El Colegio de la Frontera Sur

Efecto de la dieta sobre el crecimiento y la metamorfosis de los renacuajos de *Triprion petasatus* (Anura: Hylidae)

Tesis

Presentada como requisito parcial para optar al grado de
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Con orientación en Ecología y Sistemática

Por

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para obtener el grado de **Maestra en Ciencias en Recursos Naturales y Desarrollo Rural**

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Resumen

Los anfibios son un grupo de vertebrados altamente amenazado, por lo cual es esencial realizar estudios acerca de su biología y ecología, especialmente de su estado larvario, el renacuajo. De muchas especies de anfibios neotropicales aún se desconoce la dieta, la duración del periodo larval y la metamorfosis de los renacuajos. Por esta razón, se determinó experimentalmente el efecto que tiene la dieta sobre el crecimiento y metamorfosis de los renacuajos de *Triprion petasatus*, una rana arborícola poco estudiada, endémica de la Península de Yucatán. Se alimentó un conjunto de renacuajos con una dieta rica en proteína (comida para tilapia) y otro con una dieta más natural (detritus, hojarasca, cladóceros y ostrácodos). También, se describió la dieta de renacuajos de una población silvestre. Los renacuajos criados con la dieta rica en proteína alcanzaron tallas más grandes al llegar a la metamorfosis y como juveniles; tuvieron poca mortalidad; su periodo larval fue corto y menos variable, pero la duración de la metamorfosis fue más larga para algunos individuos, en comparación con los renacuajos criados con la dieta más natural. La dieta de la población silvestre estuvo compuesta principalmente por detritus y algas, mientras que zooplancton y otros microinvertebrados estuvieron presentes en menor proporción. Los resultados del análisis de la dieta y del experimento revelaron que los renacuajos de esta especie, además de ser omnívoros, son caníbales facultativos. Además, se observó que la dieta de los renacuajos fue diferente entre etapas de su desarrollo. Este estudio es el primero en aportar información sobre la dieta y su efecto sobre el crecimiento, duración del periodo larval y la metamorfosis para *T. petasatus*. Los resultados tienen importancia para la conservación de esta especie, ya que forman la base para el desarrollo de programas de crianza en cautiverio, si se requiere tomar esa medida para asegurar su sobrevivencia en el futuro.

Palabras clave: ecología de renacuajos, especies endémicas, necesidades alimenticias, periodo larval, rana cabeza de casco

Capítulo I. Introducción

Los anfibios son un grupo de vertebrados altamente amenazado, debido a la pérdida de su hábitat, infección por el hongo patógeno *Batrachochytrium dendrobatidis* y el cambio climático global (Lips et al. 2005), lo que ha generado tendencias alarmantes de extinción y disminución en sus poblaciones a nivel global (Stuart et al. 2004). Esto genera una necesidad urgente para realizar estudios acerca de su biología y ecología, especialmente en el caso de los anuros (anfibios sin cola: ranas y sapos) y sus renacuajos (McDiarmid y Altig 1999a).

Los renacuajos representan la fase larvaria del desarrollo indirecto que experimentan la mayoría de los anuros (Cedeño-Vázquez y Calderón-Mandujano 2011). Son completamente acuáticos, en contraste con los adultos que son terrestres (Calderón-Mandujano et al. 2008), y tienen influencia en la estructura y función de los sistemas acuáticos que habitan, debido a que son depredadores macrófagos y a la vez presas para peces, salamandras e invertebrados acuáticos (Alford 1999; Petranka y Kennedy 1999; Ranvestel et al. 2004). A pesar de su importancia, los renacuajos han sido menos estudiados que los individuos adultos y otros organismos acuáticos, como peces, por lo que se sabe relativamente poco acerca de ellos (McDiarmid y Altig 1999a; Altig et al. 2007). Si consideramos que tres cuartas partes de las especies de ranas tienen una etapa larval (renacuajo), es evidente que queda mucho por descubrir (McDiarmid y Altig 1999a).

Durante el ciclo de vida de los anuros, el renacuajo pasa por un periodo de transición conocido como metamorfosis, el cual es clave porque es cuando adquiere las características morfológicas, fisiológicas, ecológicas y conductuales necesarias para vivir en el ámbito terrestre (McDiarmid y Altig 1999b; Semlitsch 2003). La metamorfosis es influenciada y regulada por un conjunto de factores externos (ambientales) e internos (hormonas tiroideas y esteroideas) (Hayes 1997). El proceso es sumamente importante, ya que una metamorfosis exitosa es crítica para mantener y establecer poblaciones locales y, mediante la dispersión, reestablecer poblaciones extintas (Semlitsch 2003; Semlitsch y Skelly 2007). Sin embargo, este proceso ha sido estudiado mayormente en tres especies de anuros: *Rana catesbeiana* (Shaw, 1802), *R*.

pipiens (Schreber, 1782) y *Xenopus laevis* (Daudin, 1802), lo que implica que no se ha podido comparar la metamorfosis entre diferentes especies (Duellman y Trueb 1994; Wells 2007).

Desde una perspectiva ecológica, el momento en el que inicia la metamorfosis puede variar, ya que está sujeto a factores como la disponibilidad y calidad de alimento (dieta), la temperatura, el volumen de agua (desecación), la densidad poblacional, la presencia de depredadores y el tipo de competidores existentes (Hayes 1997; Rose 2005). De estos factores, la dieta ha generado un gran interés de investigación acerca del rol que juega, no solamente en la metamorfosis, sino también en el crecimiento larval. La dieta está correlacionada con las tasas de crecimiento y desarrollo, la duración del periodo larval y el tamaño al que llega el individuo al iniciar la metamorfosis y como rana juvenil (Kupferberg 1997; Harris 1999, Álvarez y Nicieza 2002). Es por eso que la dieta tiene consecuencias importantes para los individuos, ya que al metamorfosearse a temprana edad y adquirir un mayor tamaño, disminuye el riesgo de ser depredado y aumenta su fecundidad, lo que le permitirá reproducirse más joven y a una talla más grande (Rose 2005). El estudio de la dieta también proporciona información acerca del comportamiento alimenticio, el papel ecológico y las relaciones ecológicas inter e intraespecíficas de los renacuajos (Alford 1999; Altig et al. 2007). Su dieta varía según la morfología de su aparato oral, lo que los lleva a ser herbívoros, omnívoros, carnívoros e incluso caníbales (Alford 1999).

No obstante, la mayoría de las investigaciones acerca de la dieta de los renacuajos y su rol en el crecimiento, el desarrollo y la metamorfosis solo han sido realizadas con especies de climas templados o de importancia para la acuicultura, dejando a un lado a las especies neotropicales (Alford y Harris 1988; Kupferberg 1997; Skelly 1997; Richter-Boix et al. 2007; Martins et al. 2013). Con estudios que evalúen el efecto que tienen diferentes dietas sobre el crecimiento y desarrollo se puede determinar el tipo de alimento con el que los renacuajos crecen mejor y experimentan una metamorfosis más exitosa, lo que a su vez aumenta la sobrevivencia de los juveniles. Esta información es crucial para el establecimiento de sistemas de crianza de renacuajos en cautiverio que busquen incrementar la adecuación de los individuos para asegurar su sobrevivencia

(O'Rourke 2007; Martins et al. 2013). Para muchas especies de anuros aún se desconoce el papel ecológico de los renacuajos, por lo que estudios sobre la dieta son una prioridad (Altig et al. 2007).

