Digestive Plasticity in Tadpoles of the Chilean Giant Frog (*Caudiverbera caudiverbera*): Factorial Effects of Diet and Temperature

Luis E. Castañeda[1]
Pablo Sabat[2]
Sandra P. Gonzalez[2]
Roberto F. Nespolo[1,*]
[1]Instituto de Ecología y Evolución, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile; [2]Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile

ABSTRACT

Anuran metamorphosis is one of the most spectacular processes in nature. Metamorphosis entails morphological transformations and extensive changes in feeding habits, such as transforming from an herbivore to a carnivore. This phenomenon is especially sensitive to environmental cues. We studied the phenotypic plasticity of intestinal morphology and enzyme activity in tadpoles of the Chilean giant frog *Caudiverbera caudiverbera*. We tested the effects of diet and temperature using a factorial design, which included a control of nontreated individuals. There was no significant effect of diet treatment (i.e., low- vs. high-quality diet) on any of the measured variables, including external morphology. We found significant effects of temperature on morphological traits. Temperature treatment also had significant effects on aminopeptidase-N and maltase activity. Both enzymes exhibited complex interactions with temperature along the intestine. Gut size varied significantly among temperatures, with intestines from warm-treated individuals smaller than the intestines from control and cold-treated tadpoles. Our findings suggest that phenotypic plasticity of intestinal morphology and physiology exists in larvae of this species, at least in response to temperature. However, we did not detect clear effects of diet or temperature on the timing of metamorphosis.

* Corresponding author; e-mail: robertonespolo@uach.cl.

Introduction

One of the most spectacular examples of short-term phenotypic transformations in animals is anuran metamorphosis. During this process, entire organs, limbs, and tissues are either generated or absorbed, resulting in drastic changes from an aquatic larva to a terrestrial adult. Additional changes occur in feeding habits, because most amphibians shift from an herbivorous larval stage to a carnivorous adult stage, concurrent with corresponding changes in digestive physiology and morphology (Toloza and Diamond 1990*a*, 1990*b*; Sabat and Bozinovic 1996). For example, amphibian larvae have long (several times their body length) and undifferentiated guts, which are spirally arranged in the pleuroperitoneal cavity (Alford 1999; Rot-Nikcevic and Wassersug 2004). In contrast, adults possess short and differentiated intestines, which is typical of carnivorous vertebrates (Romer and Parsons 1981; Alford 1999). There are also transformations in the buccal structure, which changes from a small sucking apparatus in tadpoles to a wide and usually toothed jaw in adults (Romer and Parsons 1981; Vences et al. 2002). These major modifications can be completed in a few months and are the result of cell proliferation controlled by changes in hormone levels, especially thyroxin (Denver 1997). Such hormonal changes are mediated, in part, by the environment, where the most important cues are intra- and interspecific competition, predation, temperature, and diet (Wilbur and Collins 1973; Kupferberg 1997; Wassersug 1997; Relyea 2004). Specifically, diet (quality and quantity) generally influences rates of growth, whereas temperature generally influences the timing of metamorphosis (Marian and Pandian 1985). In addition, if these phenotypic changes increase survival and/or fecundity (i.e., fitness), metamorphosis can be considered a case of adaptive phenotypic plasticity (Hayes 1997; Reques and Tejedo 1997; Laurila et al. 2002).

Several authors have characterized the physiology of anuran metamorphosis (reviewed in Alford 1999), but few studies have investigated the environmental sensitivity of such changes, especially regarding intestinal physiology. In vertebrates, it is well known that digestive physiology and morphology are dramatically affected in the short term by variations in metabolic load and/or diet quality (Toloza and Diamond 1990*a*, 1990*b*; Buddington 1994; Secor and Diamond 2000; Nespolo et al. 2002). It is well known that anuran metamorphosis is affected by abiotic factors such as food quality and quantity, temperature,

and pond duration (Newman 1989, 1994; Blouin and Brown 2000; Merilä et al. 2000; Álvarez and Nicieza 2002; Loman 2002; Vences et al. 2002; Merilä et al. 2004). If major transformations are controlled by environmental factors, it is reasonable to expect that tadpoles should exhibit phenotypic plasticity in organs. Though it is an interesting topic, few studies have addressed whether intestinal morphology and physiology in anuran larvae respond to environmental variation. An appropriate experimental design for studying environmentally driven changes in phenotypic traits during metamorphosis should separate the treatment effects from the changes induced by metamorphosis. One way to separate these effects is to begin with premetamorphic tadpoles of similar body mass and life stage (including a control sample taken before the experiment) and then track intestinal attributes along with external morphology.

The Chilean giant frog (*Caudiverbera caudiverbera*, Leptodactylidae) is an excellent subject to study intestinal plasticity in tadpoles. Tadpoles of *C. caudiverbera* are probably one of the largest in the world (body mass >30 g and body length >10 cm). In this species, several metamorphic stages can coexist in small temporary ponds that can experience large variations in water temperature. Our qualitative observations indicate that these ponds can change water levels as much as 70% (in volume) between summer and winter. The typical larval period of *C. caudiverbera* ranges from 5 to 12 mo, with considerable variability (Diaz and Valencia [1985] reported tadpoles of 2 yr old). The diet of tadpoles in nature is mainly vegetal detritus with considerable variation depending on food availability (Diaz and Valencia 1985).

