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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 diets on growth and metamorphosis of tadpoles of the endemic Yucatan Casque-Headed Treefrog (*Triprion petasatus*). Individuals raised on a high-quality commercial fish diet experienced increased growth, a short larval period, low mortality, and large size at metamorphosis and as juveniles; however, the duration of metamorphosis was slightly 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 appears to be omnivorous and ingests mostly detritus, algae, and zooplankton. There was also a significant difference in the diet among developmental stages and between breeding seasons with a significant interaction between both of these factors. This is the first time that diet, growth rate, length of larval period, and duration of metamorphosis have been studied for *T. petasatus* tadpoles. The results of this study contribute new information on the larval 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 criados con una dieta de alta calidad crecieron más rápido, contaron con un periodo larval corto, baja mortalidad y alcanzaron una talla más grande (como metamorfos y ranas juveniles). Sin embargo, el proceso de metamorfosis fue un poco más corto para los individuos que tuvieron 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 indica que son omnívoros e ingieren mayormente detritus, algas y zooplancton. Además, la dieta fue diferente en las etapas de desarrollo y entre dos temporadas reproductivas y ambos factores interactuaron de una manera significativa. 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 información nueva acerca de la ecología larval 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) because it requires that most anurans spend part of their lives as tadpoles (McDiarmid and Altig 1999a). Tadpoles must grow, develop, and undergo metamorphosis to transition into terrestrial adults (Wells 2007). The endocrine system regulates these processes internally and is simultaneously 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 (Kupferberg et al. 1994) and can influence individual post-metamorphic performance as juvenile frogs and adults in the terrestrial environment (Ramamonjisoa et al. 2016). Tadpoles that eat high-quality diets often experience enhanced growth and development and initiate metamorphosis early at optimal sizes (Kupferberg 1997; Álvarez and Nicieza 2002). Minimizing time to metamorphosis while maximizing

Figure 1. Adult female Yucatan Casque-Headed treefrog (*Triprion petasatus*) from the study site at El Colegio de la Frontera Sur (ECOSUR) campus Chetumal, Quintana Roo, Mexico. (Photographed by Humberto Bahena-Basave).

mass at metamorphosis reduces the risk of juvenile predation and when individuals reach maturity rapidly, there is a positive effect on adult fecundity (Smith 1987; Rose 2005).

Despite the important role of larval diet in the life of a frog, diet is under-studied and poorly understood. The nutritional requirements and feeding behavior of many species remain unknown (Altig et al. 2007), yet this knowledge is essential to implement breeding programs for threatened species (Martins et al. 2013). Protein content in particular, greatly affects all facets of larval growth, because high-protein diets produce larger tadpoles with a faster growth rate than low-protein diets (Crump 1990; Kupferberg et al. 1994). The amount of protein required for growth and development is variable among taxa (Martins et al. 2013; Ramamonjisoa et al. 2016). Until recently, dietary studies have focused primarily on temperate species, or those used in aquaculture, while neotropical species have largely been neglected (Alford and Harris 1988; Kupferberg 1997; Skelly 1997; Richter-Boix et al. 2007; Martins et al. 2013). Considering that the majority of recent amphibian declines have occurred in the neotropics (Stuart et al. 2004), it is critical for conservation to carry out investigations with species native to the region.

Consequently, we investigated the effects of diet on the larval growth and development of a poorly studied hylid species, the Yucatan Casque-Headed treefrog *Triprion petasatus* (Fig. 1). This arboreal species is endemic to the Yucatan Peninsula (Duellman 2001) and receives special protection under national Mexican environmental law NOM-059 (Secretaría de Medio Ambiente y Recursos Naturales 2010). This species is currently considered to have stable population sizes, and assessment by the International Union for Conservation of Nature Red List identifies this species as being of Least Concern with regards to their risk of extinction (Santos-Barrera et al. 2004); however, considering this is an endemic hylid species (one of the four anuran groups that have experienced significant population declines; Stuart et al. 2004); this is a species of special interest. *Triprion petasatus* tadpoles are 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).

In this study, we evaluated the effects of a high-quality diet formulated to promote accelerated growth (commercial fish food) and a more natural diet (leaf litter, cladocerans, and ostracods) on growth rate, length of larval period and of metamorphosis, size at metamorphosis and as a juvenile frog. We hypothesized that *T. petasatus* tadpoles are affected by the quality of their diet. We predicted that tadpoles raised on a high-quality diet would experience a faster growth rate, achieve a larger size, and shortened larval and metamorphic periods than those provided putative dietary resources that naturally occur in the natal wetlands of this species (e.g., detritus, zooplankton, etc.). Additionally, we identified the ingested material of wild tadpoles, improving our understanding of their diet and feeding habits in a natural setting.

Materials and Methods

Study site.—We carried out this study 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 harbors Semi-deciduous Forest (Ek Díaz 2011; Herrera Sansores 2011). For the rearing experiment and dietary analysis, we collected egg masses and tadpoles of *Triprion petasatus* from a decommissioned boat now permanently filled with rainwater, organic material, and communities of zooplankton and insect larvae and used by frogs for breeding (Fig. 2). We chose to use this unusual aquatic system because every year wild *T. petasatus* adults naturally spawned here, it successfully supported the growth and development of their tadpoles (pers. obs.), and it is representative of natural small pools where *T. petasatus* tadpoles would occur in this region. This species breeds in shallow *aguadas*, temporary ponds, and solution pits (shallow craters in limestone rock) enclosed by secondary vegetation (Duellman and Trueb 1964; Carbajal-Márquez et al. 2017). These aquatic systems usually contain organic leaf material, sediments, and are home to invertebrates such as zooplankton, Diptera larvae, insects, phytoplankton, and other anuran tadpoles (Duellman and Trueb 1964; Schmitter-Soto et al. 2002; Carbajal-Márquez et al. 2017), much the same as we observed in the abandoned boat. We conducted