La rana arborícola cabeza de casco *Triprion petasatus* (Cope, 1865) se encuentra sujeta a protección especial en la NOM-059 (SEMARNAT 2010) y es endémica de la Península de Yucatán (Duellman y Trueb Klass 1964). Es una de las especies de las cuales se desconoce la composición de la dieta, tasa de crecimiento y tiempo del periodo larval, así como del proceso de metamorfosis de su renacuajo (Duellman y Trueb Klass 1964). Es una especie neotropical moderadamente grande (machos: 60.8 mm de longitud hocico-cloaca [LHC], hembras: 74.2 mm LHC) (Duellman y Trueb Klass 1964). Su renacuajo mide 27 mm de largo (etapa 30), es béntico y se encuentra en cuerpos de agua someros (ej. aguadas y charcas), con fondos ricos en materia orgánica (Altig y McDiarmid 1999; Duellman 2001). El tamaño de los juveniles recién metamorfoseados alcanza 15.5-16.1 mm de LHC (Duellman 2001).

Debido a que *T. petasatus* es una especie endémica, protegida y a que se desconocen varios aspectos de su ciclo larvario (metamorfosis, crecimiento y dieta), en la presente investigación se evaluó experimentalmente el efecto de una dieta rica en proteína (alimento comercial para tilapia) y una semejante a lo que se encontrarían en un ambiente natural (compuesta de detritus, hojarasca, cladóceros y ostrácodos) sobre el crecimiento, la duración del periodo larval y la metamorfosis, así como el tamaño de los individuos al llegar a la metamorfosis y a ser ranas juveniles. Al mismo tiempo, se describió la dieta de renacuajos de una población silvestre que se encontró viviendo en un sistema acuático formado durante cuatro años en una lancha, con agua de lluvia y aporte principal de hojas, donde se ha desarrollado una comunidad de zooplancton y algas (obs. pers.).

Con esta investigación se espera poder contestar las siguientes preguntas: ¿Con qué tipo de alimento los renacuajos experimentan una mayor tasa de crecimiento y alcanzan tallas más grandes? ¿Cómo cambia la duración del periodo larval y de metamorfosis con ambos tipos de dieta? ¿Cómo se compone la dieta natural de los renacuajos de *T. petasatus*?

La hipótesis implica que una dieta con alto contenido de proteína favorecerá el crecimiento y el desarrollo de los renacuajos y acortará el periodo larval y de la metamorfosis. Además, la dieta de los individuos de la población silvestre estará compuesta principalmente por detritus y algas, así como por zooplancton, alimentos que son más frecuentes en sistemas acuáticos donde habitan los renacuajos de esta especie.

Capítulo II. Publicación enviada

Short title.—*Triprion petasatus* diet, growth and metamorphosis

THE EFFECT OF DIET ON GROWTH AND METAMORPHOSIS OF *TRIPRION PETASATUS* (ANURA: HYLIDAE) TADPOLES

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Abstract.—Diet is an important factor that influences tadpole growth, development, and ultimately metamorphosis. Nevertheless, it has been poorly studied in neotropical tadpoles. We experimentally tested the effect of two different diets on growth and metamorphosis of tadpoles of the endemic Yucatan Casque-Headed treefrog (*Triprion petasatus*). Individuals raised on a high-protein diet experienced increased growth, large size at metamorphosis and as froglets, a short larval period, and low mortality. However, the duration of metamorphosis was shorter in individuals fed a more natural diet. These same tadpoles also engaged in cannibalistic behavior in response to limited food resources. Diet analysis of tadpoles from a wild population revealed that this species is omnivorous and consumes mostly detritus, algae, and zooplankton. There was also a significant difference in the diet between developmental stages. This is the first time that diet, growth rate, length of larval period and duration of metamorphosis have been studied for *Triprion petasatus* tadpoles. The results of this study contribute new information on the

tadpole ecology of this poorly studied hylid species, which may be used for future captive breeding programs.

Key Words.—dietary needs; endemic species; larval period; tadpole ecology; Yucatan Casque-Headed treefrog

Resumen.—La dieta es un factor importante en el crecimiento, desarrollo y metamorfosis de los renacuajos. No obstante, este factor ha sido poco estudiado en renacuajos de especies neotropicales. Por lo tanto, en este estudio se evaluó en condiciones experimentales el efecto que tienen dos tipos de dieta en el crecimiento y metamorfosis de los renacuajos de *Triprion petasatus*, una rana endémica de la Península de Yucatán. Los renacuajos que contaron con una dieta alta en proteína crecieron más rápido, alcanzaron una talla más grande (como metamorfos y ranas juveniles), un periodo larval corto y baja mortalidad. Sin embargo, el proceso de metamorfosis fue más corto para los individuos que comieron una dieta más natural. Estos mismos renacuajos mostraron comportamiento canibalístico como respuesta ante una limitación de los recursos alimenticios. El análisis de la dieta de renacuajos de una población silvestre reveló que son omnívoros, consumen mayormente detritus, algas y zooplancton. Además, la dieta fue diferente en las etapas de desarrollo. Esta es la primera vez que se estudia la dieta, tasa de crecimiento, duración del periodo larval y de la metamorfosis para los renacuajos de *T. petasatus*. Los resultados de este estudio contribuyen con nueva información acerca de la ecología del renacuajo de esta especie de hílido poco conocida, la cual puede ser usada para desarrollar programas de reproducción en cautiverio en el futuro.

*Palabras clave***.**—ecología de renacuajos; especie endémica; necesidades alimenticias; periodo larval; rana cabeza de casco

INTRODUCTION

The complex amphibian life cycle is highly susceptible to changes in environmental conditions (Wells 2007), since it requires that most anurans spend part of their lives as tadpoles (McDiarmid and Altig 1999a). Tadpoles must grow, develop and undergo metamorphosis in order to transition into terrestrial adults (Wells 2007). These processes are regulated by the endocrine system, which at the same time is influenced by external environmental factors (Hayes 1997; Viertel and Richter 1999; Wells 2007).

Diet quality is one of the principal external factors that affect growth and developmental rates, the length of the larval period, the timing of metamorphosis as well as individual post-metamorphic performance as juveniles and adults in the terrestrial environment (Kupferberg et al. 1994; Ramamonjisoa et al. 2016). Tadpoles that are fed high-quality diets often experience enhanced growth and developmental rates and are able to initiate metamorphosis early at optimal sizes (Kupferberg 1997; Álvarez and Nicieza 2002). This is of upmost importance since the sooner an individual can undergo metamorphosis at the largest size, the risk of being predated as a juvenile decreases. Moreover, they reach maturity at a younger age, which has a positive effect on their fecundity as adults (Smith 1987; Rose 2005).

Although diet plays an important role in the life of a tadpole it remains largely unstudied and misunderstood; for many species their nutritional requirements and feeding behaviour are still unknown, which emphasises the need for more research in this area (Altig et al. 2007). The protein content of diets greatly affects all aspects of larval growth, since high-protein diets produce larger tadpoles with a faster growth rate than low-protein diets (Crump 1990;

Kupferberg et al. 1994); although the amount of protein required may vary (Martins et al. 2013; Ramamonjisoa et al. 2016).

Understanding how a diet affects larval growth, development and metamorphosis is also essential for the implementation of breeding programs (Martins et al. 2013). Until recently, these studies were mostly performed with temperate species or those used in aquaculture (Alford and Harris 1988; Kupferberg 1997; Skelly 1997; Richter-Boix et al. 2007; Martins et al. 2013), while neotropical species have largely been neglected. Considering that most amphibian declines have occurred in recent years in the neotropical region (Stuart et al. 2004) it is critical to carry out investigations with native species.