The aim of this study with *C. caudiverbera* was (1) to test morphological changes in digestive tract morphology and enzyme activity in response to temperature treatment and diet quality and (2) to test the effects of these treatments on the time course of metamorphosis. These objectives are not mutually exclusive, because the acceleration of metamorphosis coincides with gut size reduction and, likely, differential enzyme activities. To control for this dependency, we designed a three-way factorial experiment including longitudinal and cross-sectional comparisons (see "Material and Methods") and a control treatment.

Material and Methods

Study Animals, Collection, and Maintenance

The Chilean giant frog (*Caudiverbera caudiverbera*) is endemic from central and south Chile from 29° to 41°S (Formas 1979). Adults of this species can reach 800–1,000 g; they spend most of their time in water and are strictly carnivorous, feeding on insects, fish, small mammals, and birds (Formas 1979; Diaz and Valencia 1985). During December 2003, we caught live tadpoles in a pond located in Valdivia, Chile (39°48′S, 73°14′W; 9 m), using entomological nets. Individuals were placed in plastic containers with pond water and transferred to the laboratory at the Universidad Austral de Chile. We caught 120 tadpoles of about 30 g and stage 38 of development (Gosner 1960). We randomly assigned 12 individuals to a "control" group (i.e., nontreated, immediately killed and processed after capture), and 48 animals were preacclimated. During the preacclimation period of 7 d, the tadpoles were maintained in a glass aquarium (100 cm × 75 cm × 50 cm) in a room kept at 21°C with a 12L : 12D photoperiod. During this time, we did not provide food to the tadpoles to assure that their guts were completely empty.

Experimental Design

We used six glass tanks (40 cm × 20 cm × 25 cm), each divided into four compartments of 25 cm × 20 cm × 10 cm. Each compartment was filled with 2.4 L of fresh water, which was changed every 2 d. The water in each compartment was aerated during the entire experimental period. Experimental design considered two orthogonal fixed factors: temperature (two levels: 16° and 26° ± 1°C; cold and warm treatment, respectively) and diet (two quality levels: fresh spinach and rat pellet, low- and high-quality diet, respectively [see Table 1]). These diets were selected following numerous trials with several other experimental foods that were undesirable, either because they killed the tadpoles (e.g., artificially composed diets, meat) or were not consumed by the tadpoles (e.g., lettuce, fish food). Ration of diet was approximately 4 g d^{-1} (dry mass) $compartment^{-1}$, which was in excess for all cases (there were always leftovers). We confirmed that all animals ate the experimental food by examining their intestinal contents via dissection at the end of the experiment. Because each tank was divided into four compartments, we interspersed the diet quality in each compartment (i.e., both diet treatments were represented twice in each tank). Three tanks were maintained at 16°C and three tanks at 26°C, which is similar to the extreme values that ponds experience during spring/summer, respectively (15°–30°C, qualitative values from 1 wk of sampling). However, because of limitations of the temperature chambers, the levels of the temperature factor could not be interspersed, thus increasing the risk of pseudoreplication (Hurlbert 1984). To statistically

Table 1: Chemical composition of 30 g of each experimental diet (dry mass)

	High-Quality Diet (Rat Chow)	Low-Quality Diet (Spinach)
Energy (kJ)	57.3	27.6
Protein (g)	7.35	.86
Total lipids (g)	1.44	.11
Crude fiber (g)	1.38	.81

Note. Values for the vegetal diet and animal diet were obtained from the corresponding commercial facility (http://www.infoagro.com and http://www.champion.cl, respectively).

control for this potential limitation, we used aquaria as blocks nested in temperature (Newman et al. 1997; Quinn and Keough 2002). We randomly selected two tadpoles for each compartment (total sample size = 48) and maintained them on the experimental treatment for 21 d. At the end of the experiment, we used one tadpole from each compartment for intestinal enzyme assays and the other for evaluating intestinal morphology. At weekly intervals, we measured the external morphology: body mass, body length, snout-vent length, and tail length. Given that metamorphic tadpoles have shorter tails than premetamorphic individuals, we used the ratio between snout-vent length and tail length as a measure of the stage of metamorphosis. We took external measurements of each individual at 0, 7, 14, and 21 d. To record body mass, we used an electronic balance (sensitivity ± 0.1 g). The three length measurements were taken from digital images, which were performed using Image J software (National Institutes of Health).