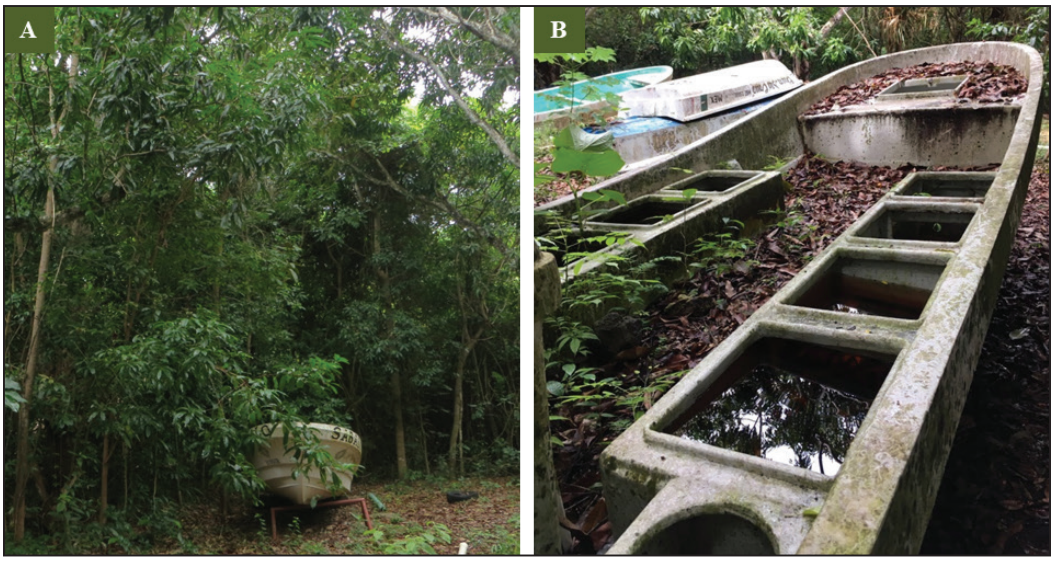

Figure 2. Study site of the Yucatan Casque-Headed treefrog (*Triprion petasatus*) from El Colegio de la Frontera Sur (ECOSUR) campus Chetumal, Quintana Roo, Mexico. (A) The decommissioned boat that acted as a breeding site for *Triprion petasatus*. (Photographed by Brianna Jacobson). (B) Interior of the boat in which we collected egg masses and tadpoles from the compartments. (Photographed by José Rogelio Cedeño-Vázquez).

the diet experiment from 15 May to 28 July 2017 in an open-air laboratory with a sheet metal roof enclosed with wire mesh.

Experimental design.—We used two treatments to determine how diet affects growth and metamorphosis. In treatment one (T1) we raised tadpoles on a commercial fish food diet of Winfish-Zeigler ® (PRONUA S.A. de C.V., Los Belenes Zapopan, Jalisco, Mexico) pellets for Tilapia (*Oreochromis* spp.), which contains 36% protein, 6% fat, 6% ash, and which is known for promoting rapid growth. For treatment two (T2), we fed tadpoles a diet more reflective of a natural setting (leaf litter, cladocerans, and ostracods). Each treatment consisted of three replicates (containers measuring 43 × 23 × 14.8 cm) filled with 8.4 L of rainwater from a holding tank. For T2, we added 30 g of dry leaf litter, cladocerans, and ostracods from the abandoned boat.

We exposed all six containers to ambient temperature (25–30° C) and natural photoperiods (13L:11D) and we aerated them with aquarium bubblers. We kept water volume constant throughout the experiment in both treatments and covered each container with mosquito netting to prevent the entrance of insects and predators. We also measured pH, which ranged from 7.2–8.4, throughout the duration of the experiment and never differed more than 1 pH unit between treatments.

On 15 May 2017, we collected *T. petasatus* eggs from four clutches (three from the decommissioned boat and one from a water tank in the open-air laboratory). Within two days, all the tadpoles had hatched and had achieved the free feeding developmental stage 25 (Gosner 1960). We randomly selected 96 of these tadpoles and placed 16 individuals in each container in both treatments. Additionally, we placed 74 tadpoles in a 29.6 L divided fish tank in conditions similar to T1 and T2. We used these tadpoles to replace dead individuals in either of the two treatments, thus density remained constant until tadpoles began to metamorphose. We replaced tadpoles that died with individuals of similar mass and stage of development.

We provided food to tadpoles in both treatments in proportion to body mass. Initially, we gave tadpoles in T1 a quantity of food equivalent to 2.5% of their total body mass twice a day, and 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 boat when individuals had consumed nearly all that had initially been provided and we added 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. In T2 containers, we replenished, (rather than replaced) water in containers every 2 d to compensate for water loss due to evaporation. This simulates conditions in the boat in which rain adds to but does not replace the water.

When tadpoles were on the verge of initiating metamorphosis, we checked containers throughout the day and removed any individuals that had reached stage 42 (fore-limb emergence) and placed them in plastic containers (22 × 22 × 15.5 cm) filled with 3 cm of water until they fully emerged from the aquatic environment. Then we transferred them to plastic bags with 1 cm of water where they remained until they completely absorbed their tails (stage 46), thus signaling

the completion of metamorphosis and the transition to a juvenile frog. We released all juveniles at dusk close to the boat.

Growth and metamorphosis.—To establish mean initial body mass and total length (TL), we 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, because tadpoles in this stage are extremely fragile (Kupferberg et al. 1994). We measured tadpoles in both treatments weekly, when they reached stage 42, and we recorded the time to metamorphosis (TTM) as the number of days from stage 25 to stage 42. We calculated individual growth rates as body mass at stage 42/TTM (Richter-Boix et al. 2011; Székely et al. 2017). Once individuals reached stage 46, we recorded mass and snout-vent length (SVL) and calculated the duration of metamorphosis as the time elapsed from fore-limb emergence to complete tail reabsorption.

Diet analysis.—We collected 120 individuals from eight developmental stages (15 individuals in stages: 27, 28, 31, 33, 34, 35, 39, and 42) across two breeding seasons (August to September 2016 and May to July 2017). In 2016 we collected 41 tadpoles (15 individuals in stage 27 and fewer than 10 individuals in each of the other stages). To equalize sample size, we collected 80 additional tadpoles from all stages (except stage 27) in 2017. We preserved 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 then carefully dissected the tadpoles under a stereoscopic microscope and removed and measured the intestinal tract to the nearest 0.01 mm as recommended by McDiarmid and Altig (1999b). We examined the foregut and midgut because the effects of digestion are less pronounced in these regions, thus facilitating the identification of ingested items. We gently scraped the intestinal tissue to remove the digested material. We carefully mixed the gut contents with two drops of 4% formalin-glycerin on microscope slides. For tadpoles in later developmental stages that contained a high concentration of digested material, we cut the foregut and midgut into 3-mm fragments and prepared a slide for each piece. We examined all samples using a compound light microscope under ×100 and ×400 magnification and proceeded to count each visible food item on every slide. We counted individual cells of unicellular algae, strands of filamentous algae and colonies of colonial algae (groups of 2–4 cells). When we only found pieces of insects and zooplankton, we looked for distinguishing features (e.g., a cladoceran post-abdomen, dipteran larva head), which we took to indicate that one entire individual had been ingested.

