible that *L. albilabris* activity increases when there are abundant resources available, exploiting them to the point where *R. marina* is incapable of

acquiring sufficient food for early survival. The presence of *L. albilabris* reduced early survival for *R. marina* during this early period of development, which in turn resulted in detrimental effects on survival to metamorphosis.

In this study I observed a significant correlation between survival to metamorphosis and early growth, early developmental rate and early survival of *R. marina* (Table 5). In contrast, this correlation was significant for *L. albilabris* only between survival to metamorphosis and early survival (Table 6). Overall, the differences in the observed pattern of correlation between survival and other measures of larval development in the two species suggest that early development is particularly important for *R. marina* when there are competitors present, and that survival to metamorphosis depends on more aspects affected during the early stages of development than for *L. albilabris*. These correlations could explain the trends seen in this experiment, when survival of *R. marina* was reduced because of interactions with *L. albilabris* during the early stages of development. *L. albilabris* tadpoles seemed to be better competitors during this important early stage for *R. marina*, and toad tadpoles that could not gain the necessary growth and development experienced increased mortality. All of the effects seen during early development and early survival had a direct effect on metamorphosis (discussed below), as in accordance to previous studies (Gollmann and Gollmann, 1993; Browne *et al.*, 2003).

Late development and survival

For *L. albilabris*, food availability is important for survival to metamorphosis and time to metamorphosis. Lower levels of food retard metamorphosis by reducing size during the early stages of development (Wilbur and Collins, 1973; Denver, 1997). Similar to early development

results, larvae of *L. albilabris* suffered more from intra- rather than interspecific competition. The interaction between food availability and species composition is also important for this native species. Since they suffer more from intraspecific competition, a low density of conspecifics, combined with a high resource level, produced the optimal condition for this species growth and survival to metamorphosis. In the 6 L: 6 R treatment, intraspecific competition was reduced and tadpoles took advantage of the available food resource to accelerate their growth and development and reach metamorphosis faster (Fig. 8). These results suggest that when food resource is available, *L. albilabris* larvae will exploit them to reduce their larval period and quickly gain the necessary growth to leave the aquatic habitat. The results from this treatment are further evidence of interspecific competition between *L. albilabris* and *R. marina* during the larval phase. In this treatment, competitive interactions between both species were clearly seen as *L. albilabris* gained competitive advantage over *R. marina*, probably by means of exploitation competition.

Growth at metamorphosis and survival to metamorphosis in *R. marina* were greatly affected by the presence of *L. albilabris*. Although food availability had no significant effect on size at metamorphosis and larval period, species composition reduced size and survival of *R. marina* metamorphs and forced surviving tadpoles to accelerate initiation of metamorphosis (Fig. 9). Apparently, the presence of a superior competitor forced *R. marina* to initiate metamorphosis at a smaller size (see Wilbur and Collins, 1973; Denver, 1997).

Body size and activity levels are two important factors in competitive interactions in freshwater systems (Richter-Boix *et al.*, 2007). Regarding body size, larger tadpoles are generally better competitors than smaller ones (Wilbur and Collins, 1973; Griffiths, 1991; Gollmann and Gollmann, 1993). A larger body size can give the advantage of suppressing

growth and reducing survival of smaller tadpoles by direct or indirect mechanisms (Wilbur and Collins, 1973; Alford and Crump, 1982; Laurila, 2000; Katzmann *et al.*, 2003; Richter-Boix *et al.*, 2007; Cabrera-Guzmán *et al.*, 2011). Also, smaller species can be greatly affected by interspecific competition when compared to larger ones (Richter-Boix *et al.*, 2007). Activity levels can also influence competitive balance in tadpoles. More active tadpoles are thought to be better competitors because activity increases foraging efficiency (Laurila, 2000; Richter-Boix *et al.*, 2007; Dayton and Fitzgerald, 2001). The effects of body size and activity levels can give insight to the competitive interactions between *L. albilabris* and *R. marina*. *L. albilabris* tadpoles are larger than those of *R. marina*, and in this experiment I could see that *L. albilabris* had the competitive advantage over the smaller species. Also, *R. marina* smaller size could have made it more vulnerable to the effects of interspecific competition. Activity level, on the other hand, was not directly measured in this experiment. During weekly measurements, however, tadpoles of *L. albilabris* were harder to manipulate because they moved faster and tended to jump constantly, which suggests that they are more active than those of *R. marina*. This higher level of activity could also explain why *L. albilabris* gained the competitive advantage during this study.