Intestines

Tadpoles from the control and experimental treatments were weighed and killed via cervical dislocation, and their guts were immediately removed. The full intestines were first weighed, then the guts were cut longitudinally, and the contents were removed by washing them with 0.9% NaCl solution. Finally, we measured the total length and wet mass of the empty gut, and the mass of the intestinal contents was measured by the difference between full gut mass and empty intestine mass. Guts from tadpoles (control: $n = 5$; treated: $n = 24$) used to assess intestinal morphology were dried at 60°C for 48 h and then weighed to obtain dry mass. Intestines from animals designated for enzyme assays (control: $n = 7$; treated: $n = 24$) were divided into four sections of similar length, washed with 0.9% NaCl solution, and immediately frozen in liquid nitrogen. This solution was used only for dilution purposes. The enzyme reaction was performed at controlled pH, the validity of which has been proven in other amphibians (see Sabat and Bozinovic 1996; Sabat et al. 2005). For analysis, tissues were thawed and homogenized (30 s in an ULTRA TURRAX T25 homogenizer at maximum setting) in 20 vol of 0.9% NaCl solution. Sucrase and maltase activities were determined according to the method described by Martínez del Río et al. (1995). Briefly, tissue homogenates (100 μL) were incubated at 25°C with 100 μL of 56 mmol L^{-1} sugar solutions (maltose and sucrose) in 0.1 M maleate/NaOH buffer, pH 6.5. After 10 min of incubation, reactions were stopped by adding 3 mL of stop/develop Glucose-Trinder (one bottle of Glucose Trinder 500 reagent [Sigma, St. Louis, MO] in 250 mL 0.1 mol L^{-1} TRIS/HCl, pH 7, plus 250 mL of 0.5 mol NaH_2PO_4, pH 7). Absorbance was measured at 505 nm with a Spectronic 21D spectrophotometer after 18 min at 20°C. Aminopeptidase-N assays were conducted using L-alanine-p-nitroanilide as a substrate. Briefly, 100 μL of homogenate diluted with 0.9% NaCl solution was mixed with 1 mL of assay mix (2.04 mmol L^{-1} L-alanine-p-nitroanilide in 0.2 mol L^{-1} NaH_2PO_4/Na_2HPO_4, pH 7). The reaction was incubated at 25°C and arrested after 10 min with 3 mL of ice-cold 2 N acetic acid, then absorbance was measured at 384 nm. The selected pH's for measuring the activities were the optima for each enzyme. On the basis of absorbance, standardized intestinal enzymatic activities were calculated. The activities of all enzymes are presented as standardized hydrolytic activity (UI g^{-1} wet tissue, where UI = mmol hydrolyzed min^{-1}; for an explanation of the use of this standardization, see Martínez del Río et al. 1995). We measured enzyme activity in whole-tissue homogenate to avoid the underestimation of activity.

Statistical Analyses

Overall, we had two orthogonal fixed factors and several dependent variables that were measured either weekly or at the end of the experiment. In some cases, these dependent variables were correlated with body mass, according to linear correlations (i.e., external morphology, gut morphology, gut mass, and total enzyme activity). For these variables, we performed ANCOVAs using body mass as the covariate. Variables measured on a weekly basis (e.g., body mass, body length, etc.) were included as a repeated-measures factor. Because enzyme activity (i.e., UI g^{-1} wet tissue; independent of body mass [m_b]) was measured in four sections of the intestine, it was also considered a repeated-measures factor, given that it was recorded in the same experimental individual. A two-way ANOVA demonstrated that there were no differences among treatment groups at the start of the experiment. Assumptions of statistical tests were evaluated using the Kolmogorov-Smirnov test for normality, the Cochran C test for homoscedasticity, and parallelism tests for interactions between factors and the covariate (body mass). The assumption of homogeneity of variances in the repeated-measures factor (i.e., sphericity; Quinn and Keough 2002) was evaluated using a Mauchley test (StatSoft 2004). No transformation was necessary in any case. When the sphericity assumption was violated (i.e., enzyme activities), we performed a Greenhouse-Geisser adjustment to calculate the appropriate degrees of freedom (Davis 2002).

Results

We found no significant effects of diet treatment on any response variable ($P > 0.10$ for all comparisons). Also, there were no significant effects of blocks (i.e., aquaria) on any response variable either by including this effect in the full analysis or by analyzing it by a one-way ANOVA ($P > 0.35$ in all cases). Hence, to gain statistical power, we pooled data from both diet groups. Comparisons of tadpoles measured at the beginning and end of experiments showed small differences in external morphology during the experimental period (Table 2).

External Morphology

There was a significant interaction between temperature treatments and acclimation time (repeated measures) on body mass ($F_{3,129} = 20.0$; $P < 0.0001$, repeated-measures ANOVA). Warm-treated individuals lost body mass at a constant rate, whereas cold-treated tadpoles lost body mass during the first week but maintained body mass thereafter (Fig. 1). The snout-vent length/tail length ratio also had an interaction between temperature and time ($F_{3,120} = 12.8$; $P < 0.0001$, repeated-measures ANOVA). During the first week, the ratio increased in both temperature treatments. Then, for cold-treated individuals, the ratio increased to reach a peak at 14 d and then decreased slightly. In contrast, this ratio in warm-treated tadpoles decreased at a constant rate (Fig. 2).