Nutritional analyses.—To evaluate the quality of the experimental T2 diet, we determined the total concentration of protein, fats, and oils of sediment and water (two 150 mL samples) from the boat. We followed the persulfate method 10072 Test N for protein analysis (Complete Kit for Program 394 N Total HR TNT, 2 – 150 mg/L Nitrogen; Hach Company 2013). We used a UV spectrophotometer (Hach-DR600; Hach Company, Loveland, Colorado, USA), digestor (Hach-DRB 200), and certified reference material ERA-525 for total N and sample blanks to determine the accuracy of the analyses. Recovery percentages were within 80–110%. We followed Method 5520 E of the Soxhlet Extraction Method (American Public Health Association 1995) using a Labconco extractor (Labconco Corporation, Kansas City, Missouri, USA) to determine fats and oils content. Accuracy of the analysis was determined using certified reference material included in Mexican law NMX-AA-005-SCFI-2000.

Statistical analyses.—The response variables for the rearing experiment were tadpole growth rate, size of metamorphs and juveniles, length of the larval period, and duration of metamorphosis. We tested all the data for normality and homogeneity of variances using the Shapiro-Wilks and Levene tests, respectively. For normal and homoscedastic data, we applied independent sample Student´s *t*-tests (IBM® SPSS® Statistics, Armonk, New York, USA) to determine differences between metamorph and juvenile frog body mass and length of individuals in each treatment. To calculate differences in growth rate, we used the Welch's *t*-test. Where the data violated the assumptions of parametric testing procedures, we used non-parametric Mann-Whitney tests to evaluate differences in the duration of the larval period and metamorphosis. We verified the significance of multiple comparisons with the *post hoc* Bonferroni method.

We characterized the tadpole diet (all the ingested material) by using a variation of the Kawakami and Vazzoler (1980) alimentary index as implemented by Huckembeck et al. (2015). This index uses percentage frequency of occurrence (FO) and percentage numerical frequency (NP) to determine which ingested items are more dominant in the tadpole diet and is denoted as follows:

$$IA_i = (FOi \times NPi)/\sum_{i=1}^{n}(FOi \times NPi) \times 100$$

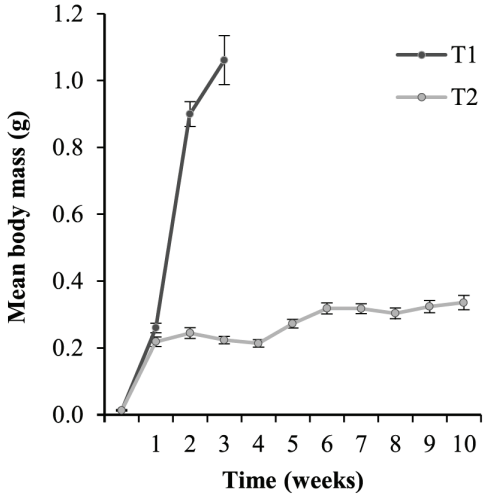

Figure 3. Mean body mass (± SE) of tadpoles of the Yucatan Casque-Headed treefrog (*Triprion petasatus*) from El Colegio de la Frontera Sur (ECOSUR) campus Chetumal, Quintana Roo, Mexico, in the two treatments (T1 and T2) measured weekly until they reached Gosner stage 42.

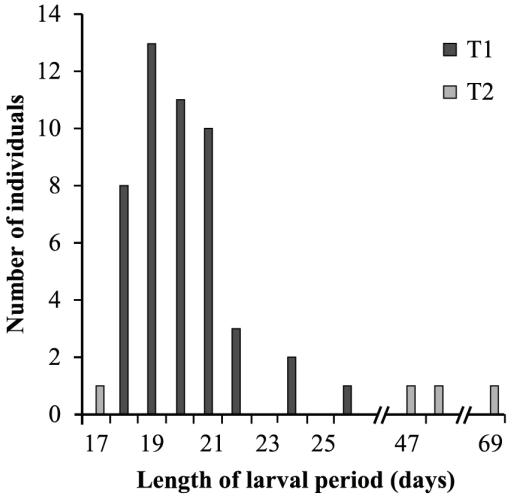

Figure 4. Length of larval period for tadpoles of the Yucatan Casque-Headed treefrog (*Triprion petasatus*) from El Colegio de la Frontera Sur (ECOSUR) campus Chetumal, Quintana Roo, Mexico, in the two treatments (T1 and T2).

where FO*i* is the percentage frequency of a particular ingested item *i* (the number of stomachs in which item *i* was found) and NP*i* is the numerical frequency (the abundance of item *i* in the gut contents divided by the abundance of all ingested items present). This way we avoided the bias that is associated with calculating numerical frequency and frequency of occurrence separately.

To determine if there were differences in the tadpole diet between developmental stages and between breeding seasons, we performed Non-metric Multidimensional Scaling (NMDS) and then carried out two one-way Permutational Multivariate Analysis of Variance (PERMANOVA) in PAST 3.10 (Hammer et al. 2001). We then performed a two-way PERMANOVA to determine if there was any interaction between these two factors (breeding season and developmental stage) in PRIMER v7 (PRIMER-e Quest Research Ltd, Auckland, New Zealand). We proceeded to identify which ingested items contributed most to the differences observed in the diet of tadpoles in the different development stages and from the different breeding seasons through the use of a SIMPER analysis. We used square root transformed data and the Bray-Curtis similarity index with 9999 permutations to perform these analyses (Hammer et al. 2001; Clarke and Gorley 2015). We also used a pairwise analysis as a *post hoc* test and corrected *P*-values with the Holm-Bonferroni method (Marcus et al. 1976). We accepted *P*-values ≤ 0.05 to indicate significant difference.

Results

Growth and metamorphosis.—Tadpole mean ± (standard error) initial body mass was 0.01 ± 0.002 g (range, 0.01–0.08 g). During the first week, individuals in both treatments reached similar sizes, differing only by 0.04 g; however, dramatic differences in size became apparent by week two (Fig. 3), when mean body mass in T1 tripled (0.90 ± 0.04 g; range, 0.34–1.41 g), while tadpoles in T2 only marginally increased in mean body mass (0.24 ± 0.02 g; range, 0.07–0.48 g). Individuals in T2 experienced only slight increases in mean body mass over the course of 10 weeks (Fig. 3), while tadpoles in T1 continued to rapidly increase in size and by week three only four individuals that had not initiated metamorphosis remained, and they achieved the largest mean body mass (1.06 ± 0.07 g; range, 0.77–1.40 g). Between weeks three and four, all tadpoles in T1 had reached the end of the larval period and initiated metamorphosis (Fig. 3).