Consequently, we decided to investigate the effect of diet on the larval growth and metamorphosis of tadpoles of a poorly studied hylid species, the Yucatan Casque-Headed treefrog *Triprion petasatus* (Cope, 1865). This arboreal species is endemic to the Yucatan Peninsula (Duellman 2001) and subjected to special protection under national Mexican environmental law NOM-059 (SEMARNAT 2010). Even though this anuran species currently has stable population sizes and is categorized as Least Concerned on the IUCN Red List (Santos-Barrera et al. 2004), its endemic status and position as a member of the family Hylidae, one of the four anuran groups that have experienced significant population declines (Stuart et al. 2004), make it a species of special interest. *Triprion petasatus* tadpoles are known to be benthic (Altig and McDiarmid 1999), but their natural diet, growth rate, duration of the larval period and metamorphosis have yet to be determined (Duellman and Trueb Klass 1964).

We experimentally evaluated the effects of a high-protein diet (fish food) and a more natural diet (leaf litter, detritus, cladocerans, and ostracods) on: growth rate, length of larval period and

of metamorphosis, size at metamorphosis and as a froglet. We also described the diet of wild tadpoles to provide new information about their feeding habits in a natural setting.

We hypothesized that *T. petasatus* tadpoles raised on a high-protein diet would experience a faster growth rate, shortened larval and metamorphic periods, and reach a larger size than those fed a more natural diet. We also assumed that the diet of wild tadpoles would be composed of a mixture of detritus, algae, and zooplankton, all of which are readily available in aquatic ecosystems.

MATERIALS AND METHODS

Study site.—We carried out all aspects of this study from the experiment to the collection of tadpoles for the diet analysis at El Colegio de la Frontera Sur (ECOSUR), campus Chetumal, Quintana Roo, Mexico (18°32'40.90"N, 88°15'49.88"W). This region of Mexico has a warm sub-humid climate with rains in the summer and semi-deciduous forest (Ek Díaz 2011; Herrera Sansores 2011). On campus, there is a decommissioned boat that has been left untouched for four years and has since permanently filled with rainwater, organic material, communities of zooplankton, and insect larvae (Fig. 1A). *Triprion petasatus* adults naturally come to this aquatic ecosystem to breed and spawn, as it is surrounded by abundant foliage (Brianna Jacobson, pers. obs.). From this artificial aquatic system (Fig. 1B) we collected egg masses for the experiment and individuals for the diet analysis. The diet experiment was carried out from 15 May 2017 to 28 July 2017 in an open-air laboratory, also on campus, with a sheet metal roof, enclosed with wire mesh.

Experimental design.—We used two different treatments to determine how diet affects growth and metamorphosis. In treatment one (T1) we raised tadpoles on a high-protein diet composed

of Winfish-Zeigler ® pellets for tilapia (36% protein, 6% fat, 6% ash), while in treatment two (T2) tadpoles were fed a more natural diet (leaf litter, detritus, cladocerans, and ostracods). Each treatment consisted of three replicates (containers measuring 43 cm x 23 cm x 14.8 cm), which in T1 we filled with 8.4 L of rainwater from a holding tank. To simulate natural conditions, T2 containers were filled with 8.4 L of water, 30 g of dry leaf litter, detritus, cladocerans, and ostracods from the artificial aquatic system (decommissioned boat).

We exposed all containers to ambient temperature (25–30° C) and natural photoperiods, but controlled dissolved oxygen levels with an oxygen pump for aquariums (Hagen® Elite 802). Water volume was kept constant throughout the experiment in both treatments, and we covered each container with mosquito netting to prevent the entrance of insects and predators.

On 15 May 2017, we collected *T. petasatus* eggs from four different clutches (three from the decommissioned boat and one from a water tank in the open-air laboratory). The Gosner staging system (Gosner 1960) was used to classify tadpole development. By the morning of 17 May 2017 all tadpoles had hatched and reached developmental stage 25 (feeding tadpole with spiracle present). We randomly selected 96 of these tadpoles and placed 16 individuals in each container in both treatments. Additionally, 74 tadpoles were placed in a 29.6 L divided fish tank in conditions similar to T1 and T2. These tadpoles were used to replace dead individuals in either of the two treatments, thus density remained constant until tadpoles began to metamorphose.

We provided food to tadpoles in both treatments as close to *ad libitum* as possible. Tadpoles in T1 were initially fed a quantity of food equivalent to 2.5 % of their total body mass twice a day, but we increased this amount to 3.5 % during the first week. For tadpoles in T2 we added partially decomposed leaf litter (3.75 g per individual) from the artificial aquatic system when

individuals had consumed nearly all that had initially been provided and more cladocerans to each container when their abundance decreased. We cleaned and changed 50 % of the water in every container in T1 once in the first week and every 2 d in the following weeks, while in T2 containers were left relatively untouched.

When tadpoles were on the verge of initiating metamorphosis we checked containers throughout the day and removed any individual that had reached stage 42 (fore limbs emerge and tadpoles become metamorphs) and placed them in plastic containers (22 cm x 22 cm x 15.5 cm) filled with 3 cm of water until they fully emerged from the aquatic environment. They were then transferred to plastic bags with 1 cm of water where they remained until their tails were completely absorbed (stage 46), thus signalling the end of metamorphosis and the emergence of a juvenile frog (froglet). We released all froglets at dusk close to the artificial aquatic system previously mentioned.

Growth and metamorphosis.—To establish mean initial body mass and total length (TL) we weighed and measured 40 randomly selected individuals (stage 25) using an analytical balance (0.0001 g precision) and a digital caliper (0.01 mm precision). These individuals were from the same four egg masses but were not part of the experiment, thus avoiding unnecessary fatalities, since tadpoles in this stage are extremely fragile (Kupferberg et al. 1994).

We weighed and measured tadpoles in both treatments weekly and when they initiated metamorphosis (stage 42), whereupon we recorded the number of days it took each tadpole to reach this stage (length of the larval period). Individual growth rates were calculated for both treatments as: body mass at stage 42/length of the larval period (Richter-Boix et al. 2011; Székely et al. 2017). Once individuals completed metamorphosis we recorded froglet weight

and snout-vent length (SVL) and annotated the time elapsed from the onset of this process to determine the duration of metamorphosis.

Diet analysis.—We collected 120 individuals in eight different developmental stages (15 individuals in stages: 27, 28, 31, 33, 34, 35, 39, and 42) from two breeding seasons (August to September 2016 and May to July 2017). We preserved all tadpoles in 10% neutralized formalin (McDiarmid and Altig 1999b), and transferred them to 4% neutralized formalin to avoid loss of algae pigmentation. We recorded total length, body and tail length, internarial and interorbital distance, and intestinal length to the nearest 0.01 mm as recommended by McDiarmid and Altig (1999b). We then carefully dissected the tadpoles under a stereoscopic microscope and removed the intestinal tract. Only the foregut and midgut were examined as the effects of digestion are less pronounced in these regions, thus facilitating the identification of food items. We placed the gut contents on microscope slides with 4% formalin-glycerin and proceeded to quantify food items under a compound light microscope.

Statistical analysis.—We tested all the data for normality and homogeneity of variances using the Shapiro-Wilks and Levine tests. For normal and homoscedastic data, we applied independent sample Student´s *t*-tests (IBM® SPSS® Statistics, USA) to determine differences between metamorph and froglet body mass and length of individuals in each treatment. To calculate differences in growth rate (normal, heteroscedastic data) we used the Welch's *t*-test. To evaluate differences in the duration of the larval period and metamorphosis (non-normal and heteroscedastic data) we used nonparametric Mann-Whitney tests.

To characterize the tadpole diet we calculated frequency of occurrence and numeric frequency for all food items (see Hyslop 1980). We carried out a one-way permutational multivariate

analysis of variance (PERMANOVA) in PAST 3.10 (Hammer et al. 2001) to detect differences in the diet of tadpoles in the eight development stages. We used square root transformed data and the Bray-Curtis similarity index with 9999 permutations to perform this analysis. We then used a pairwise analysis as a *post hoc* test and corrected P-values with the sequential Bonferroni method. We took P-values less than 0.05 to indicate significant difference and used a SIMPER analysis using the Bray-Curtis similarity measure to determine which food item groups contributed most to the differences observed in the diet of tadpoles in the different development stages.