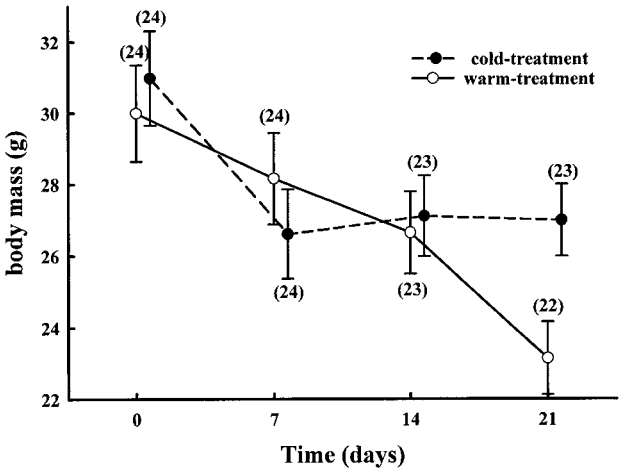

Figure 1. Body mass variation of *Caudiverbera caudiverbera* tadpoles treated to 16°C (cold treatment) and 26°C (warm treatment) during the experiment. Values are represented as mean ± 1 SE (sample sizes in parentheses).

Enzyme Activity and Intestinal Morphology

No sucrase activity was detected, which suggests that this enzyme may not be present in tadpoles from this species and that the marginal amount detected was probably due to exogenous material. There were no significant effects of temperature treatment on total maltase activity ($F_{2,27} = 1.54$; $P = 0.23$, one-way ANOVA; Fig. 3). We found significant effects of temperature treatment on total aminopeptidase-N activity ($F_{2,27} = 6.27$, $P = 0.006$, one-way ANOVA), with enzyme activity of warm-treated animals being lower than that of control (field) and cold-treated individuals ($P < 0.01$, Tukey a posteriori tests; Fig. 3). Also, aminopeptidase-N activity varied significantly among different intestinal segments, but not between temperature treatments ($F_{3,42} = 4.3$; $P = 0.01$, repeated-measures ANOVA; Fig. 4). For maltase activity, a significant interaction was found between gut sections and temperature treatment ($F_{6,39} = 3.9$; $P = 0.004$, repeated-measures ANOVA; Fig. 4). We tested for a correlation between aminopeptidase-N and maltase activity, but this was not significant ($P > 0.15$). For intestinal morphology, guts were smaller, in both length and mass, in warm-treated individuals compared with control and cold-treated tadpoles (Fig. 5). Similarly, intestinal content was lower in warm-treated individuals (Fig. 5).

Discussion

The intestinal morphology and physiology of vertebrates is highly plastic (Piersma and Lindström 1997), with changes reported in enzyme activities, gut dimensions, and nutrient transport following exposure to temperature, diet, or metabolic load (Toloza and Diamond 1990*a*, 1990*b*; Sabat et al. 1995; Secor and Diamond 2000; Nespolo et al. 2002). This response has been reported in a range of animals, both ectotherms (Buddington 1987, 1994; Secor and Diamond 2000) and endotherms (Geluso and Hayes 1999; Nespolo et al. 2002), but rarely for larval anurans (Toloza and Diamond 1990*a*, 1990*b*; Feder 1992). According to some authors, the guts of tadpoles should be highly plastic because they have a more generalist diet than adult anurans (Feder 1992; Karasov 1992). However, in *Cau-*

Table 2: Descriptive data (means ± SE)

Variable	Initial Value	*N*	Final Value	*N*
Body mass (g)	30.6 ± 2.01	48	25.3 ± 1.40	48
Snout-vent length (cm)	5.32 ± .15	48	5.26 ± .13	48
Tail length (cm)	6.67 ± .17	48	6.43 ± .13	48
Aminopeptidase-N activity (UI g^{-1} wet tissue)	2.22 ± .16	7	1.79 ± .22	18
Maltase activity (UI g^{-1} wet tissue)	5.89 ± 1.17	7	5.60 ± .56	23
Intestine wet mass (g)	.81 ± .16	11	.76 ± .04	40
Intestine dry mass (g)	.05 ± .02	5	.08 ± .01	19
Intestine length (cm)	89.7 ± 9.58	12	66.4 ± 3.83	44

Note. *N* indicates sample size. Initial values for enzyme activity and intestine morphology are from field animals (i.e., control animals). UI = mmol hydrolyzed min^{-1}.

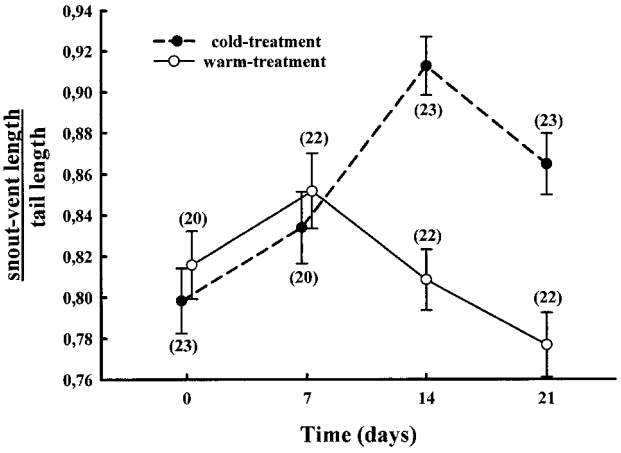

Figure 2. Temporal variation of the snout-vent length to tail length ratio in *Caudiverbera caudiverbera* tadpoles treated to 16°C (cold treatment) and 26°C (warm treatment). Values are represented as mean ± 1 SE (sample sizes in parentheses).

diverbera caudiverbera, digestive plasticity was found to be highly sensitive to temperature but not to diet quality.