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. 4). 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 = 27.52, df = 30.27, P < 0.001). T1 individuals experienced higher growth rates than the four individuals that metamorphosed in T2 (0.03–0.06 g/day vs. 0.00–0.01 g/day; Fig. 5).

Mortality during the larval period was higher in T2 (13 died, 27%) than in T1 (two died, 4%). Events of cannibalism where individuals consumed conspecifics

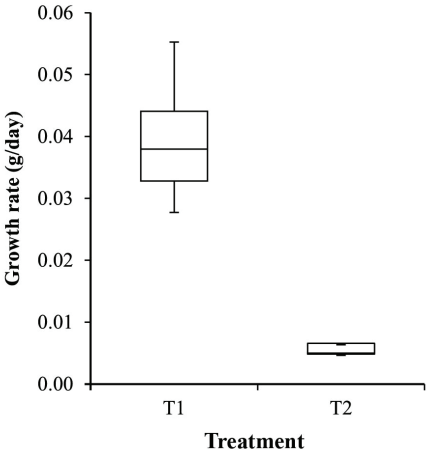

Figure 5. Growth rate of tadpoles of the Yucatan Casque-Headed treefrog (*Triprion petasatus*) from El Colegio de la Frontera Sur (ECOSUR) campus Chetumal, Quintana Roo, Mexico, during the larval period in the two treatments (T1 and T2). The boxplot reveals the distribution of data where boxes include data in the 25–75 percentiles and median values (middle lines), while whiskers represent minimum and maximum values.

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. Because mortality continued to increase in T2 each week, we decided to terminate the experiment after week 10, when it was evident that we would eventually reach a point where we would have had to replace more than half of the individuals to continue, and the integrity of our experiment would have been compromised.

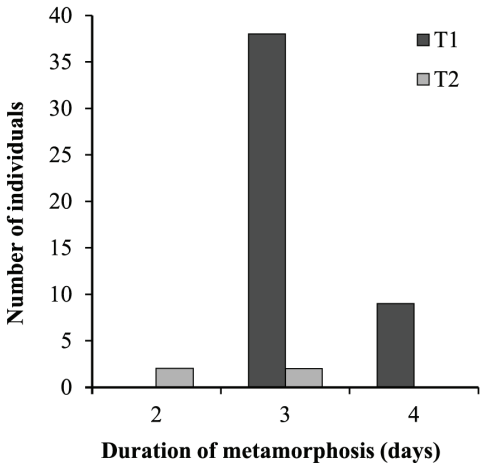

Figure 7. The duration of metamorphosis for tadpoles of the Yucatan Casque-Headed treefrog (*Triprion petasatus*) from El Colegio de la Frontera Sur (ECOSUR) campus Chetumal, Quintana Roo, Mexico, in the two treatments (T1 and T2).

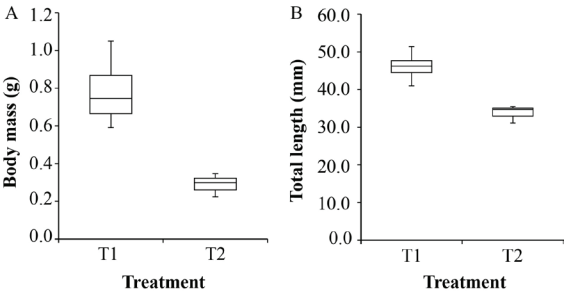

Figure 6. Size at metamorphosis of tadpoles of the Yucatan Casque-Headed treefrog (*Triprion petasatus*) from El Colegio de la Frontera Sur (ECOSUR) campus Chetumal, Quintana Roo, Mexico. (A) Body mass of individuals in the two treatments (T1 and T2). (B) Total length of individuals in T1 and T2. Boxplots reveal the distribution of data just as in Fig. 5.

Size at metamorphosis was significantly different between T1 and T2 (body mass: $t = 6.77$, df = 49, $P < 0.001$ and TL: $t = 7.88$, df = 49, $P < 0.001$). Individuals from T1 were heavier and longer than those in T2 (0.59–1.05 g and 40.96–51.38 mm TL vs. 0.22–0.35 g and 31.11–35.44 mm TL; Fig. 6). The duration of metamorphosis in both treatments was only significantly different ($U = 36.0$, $P < 0.05$) before *post hoc* verification. Individuals from T1 completed this phase within 3–4 d, whereas the four individuals that metamorphosed in T2 emerged as juvenile frogs within 2–3 d (Fig. 7).

The size of the juvenile frogs was also significantly different (body mass: $t = 10.39$, df = 48, $P < 0.001$ and SVL: $t = 10.42$, df = 48, $P < 0.001$). Individuals from T1 (n = 46) were both heavier and longer (0.34–0.58 g and 15.65–19.92 mm SVL) than those from T2 (n = 4; 0.11–0.18 g and 11.97–14.19 mm SVL; Fig. 8). Visually, juveniles from T1 appeared to be much more robust in comparison with those from T2 which seemed almost emaciated (Fig. 9).

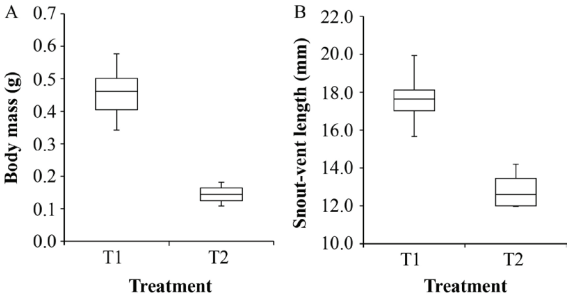

Figure 8. Size of juvenile frogs of the Yucatan Casque-Headed treefrog (*Triprion petasatus*) from El Colegio de la Frontera Sur (ECOSUR) campus Chetumal, Quintana Roo, Mexico. (A) Juvenile body mass for individuals in both treatments (T1 and T2). (B) Juvenile snout-vent length for individuals in T1 and T2. Boxplots reveal the distribution of data just as in Fig. 5.

Table 1. Mean (± standard error) measurements of tadpoles of the Yucatan Casque-Headed treefrog (*Triprion petasatus*) from El Colegio de la Frontera Sur (ECOSUR) campus Chetumal, Quintana Roo, Mexico, for each Gosner development stage examined in the diet analysis. Samples sizes = 15 per stage. Abbreviations are 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

Diet analysis.—The 120 tadpoles (stages 27–42) that we examined ranged from a mean of 16.71 ± 0.72 mm (range, 12.71–21.75 mm) TL to 36.86 ± 0.64 mm (range, 30.98–41.35 mm) TL (Table 1). The average intestinal length of tadpoles dramatically changed in the later stages of development. The intestines of tadpoles in stage 35 measured an average of 170.70 ± 5.35 mm whereas by stage 42 intestines were only 29.27 ± 2.07 mm long. We identified 32 different ingested materials and organized them into 13 groups (Table 2). Detritus (mainly unidentified plant material and parts of arthropods, but also invertebrate eggs, pollen, and spores) was present in every individual in all developmental stages and proved to be a commonly ingested resource. Of this group, plant material together with arthropod parts were the most important ingested items in stages 31, 33, 34, 35, and 42.