RESULTS

Growth and metamorphosis.—Tadpole mean initial body mass was 0.0129 ± (SE) 0.0002 g (range, 0.0058–0.078 g). During the first week, individuals in both treatments reached similar sizes, differing only by 0.04 g. However, drastic differences in size became apparent by week two (Fig. 2), when mean body mass in T1 tripled (0.8997 ± [SE] 0.0369 g [range, 0.3356–1.406 g]), while tadpoles in T2 only marginally increased in mean body mass (0.2447 ± [SE] 0.0161 g [range, 0.0742–0.4783 g]). Individuals in T2 experienced only slight increases in mean body mass over the course of 10 weeks (Fig. 2), while tadpoles in T1 continued to rapidly increase in size and by week three only four individuals were left that had not initiated metamorphosis, and they achieved the largest mean body mass (1.061 ± [SE] 0.0737 g [range, 0.7658–1.396 g]). Between weeks three and four all tadpoles in T1 had reached the end of the larval period and initiated metamorphosis (Fig. 2).

One individual in T2 with the shortest larval period was the first to initiate metamorphosis 17 d after the experiment started. Only three more tadpoles from this treatment metamorphosed, but

not until days 47, 49 and 69. For tadpoles in T1, eight individuals initiated metamorphosis on day 18, while the remaining 30 individuals metamorphosed within the next 8 d, thus the larval period for individuals in T1 was, in general, less variable and shorter than for tadpoles in T2 (Fig. 3). The duration of the larval period was significantly different between treatments (U = 144, $P < 0.001$) after we excluded the individual that metamorphosed on day 17.

The growth rates of individuals from both treatments were significantly different (t = 27.518, df = 30.266, $P < 0.001$). T1 individuals experienced higher growth rates than the four individuals that metamorphosed in T2 (0.028–0.056 g/day vs. 0.0047–0.0073 g/day; Fig. 4).

Mortality during the larval period was higher in T2 (13 died, 27%) than in T1 (2 died, 4%). Events of cannibalism where individuals consumed conspecifics that were alive or that had recently died occurred on more than one occasion in T2, but never in T1. Some individuals also experienced morphological changes as their intestines shrunk, their bodies took on a triangular appearance, and their gills turned grey. These individuals lost control of their buoyancy, died and were eaten by other tadpoles. Although mortality was lower in T1, we noticed that two individuals developed abnormalities (crooked tails), but did not die over the course of the experiment.

Size at metamorphosis was significantly different between T1 and T2 (body mass: t = 6.773, df = 49, $P < 0.001$ and TL: t = 7.881, df = 49, $P < 0.001$). Individuals from T1 were heavier and longer than those in T2 (0.5915–1.0499 g and 40.96–51.38 mm TL vs. 0.2235–0.3461 g and 31.11–35.44 mm TL) (Fig. 5A, B).

The duration of metamorphosis in both treatments was significantly different ($U = 36$, $P < 0.05$). Individuals from T1 completed this phase within 3–4 d, whereas the four individuals that metamorphosed in T2 emerged as froglets within 2–3 d (Fig. 6).

Froglet size was also significantly different (body mass: $t = 10.39$, df = 48, $P < 0.001$ and SVL: $t = 10.419$, df = 48, $P < 0.001$). Individuals from T1 (n = 46) were both heavier and longer (0.3422–0.5769 g and 15.65–19.92 mm SVL) than those from T2 (n = 4; 0.1087–0.1817 g and 11.97–14.19 mm SVL) (Fig. 7A, B). Visually, froglets from T1 appeared to be much more robust in comparison with those from T2 which seemed almost emaciated (Fig. 8A, B).

Diet analysis.—The 120 tadpoles (stages 27–42) that we examined ranged from a mean of 16.71 ± (SE) 0.72 mm (range, 12.71–21.75 mm) TL to 36.86 ± (SE) 0.64 mm (range, 30.98–41.35 mm) TL (Table 1).

We identified 32 different food items organized into 13 groups (Table 2). Detritus (mainly unidentified plant material and parts of arthropods, but also eggs, pollen, and spores) was the most frequent group and occurred in every individual in all developmental stages. Of this group, plant material together with arthropod parts were the most important in terms of numerical frequency in stages 31, 34, 35, and 42.

The algae and fungi groups were also present in all developmental stages, although not in all individuals. The euglenoid *Phacus* was the most important food item in terms of numerical frequency for four development stages (28, 33, and 39). Meanwhile, the cyanophyceans *Chroococcus* and *Lyngbya* were the most important food items for individuals in stage 27 and they were also consistently present in all developmental stages, with the exception of stage 42.

The cladoceran *Moina*, ostracods (Cyprididae), rotifers (Bdelloidea and *Lecane bulla*), Ciliophora, and dipteran larvae were important animal components of the tadpole diet. These organisms were present in all developmental stages with the exception of Bdelloidea and Ciliophora. Two individuals in stage 27 also had tadpole tissue in their gut contents.

The diet was significantly different in abundance among the distinct developmental stages (*pseudo-F* = 9.635, df =7, $P < 0.001$). The pairwise *post hoc* analysis revealed that the diets of stages 27 and 42 were both different from each other and all six other stages. The diets of stages 35 and 39 were both different from each other and the five other stages (stage 35 was similar to 34; stage 39 was similar to 28). The SIMPER analysis indicated that these differences were mostly due to the abundance of algae (*Chrooccocus*, *Lyngbya*, and *Phacus*) and detritus (plant material and arthropod parts). Stage 27 had the greatest variety of food items (26) and the elevated abundance of *Chrooccocus* and *Lyngbya* and the absence of *Phacus* is what set this stage apart from all others. Conversely, stage 42 had the lowest variety of food items (11) and the highest abundance of plant material and arthropod parts and reduced *Phacus* abundance, which caused the diet in this stage to be different. The high abundance of plant material and arthropod parts, and low *Phacus* abundance is what contributed most to the differences in the diet between stage 35 and the six other stages. For individuals in stage 39, the elevated abundance of *Phacus* is what caused this stage to be similar to stage 28, but different from all others (Table 2).

DISCUSSION

Growth and metamorphosis.—Our results demonstrate that diet plays an important role in the growth and metamorphosis of *T. petasatus* tadpoles. The high-protein experimental diet

minimized larval period variability and mortality and increased growth, thus allowing individuals to metamorphose early at large sizes. These positive effects have also been documented in other anuran species raised on high-protein diets, such as *Epidalea calamita* and *Osteopilus septentrionalis* (Kupferberg et al. 1994; Babbit and Meshaka Jr. 2000; Martins et al. 2013; Ramamonjisoa et al. 2016) and support the assumption that an increase in the protein content of a diet will improve tadpole growth (Kupferberg 1997).

Conversely, low-protein diets have been known to cause prolonged larval periods, low growth rates, increased mortality and small sized individuals (Kupferberg et al. 1994; Álvarez and Nicieza 2002; Ramamonjisoa et al. 2016), just as we observed in tadpoles fed the more natural diet. A low-protein diet strictly composed of leaf litter caused *Rhacophorus arboreus* tadpoles to experience a long larval period, slow growth rate, small mass at metamorphosis, and only some individuals metamorphosed (Ramamonjisoa et al. 2016). *Hyla regilla* tadpoles also experienced low growth and development rates and high mortality even when fed suspended detritus mixed with algae cells (Kupferberg et al. 1994). Even though tadpoles in T2 had the opportunity to consume zooplankton, an additional source of protein, they experienced the same growth trends as these two other species raised on similar low-protein diets, and were not able to grow and develop at the same rate as individuals fed the high-protein diet in T1. This can be attributed to a lack of other important diet components, which we explain in further detail in the discussion concerning the diet analysis.