Another important aspect that can explain the interaction seen in this experiment with both species is habitat adaptation. *L. albilabris* and *R. marina* seemed to be adapted to different types of habitats. *L. albilabris* adults lay eggs in terrestrial foam nests that develop in temporary ponds or channels after being washed away by water runoff during heavy rains (Rivero, 1998). This species is adapted to ephemeral habitats (Heatwole *et al.*, 1968; Joglar, 2005). Tadpoles that develop in ephemeral water bodies need to grow quickly to escape the risk of death due to habitat degradation (Heatwole *et al.*, 1968; Wellborn *et al.*, 1996; Loman 1999; Richter-Boix *et al.*, 2007). Time to acquire resources can be limited in ephemeral habitats, thus, tadpoles that

develop in these systems tend to be more active feeders and to grow faster (Wellborn *et al.*, 1996; Loman, 1999; Richter-Boix *et al.*, 2007). As demonstrated in this experiment, *L. albilabris* exploited resources to increase their developmental rate, probably as a consequence of adaptation to life in ephemeral habitats. Tadpoles that develop in ephemeral water bodies may be superior competitors over those that are more adapted to life in permanent water habitats (Wilbur, 1987; Wellborn *et al.*, 1996), as was demonstrated in the present study.

Species adapted to life in permanent ponds can experience reduction in exploitation competition (Richter-Boix *et al.*, 2007) and a relaxed restriction on developmental rates (Wellborn *et al.*, 1996). For these reasons, species living in permanent habitats tend to have longer larval periods than those from ephemeral habitats (Denver, 1997). This would be the case for *R. marina*, which seems to be more adapted to life on permanent water bodies. This species seems to rely on its unpalatability and toxicity to escape predation risk, which is more likely to occur in permanent water bodies than in ephemeral ones (Heyer *et al.*, 1975; Wellborn *et al.*, 1996). Thus, when competition interactions are important and the competitor is not affected by unpalatability or toxicity of *R. marina*, *R. marina* can lose competitive advantages. Also, Alford's (1999) suggestion that larvae of *R. marina* can have the competitive advantage by being more aggressive feeders was not observed in this study. It would seem that when there is a more active ephemeral pond species present, *R. marina* can lose this advantage. The differences in *L. albilabris* and *R. marina* habitat adaptations may explain why *R. marina* was the inferior competitor when reared in the presence of *L. albilabris*.

In this study I demonstrated that interspecific competition, probably by means of exploitation, is possible between *L. albilabris* and *R. marina* larvae. I also demonstrated how interference competition affects development and survival in the two species. This competitive

interaction can be complex and is dependant of several factors. Under the conditions used in this experiment, *L. albilabris* seemed to have the competitive advantage over *R. marina*. Under more natural conditions, however, this interaction can be non-existent or reversed. Previous studies have shown that although competition can be proven with a laboratory experiment, other factors present under natural conditions can change these interactions and either reduce competitive importance (Pavignano, 1990; Wellborn *et al.*, 1996; Williamson, 1999; Schiesari *et al.*, 2009) or increase competitive interactions (Alford and Crump, 1982; Scott, 1990; Griffiths, 1991; Crossland *et al.*, 2009). Predation is seen as one important factor in larval amphibian communities. *L. albilabris* may be better competitors than *R. marina* because of activity levels, but this also makes them more vulnerable to predation (Van Buskirk and Yurewicz, 1998; Van Buskirk and McCollum, 2000; Dayton and Fitzgerald, 2001).