The algae and fungi groups were also present in all developmental stages. The euglenoid *Phacus* was the most dominant ingested item for two developmental stages (28 and 39). The cyanophyceans *Chroococcus* and *Lyngbya* were the most dominant ingested items by 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 commonly ingested by tadpoles. These organisms were present in all developmental stages with the exception of Bdelloidea and Ciliophora. Of these animal groups, *Moina* was dominant across all the developmental stages. Two individuals in stage 27 also had tadpole tissue in their gut contents.

Differences and similarities in the diet among tadpole developmental stages became somewhat visible through the use of NMDS. Individuals from stage 27 clearly grouped together and individuals from stage 39 also formed a group that somewhat separated from the rest, although there was a high degree of overlap between all stages (Fig. 10A). Separation was most apparent when we grouped diets according to the breeding season when we collected the tadpoles. The individuals from 2016 clearly clustered together while individuals from 2017 formed another distinct group, although there was some overlap here as well (Fig. 10B).

The abundance of individual dietary resources differed significantly among developmental stages

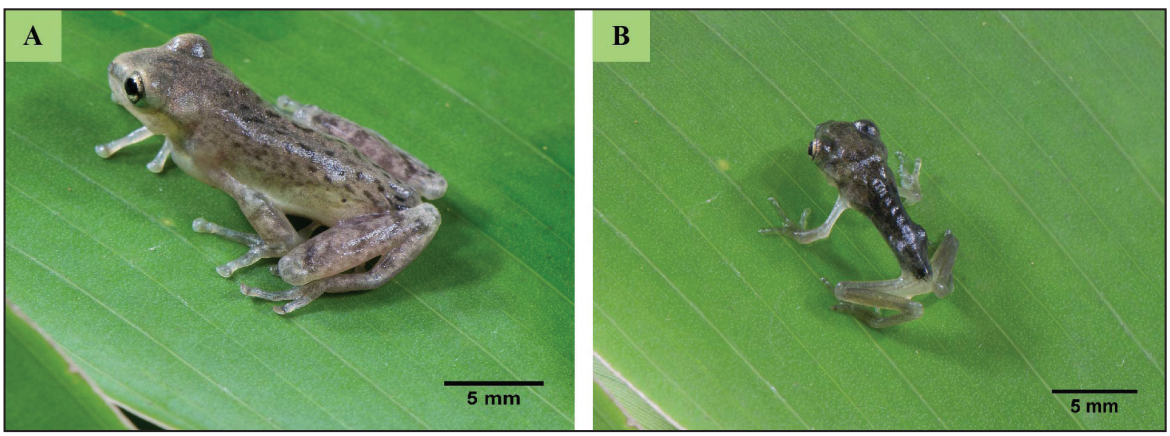

Figure 9. Appearance of juvenile frogs of the Yucatan Casque-Headed treefrog (*Triprion petasatus*) from El Colegio de la Frontera Sur (ECOSUR) campus Chetumal, Quintana Roo, Mexico. (A) Dorsal-lateral view of a juvenile from treatment T1. (B) Dorsal view of a juvenile from treatment T2. (Photographed by Humberto Bahena-Basave).

Table 2. Alimentary index values for items ingested by tadpoles (n = 15 per stage) of the Yucatan Casque-Headed treefrog (*Triprion petasatus*) from El Colegio de la Frontera Sur (ECOSUR) campus Chetumal, Quintana Roo, Mexico, from eight Gosner development stages. Food items identified to varying taxonomic group levels: Fungi, family; Algae, order or genus; Invertebrates, phylum, class, subclass, order, family or genus. For eggs, the plus sign (+) = invertebrate eggs (e.g., zooplankton) and for resting eggs = dormant zooplankton eggs.

		Stage							
Taxonomic group		27	28	31	33	34	35	39	42
Fungi	Trichocomaceae	0.58	0.05	0.02	0.05	0.04	0.23	0.28	0.44
Algae	Unidentified	0.12	—	—	—	—	—	—	—
Cyanophyceae	*Chroococcus*	73.6	0.21	0.12	0.09	0.01	0.05	0.003	—
	Anabaena	0.04	—	—	—	—	—	0.001	—
	Lyngbya	14.7	0.36	0.24	0.05	0.01	0.12	0.17	—
	Oscillatoriales	0.01	—	—	—	—	—	—	—
Euglenophyceae	*Phacus*	—	78.5	0.04	23.4	16.3	0.02	83.3	0.11
Chlorophyceae	*Gloeocystis*	0.003	—	—	—	—	—	—	—
	Chlorella	0.001	—	—	—	—	—	—	—
	Ulothrix	0.003	—	—	—	0.01	—	—	—
	Oedogonium	0.05	—	—	—	—	—	—	—
	Cosmarium	1.03	—	—	—	—	0.004	0.001	—
Ciliophora	Unidentified	0.28	0.67	0.12	0.15	0.03	0.41	0.33	—
Rotifera	Unidentified	0.01	—	—	0.01	—	0.004	0.003	—
	Bdelloidea	0.001	0.05	0.01	0.02	—	—	0.13	—
	Lecane bulla	0.04	0.01	0.09	0.14	0.6	0.6	0.01	0.44
	Lepadella	—	—	—	—	—	0.004	0.001	—
Nematoda	Unidentified	—	—	—	—	—	0.02	—	—
	Dorylaiminae	0.001	—	—	—	—	—	—	—
Oligochaeta	Unidentified	—	—	—	—	—	0.004	0.001	—
Cladocera	*Moina*	0.54	0.76	4.21	2.37	3.93	4.91	0.71	3.07
Ostracoda	Cyprididae	0.02	0.04	0.36	0.24	0.56	0.52	0.04	0.11
Copepoda	Nauplius	0.001	—	—	—	—	—	—	—
Diptera	Unidentified	0.01	0.01	0.16	0.05	0.15	0.07	0.02	0.11
Acari	Unidentified	—	0.002	0.01	—	—	0.03	0.001	—
	Oribatida	—	—	0.01	—	—	—	—	—
Tadpole tissue		0.12	—	—	—	—	—	—	—
Detritus	Plant material	3.60	7.86	34.0	35.2	36.5	46.2	6.53	48.2
	Arthropod parts	4.33	10.3	58.0	35.9	39.3	42.0	7.48	40.4
	Eggs^{+}	0.08	0.12	0.12	0.23	0.48	1.12	0.21	0.11
	Resting eggs^{+}	0.001	0.002	0.01	0.01	—	0.03	0.001	0.44
	Pollen/spores	0.80	1.02	2.49	2.15	2.11	3.64	0.77	6.57