Diet Acclimation

We found that diet has a minimal effect on growth and intestinal gross morphology and two enzyme activities in *C. caudiverbera*. This result contrasts with other studies that have demonstrated that dietary protein accelerates metamorphosis in tadpoles (Denver 1997; Kupferberg 1997; Álvarez and Nicieza 2002) by stimulating an increase in thyroid hormone production (Kupferberg 1997). A reason for not detecting such an effect in our study could be that animals did not consume the food that we provided. However, we confirmed consumption of both diets by examining and weighing intestinal contents. In addition, we were focusing on intestinal response; our experiments may have not been long enough to observe a significant effect of the diet on metamorphosis. Nevertheless, in *Discoglossus galganoi*, Álvarez and Nicieza (2002) reported a significant effect of diet quality over a short time period, but this effect was temperature dependent. Therefore, we cannot completely exclude the potential effect of diet quality on the timing of metamorphosis for *C. caudiverbera*. We can conclude only that these tadpoles do not exhibit short-term plasticity (3 wk) to these diets.

Temperature Treatments

For aquatic ectotherms that undergo metamorphosis, an increase in ambient temperature—and therefore body temperature—results in an increase in metabolic rate, which is directly linked to ectotherm bioenergetics (McNab 2002). Therefore, temperature is considered to be one of the most important proximal causes of variation in size and age of metamorphosis (Álvarez and Nicieza 2002). In anuran metamorphosis, shifts in digestive physiology translate to a reduction in gut size, an increase in enzyme activity associated with the breakdown of proteins (i.e., aminopeptidase-N activity), and a reduction in the activity of enzymes that hydrolyze carbohydrates (e.g., sucrase and maltase; Toloza and Diamond 1990*b*; Feder 1992; Kupferberg 1997). However, this is complicated by the fact that in vertebrates, digestive physiology is also affected by phenotypic plasticity. In our experiment, a completely plastic response would be denoted by differential enzyme activities as a function of diet composition (i.e., higher aminopeptidase-N activity for individuals treated to the high-quality diet, and higher activity of maltase for individuals treated to the low-quality diet). Similarly, temperature should produce larger intestines in warm-treated individuals (compared with cold-treated tadpoles) because they have higher metabolic requirements. However, we observed a mixed response (i.e., a consequence of temperature treatment together with a phenotypically plastic response). Also, external morphology did respond to the rate of metamorphosis (i.e., warm-treated individuals were shorter and smaller), as in previous studies (Blouin and Brown 2000; Álvarez and Nicieza 2002; Loman 2002). In addition, warm-

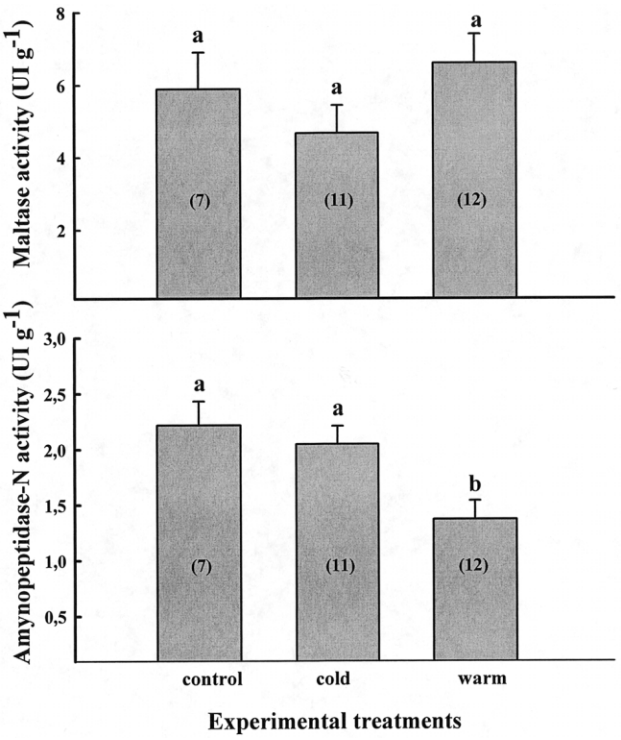

Figure 3. Maltase (*top*) and aminopeptidase-N (*bottom*) activities from control (field), warm treatment, and cold treatment tadpoles. Different letters indicate significant differences following a Tukey post hoc test. Values are represented as mean ± 1 SE (sample sizes in parentheses).

