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Stable isotopes document mainland–island divergence in resource use without concomitant physiological changes in the lizard *Liolaemus pictus*

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ABSTRACT

Shifts in feeding ecology are believed to promote island–mainland divergence. The lizard *Liolaemus pictus* has several different subspecies on Chilean islands and mainland. These subspecies inhabit contrastingly different habitats both in different islands and mainland, which suggests the potential for habitat related dietary variation. We investigated the dietary habits of *L. pictus* by both stomach content analyses and by nitrogen stable isotope analyses (δ^{15}N), which we used as a proxy variable for trophic level. We also compared the morphology of the digestive tract and the activity of intestinal digestive enzymes of mainland and island lizards. We hypothesized differences in diet and trophic level among populations and that these differences would predict the expression of the morphological and biochemical features of the digestive tract. More specifically, we predicted shorter intestines and higher levels of peptidases in more insectivorous than in more frugivorous/herbivorous lizards. The diet of *L. pictus* was characterized by the consumption of a wide diversity of food types, including fruit and insects, in all populations. Stable isotopes revealed higher trophic level, and hence probably higher protein intake, in mainland than in island populations, but contrary to our prediction, they had shorter intestines and higher relative activity of intestinal peptidases than mainland lizards. Furthermore, the proportion of fruit items in the stomach content was higher in the population that exhibited the lowest tropic level. These results suggest that morphological and physiological differences among populations of *L. pictus* are not correlated with feeding ecology, suggesting that the lizard's first responses to the selective pressure represented by a diet shift are behavioral.

1. Introduction

It is widely believed that shifts in feeding ecology promote island–mainland phenotypic divergence in vertebrates (Clegg and Owens, 2002; Rocha et al., 2004). As a result of higher densities, diet expansion, and low predation levels (Olesen and Valido, 2003), lizards that live in islands are often more herbivorous than mainland lizards (Van Damme, 1999; Cooper and Vitt, 2002; Valido and Nogales, 2003).

Liolaemus lizards are a speciose (more than 160 spp, Etheridge and Espinoza, 2000; Schulte et al., 2000) and widely distributed South American taxon (Donoso-Barros, 1966; Frost and Etheridge, 1989). *Liolaemus pictus* has one of the southernmost distributions of all Chilean lizards (37°S–43°S). Various subspecies of *L. pictus* inhabit different habitats on islands and the continental mainland. Because the foraging ecology of reptiles can be affected by changes in prey availability and by specific nutrient requirements (Hurtubia, 1973; Ortiz and Riveros, 1976; Hirai, 2002), which are likely to differ between habitats, it is likely that different populations vary in diet. Anecdotal evidence indicates that mainland *Liolaemus* feed primarily on invertebrates, whereas island populations feed on fruit (Ortiz, 1974; Willson et al., 1996). Vidal et al. (2006) hypothesized that morphometric differences among *L. pictus* from the continental mainland and Chiloé Island are evidence of insular divergence resulting from diet differences (see also Ortiz, 1974; Willson et al., 1996).

The morphology and physiology of the gastrointestinal tract can constrain the ability of animals to digest specific substrates and hence determine their ecological niche (Karasov, 1996). Digestion rates can influence feeding rates, time-budgets, growth, survival, and reproduction (Kersten and Visser, 1996; Karasov, 1990; Secor, 2001). In spite of the importance of digestive processes on food processing and energy and nutrient intake of reptiles feeding different diets, no studies have assessed intraspecific differences in the digestive physiology of geographically separated populations. We evaluated the hypothesis that the morphology and digestive physiology are correlated with dietary habits in five populations of *L. pictus*. We used δ^{15}N of tissues and gut content analysis to investigate whether the relative reliance of lizards on animal prey and plants varies among lizard populations and

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whether it has an effect on gut morphology and physiology. Although the use of stable isotope tools has been widely used in ecological studies of vertebrates, few studies have been conducted to study the trophic ecology of reptiles (Dalerum and Angerbjörn, 2005, Kupfer et al., 2006). Because we simultaneously used dietary and isotopic analysis, our study also might contribute to the validation of this tool to infer dietary habits in reptiles.