(pseudo-F = 9.64, df = 7, P < 0.001) as well as between breeding seasons (pseudo-F = 21.35, df = 1, P < 0.001). Developmental stage and breeding season interacted significantly (pseudo-F = 5.3, df = 6, P < 0.001). The pairwise *post hoc* analysis (Appendix Table 1) revealed that the diet in stages 27 and 42 (when individuals abstain from eating) was 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 (Appendix Table 2) indicated that these differences were mostly due to the abundance of algae (*Chrooccocus*, *Lyngbya*, and *Phacus*) and detritus (plant material and arthropod parts). Diet of stage 27 tadpoles was distinct from all others because it had the greatest variety of ingested items (26), greater abundance of *Chrooccocus* and *Lyngbya*, and an

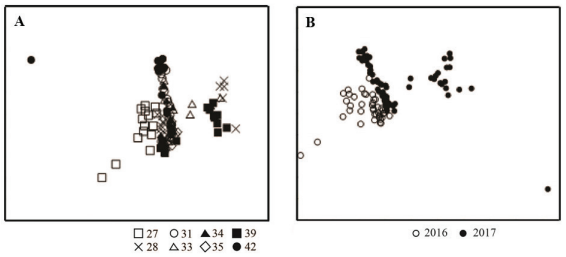

Figure 10. Non-metric Multidimensional Scaling (NMDS) ordination plots for diet composition data. (A) Plot of when developmental stage was used as the grouping factor. (B) Plot of when breeding season was used as the grouping factor. Stress values for both plots were 0.14.

absence of *Phacus*. In contrast, stage 42 had the lowest variety of ingested items (11), highest abundance of plant material and arthropod parts, and reduced *Phacus* abundance. The high abundance of plant material and arthropod parts, and low *Phacus* abundance contributed most to the differences in diet between stage 35 and the six other stages. For individuals in stage 39, the elevated abundance of *Phacus* was similar to stage 28, but different from all others. The abundances of these same groups (*Phacus*, plant material, arthropod parts, *Lyngbya* and *Chrooccocus*) also contributed the most to the differences observed in the diets of the tadpoles from the 2016 breeding season and those collected in 2017. *Phacus* was also only present in the stomach contents of tadpoles collected in 2017.

Nutritional analyses.—The sediment and water from the boat contained different concentrations of both protein and fats and oils. Protein was absent from the sediment and constituted only 0.01% of the water. The concentration of fats and oils in the sediment was 72.57 mg/g and 198 mg/L in the water.

Discussion

Growth and metamorphosis.—Our results demonstrate that diet quality influences the growth and metamorphosis of *T. petasatus* tadpoles. The high-protein commercial fish diet minimized larval period variability and mortality and increased growth, thus allowing individuals to metamorphose early at large sizes. The positive effects of protein have been documented in a diverse array of anurans raised on high-quality diets (Kupferberg et al. 1994; Babbit and Meshaka Jr. 2000; McCallum and Trauth 2002; Martins et al. 2013). Conversely, as we observed in tadpoles fed the more natural diet, low-protein foods (e.g., leaf litter, suspended detritus, and corn meal) cause prolonged larval periods, low growth rates, increased mortality and small individuals (Kupferberg et al. 1994; Álvarez and Nicieza 2002; McCallum and Trauth 2002; Ramamonjisoa et al. 2016).

The results of the nutritional analyses partly confirmed the poor quality of the natural diet. If we consider the nutritional content of the sediment analysis to be representative of the leaf litter used in T2, the fat content varied little between this diet and the commercial food used in T1 (sediment: 7.2 g of fat in a 100 g sample; commercial diet: 6 g of fat in a 100 g sample). There is a dramatic difference in protein content, however (36% in the commercial diet vs 0–0.01% in the sediment and water). Tadpoles require more than the 10% protein to grow and develop adequately (McCallum and Trauth 2002). Therefore, it is not surprising that *T. petasatus* tadpoles performed so poorly when fed leaf litter. We also suspect that the quantity of food provided to tadpoles in T2 also played a role in the poor growth and slow development.

Individuals fed the low-quality natural diet were smaller as metamorphs and juvenile frogs than those reported by Duellman and Trueb Klass (1964; metamorphs: 29.40 mm vs. 35 mm TL; juveniles: 12.83 mm vs. 15.8 mm SVL). In contrast, the high-quality diet produced larger metamorphs and juveniles (metamorphs: 46.12 mm TL; juvenile frogs: 17.66 mm SVL) than the *T. petasatus* Duellman and Trueb Klass (1964) measured.

These differences in metamorph size, and length and variability of the larval period between treatments are consistent with the ecological model of metamorphosis developed by 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 continue to grow in the aquatic environment if resources are abundant and metamorphose at 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 took advantage of the high-quality food and metamorphosed at a larger size. Variation in the length of the larval period can occur when individuals differ in their competitive abilities and thus are not able to reach minimum body size at the same time (Wilbur and Collins 1973). Our observation that one individual in T2 metamorphosed before all others is consistent with this differential competitiveness mechanism.

Contrary to our initial hypothesis, the duration of metamorphosis was slightly shorter for the smaller individuals in T2 than in T1. This could be due to differences in tadpole size, because smaller individuals might metamorphose faster than larger ones simply because less tissue needs to be modified and reabsorbed (Downe et al. 2004). Rapid development in small tadpoles may be a result of individuals selecting food items that favor development over growth, allowing them to escape from adverse environmental conditions

(Richter-Boix et al. 2007). Székely et al. (2017) also suggested that a short duration of metamorphosis allows tadpoles to transition faster to the terrestrial environment to avoid pond desiccation.