The high-protein diet also produced record size *T. petasatus* metamorphs and froglets in comparison with those reported by Duellman and Trueb Klass (1964) (metamorphs: 46.12 mm vs. 35 mm TL; froglets: 17.66 mm vs. 15.8 mm SVL). On the other hand, individuals fed the

more natural diet were smaller (metamorphs: 29.40 mm; froglets: 12.83 mm) than those that Duellman and Trueb Klass (1964) measured.

These differences in metamorph size, and length and variability of the larval period among treatments can be explained in an ecological context with the Wilbur-Collins model (Wilbur and Collins 1973). This model assumes that once an individual reaches a minimum body size it can either metamorphose at this small size if environmental conditions are unfavorable or stay in the aquatic environment if resources are abundant and continue to grow and metamorphose at a larger maximum size (Wilbur and Collins 1973). The small size of metamorphs in T2 indicates that tadpoles metamorphosed once they reached minimum size, whereas tadpoles in T1 were able to take advantage of the high quality food and metamorphose at a larger maximum size. Variation in the length of the larval period occurs because individuals are not able to reach minimum body size at the same time due to differences in their competitive abilities (Wilbur and Collins 1973). This explains why the length of the larval period was so different in T2 where one individual metamorphosed before all others, while the majority remained as larval tadpoles.

Contrary to our initial hypothesis, the duration of metamorphosis was shorter for the smaller individuals in T2 than in T1. This could be due to differences in tadpole size, since smaller individuals might metamorphose faster than larger ones simply because there is less tissue that needs to be modified and reabsorbed (Downe et al. 2004). This could also be a strategy to escape unfavorable environments since tadpoles in T2 that metamorphosed in a shorter period of time were able to leave adverse growing conditions in the aquatic environment and begin feeding sooner in the terrestrial habitat. Székely et al. (2017) also suggested that a short duration of metamorphosis allowed *Ceratophrys stolzmanni* tadpoles to transition faster to the terrestrial environment to avoid pond desiccation.

The events of cannibalism that we observed in this study suggest that *T. petasatus* tadpoles are facultative cannibals. Tadpoles often resort to cannibalism when confronted with unfavorable food conditions or high tadpole population densities (Babbit and Meshaka Jr. 2000). Even though population densities were low in T2, we assume that a diet based almost entirely on leaf litter does not meet tadpole nutritional requirements and individuals began to practice cannibalism in order to survive. Resorting to cannibalism has been known to shorten larval periods and increase size at metamorphosis for the treefrog *Osteopilus septentrionalis* (Babbit and Meshaka Jr. 2000).

Diet analysis.—Evidently, *T. petasatus* tadpoles are omnivores and generalists since they consumed a wide variety of plant and animal material, similar to *Pseudis paradoxa platensis*, *Leptodactylus fuscus*, and *Scinax angrensis* tadpoles (Arias et al. 2002; Rossa-Feres et al. 2004; Sousa Filho et al. 2007).

Detritus and algae are particularly important elements of the tadpole diet considering how frequently individuals consumed these food groups. The fact that *T. petasatus* tadpoles are benthic (Altig and McDiarmid 1999) explains why benthic items, such as detritus, bdelloid rotifers, and ostracods were usually present in the gut contents. Detritus and rotifers could be important sources of nutrients for these tadpoles since many microorganisms, such as bacteria, are associated with the former (Akers et al. 2008) and the latter is recognized as an essential component of planktivorous fish, other omnivorous tadpoles, and aquatic invertebrate diets (Rossa-Feres et al. 2004; Wallace and Snell 2010).

Moina and dipteran larvae are another important source of animal protein that we found in the gut contents, although they were less abundant than detritus and algae. *Moina* is often used as

live food for aquaculture systems due to its nutritional value (Islam et al. 2017). The presence of dipteran larvae in the gut contents suggests that *T. petasatus* tadpoles could act as a form of biological control of insect populations, such as mosquitos, like in other anuran species (Mokany and Shine 2003).

The diet among developmental stages differed in composition and abundance of food items, although a potential explanation is not clear. Sousa Filho et al. (2007) attributed changes in the abundance of food items in the diet of *Scinax angrensis* tadpoles to an increase in tadpole mouth size in later developmental stages (food consumption increased with age). We could not determine if such a relationship occurred because tadpoles were from two different breeding seasons (2016 and 2017). Stage 27 tadpoles were only from 2016, whereas all other stages contained a mix of individuals from both seasons. Food availability changes frequently because phytoplankton and zooplankton experience temporal and spatial variations in population abundance in aquatic systems (Lampert and Sommer 2007). Although we did not examine water samples from the aquatic system, we noticed that tadpoles from 2016 contained a wide variety of cyanobacteria, while *Phacus* dominated gut contents from 2017. Changes in the availability of food from one year to another made it impossible to attribute differences in food abundance or diet composition to tadpole size or age. We could not determine if one stage consumed less of a specific food item or even a certain type by choice or simply because it was not as abundant or even present in the aquatic system as it had been the year before. However, the difference in diet between stage 42 and all other stages can be partially explained. Individuals had little to no food items in the fore and midguts because tadpoles stop eating in this stage (42) due to morphological changes in the digestive system associated with metamorphosis (Kupferberg et al. 1994).

Even though individuals in T2 consumed a similar diet as the wild population of *T. petasatus* tadpoles (detritus, leaf litter, cladocerans, and ostracods) they still suffered poor growth. This could be because algae were not actively provided to individuals, which represented a large and important part of the diet of the wild population, thus preventing tadpoles from achieving higher growth and development rates. Algae is a main component of the diet of omnivorous tadpoles and individuals can grow and develop effectively when raised on a diet composed entirely of this food group (Kupferberg et al. 1994; Pryor 2009).

We also noticed that a population of *T. petasatus* tadpoles in the artificial aquatic system (decommissioned boat) also experienced slow growth and development, and individuals were also prone to cannibalism and developed the same triangular body shape that we observed in T2 and eventually died. Babbit and Meshaka Jr. (2000) also noticed this phenomenon in wild populations of Cuban treefrogs and associated it with poor environmental conditions (low quality diet). This suggests that even a natural diet, if not properly balanced, may not always promote successful growth and development, just like what occurred with tadpoles in T2.

Our results suggest that *T. petasatus* tadpoles require a certain level of protein to reach optimum growth and development. However, determining the minimum and maximum amounts of necessary protein is a subject for future investigations. High-protein diets have been known to cause abnormalities, such as the development of short legs, in omnivorous tadpoles, as well as subpar fitness and slow growth in post-metamorphic stages (Tarvin et al. 2015; Ramamonjisoa et al. 2016). The presence of tadpoles with curved tails in T1 seems to support this fact, although it is necessary to evaluate post-metamorphic performance in order to reach a definitive conclusion.

This is the first study to provide information concerning *T. petasatus* tadpole diet, growth rate, duration of the larval period and of metamorphosis. Our results showed that high-protein diets positively affect growth and development, enabling tadpoles of this species to metamorphose at a larger size and in a short period of time. Given the endemic and protected status of this species, the results of this study may be useful for future conservation efforts, especially if captive breeding is required.

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TABLES

TABLE 1. Mean (± SE) *Triprion petasatus* tadpole measurements for each Gosner development stage examined in the diet analysis. Sample size was the same for all stages (n=15). BL = body length, IND = internarial distance, INT = total intestine length, IOD = interorbital distance, TAL= tail length, TL = total length.