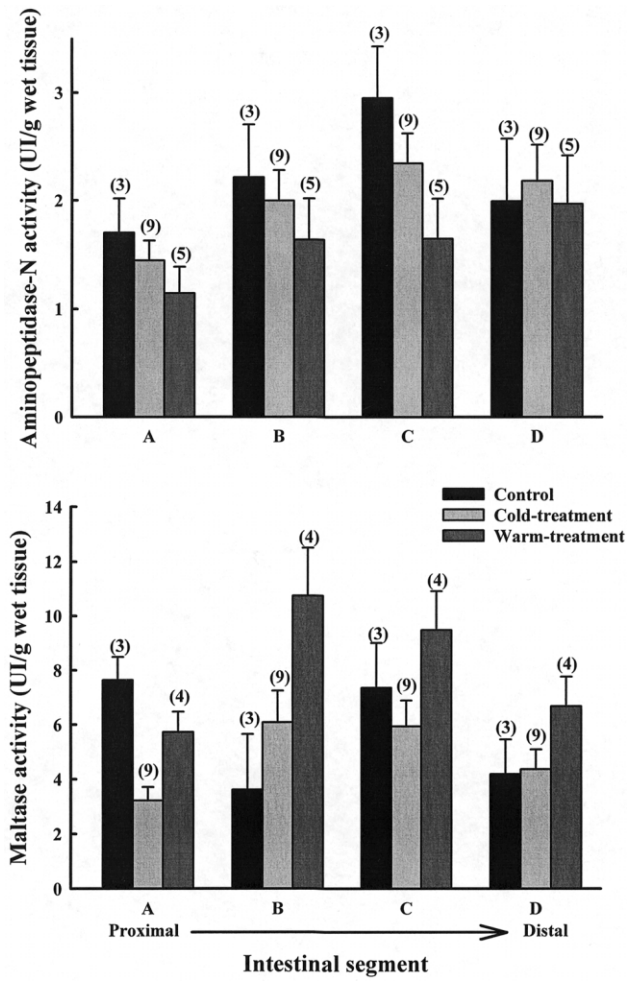

Figure 4. Enzyme activities along the digestive tract of aminopeptidase-N (*top*) and maltase (*bottom*) activities in control (field), cold treatment, and warm treatment tadpoles. Different segments of the gut are designated with a letter: *A*, proximal; *B*, *C*, middle; *D*, distal segment (sample sizes in parentheses). See "Results" for details of the statistical analyses.

treated tadpoles showed a reduction in intestine mass and length compared with control and cold-treated individuals. The repeated-measures factor showed a complex response in external morphology because acclimation time interacted with temperature. This interaction (Fig. 2) shows that differences in mass between groups appeared only at the fourth week of treatment (i.e., a shorter experiment would not have detected differences). Enzyme activities showed somewhat contrasting responses to temperature treatment. No tadpole (from the field or thermally treated in the laboratory) had any indication of sucrase activity, which suggests that they are not complete generalists. However, there could have been problems with the methodology, given that it has not been previously used with tadpoles. An examination of total enzyme activity showed a clear effect of temperature treatment on activity (i.e., warm-treated individuals had lower activity) for maltase, but not for aminopeptidase-N. When including intestinal section as a repeated-measures factor, the effect of temperature on aminopeptidase-N activity was not significant. This apparently contradictory result is due to a loss of power when we include the additional factor in the analysis (i.e., the repeated measures), which reduced the sample size at the lowest-level combination

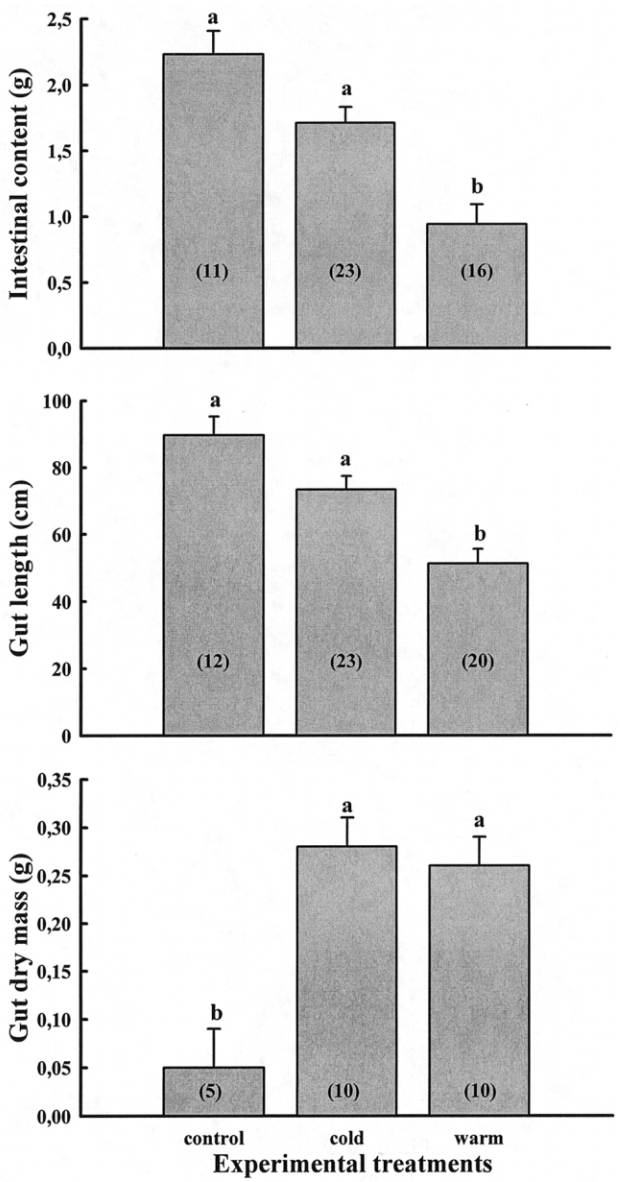

Figure 5. Intestinal variables: intestinal contents (*top*), gut length (*middle*), and gut mass (*bottom*) from control (field), cold treatment, and warm treatment tadpoles. Different letters indicate significant differences following a Tukey post hoc test. Values are represented as mean ± 1 SE (sample sizes in parentheses).

of treatments. Nevertheless, for maltase activity, we did observe an interaction between intestinal section and temperature.