Water quality (e.g., ammonium, nitrate and nitrite concentrations, pH, and dissolved oxygen) can also affect tadpole growth and development (Alford 1999; Ortiz et al. 2004; Burgett et al. 2007). In our experiment, it is unlikely that pH and dissolved oxygen contributed to the treatment effects we observed because we either controlled the levels or they varied little between treatments; however, we did not measure ammonium, nitrates or nitrites, which at high concentrations can cause slow growth, reduced activity, low survivorship, and deformities in tadpoles (Ortiz et al. 2004; Burgett et al. 2007). We observed two individuals with curved tails in T1, which occurs when tadpoles are exposed to elevated concentrations of ammonium nitrate (Ortiz et al. 2004). This mechanism is unlikely because we regularly changed the water, mortality was low, and tail abnormalities are also associated with elevated levels of protein in the diet (McCallum and Trauth 2002). Ammonium effects in T2 are also unlikely because we added clean water regularly and no deformities were observed, other than the triangular body shape, which indicates starvation (Babbit and Meshaka Jr. 2000).

The cannibalism we observed in T2 is likely a result of starvation or intense competition for limited food resources and suggests that *T. petasatus* tadpoles are facultative cannibals. Wild tadpoles from the boat were also prone to necrophagy, and actively consumed living conspecifics. Tadpoles often resort to cannibalism when diets do not meet their nutritional requirements or when competition is high (Jefferson et al. 2014a,b). When quantity or quality of food is insufficient, tadpoles that practice cannibalism increase their chances of survival (Babbit and Meshaka 2002). Jefferson et al. (2014b) found that cannibalism increased the likelihood of reaching metamorphosis, although an exclusively conspecific diet prolonged the larval period for tadpoles of Wood Frogs (*Lithobates sylvaticus*) and still produced small individuals relative to those fed a high-protein diet. This seems to be the case for tadpoles in T2 who consumed conspecifics but still did not match the growth and developmental rate of tadpoles in T1.

Diet analysis.—*Triprion petasatus* tadpoles appear to be omnivores and generalists throughout their development because they ingested a wide variety of plant and animal material in each stage, similar to other neotropical tadpoles that also live in small ponds (e.g., Swimming Frog, *Pseudis paradoxa platensis*, South American White-lipped Grass Frog, *Leptodactylus fuscus*, and Serra da Bocaina Snouted Treefrog, *Scinax angrensis*; Arias et al. 2002; Rossa-Feres et al. 2004; de Sousa Filho et al. 2007). The high frequency of detritus and algae as well as benthic invertebrates (bdelloid rotifers and ostracods) is consistent with the benthic habits of *T. petasatus* tadpoles (Altig and McDiarmid 1999). Detritus and rotifers could be important sources of nutrients because many microorganisms, such as bacteria, are associated with the former (Akers et al. 2008) and the latter is recognized as an essential diet component for planktivorous fish, other omnivorous tadpoles, and aquatic invertebrates (Rossa-Feres et al. 2004; Wallace and Snell 2010). Stable isotope analyses of tadpole gut contents and body tissues have demonstrated that detritus can be assimilated (Trakimas et al. 2011) and that the numerically most important ingested items identified by visual inspection of gut contents were also the most assimilated (Huckembeck et al. 2016).

Other sources of animal protein we found in the gut contents were the cladoceran *Moina* and dipteran larvae. *Moina* is often used as live food for aquaculture due to its nutritional value (Islam et al. 2017). Dipteran larvae, specifically chironomids, also contain a high concentration of protein and can be used to rear tadpoles (Ramamonjisoa et al. 2016). 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, as is done with other anuran species (Mokany and Shine 2003). Although traditional gut content analysis is uninformative with respect to assimilation (Altig et al. 2007), our results are useful indicators of omnivorous feeding behaviors and diet breadth.

The differences in composition and abundance of the ingested items we observed are likely a function of both temporal variation in resources and changes during tadpole development. Tadpoles can shift from being primarily herbivorous in early developmental stages to mostly carnivorous in the later stages (Schriever and Williams 2012). These changes are linked to alterations in digestive enzyme activity and relate to morphological changes in the transition from a mostly herbivorous tadpole to a carnivorous adult (Santos et al. 2016). In our study, this trend of herbivory in early developmental stages and carnivory in the later stages was not as pronounced because the tadpole diet varied significantly between the two different breeding years as indicated by the NMDS plots. The significant interaction between developmental stage and breeding season supports the conclusion that differences in the diet of tadpoles between developmental stages could not be strictly attributed to morphological changes that occur throughout metamorphosis. The diet between breeding seasons likely differed due to changes in food availability, which fluctuates because phytoplankton and zooplankton frequently experience temporal and

spatial variations in population abundance (Lampert and Sommer 2007). Although we did not examine water samples from the boat, we noticed that tadpoles from 2016 contained a wide variety of cyanobacteria, while *Phacus* dominated gut contents from 2017. We collected tadpoles at stage 27 only in 2016 and they did not consume any *Phacus*. A future analysis is required in which individuals in different stages of development are collected from the same breeding season.

Although variations in tadpole diet could not be fully attributed to developmental changes, the difference in the diet between stage 42 and all other stages was an exception. Individuals had little to no ingested material in the fore and midguts because tadpoles stop eating in this stage (42) due to morphological changes in the digestive system associated with metamorphosis when the pancreas, stomach, and intestines all undergo significant restructuring (Kupferberg et al. 1994; Santos et al. 2016). These changes were evident in the differences that we observed in the intestinal length of tadpoles in early (31–35) versus later developmental stages (42).

Food quantity is important especially in the case of a low-quality diet and having food *ad libitum* can allow tadpoles to make up the poor nutritional content of a diet by consuming more of it (Kupferberg 1997). We provided tadpoles with a certain quantity of leaf litter and more was only added when it had almost been completely consumed. Individuals collected from the boat for the diet analysis received a continual supply of leaves and fallen insects (a potential source of protein) on a daily basis, which provides tadpoles with a greater quantity of food and were even larger than tadpoles from T2 (stage 31: 29.52 mm vs 26.38 mm; stage 35: 33.80 mm vs 31.66 mm; stage 42: 36.86 mm vs 33.74 mm) and comparable to those in T1 (stage 31: 28.48 mm; stage 35: 39.16 mm; stage 42: 46.16 mm). This indicates that a natural diet can support successful growth and development and poor tadpole performance in T2 may be attributed to the quantity of food provided. If tadpoles in T2 had been provided with a greater quantity of leaf litter, on a more consistent basis, they may have been able to compensate for the low-quality diet and experience higher growth and development rates just like their wild counterparts.