The use of native species to control invasive *R. marina* has been already considered (Cabrera-Guzmán *et al.*, 2011). Even if *L. albilabris* seems to be a superior competitor during the larval phase, other aspects of natural history of the species need to be better understood in order to think about this possibility. For instance, both species may not share the same breeding sites at the same time. Also, larvae of *R. marina* can be found at higher densities than native species, and this could have a negative effect on native species development. Toxicity of *R. marina* can also have detrimental effects on native species (Crossland, 2000; Crossland *et al.*, 2008; Crossland and Shine, 2010). During this study, *L. albilabris* scavenged dead *R. marina* tadpoles without apparent ill effect. However, toxicity of *R. marina* varies during ontogeny, and eggs are known to be more toxic than larvae (Hayes *et al.*, 2009). In addition, priority effects are known to alter interactions of species during the larval phase (Lawler and Morin, 1993). The impact of the invasive species over the native one and *vice versa* are highly sensitive to priority effects

(Crossland *et al.*, 2009). Thus, because of the complexity of larval amphibian interactions, further studies are needed to conclusively determine if *R. marina* is having a negative effect on larval development in *L. albilabris*.

THE NATURAL HISTORY OF *LEPTODACTYLUS ALBILABRIS*

In this section I report previously undocumented aspects on the development and behavior of larval *L. albilabris*. I trust that this study will increase our knowledge of the larval phase of this native species, and that it will encourage other researchers to continue studying it.

Larval diet

Tadpoles of *L. albilabris* have been reported feeding on a dead earthworm and an anole carcass (Lebrón *et al.*, 1995) and on an adult carcass of *R. marina*, only avoiding the area of the parotid glands (Joglar, 2005). In this study, larval *L. albilabris* scavenged on dead conspecifics and *R. marina* larvae. Dead conspecifics were quickly scavenged, and in less than ten minutes there was almost nothing left of the dead individual. *R. marina* larvae were not as quickly scavenged, so it seems *L. albilabris* prefers to feed on dead conspecifics than on *R. marina*. This trend, however, was observed in different aquaria. It would be interesting then to see if this behavior can change if carcasses of both species are encountered at the same time by the same individual.

Size

At developmental stage 26, the smallest *L. albilabris* larvae measured 9.7 mm, and the largest 17.5 mm. Average size for larvae at stage 26 was 14.8 mm (S.D. $\pm$ 0.195).

At developmental stage 42, the smallest larvae measured 19 mm (4 L : 8 R treatment, high food), and the largest, 44 mm (4 L : 8 R treatment, low food). Average size for larvae at stage 42 was 32.5 mm (S.D. $\pm$ 0.433).

Mass

At developmental stage 26, the smallest *L. albilabris* larvae weighted 0.0155 g, and the largest, 0.0701 g. Average mass for larvae at stage 26 was 0.0376 g (S.D. $\pm$ 0.011).

At developmental stage 42, the smallest larvae weighted 0.0979 g (4 L : 8 R treatment, high food), and the largest, 0.7677 g (4 L : 8 R treatment, low food). Average mass for larvae at stage 42 was 0.2986 g (S.D. $\pm$ 0.109).

These measurements of mass do not necessarily correspond to the smallest or largest tadpoles measured for the *Size* section.

Larval period

In this experiment, the longest larval period (from developmental stages 26 to 42) was 87 days (12 L: 0 R treatment, low food). The shortest larval period was 10 days (12 L: 0 R treatment, high food). The average larval period was 24.22 days (S.D. $\pm$ 8.855).

Dent (1956) observed that deprivation of nutrition can extend *L. albilabris* metamorphosis to 73 days. His observations were based on four individuals kept in a glass container with little food. In this experiment I demonstrated that this effect is variable and can depend on number of conspecifics present. Larvae in control groups tended to metamorphose

faster when there were many conspecific present, and they could extend time to metamorphosis when they were alone and with enough resources. Thus, food deprivation alone is not responsible for extended larval periods in this species.