Studies addressing changes in enzyme activity following temperature or diet exposure in anuran larvae are very scarce (Hunt and Farrar 2002). This contrasts with the abundance of studies characterizing the plastic response of tadpoles in terms of life-history traits (Newman 1989, 1994; Blouin and Brown 2000; Merilä et al. 2000, 2004; Álvarez and Nicieza 2002; Loman 2002; Vences et al. 2002). Moreover, plasticity in intestinal enzymes is widely known for a variety of ectothermic animals, mostly in response to diet treatment (Brito et al. 2001; Guzman et al. 2001; Lundstedt et al. 2004). In *C. caudiverbera* tadpoles, we found enzyme plasticity associated with temperature treatment, but not with diet treatment. Because anuran larvae exhibit intermediate physiological features between fish and amphibians, and given the plasticity present in intestinal features of fish (Buddington 1987, 1994; Lundstedt et al. 2004), our results are interesting and call for more detailed studies on the intestinal physiology of anuran larvae.

Acknowledgments

This work was funded by Fondo Nacional de Desarrollo Científico y Technologico (FONDECYT) grant 3030032 to R.N. and FONDECYT grant 1010647 to P.S. L.E.C. acknowledges a PhD fellowship from the Comisión Nacional de Investigación Científica y Tecnológica. We thank Elias Barriga and Leonardo Almonacid for assistance in capturing and maintaining experimental animals and Oscar Chaparro for the facilities to work in his laboratory.

Literature Cited

Alford R.A. 1999. Ecology: resource use, competition, and predation. Pp. 240–278 in R.W. McDiarmid and R. Altig, eds. Tadpoles: The Biology of Anuran Larvae. University of Chicago Press, Chicago.

Álvarez D. and A.G. Nicieza. 2002. Effects of temperature and food quality on anuran larval growth and metamorphosis. Funct Ecol 16:640–648.

Blouin M.S. and S.T. Brown. 2000. Effects of temperature-induced variation in anuran larval growth rate on head width and length at metamorphosis. Oecologia 125:358–361.

Brito R., C. Rosas, M.E. Chimal, and G. Gaxiola. 2001. Effects of different diets on growth and digestive enzyme activity in *Litopenaeus vannamel* (Boone, 1931) early post-larvae. Aquac Res 32:257–266.

Buddington R.K. 1987. Does the natural diet influence the intestine's ability to regulate glucose absorption? J Comp Physiol B 157:677–688.

———. 1994. Nutrition and ontogenic development of the intestine. Can J Physiol Pharmacol 72:251–259.

Davis C.S. 2002. Statistical Methods for the Analysis of Repeated Measurements. Springer, New York.

Denver R.J. 1997. Proximate mechanisms of phenotypic plasticity in amphibian metamorphosis. Am Zool 37:172–184.

Diaz N.F. and J. Valencia. 1985. Larval morphology and phenetic relationships of the Chilean *Alsodes*, *Telmatobius*, *Caudiverbera* and *Insuetophrynus* (Anura, Leptodactylidae). Copeia 1985:175–181.

Feder M.E. 1992. A perspective on environmental physiology of the amphibians. Pp. 447–473 in M.E. Feder and W.W. Burggren, eds. Environmental Physiology of the Amphibians. University of Chicago Press, Chicago.

Formas J.R. 1979. Los anfibios del bosque temperado del sur de Chile: una aproximación sobre su origen. Arch Biol Med Exp 12:191–196.

Geluso K. and J.P. Hayes. 1999. Effects of dietary quality on basal metabolic rate and internal morphology of European starlings (*Sturnus vulgaris*). Physiol Biochem Zool 72:189–197.

Gosner K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16:183–190.

Guzman C., G. Gaxiola, C. Rosa, and A. Torre-Blanco. 2001. The effect of dietary protein and total energy content on digestive enzyme activities, growth and survival of *Litopenaeus setiferus* (Linneaus 1767) postlarvae. Aquac Nutr 7:113–122.

Hayes T.B. 1997. Amphibian metamorphosis: an integrative approach. Am Zool 37:121–123.

Hunt J.A. and E.S. Farrar. 2002. Phenotypic plasticity of the digestive system of tadpoles in response to diet. Integr Comp Biol 43:934.

Hurlbert S.H. 1984. Pseudoreplication and the design of ecological field experiments. Ecol Monogr 54:187–211.

Karasov W.H. 1992. Test of adaptive modulation hypothesis for dietary control of intestinal transport. Am J Physiol 263: R496–R502.