Stage	TL (mm)	BL (mm)	TAL (mm)	IOD (mm)	IND (mm)	INT (mm)
27	16.71 ± 0.72	7.22 ± 0.34	9.30 ± 0.46	3.27 ± 0.14	1.59 ± 0.08	48.69 ± 4.39
28	25.86 ± 0.76	10.55 ± 0.28	15.23 ± 0.51	5.41 ± 0.13	2.41 ± 0.04	103.92 ± 10.9

31	29.52 ± 0.11	11.69 ± 0.03	17.80 ± 0.08	5.92 ± 0.03	2.60 ± 0.02	124.40 ± 1.26
33	30.60 ± 0.43	12.30 ± 0.21	18.34 ± 0.28	6.24 ± 0.12	2.72 ± 0.06	128.47 ± 6.45
34	31.21 ± 0.42	12.29 ± 0.25	18.73 ± 0.21	6.14 ± 0.12	2.54 ± 0.09	140.16 ± 6.08
35	33.80 ± 0.37	13.24 ± 0.15	20.54 ± 0.27	6.65 ± 0.1	2.68 ± 0.1	170.70 ± 5.35
39	36.83 ± 0.51	13.06 ± 0.16	23.64 ± 0.46	6.90 ± 0.06	2.43 ± 0.1	119.95 ± 6.37
42	36.86 ± 0.64	13.02 ± 0.23	24.08 ± 0.49	5.94 ± 0.11	1.35 ± 0.04	29.27 ± 2.07

TABLE 2. Diet composition of *Triprion petasatus* tadpoles from eight different Gosner development stages (27–42). FO = % of frequency of occurrence, N = % of numeric frequency, and * = food item that contributed the most to differences in the diet among stages (result of the SIMPER analysis).

Food items	**Stages**															
	27		28		31		33		34		35		39		42	
	FO	N	FO	N	FO	N	FO	N	FO	N	FO	N	FO	N	FO	N
FUNGI																
Trichocomaceae	80	0.71	20	0.1	7	0.27	13	0.2	7	0.40	20	1.09	33	0.53	13	1.6
ALGAE																
Unidentified	27	0.43	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Cyanophyceae																
Chroococcus *	100	72.01	47	0.18	7	1.61	13	0.4	7	0.05	13	0.36	7	0.03	—	—
Anabaena	20	0.2	—	—	—	—	—	—	—	—	—	—	7	0.01	—	—
Lyngbya *	100	14.41	53	0.26	13	1.61	20	0.15	7	0.1	20	0.57	33	0.32	—	—
Oscillatoriales	7	0.08	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Euglenophyceae																
Phacus *	—	—	33	90.53	13	0.27	27	50.47	27	41.19	13	0.1	60	88.03	7	0.81
Chlorophyceae																
Gloeocystis	7	0.04	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Chlorella	7	0.02	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Ulothrix	7	0.04	—	—	—	—	—	—	7	0.05	—	—	—	—	—	—
Oedogonium	13	0.33	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Cosmarium	67	1.52	—	—	—	—	—	—	—	—	7	0.05	7	0.01	—	—
CILIOPHORA	73	0.37	67	0.39	7	0.13	20	0.45	7	0.25	40	0.99	33	0.63	—	—
ROTIFERA																

Unidentified	13	0.06	—	—	—	—	7	0.05	—	—	7	0.05	13	0.01	—	—
Bdelloidea	7	0.02	20	0.1	7	0.13	13	0.1	—	—	—	—	60	0.13	—	—
Lecane bulla	33	0.12	13	0.03	20	0.4	27	0.3	53	0.76	73	0.78	20	0.04	13	1.63
Lepadella	—	—	—	—	—	—	—	—	—	—	7	0.05	7	0.01	—	—
NEMATODA																
Unidentified	—	—	—	—	—	—	—	—	—	—	13	0.1	—	—	—	—
Dorylaiminae	7	0.02	—	—	—	—	—	—	—	—	—	—	—	—	—	—
OLIGOCHAETA	—	—	—	—	—	—	—	—	—	—	7	0.05	7	0.01	—	—
CLADOCERA																
Moina	100	0.53	73	0.4	93	4.03	60	2.27	93	2.83	100	4.69	80	0.56	27	5.69
OSTRACODA																
Cyprididae	27	0.08	27	0.06	40	0.81	40	0.35	53	0.71	73	0.68	47	0.06	7	0.81
COPEPODA																
Nauplius	7	0.02	—	—	—	—	—	—	—	—	—	—	—	—	—	—
DIPTERA																
Larvae	13	0.04	13	0.03	27	0.54	20	0.15	33	0.3	27	0.26	27	0.04	7	0.81
ACARI																
Unidentified	—	—	7	0.01	7	0.13	—	—	—	—	20	0.16	7	0.01	—	—
Oribatida	—	—	—	—	7	0.13	—	—	—	—	—	—	—	—	—	—
TADPOLE TISSUE	13	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
DETRITUS																
Plant material *	100	3.52	100	3.02	93	32.53	93	21.7	100	24.54	100	44.16	100	4.14	53	44.72
Arthopod parts*	100	4.23	93	4.23	100	51.88	100	20.66	100	26.52	100	40.2	100	4.74	60	33.33
Eggs	40	0.2	40	0.11	20	0.54	33	0.4	53	0.61	73	1.46	87	0.16	7	0.81
Resistance eggs	7	0.02	7	0.01	7	0.13	7	0.05	—	—	20	0.16	7	0.01	13	1.63
Pollen/spores	80	0.98	73	0.54	53	4.17	53	2.32	80	1.77	87	4.01	93	0.52	40	8.13

FIGURES

FIGURE 1. Study site. A) The decommissioned boat that now acts as a breeding site for *Triprion petasatus*. (Photographed by Brianna Jacobson). B) Interior of the boat. We collected egg masses and tadpoles from the compartments. (Photographed by José Rogelio Cedeño-Vázquez).

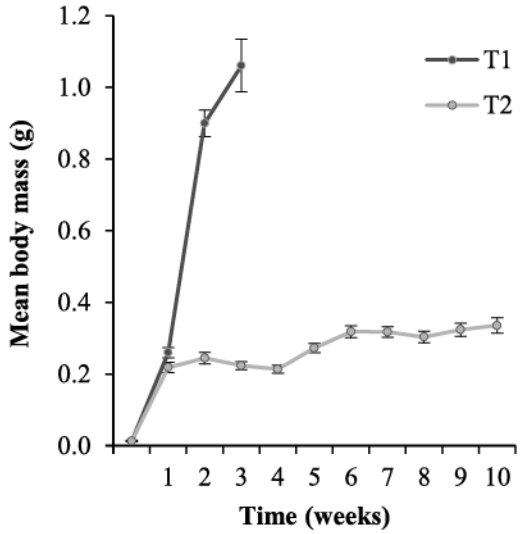

FIGURE 2. Mean body mass (± SE) of *Triprion petasatus* tadpoles in the two treatments (T1 and T2) measured weekly until they reached Gosner stage 42.

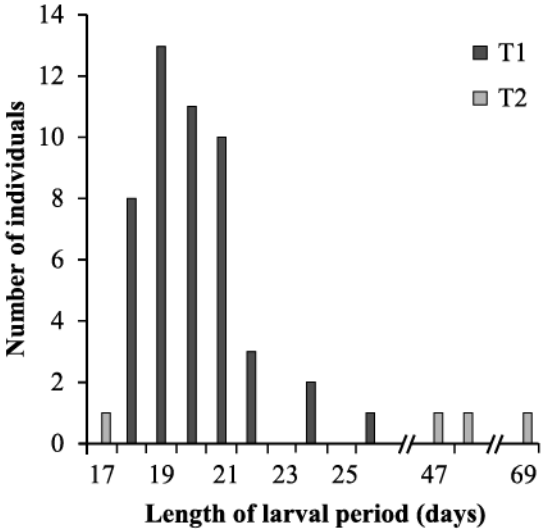

FIGURE 3. Length of larval period for *Triprion petasatus* tadpoles in the two treatments (T1 and T2).