Activity

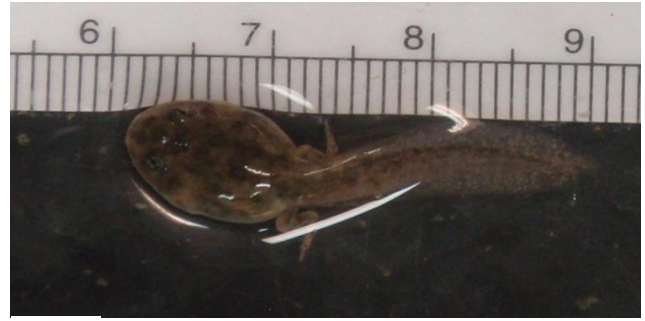
Tadpoles of *L. albilabris* were hard to manipulate because of activity levels. They were fast swimmers and tended to jump constantly in the Petri dish. Some tadpoles jumped out of the Petri dish and continued to jump away from it afterwards.

Coloration patterns

Patterns in coloration have been described for adult *L. albilabris* (Joglar, 2005). In this study we observed different coloration patterns in tadpoles that were noticeable after stage 31 of development. Tadpoles of *L. albilabris* seen in this experiment ranged from light brown to dark brown, and some exhibited dark brown or black spots in the tail and body (Fig. 10). All photos were taken with an OLYMPUS Stylus 840.



(a)



(b)



(c)



(d)



(e)



(f)

Figure 10. Differences in coloration patterns on tadpoles of *Leptodactylus albilabris*. Gosner stage (G) and treatment (t) are provided for each tadpole. Treatments: Control A (CA; 12 *L. albilabris*), Experimental A (EA; 6 *L. albilabris* and 6 *R. marina*), Experimental B (EB; 4 *L. albilabris* and 8 *R. marina*); 0 = low food, 1 = high food. (a) G-38, t-EB-0; (b) G-40, t-EB-1; (c) G-40, t-EB-0; (d) G-37, t-CA-0; (e) G-37, t-CA-0; (f) G-39, t-CA-0.



(g)



(h)



(i)



(j)



(k)



(l)

Figure 10 cont. Differences in coloration patterns on tadpoles of *Leptodactylus albilabris*. Gosner stage (G) and treatment (t) are provided for each tadpole. Treatments: Control A (CA; 12 *L. albilabris*), Experimental A (EA; 6 *L. albilabris* and 6 *R. marina*), Experimental B (EB; 4 *L. albilabris* and 8 *R. marina*); 0 = low food, 1 = high food. (g) G-38, t-CA-0; (h) G-38, t-CA-1; (i) G-38, t-CA-1; (j) G-36, t-CA-0; (k) G-40, t-CA-1; (l) G-39, t-CA-0.

RECOMMENDATIONS AND FUTURE STUDIES

Several aspects of *L. albilabris* tadpole development are still needed to gain insight on the natural history of this native species. The maximum and minimum larval size needed to reach metamorphosis is species specific and determined by genetics (Denver, 1997), and this would be an interesting aspect to look at. It would also be interesting to determine how larval interactions between *L. albilabris* and *R. marina* could affect adult survival and fitness of both species.

It would be valuable to determine the natural densities of *L. albilabris* and *R. marina* and observe interactions under more realistic natural conditions. One of these natural conditions could be water level. For example, a higher water volume would allow use of increased levels of food, and these higher resource levels could show different patterns on larval development. Conducting these types of experiments under natural conditions could also help us determine a more realistic time and size at metamorphosis for *L. albilabris*, since it has been reported that larvae grow faster (Williamson, 1999) and bigger (Katzmann *et al.*, 2003) in enclosures when compared to more natural conditions. Predator presence could also affect larval development under natural conditions. Since activity levels of *L. albilabris* can make them more susceptible to predation, a future study should concentrate on how competitive interactions change in the presence of predators.