2. Material and methods

A total of 97 lizards were collected during the austral summer from five locations (see Fig. 1) on the Chiloé Archipelago: *L. p. pictus* from Antillanca (40°46′S, 72°15′W, $n=22$), and Hornopirén (41°56′S; 72°23′W, $n=14$); *L. p. chiloensis* from Chiloé Island (42°23′S; 73°51′W, $n=30$); *L. p. talcanensis* from Talcán Island (42°44′S; 72°57′W, $n=14$), and *L. p. codoceae* from Butachauques Island (42°18′S; 73°08′W, $n=17$). Animals were weighed (±0.01 g), sacrificed and stored in liquid nitrogen for posterior analysis. Samples were transported to the laboratory in Santiago where all analyses were performed.

The Chilotan Archipelago includes Isla Grande de Chiloé (IGC) and a series of small islands located along the western margin of the SE Pacific Ocean. This area has been affected by repeated glaciations during Plio-Pleistocene times, which have shaped its present physical and biological configuration (Villagrán et al., 1986; Denton et al., 1999; Moreno and León, 2003). As Patagonian glaciers withdrew back into the Andes, a series of small islands located east of IGC were either uncovered or formed sometime between ~17,500 and ~14,700 cal yr BP, judging from minimum radiocarbon dates for ice free conditions in continental Chiloé (Heusser, 1990; Denton et al., 1999). According to Villagrán et al. (1986), the relief of the archipelago and mainland determines differences in forests after de last glaciation. At the IGC, three kind of forest are shared with the mainland, while the islands show valdivian–Northpatagonian forest, which suggests a diet differential between different islands and mainland.

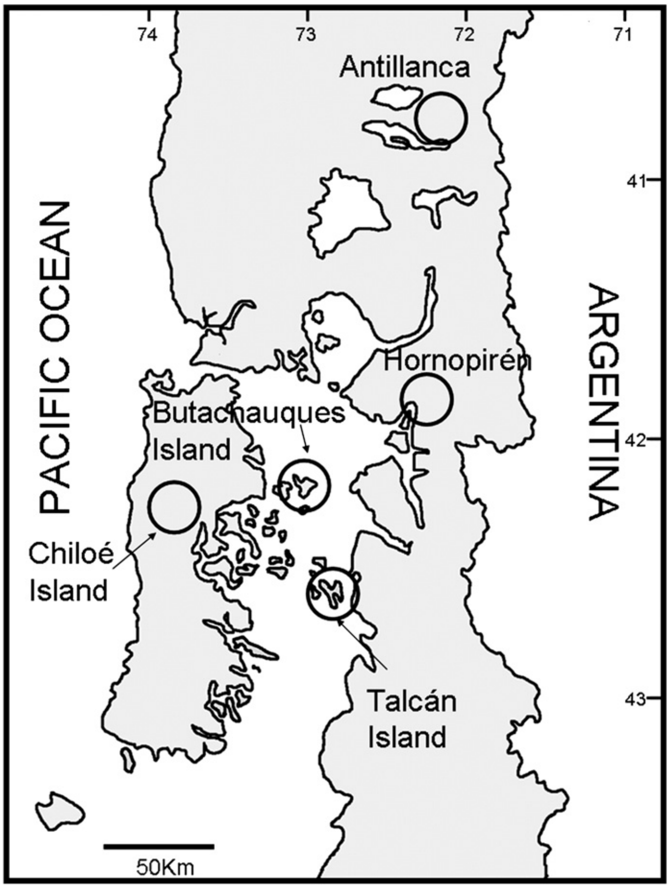

Fig. 1. Geographic distribution of *Liolaemus pictus* included in this study within archipelago area.

2.1. Diet composition

In the laboratory, specimens were thawed and the entire digestive tract was removed. Gut contents were thawed and prey items were separated, counted and identified to the lowest possible taxonomic level. For each sample, the frequency of occurrence and the percent composition of prey taxa by number (percent number) were calculated. Frequency of occurrence is the percentage of all non-empty stomachs containing food in which prey taxon *i* was found and percent number is the percentage that prey taxon *i* contributes to the total number of food items in all stomachs (Bowen, 1996). The relative importance index, RI (George and Hadley, 1979) was calculated as:

$$\mathrm{RI}i = 100\mathrm{AI} / \sum \mathrm{AI}$$

where AI = (% frequency of occurrence + % total number) is the absolute importance of prey taxon *i*). This index reduces the amount of bias that may occur if measures are used separately (Wallace, 1981).