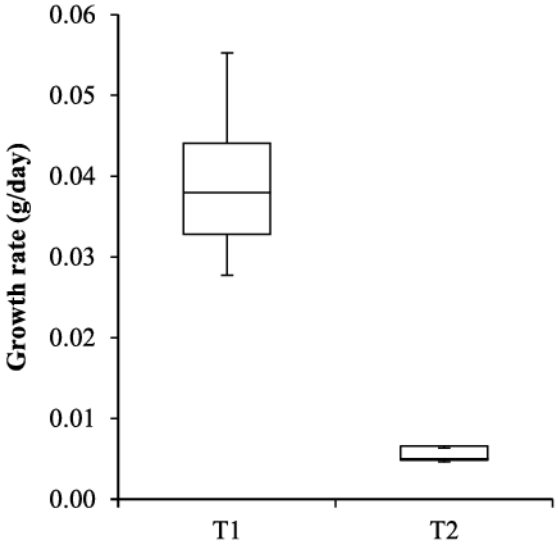

FIGURE 4. *Triprion petasatus* tadpole growth rate during the larval period in the two treatments (T1 and T2). The boxplot reveals the distribution of data where boxes include data in the 25–75 percentiles and median values (middle lines), while whiskers represent minimum and maximum values.

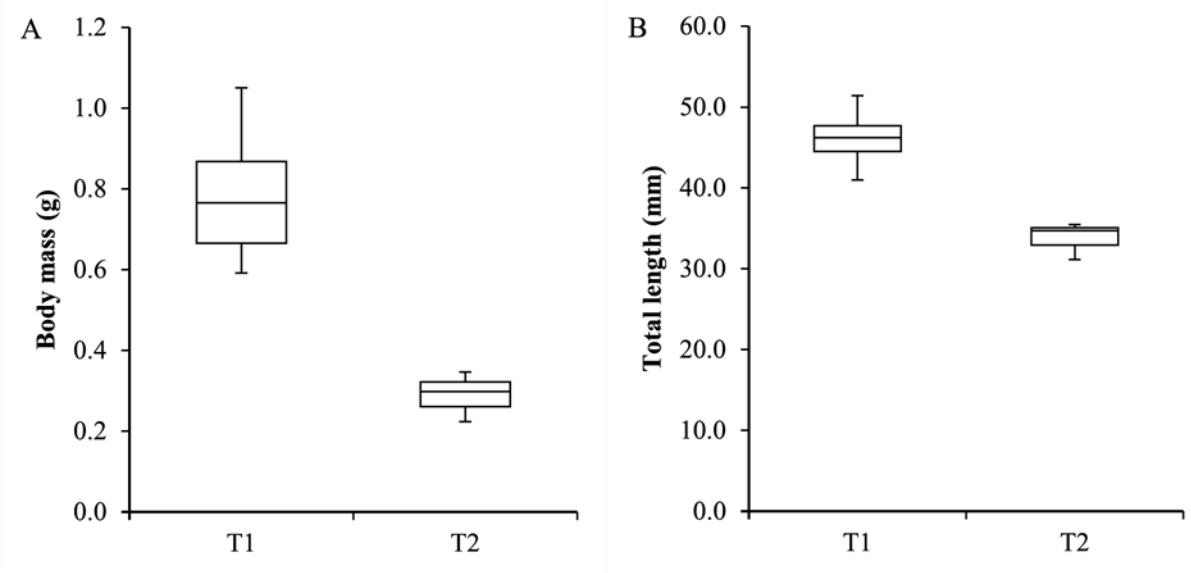

FIGURE 5. *Triprion petasatus* size at metamorphosis. A) Body mass of individuals in the two treatments (T1 and T2). B) Total length of individuals in T1 and T2. Boxplots reveal the distribution of data just as in Fig. 4.

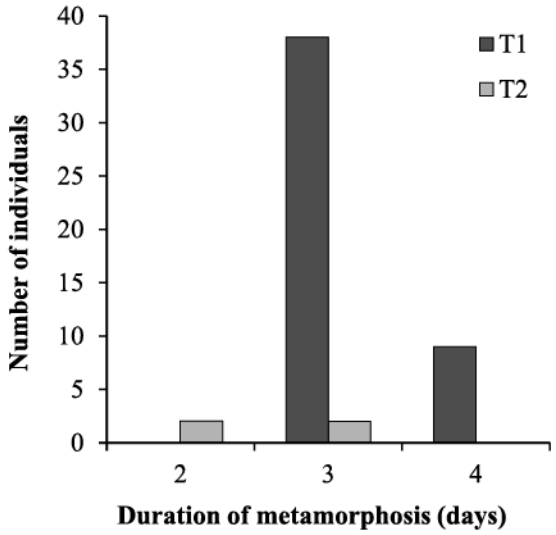

FIGURE 6. The duration of metamorphosis for *Triprion petasatus* tadpoles in the two treatments (T1 and T2).

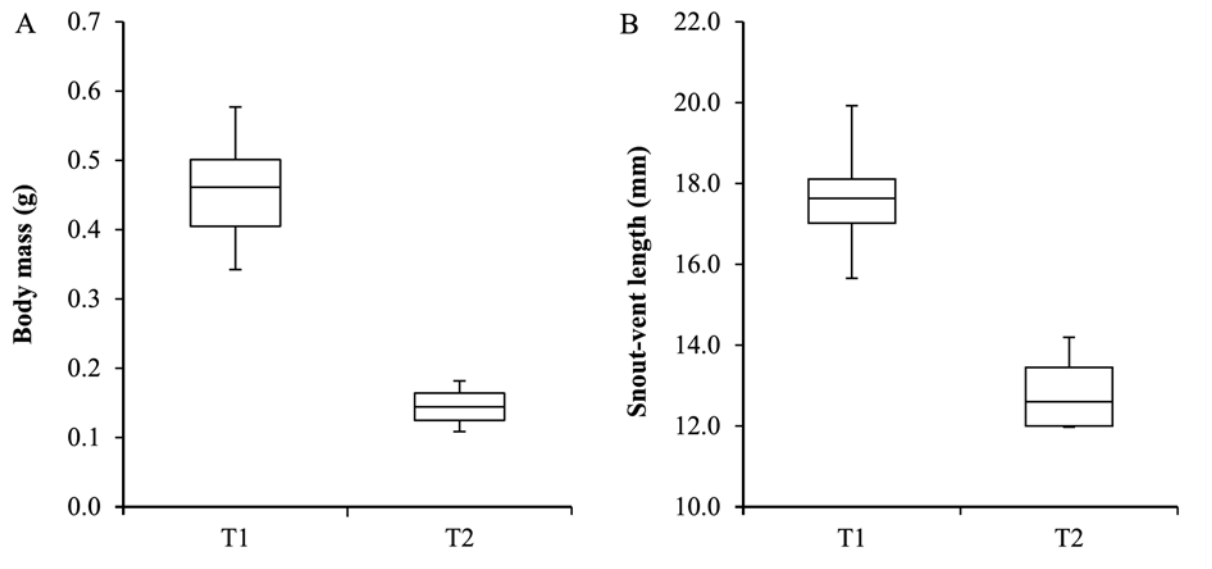

FIGURE 7. Size of *Triprion petasatus* froglets. A) Froglet body mass for individuals in both treatments (T1 and T2). B) Froglet snout-vent length for individuals in T1 and T2. Boxplots reveal the distribution of data just as in Fig. 4.

