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J. A. Lemos-Espinal

Geoffrey R. Smith

G. A. Woolrich-Pina

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Diet of Larval *Ambystoma altamiranoi* from Llano de los Axolotes, Mexico

Julio A. LEMOS-ESPINAL[1]*, Geoffrey R. SMITH[2], and Guillermo A. WOOLRICH-PIÑA[3]

[1]*Laboratorio de Ecología—UBIPRO, Facultad de Estudios Superiores Iztacala, Av. Los Barrios 1, Los Reyes Iztacala, Tlalnepantla, Estado de México, 54090, MEXICO*
[2]*Department of Biology, Denison University, Granville, Ohio 43023, USA*
[3]*Laboratorio de Paleontología y Geobiología, ESIA Ticomá–IPN, Av. Ticomán 600, Col. Ticomán, Mexico, D.F. 07340, MEXICO*

Abstract: *Ambystoma altamiranoi* is an endangered endemic salamander found in the Transvolcanic Belt of Mexico. Unfortunately, relatively little is known about its ecology. Here we report on the diet of larval *A. altamiranoi* from a population in Llano de los Axolotes, Sierra de las Cruces, State of México, Mexico. Empty stomachs were found in 13.3% of individuals. Ostracods and gastropods dominated the diet of *A. altamiranoi*, together accounting for 89.9% of prey items consumed. The remainder of the diet consisted primarily of insects. Our observations suggest that the diet of *A. altamiranoi* is relatively narrow and that resources may be limited (relatively high frequency of empty stomachs), suggesting that factors that could impact the availability of ostracods and gastropods might have serious consequences for these endangered salamanders.

Key words: Ambystomatidae; Diet; Mexico; Salamanders; Transvolcanic Belt

Introduction

Ambystoma altamiranoi is an endemic species of salamander found in the Transvolcanic Belt of Mexico (Taylor and Smith, 1945; Lemos-Espinal et al., 1999). It is currently considered to be Endangered by the IUCN and Threatened by the Mexican government (Frías-Alvarez et al., 2010). Many of the sites where *A. altamiranoi* are found, as well as other Mexican *Ambystoma*, are being impacted by humans, especially by the encroachment of residential areas, pollution, conversion to agriculture, and the introduction of fish (e.g., Lemos-Espinal et al., 1999; Griffiths et al., 2004; Frías-Alvarez et al., 2010). In addition, *A. altamiranoi* has been found to be infected with *Batrachochytrium dendrobatidis* (Frías-Alvarez et al., 2008). Genetic studies of *A. altamiranoi* populations found evidence for low levels of gene flow and low population sizes, and suggest that populations of this species could be particularly susceptible to extinction due to isolated populations within its broader distribution range (Parra-Olea et al., 2011). Unfortunately, relatively little is known about the ecology or natural history of this rare and endangered salamander, beyond a few published anecdotal

* Corresponding author. Tel: +01–52–55–563–1333 ex7914; FAX: +1–740–587–5634;
E-mail address: lemos@unam.mx

observations (e.g., Taylor, 1938; Taylor and Smith, 1945; Maldonado Koerdell, 1947; Lemos-Espinal et al., 1999) and studies on their eggs and larval growth (Campbell and Simmons, 1962; Brandon and Altig, 1973). What is known suggests that breeding may take place throughout the year (Campbell and Simmons, 1962; Brandon and Altig, 1973), with larvae taking six months (in lab; Brandon and Altig, 1973) to at least a year to metamorphose (J. Lemos-Espinal, pers. comm). Once individuals metamorphose, they remain in, or along the edges of, the aquatic habitat (Lemos-Espinal et al., 1999). Such a lack of knowledge may hinder conservation efforts.

Here we report on the diet of larval *A. altamiranoi* from a population in Llano de los Axolotes, Sierra de las Cruces, State of México, Mexico. Diets of *Ambystoma* from Mexico have been shown to differ between streams and lakes affected or not affected by anthropogenic disturbance (e.g., Chaparro-Herrera et al., 2013; Ruíz-Martínez et al., 2014). In addition, population growth in some Mexican *Ambystoma* is most affected by the egg and larval stages (Zambrano et al., 2007), so an understanding of the ecology of the larval stage may be critical to conserving these species.

Materials and Methods

Our study was conducted at Llano de los Axolotes, Mpio. Isidro Fabela, Sierra de las Cruces, State of México (19°32'19"N; 99°29'97.8"W; 3475 m a.s.l). This area is an alpine grassland surrounded by coniferous forest. In the middle of this grassland runs a small permanent stream. The grassland extends on the sides of the stream for 100–150 m on the south side and 350–400 m on the north side. This grassland is grazed by cows and sheep. The stream is inhabited by a population of *A. altarmiranoi*, and most individuals (both adults and larvae) of this population aggregate in the portion of the stream that runs through the grassland, although we have seen some individuals in the parts of the stream that run through the forest. At this site breeding takes place in July, and larvae appear to take at least a year to reach metamorphosis (J. Lemos-Espinal, pers. comm.). Adults remain in the stream after metamorphosis. The smallest adults observed were >70 mm and were only found in July. There are no fishes (possible predators of the salamanders), and infection with *Batrachochytrium dendrobatidis* has never been documented in this population.

We visited the study site monthly from ca. 10:00 to 15:00 h from January through July 2014. Individuals of *A. altamiranoi* were observed in all months except January. During each visit we searched a 1 km section of the stream that runs in the grassland for 5 h per visit. All individuals detected were caught by hand or dipnet. We captured a total of 45 different larvae. Using the stomach flushing technique (Legler and Sullivan, 1979; Cecala et al., 2007), we obtained stomach contents for individuals of at least 35 mm SVL or larger (smaller individuals were too small to stomach flush). Once we obtained the stomach contents, individuals were released at the site of their capture. Stomach contents were preserved in ethanol and were later identified to the lowest taxonomic level possible and counted. We calculated diet breadth using Levin's B (Krebs, 1989), as well as the standardized Levin's B, which ranges from 0 (specialist) to 1 (generalist) (Hurlbert, 1978).