Although tadpoles from the boat demonstrated superior growth to those in T2, late season tadpoles developed the same triangular bodies as individuals in this treatment. This phenomenon is associated with poor environmental conditions (Babbit and Meshaka Jr. 2000) brought on by nutrient depletion. The first individuals to metamorphose remove nutrients from ephemeral ponds, leaving the remaining tadpoles with little resources to feed upon (Griffiths and Foster 1998). Both the aquatic system in the boat and T2 likely experienced nutrient depletion in this manner. The quantity of food provided may have been ideal in the first week in T2 but was insufficient for the remainder of the experiment, which explains why so few individuals reached metamorphosis and why the remaining tadpoles experienced such poor growth. Growth and development in T2 may also have been compromised by a lack of other important diet components, such as algae. Algae constitutes a main part of the diet of *T. petasatus* tadpoles, as evidenced by our gut content analysis. Omnivorous tadpoles can grow and develop effectively when raised on a diet composed entirely of this food group (Kupferberg et al. 1994; Pryor 2009). Including algae potentially could have enhanced the quality of the natural diet and increased tadpole performance in T2.

Although this is the first and only study evaluating diet effects on *T. petasatus* tadpoles, the results are consistent with previous findings regarding food quality in temperate ecosystems (Steinwascher and Travis 1983; Kupferberg 1997; McCallum and Trauth 2002). Our results suggest that *T. petasatus* tadpoles are able to accelerate growth and development when fed a high-quality diet with a relatively high percentage of protein (36%). The next steps are to determine the minimum amount of necessary protein required by this species to reach optimum growth and development and the maximum amount that can be tolerated without causing morphological abnormalities and subpar fitness. Because larval diets also influence terrestrial post-metamorphic development (Tarvin et al. 2015; Ramamonjisoa et al. 2016), it is necessary to evaluate post-metamorphic performance to reach a definitive conclusion as to which diet (natural or commercial) is the most beneficial. 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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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 pursued a Master of Science degree in Natural Resources and Rural Development at El Colegio de la Frontera Sur in Chetumal, Mexico. This investigation concerning *Triprion petasatus* tadpoles is a product of her Master's thesis. (Photographed by N. Gabriela Blanco Campos).

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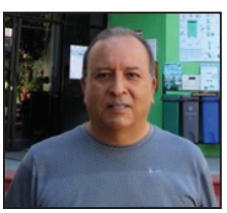

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David González-Solís is a full-time researcher at El Colegio de la Frontera Sur, Chetumal, Mexico. He received his Ph.D. in fish parasitology from the Academy of Sciences of the Czech Republic in 2001 and his research focuses on the taxonomy, systematics, and ecology of helminths in fish and other aquatic and terrestrial vertebrates. Currently, he is studying the parasites of marine fishes, crocodiles, and marine and freshwater turtles from the southern region of the Mexican Caribbean. (Photographed by Brianna Jacobson).

Appendix Table 1. Results of the PERMANOVA analysis that we performed with the data gathered for the diet analysis.

	pseudo-F	*P*
Stages x Diet (*Global*)	9.64	0.001
Pairwise Comparisons		
27 vs		
28	6.42	0.003
31	13.21	0.003
33	9.53	0.003
34	13.94	0.003
35	17.51	0.003
39	14.55	0.003
42	18.35	0.003
35 vs		
28	6.90	0.003
31	12.61	0.003
33	5.58	0.039
39	11.9	0.019
42	24.8	0.003
39 vs		
31	16.63	0.003
33	8.17	0.033
34	9.82	0.017
42	23.14	0.019
42 vs		
28	9.49	0.003
31	6.96	0.006
33	7.21	0.006
34	14.12	0.003
Breeding season		
2016 vs 2017	21.35	0.0001
Breeding season x Stages	5.3	0.0002

Appendix Table 2. SIMPER analysis results showing average dissimilarity and contribution of ingested items. We present only stages that are significantly different from one another, and only ingested items that contribute more than 10% to the dissimilarity.

		Average dissimilarity	Contribution (%)
Comparison (stages or year)			
27 vs 28	Overall	45.99	
	Phacus	13.15	28.6
	Chroococcus	7.765	16.89
	Lyngbya	5.944	12.93
27 vs 31	Overall	39.87	
	Chroococcus	9.83	24.65
	Lyngbya	8.137	20.41
27 vs 33	Overall	44.53	
	Phacus	4.612	10.36
	Chroococcus	9.221	20.71
	Lyngbya	7.665	17.21
27 vs 34	Overall	41.93	
	Chroococcus	9.06	21.6
	Lyngbya	7.485	17.85
27 vs 35	Overall	42.56	
	Chroococcus	8.418	19.78
	Lyngbya	6.733	15.82
	Plant material	5.59	13.12
	Arthropod parts	4.757	11.18
27 vs 39	Overall	56.11	
	Phacus	22.52	40.13
	Chroococcus	6.998	12.47
27 vs 42	Overall	53.25	
	Chroococcus	11.44	21.47
	Lyngbya	9.959	18.7
	Arthropod parts	5.687	10.68
35 vs 28	Overall	39.51	
	Phacus	13.89	35.16
	Plant material	5.318	13.46
	Arthropod parts	4.399	11.14
35 vs 31	Overall	29.53	
	Plant material	7.675	25.99
	Arthropod parts	5.293	17.92
35 vs 33	Overall	32.85	
	Phacus	4.99	15.19
	Plant material	6.647	20.24
	Arthropod parts	5.225	15.9

Appendix Table 2 (continued). SIMPER analysis results showing average dissimilarity and contribution of ingested items. We present only stages that are significantly different from one another, and only ingested items that contribute more than 10% to the dissimilarity.

		Average dissimilarity	Contribution (%)
Comparison (stages or year)			
35 vs 39	Overall	45.12	
	Phacus	23.54	52.17
35 vs 42	Overall	49.75	
	Plant material	13.71	27.56
	Arthropod parts	13.22	26.57
39 vs 31	Overall	51.78	
	Phacus	26.84	51.84
39 vs 33	Overall	49.17	
	Phacus	25.05	50.95
39 vs 34	Overall	45.53	
	Phacus	24.56	53.94
39 vs 42	Overall	66.77	
	Phacus	30.36	45.47
	Plant material	8.262	12.37
	Arthropod parts	7.945	11.9
42 vs 28	Overall	52.8	
	Phacus	18.89	35.78
	Plant material	7.957	15.07
	Arthropod parts	6.723	12.73
42 vs 31	Overall	33.96	
	Plant material	10.56	31.08
	Arthropod parts	7.848	23.11
42 vs 33	Overall	41.07	
	Phacus	7.068	17.21
	Plant material	9.363	22.8
	Arthropod parts	9.432	22.97
42 vs 34	Overall	43.12	
	Plant material	11.1	25.75
	Arthropod parts	11.64	26.99
2016 vs 2017	Overall	60.94	
	Phacus	8.28	18.83
	Lyngbya	4.5	10.22
	Plant material	5.22	11.87
	Arthropod parts	4.9	11.14