2.2. Isotopic composition

Samples of leg muscle were dried to constant mass at 80 °C and then de-fatted by ether extraction. Bones were demineralized in 1.0 N HCl at 4 °C in a magnetic shaker overnight. Collagen extracts were rinsed with distilled water and dried under vacuum. Samples were analyzed for stable carbon and nitrogen ratios using a continuous flow isotope ratio mass spectrometer. Stable isotope data were presented as the relative difference between ratios of the sample and standards. Stable isotope ratios were expressed using standard delta notation (δ, Beaudoin et al., 1999; Vizzini and Mazzola, 2003) in parts per thousand (‰) as:

$$\delta X = (\mathrm{Rsample} / \mathrm{Rstandard} - 1) \times 1000$$

where Rsample and Rstandard are the molar ratios of the heavy/light isotope of the sample and the reference, respectively. Samples were referenced against the international standard, the Vienna Pee Dee Belemnite (VPDB) for δ^{13}C and atmospheric nitrogen (air) for δ^{15}N.

A proper evaluation of the trophic position of individuals requires information about the specific enrichment of δ^{15}N from food to consumers and also the basal value of the producers. Thus, we also analyzed the isotopic composition of the representative seeds and fruits in all localities to obtain the basal levels of δ^{15}N. The species selected for this analysis were those that appeared in the stomach contents of individuals. Thus we calculated the relative trophic level (TL) in each specimen of lizard following Post (2002) as: $\mathrm{TL} = (1 + [\delta^{15}\mathrm{Nanimal} - \delta^{15}\mathrm{Nproducers}]/\Delta^{15}\mathrm{N})$, where the δ^{15}Nanimal represent the isotopic signature from leg muscle samples, δ^{15}Nproducers is the isotopic signature of the producers, and Δ^{15}N is the enrichment factor by trophic level. Recently, it has been reported that discrimination of ^{15}N varies among species, as well as with feeding rates and dietary protein content (see Martínez del Rio et al., 2009 for a review), so consumers at higher trophic levels have lower Δ^{15}N than consumers at lower trophic levels. For our calculations we used a mean of 2.3‰ reported for muscle of terrestrial animals (McCutchan et al., 2003) which could, to some extent, underestimate the relative position of lizards at a higher level in the food web.

2.3. Intestinal morphology

Digestive organs were washed with saline solution (0.9% NaCl), and the length measured with a digital caliper (±0.01 mm). Additionally, we measured the snout-vent length of all specimens. After morphological measurements, the stomach content was removed for diet analysis and the small intestine was used for digestive enzyme determinations.

2.4. Enzyme activity measurements

Small intestine sections were thawed, weighed (±0.01 g) and homogenized (30 s in an Ultra Turrax T25 homogenizer at maximum setting) in 20 vol of 0.9% NaCl solution. Disaccharidase activities (Maltase [EC 3.2.1.20], Sucrase [EC 3.2.1.48] and trehalase [EC 3.2.1.28]) were determined according to the method described by Martínez del Río et al. (1995). Briefly, tissue homogenates (100 µL of 56 mmol L-1 sugar solutions — maltose, sucrose and trehalose) were added to 0.1 M maleate/NaOH buffer, pH 6.5. After 10 min of incubation at 25 °C, reactions were stopped by adding 3 mL of a stop/develop glucose–Trinder (one bottle of Glucose Trinder 500 reagent (Sigma, St Louis, MO, USA) in 250 mL 0.1 mol L^{-1} TRIS/HCL, pH 7 plus 250 mL of 0.5 NaH_2PO_4, pH 7). Absorbance was measured to 505 nm with a spectrophotometer after 18 min at 20 °C. Based on a standard curve for glucose, we calculated maltase, sucrase, and trehalase activity in micromoles hydrolyzed per gram of wet tissue. Aminopeptidase-N [APN, EC 3.4.11.2] assays were done with 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 ice-cold acetic acid 2 N, and absorbance was measured at 384 nm. We calculated the liberated amount of p-nitroanilide from the absorbance at 384 nm, and then we estimated the aminopeptidase-N activity by using a standard curve constructed for p-nitroanilide. The selected pHs for measuring the activities were the optimum for each enzyme, which were determined previously by measuring enzyme activities in a range of pH from 4.0 to 9.0. Because all enzyme reactions were linear in the time range of the experiments, we are confident that reactions were under substrate saturating conditions. We measured enzyme activity in a whole-tissue homogenate to avoid underestimating the activity. Hence, the activities of all enzymes are presented as standardized hydrolytic activity (IU total, where IU = µmol hydrolyzed/min).