Results and Discussion

Of the 45 larvae caught, 39 (89.7%) contained stomach contents, and 6 (13.3%) had empty stomachs. Mean (±SE) SVL for examined larvae was 52.7±1.4 mm (n=45, range=35–67 mm). The proportion of empty stomachs that we observed is higher than many previously published reports. McCoy and Savitzky (2004) found that 2% of the larval *A. mabee* that they examined had empty stomachs. Tyler and Buscher (1980) also found that 2% of *A. tigrinum* larvae that they examined had empty stomachs. Other studies reported 2.5% empty stomachs in larval *A.*

cingulatum (Whiles et al., 2004) and 6% in larval *A. jeffersonianum* (Bardwell et al., 2007).

Numerically, ostracods dominated the diets of *A. altamiranoi*, making up 68.3% of the prey items consumed (Table 1). Gastropods were the next most frequently consumed prey item, making up 21.7% of the prey recovered. The remainder of the diet consisted primarily of insects, many of which were adults that probably fell into the water and were consumed by the salamander larvae. Ostracods and gastropods were found in every stomach that contained food items (Table 1). Adult dipterans (92.3%), lepidopterans (64.1%), and trichopterans (71.8%) were found in the majority of stomachs. The dominance of ostracods and gastropods in the diet relative to the other prey taxa is evident (Table 1). We observed numerous gastropods and ostracods among the aquatic vegetation on the stream bottom at the study site; however, we did not quantify their availability (J.A. Lemos-Espinal, pers. comm.). Levin's B value for the diet was 1.94, and the standardized Levin's B value was 0.078. These values suggest a narrow diet.

TABLE 1. Stomach contents of *Ambystoma altamiranoi* (N=39 stomachs) from Llano de los Axolotes, Sierra de las Cruces, Estado de México, Mexico. Unless otherwise indicated, prey items were adults. Percentages are given in parentheses.

Prey taxa	Number of Items	Number of stomachs
Annelida	4 (0.07)	4 (10.26)
Arachnida		
Acari	36 (0.63)	10 (25.64)
Araneae	6 (0.10)	6 (15.38)
Crustacea		
Ostracoda	3873 (68.25)	39 (100.00)
Insecta		
Coleoptera		
Larvae	8 (0.14)	5 (12.82)
Adult	95 (1.67)	12 (30.77)
Diptera	310 (5.50)	36 (92.31)
Hemiptera	1 (0.02)	1 (2.56)
Heteroptera	1 (0.02)	1 (2.56)
Lepidoptera	42 (0.74)	25 (64.10)
Orthoptera	2 (0.04)	2 (5.13)
Trichoptera	65 (1.14)	28 (71.79)
Mollusca		
Gastropoda	1231 (21.69)	39 (100.00)
Total	5674	39

Ostracods are a frequent, important component of the diets of larval *Ambystoma* (e.g., Lannoo and Bachmann, 1984; McWilliams and Bachmann, 1989; Nyman, 1991; Ghioca-Robrecht and Smith, 2008). However, ostracods are not important prey items for all larval *Ambystoma* (e.g., Bardwell et al., 2007). In particular, Ruíz-Martínez et al. (2014) found that ostracods were the most important prey item for *A. ordinarium* in undisturbed stream sections in the Transvolcanic Belt of Mexico, but were less important in stream sections showing human disturbance. Our observations suggest that if ostracods are an indication of relatively undisturbed streams, as suggested by the results of Ruíz-Martínez et al. (2014), then the streams that we sampled for *A. altamiranoi* may presently be suitable for the success of these salamanders.

In our study, gastropods were also an important prey item in the diet of *A. altamiranoi*. This result contrasts with the observation that gastropods are infrequent and relatively uncommon prey items for larval *Ambystoma* (e.g., Tyler et al., 1980; Lannoo and Bachmann, 1984; McWilliams and Bachmann, 1989; Ghioca-Robrecht and Smith, 2008). However, there are some species and populations that regularly consume snails or other molluscs (e.g., Dodson and Dodson, 1971; Brophy, 1980; Holomuzki and Collins, 1987; Benoy et al., 2002). Variation among populations in predation on snails by larval *Ambystoma* needs further examination, but may reflect variation in the availability of snails in the environment, as well as larval size. Indeed, diet composition in larval *Ambystoma* can vary with environmental availability of prey items (McWilliams and Bachmann, 1989), and the importance of molluscs in the diet can increase with larval size (Dodson and Dodson, 1971). In our case the size of salamander larvae with snails ranged from 35–67 mm

SVL (i.e., the entire range of sizes of larvae we examined); thus larval size does not appear to affect the potential to consume snails in our population.

The relatively high frequency of empty stomachs suggests that resources may be limiting in this population. This observation, when taken together with our other diet results, suggests a relatively narrow diet and limited prey availability for *A. altamiranoi* and further suggests that factors that impact the availability of the two most frequently consumed prey items, ostracods and gastropods, might have serious consequences for these endangered salamanders. Studies confirming the relative availability and value of various prey items in different habitats (i.e., those that have been impacted by human activities vs. those that are undisturbed) would be valuable for understanding the link between diet and habitat quality. In addition, data on the growth rates of larval *Ambystoma* from natural populations in streams would be very enlightening. Such knowledge might facilitate detection and/or prediction of stream conditions conducive to the maintenance of healthy populations of Mexican *Ambystoma*.

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