In this experiment, larvae of *R. marina* had no negative effect on development of *L. albilabris*, but there are other factors to be considered that could change the interactions seen in this experiment. Priority effects can change competitive advantage (Lawler and Morin, 1993; Crossland *et al.*, 2009) and a future study could focus on how these priority effects can alter the competitive interactions observed in this experiment. Another factor to be considered is toxicity

of *R. marina* eggs and larvae. Tadpoles of the genus *Leptodactylus* are known to be predators of *R. marina* (i. e. *L. pentadactylus*, Heyer *et al.*, 1975) without ill effects. *L. albilabris* was not observed predating on live *R. marina* larvae, and the possibility can be studied, including whether larvae of the native species eat the eggs of invasive cane toads and the resulting effects.

Dietary studies can also be conducted to give great insight to resource use, diet preferences, habitat partitioning, and other interspecific interactions within species sharing an aquatic habitat. If there is high resource overlap, competition may be an important factor in species interactions. The study of the morphology of the oral disc can also reflect ecological differences between species (Altig and Johnston, 1989), and the oral disc morphology of *L. albilabris* could be studied in detail to better understand ecological interactions between this native species and other invasive species.

Osteopilus septentrionalis, the Cuban tree frog, is also an invasive species in Puerto Rico. This species could be better adapted to life in ephemeral ponds than *R. marina* and could probably compete with *L. albilabris*. Tadpoles of *O. septentrionalis* are bigger than those of *L. albilabris* (personal observation), and their larger body size could give this invasive species the competitive advantage. It would be interesting to do a study similar to this one to determine the effects of *O. septentrionalis* on larval development of *L. albilabris*. Also, tadpoles of the endangered Puerto Rican crested toad, *Peltophryne lemur*, may be more similar to those of *R. marina*. For this reason, they might be negatively affected by *R. marina* larvae. Since *P. lemur* is an endangered species, a competition experiment with *R. marina* cannot be conducted for now, but if the species recovers, a similar experiment could give great insight to the interaction of the larvae between these two species.