2.5. Statistical analysis

Prior to statistical analyses, all data were examined for assumptions of normality and homogeneity of variance, using Kolmogorov–Smirnov and Levene tests, respectively. Differences of diet composition among localities were tested by one-way ANOVA, followed by Tukey test (Sokal and Rohlf, 1995). Differences of intestinal morphology among populations were evaluated by multivariate analysis of variance (MANCOVA), using body mass as the covariate, followed by a Tukey's test. For enzyme activity, a correlation matrix was performed to examine a potential relationship among disaccharidases and N-aminopeptidase. Differences in enzyme activity were evaluated in MANCOVA, using body mass as the covariate and then tested by one-way ANOVA. The differences in isotopic composition between bone and muscle and among localities were evaluated by 10,000 random permutations, because it allows us to determine the probability that the results do not represent random events. Finally, correlational evidence was evaluated by a non-parametric correlation matrix (Spearman rank order correlation) among biological (enzymes, morphology) and ecological (stomach content, trophic level) variables, using the average value of the variables from each location (Sokal and Rohlf, 1995). Because morphological and biochemical variables were correlated with body mass, we used the residuals of each variable against body mass to perform the correlation analysis. All statistical analyses were conducted using SYSTAT software (SPSS, 2000). Since some animals had no significant food in their stomach, for some individuals we could not analyze the isotopic signature or enzymatic activities. Thus, the numbers of individuals used for each analysis may differ.

3. Results

3.1. Diet composition

The diet composition of *L. pictus* included 19 taxonomic groups (Table 1). On average, 88% corresponds to invertebrate prey, while the remainder corresponds to vegetation (fruits, and seeds). Of these, mainland lizards consume on average 89.9% invertebrates and 10.1% vegetation, and island lizards consume 87.4% invertebrates and 12.6% of vegetation. We found no significant differences between mainland and island populations for both invertebrate (ANOVA, $F_{1,95} = 1.27$; $p > 0.1$) and fruit (ANOVA, $F_{1,95} = 0.07$; $p > 0.1$) consumption. However, significant differences were found when all localities were analyzed (ANOVA, $F_{4,92} = 4.24$; $p < 0.001$). The *a posteriori* test revealed significant differences between Antillanca–Hornopiren (HSD–Tukey, $p < 0.01$) and Antillanca-I. Talcan ($p < 0.01$). For each locality, the RI varied in the taxonomic origin of prey. For mainland localities, Dermaptera, Diptera, Thysanoptera, and Coleoptera were the more represented items, whereas seeds of *Nertera granadensis* were those of lower importance. For island localities, Odonata, Diptera, Coleoptera, Hymenoptera, Quilopoda, Crustacea, seeds of *Galium hypocarpium*, *Gaultheria sp*., and the rest of plants were the more consumed prey (Table 1). Although all lizards consumed a higher number of invertebrates than fruit, lizards from Talcan Island had a higher RI of fruit contents than the remainder localities (Table 1).

3.2. Isotopic composition

Values of δ^{15}N of the producers spanned from −7.04 to −1.73 in mainland locations and from −0.57 to 5.26 in island locations, demonstrating a higher mean value of δ^{15}N in mainland than in island populations (Table 2). Comparisons of isotopic composition of ^{15} N and ^{13}C between muscle and bone (Fig. 2), did not reveal significant differences (10,000 random permutations, $p = 0.413$). In both mainland and island localities, δ^{15}N signature was 4.83±2.1‰ (mean±SE) which ranged from 2.04 to 9.27‰, indicating considerable individual variation. δ^{15}N values were lower in mainland (2.75±0.4‰), than island (6.46±1.3‰) populations (Fig. 2, 10,000 random permutations, $p < 0.001$). However, when the δ^{15}N of the producers was used to estimate the tropic level, the analysis revealed a different pattern (Table 2). Indeed, the TL spanned approximately two levels showing a higher value in mainland than in island populations ($F_{(4,36)} = 66.34$, $p < 0.001$). Values of δ^{13}C in all localities were −25.15±0.66‰ (ranged from −24.20 to −26.14‰). This signature was similar between mainland (−25.08±0.51) and island (−25.19±0.75) populations (10,000 random permutations, $p < 0.001$). The δ^{13}C of the producers was similar between island and mainland populations (−28.60±0.94 and−31.01±4.92 for island and mainland locations respectively).