Kupferberg S.J. 1997. The role of larval diet in anuran metamorphosis. Am Zool 37:146–159.

Laurila A., S. Karttunen, and J. Merilä. 2002. Adaptive phenotypic plasticity and genetics of larval life histories in two *Rana temporaria* populations. Evolution 56:617–627.

Loman J. 2002. Temperature, genetic and hydroperiod effects on metamorphosis of brown frogs *Rana arvalis* and *R. temporaria* in the field. J Zool (Lond) 258:115–129.

Lundstedt L.M., J.F.B. Melo, and G. Moraes. 2004. Digestive enzymes and metabolic profile of *Pseudoplatystoma corrucans* (Teleostei: Siluriformes) in response to diet composition. Comp Biochem Physiol B 137:331–339.

Marian M.P. and T.J. Pandian. 1985. Effect of temperature on development, growth and bioenergetics of the bullfrog tadpole *Rana tigrina*. J Therm Biol 10:157–161.

Martínez del Río C., E. Brugger, J.L. Ríos, M.E. Vergara, and

M. Witmer. 1995. An experimental and comparative study of dietary modulation of intestinal enzymes in European starlings (*Sturnus vulgaris*). Physiol Zool 68:490–511.

McNab B.K. 2002. The Physiological Ecology of Vertebrates: A View from Energetics. Comstock/Cornell University Press, Ithaca, NY.

Merilä J.A., A. Laurila, A.T. Laguen, and K. Räsänen. 2000. Plasticity in age and size at metamorphosis in *Rana temporaria*: comparison of high and low latitude populations. Ecography 23:457–465.

———. 2004. Heads or tails? variation in tadpole body proportions in response to temperature and food stress. Evol Ecol Res 6:727–738.

Nespolo R.F., L.D. Bacigalupe, P. Sabat, and F. Bozinovic. 2002. Interplay among energy metabolism, organ mass and digestive enzyme activity in the mouse-opossum *Thylamys elegans*: the role of thermal acclimation. J Exp Biol 205:2697–2703.

Newman J.A., J. Bergelson, and A. Grafen. 1997. Blocking factors and hypothesis tests in ecology: is your statistics text wrong? Ecology 78:1312–1320.

Newman R.A. 1989. Developmental plasticity of *Scaphiopus couchii* tadpoles in an unpredictable environment. Ecology 70:1775–1787.

———. 1994. Effects of changing density and food levels on metamorphosis of a desert amphibian, *Scaphiopus couchii*. Ecology 75:1085–1096.

Piersma T. and A. Lindström. 1997. Rapid reversible changes in organ size as a component of adaptive behavior. Trends Ecol Evol 12:134–138.

Quinn G.P. and M.J. Keough. 2002. Experimental Design and Data Analysis for Biologists. Cambridge University Press, Cambridge.

Relyea R.A. 2004. Fine-tuned phenotypes: tadpole plasticity under 16 combinations of predators and competitors. Ecology 85:172–179.

Reques R. and M. Tejedo. 1997. Reaction norms for metamorphic traits in natterjack toads to larval density and pond duration. J Evol Biol 10:829–851.

Romer A.S. and T.S. Parsons. 1981. Anatomia Comparada. Interamericana, Ciudad de Mexico.

Rot-Nikcevic I. and R.J. Wassersug. 2004. Arrested development in *Xenopus laevis* tadpoles: how size constrains metamorphosis. J Exp Biol 207:2133–2145.

Sabat P. and F. Bozinovic. 1996. Dietary chemistry and allometry of intestinal disaccharidases in the toad *Bufo spinulosus*. Rev Chil Hist Nat 69:387–391.

Sabat P., F. Bozinovic, and F. Zambrano. 1995. Role of dietary substrates on intestinal disaccharidases, digestibility, and energetics in the insectivorous mouse-opossum (*Thylamys elegans*). J Mammal 76:603–611.

Sabat P., J.M. Riveros, and C. López-Pinto. 2005. Phenotypic flexibility in the intestinal enzymes of the African clawed frog *Xenopus laevis*. Comp Biochem Physiol A 140:135–139.

Secor S.M. and J.M. Diamond. 2000. Evolution of regulatory response to feeding in snakes. Physiol Biochem Zool 73:123–141.

StatSoft. 2004. STATISTICA (data analysis software system). Version 6.1. http://www.statistica.com.

Toloza E.M. and J.M. Diamond. 1990*a*. Ontogenetic development of nutrient transporters in bullfrog intestine. Am J Physiol 258:760–769.

———. 1990*b*. Ontogenetic development of transporter regulation in bullfrog intestine. Am J Physiol 258:770–773.

Vences M., M. Puente, S. Nieto, and D.R. Vieites. 2002. Phenotypic plasticity of anuran larvae: environmental variables influence body shape and oral morphology in *Rana temporaria* tadpoles. J Zool (Lond) 257:155–162.

Wassersug R.J. 1997. Where the tadpole meets the world: observations and speculations on biomechanical and biochemical factors that influence metamorphosis in anurans. Am Zool 37:124–136.

Wilbur H.M. and J.P. Collins. 1973. Ecological aspects of amphibian metamorphosis. Science 182:1305–1314.