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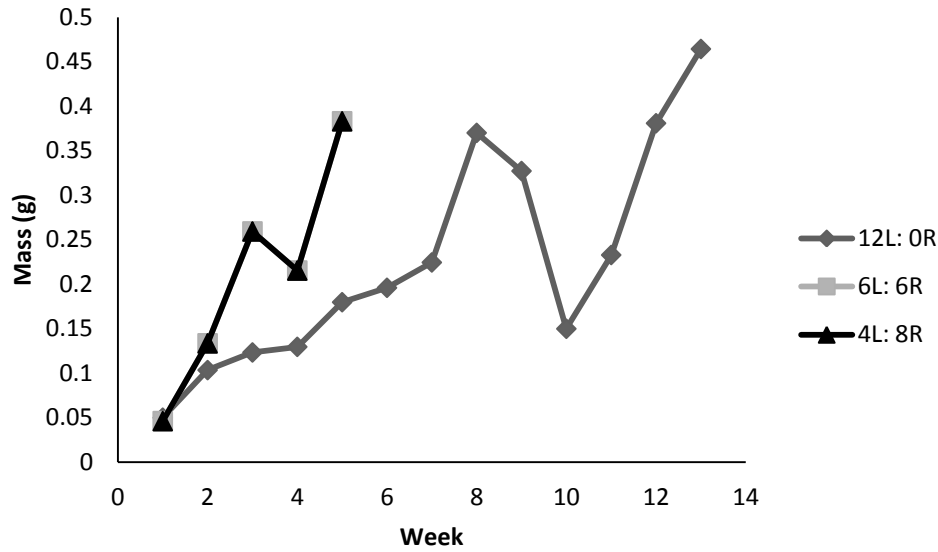
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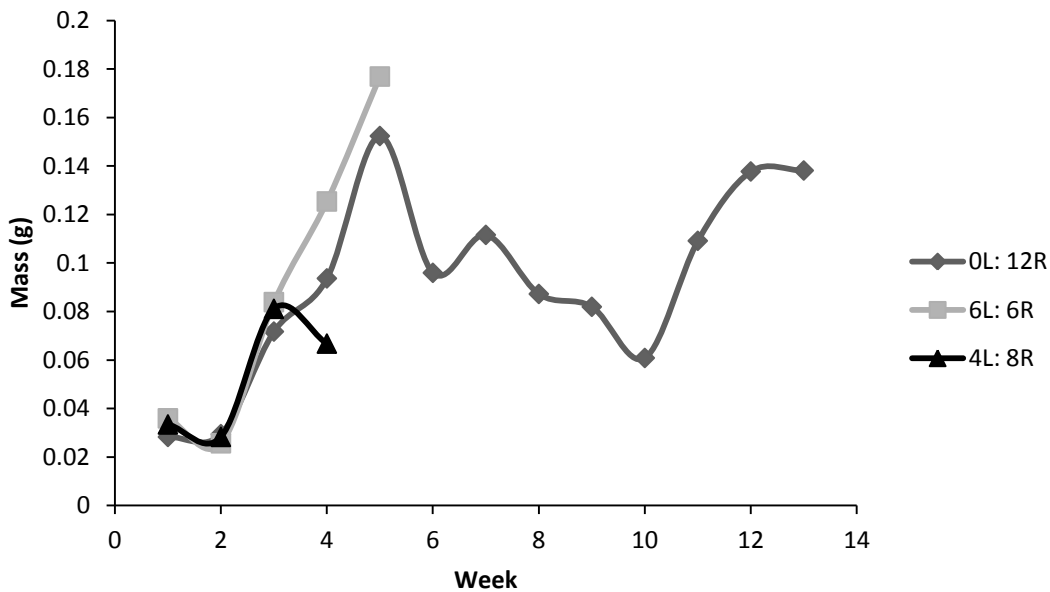
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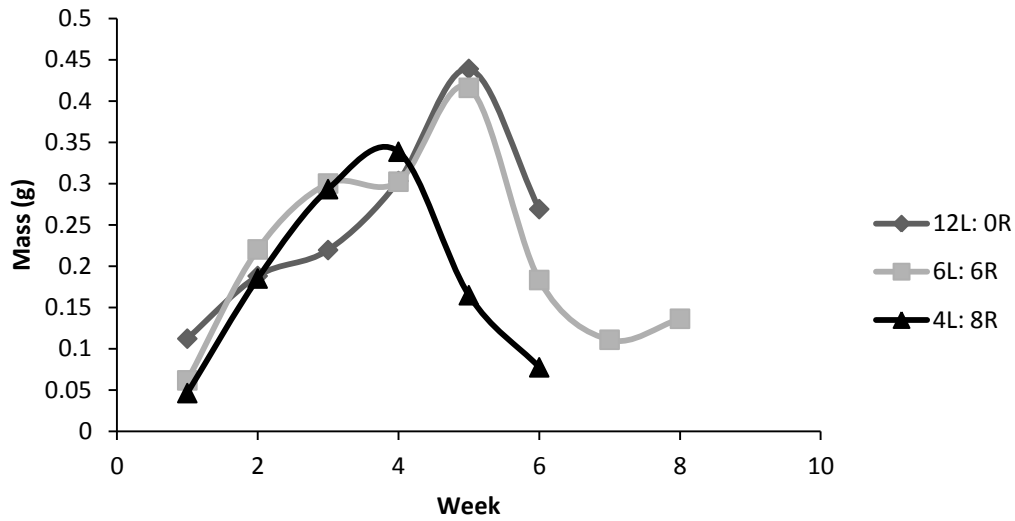
APPENDIX