3.3. Intestinal morphology

Significant and positive associations were found between body mass and the length of the stomach ($r^2 = 0.11$; $p < 0.002$), small intestine ($r^2 = 0.22$, $p < 0.001$), large intestine ($r^2 = 0.34$, $p < 0.001$) and total gut ($r^2 = 0.43$, $p < 0.001$). The MANCOVA analysis for morphometry of digestive tract indicated significant differences between mainland and island localities (Wilks' Lambda = 0.598; $p < 0.001$; Table 2). When all localities were considered, significant differences were also found (Wilks' Lambda = 0.625; $p < 0.001$). The *a posteriori* test indicated the

Table 1
Frequency of occurrence (%FO), percentage by number (%Number) and Index of relative importance (RI) of prey taxa in the diets of *Liolaemus pictus* from island and mainland localities. Chiloe Island (CHI, $n=30$); Talcan Island (TAL, $n=14$); Butachauques Island (BUT, $n=17$), Antillanca (ANT, $n=22$) and Hornopiren (HOR, $n=14$). Samples sizes (n) refer to the total number of lizard stomachs examined in each population.

	%FO					%NUMBER					RI				
Prey item	CHI	TAL	BUT	ANT	HOR	CHI	TAL	BUT	ANT	HOR	CHI	TAL	BUT	ANT	HOR
Animal															
Homoptera	23.3	21.4	23.5	22.7	14.3	6.3	2.6	2.3	3.9	2.1	7.7	5.9	6.3	5.7	4.5
Dermaptera	0	0	0	4.5	0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	1.1	0.0
Lepidoptera	10.0	0	0	18.2	0	3.6	0.0	0.0	1.9	0.0	3.5	0.0	0.0	4.3	0.0
Odonata	3.3	0	11.8	0	0	0.3	0.0	2.2	0.0	0.0	0.9	0.0	3.4	0.0	0.0
Ortoptera	10.0	7.1	0	31.8	7.1	1.2	0.8	0.0	6.7	0.7	2.9	1.9	0.0	8.2	2.1
Diptera	36.6	35.7	58.8	54.5	78.6	14.2	8.5	18.4	19.3	36.1	13.2	10.9	18.8	15.8	31.7
Thysanoptera	0	0	0	9.1	0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	2.0	0.0
Hemiptera	26.7	7.1	29.4	31.8	14.3	3.8	1.8	9.3	5.6	2.3	7.9	2.2	9.4	8.0	4.6
Coleoptera	83.3	85.7	94.1	86.4	78.6	49.0	55.3	49.4	38.4	39.5	34.2	34.7	34.8	26.7	32.6
Hymenoptera	53.3	0	0	40.9	7.1	7.7	0.0	0.0	10.0	5.4	15.8	0.0	0.0	10.9	3.4
Arachnida	16.7	7.1	17.6	4.5	7.1	7.2	0.5	3.0	0.3	1.4	6.2	1.9	5.0	1.0	2.3
Chilopoda	3.3	0	0	0	0	1.1	0.0	0.0	0.0	0.0	1.1	0.0	0.0	0.0	0.0
Gastropoda	0	0	5.9	13.6	0	0.0	0.0	0.6	1.0	0.0	0.0	0.0	1.6	3.1	0.0
Insecta larvae	0	14.3	17.6	18.2	14.3	0.0	8.0	1.6	1.9	1.2	0.0	5.5	4.7	4.3	4.3
Crustacea	0	0	23.5	0	14.3	0.0	0.0	3.6	0.0	1.5	0.0	0.0	6.6	0.0	4.4
Vegetation															
Galium hypocarpium	0	50.0	11.8	4.5	7.1	0	19	4.1	3.0	5.4	0.0	17.0	3.9	1.6	3.4
Nertera granadensis	10.0	7.1	5.9	18.2	21.4	2.6	1.2	2.8	3.8	4.5	3.3	2.0	2.1	4.7	7.2
Gaultheria sp	3.3	0	0	0	0	1.7	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0
Unidentified	6.6	71	11.8	9.1	0	1.3	2.3	2.7	3.5	0.0	2.0	18.0	3.5	2.7	0.0
Total invertebrates	100	100	100	100	100	94.4	77.5	90.3	89.7	90.1	93.4	62.9	90.5	91.0	89.4
Total vegetation	13.3	57.1	17.6	18.2	28.6	5.6	22.5	9.7	10.3	9.9	6.6	37.1	9.5	9.0	10.6

following significant differences: Antillanca and Hornopiren–Butachauques, Talcan Islands, Butachauques–Chiloé islands, and Chiloé–Talcan islands ($p<0.001$). The *a posteriori* analysis revealed that differences were found for small intestine length and for large intestine length (Table 2). No differences were found for stomach or total intestine length (Table 2).