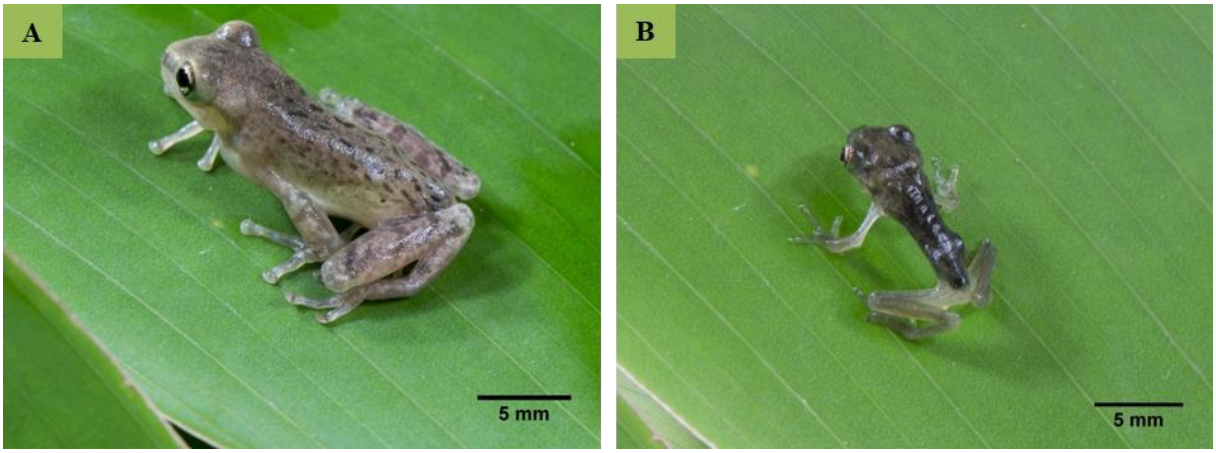

FIGURE 8. Appearance of *Triprion petasatus* froglets. A) Dorsal-lateral view of a froglet from T1. B) Dorsal view of a froglet from T2. (Photographed by Humberto Bahena-Basave).

AUTHOR BIOGRAPHIES

BRIANNA JACOBSON first became fascinated with tadpoles when she studied their relationship with zooplankton as part of her undergraduate thesis for which she received a Bachelor's degree in Natural Resource Management from the University of Quintana Roo in Cozumel, Mexico. After this first foray into the world of amphibians, she was given the opportunity to continue studying neotropical tadpoles in Mexico and is currently in the process of finishing a Master of

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JOSÉ ROGELIO CEDEÑO-VÁZQUEZ received his B.Sc. in Biology from the Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Michoacán, Mexico in 1995, and his Master's and Doctoral degrees at El Colegio de la Frontera Sur (ECOSUR) in 2002 and 2008, respectively. From 2008 to 2012 he was a researcher and biology instructor at Instituto Tecnológico de Chetumal, Quintana Roo, Mexico. He has been a professor and researcher in the Department of Systematics and Aquatic Ecology at ECOSUR, Chetumal since 2013; he is also the Curator of Herpetology at the Museo de Zoología of ECOSUR. His main research interests include systematics, ecology, conservation, and management of amphibians and reptiles. He is a member of the Sistema Nacional de Investigadores (National System of Researchers), of Mexican herpetological associations, and of the IUCN/ SSC-Amphibian and Crocodile Specialist Group. (Photographed by Valentín González-Avila).

JULIO ESPINOZA-AVALOS received his Bachelor of Science in Oceanology from the Universidad Autónoma de Baja California, Mexico, a Master of Science in Biology from Dalhousie University, Canada, and a Doctor of Philosophy from the Universidad Autónoma Metropolitana, Mexico. He has carried out studies in cold, temperate, subtropical and tropical subtidal marine waters, mainly concerning the ecological aspects of macroalgae. His recent research is focused on coral-macroalgae interactions in the Mexican Caribbean, in the context of the degradation of coral reefs presently occurring in marine tropical environments. (Photographed by Neidy P. Cetz-Navarro).

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Capítulo III. Conclusiones

A partir de este estudio se puede concluir que una dieta rica en proteína favorece el crecimiento de los renacuajos de *Triprion petasatus*. Esta dieta les permite alcanzar tallas más grandes en un corto periodo de tiempo, así como tener baja mortalidad, en contraste con individuos criados con una dieta más natural. Por lo tanto, una dieta compuesta solamente de detritus, hojarasca, cladóceros y ostrácodos no cumple con los requisitos nutricionales de esta especie.

El tipo de dieta también afecta la duración del periodo larval y de la metamorfosis. De acuerdo con una de nuestras hipótesis, una dieta alta en proteína disminuyó la variabilidad y la duración del periodo larval de los renacuajos, mientras que una dieta más natural tuvo el efecto opuesto. Sin embargo, contrario a otra de nuestras hipótesis, una dieta de alta proteína incrementó la duración de la metamorfosis, ya que fue más larga para algunos individuos que para renacuajos alimentados con la dieta más natural.

Los renacuajos de *T. petasatus* son omnívoros, generalistas y caníbales facultativos. Su dieta en el entorno natural consiste mayormente de detritus y materia vegetal (algas), mientras que fuentes de proteína animal son consumidas con menor frecuencia. Las diferencias observadas en la dieta entre etapas de desarrollo se debieron más a cambios en la disponibilidad de alimento entre temporadas reproductivas, que a la edad de los individuos. El comportamiento canibalístico observado en los renacuajos (confirmado en el análisis de la dieta), indica que esta conducta alimentaria, presente en condiciones limitantes de proteína, es una estrategia que aplican los individuos malnutridos de esta especie para incorporar la proteína que requieren a su dieta.

Los resultados obtenidos representan un valioso aporte para la conservación de *T. petasatus* debido a que es una especie poco estudiada y vulnerable por su distribución restringida. De acuerdo con las observaciones de este experimento, los renacuajos son fáciles de criar en condiciones controladas, ya que crecen rápido y presentan bajas tasas de mortalidad con una dieta relativamente económica (comida para tilapia). Esto

significa que podrían ser excelentes candidatos para un programa de crianza en cautiverio para su conservación en caso de que disminuyan sus poblaciones.

Aunque se logró ampliar el conocimiento de los renacuajos de *T. petasatus*, surgieron más temas para investigar, que al abordarlos, permitirían obtener una visión más completa del efecto de la dieta en el ciclo de vida de este anfibio. Por ejemplo, aunque se encontró que la dieta afectó la longitud total y la masa corporal de los individuos, falta comprobar si otras características morfológicas fueron afectadas como el tamaño de la cabeza, el cuerpo, la cola y las piernas traseras como encontraron Martins et al. (2013). Los renacuajos de *T. petasatus* experimentaron un mejor crecimiento y metamorfosis con una dieta con 36 % de proteína; no obstante se sugiere determinar la cantidad óptima que esta especie requiere en su dieta para alcanzar mayores tasas de crecimiento y metamorfosis y para evitar que el exceso de proteína provoque anormalidades en los individuos. Por otra parte, este estudio se enfocó en la influencia de la dieta en la etapa larval; sin embargo, es necesario examinar el efecto de la dieta sobre la adecuación, desempeño y crecimiento de individuos en las etapas post-metamórficas.

Los resultados de este estudio y los que se pueden obtener con la ampliación de otras investigaciones, como las indicadas antes, tomando en cuenta todos los aspectos de su ciclo de vida, podrán en conjunto ayudar a concluir si una dieta con alto contenido de proteína es la más adecuada para *T. petasatus*.

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