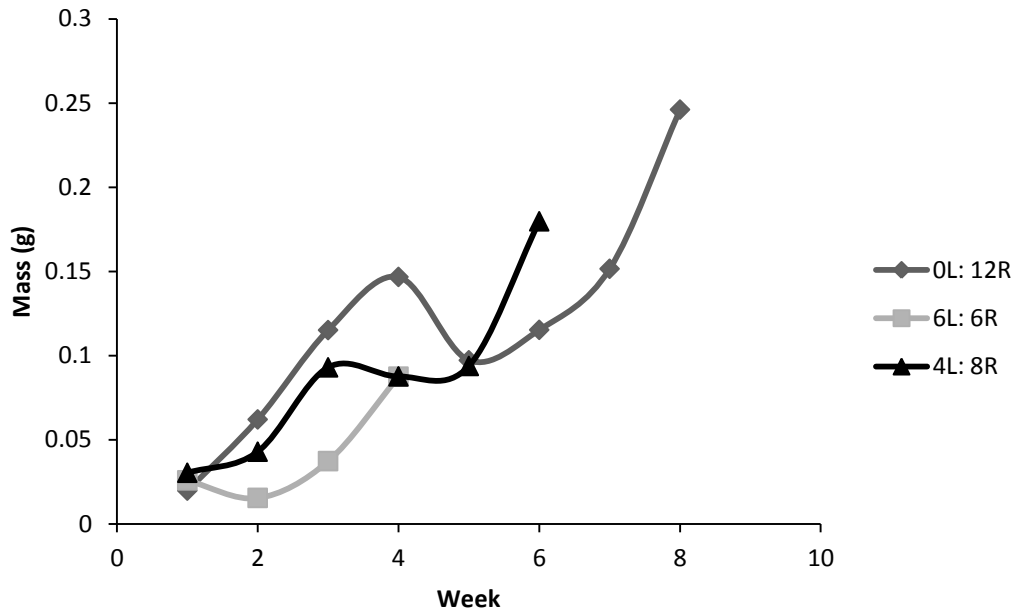
Appendix A. Change in mass for *Leptodactylus albilabris* tadpoles in the low food treatment of the competition experiment. Low food = 0.23 g. Species composition treatments: a) 12L: 0R (12 *L. albilabris* tadpoles and 0 *Rhinella marina* tadpoles); b) 6L: 6R (6 *L. albilabris* and 6 *R. marina*); c) 4L: 8 R (4 *L. albilabris* and 8 *R. marina*).



Appendix B. Change in mass for *Rhinella marina* tadpoles in the low food treatment of the competition experiment. Low food = 0.23 g. Species composition treatments: a) 0L: 12R (0 *L. albilabris* tadpoles and 12 *Rhinella marina* tadpoles); b) 6L: 6R (6 *L. albilabris* and 6 *R. marina*); c) 4L: 8 R (4 *L. albilabris* and 8 *R. marina*).



Appendix C. Change in mass for *Leptodactylus albilabris* tadpoles in the high food treatment of the competition experiment. High food = 0.68 g. Species composition treatments: a) 12L: 0R (12 *L. albilabris* tadpoles and 0 *Rhinella marina* tadpoles); b) 6L: 6R (6 *L. albilabris* and 6 *R. marina*); c) 4L: 8 R (4 *L. albilabris* and 8 *R. marina*).



Appendix D. Change in mass for *Rhinella marina* tadpoles in the high food treatment of the competition experiment. High food = 0.68 g. Species composition treatments: a) 0L: 12R (0 *L. albilabris* tadpoles and 12 *Rhinella marina* tadpoles); b) 6L: 6R (6 *L. albilabris* and 6 *R. marina*); c) 4L: 8 R (4 *L. albilabris* and 8 *R. marina*).

Appendix E. Growth rates for *Leptodactylus albilabris* and *Rhinella marina* in the competition experiment

Food treatment (g)	Species composition treatment (<i>Leptodactylus albilabris</i> : <i>Rhinella marina</i>)	<i>Leptodactylus albilabris</i> growth rate (g/days)	<i>Rhinella marina</i> growth rate (g/days)
0.23	12L: 0 R	0.007	--
0.23	6L: 6:R	0.013	0.005
0.23	4L: 8R	0.016	0.004
0.23	0L: 12R	--	0.004
0.68	12L: 0 R	0.014	--
0.68	6L: 6:R	0.018	0
0.68	4L: 8R	0.017	0.004
0.68	0L: 12R	--	0.005