3.4. Enzyme activity

The MANCOVA analysis revealed significant differences in enzymatic activity between mainland and island populations (IU total: Wilks' Lambda = 0.859, $p=0.016$). In this case, specific differences were found only for APN activity, which were higher in island than in mainland lizards. When we analyzed the data separately by localities, the a posteriori test revealed that APN was higher in individuals from Butachauques and Talcan island, whereas maltase activity was lower in lizards from Chiloe Island (Table 2). A correlation matrix among the enzyme activity of digestive tracts indicated that all total dissacharidase activities were positively correlated (all $p<0.01$). Total APN activity was not correlated with sucrase and trehalase activities (sucrase: $r=0.02$, $p=0.83$; and trehalase: $r=0.16$, $p=0.14$). However a significant, positive association between aminopeptidase-N and maltase activities was found ($r=0.31$, $p=0.003$).

3.5. Correlational evidence

The correlation analysis among the average value of morphological and biochemical data against ecological variables revealed no significant association between the TL and the RI of invertebrates and fruits in the stomach content (all $p>0.05$). We detected no significant association among the proportion of different prey and digestive variables (all $p>0.05$). Surprisingly, we found a negative correlation between the TL and the APN activitiy (Spearman $R=-0.9$, $p<0.05$).

Table 2
Morphometic measurements of the digestive tract and the total activity of digestive enzymes (μmol hydrolyzed/min) of *Liolaemus pictus* from mainland and island populations. Sample size in parenthesis. Data are shown as mean ± standard error. Different superscripts indicate significant ($p<0.05$) differences between populations, after the *a posteriori* Tukey test.

Digestive traits	Localities				
	Mainland		Island		
	Antillanca	Hornopirén	I. Chiloé	I. Butachauques	I. Talcán
	(21)	(17)	(19)	(17)	(16)
Body mass (g)	5.29 ± 1.91	6.78 ± 2.01	5.60 ± 1.78	7.55 ± 4.41	6.60 ± 3.18
Stomach (mm)	18.16 ± 2.47	18.13 ± 2.75	18.97 ± 3.01	19.91 ± 3.39	19.80 ± 5.47
Small intestine (mm)	$79.19 \pm 12.85^{a,c}$	84.50 ± 15.55^{a}	$71.89 \pm 11.69^{b,c}$	$70.71 \pm 15.81^{b,c}$	68.26 ± 17.23^{b}
Large intestine (mm)	18.70 ± 3.76^{b}	$20.34 \pm 3.61^{b,c}$	18.67 ± 3.86^{b}	24.28 ± 6.20^{a}	$22.76 \pm 4.87^{a,c}$
Total (mm)	115.77 ± 16.38	123.27 ± 18.41	109.17 ± 15.79	114.90 ± 22.39	111.26 ± 24.57
Enzyme (μmol/min)					
Sucrase	2.16 ± 0.39	1.64 ± 0.25	1.77 ± 0.40	2.00 ± 0.50	2.74 ± 1.23
Maltase	11.31 ± 1.70^{a}	$9.57 \pm 1.30^{a,b}$	5.92 ± 1.10^{b}	$9.17 \pm 1.00^{a,b}$	$8.53 \pm 1.39^{a,b}$
Trehalase	6.12 ± 1.10	7.84 ± 1.40	5.90 ± 1.10	9.44 ± 1.80	7.79 ± 1.30
Aminopeptidase-N	0.11 ± 0.01^{b}	$0.14 \pm 0.02^{a,b}$	$0.13 \pm 0.03^{a,b}$	0.20 ± 0.04^{a}	0.21 ± 0.06^{a}
Plant $\delta^{15}N$	−5.25 ± 2.33		2.02 ± 2.18		
Trophic level	4.51 ± 0.21^{c}	4.41 ± 0.02^{c}	2.86 ± 0.27^{b}	3.60 ± 0.80^{a}	2.43 ± 0.37^{b}

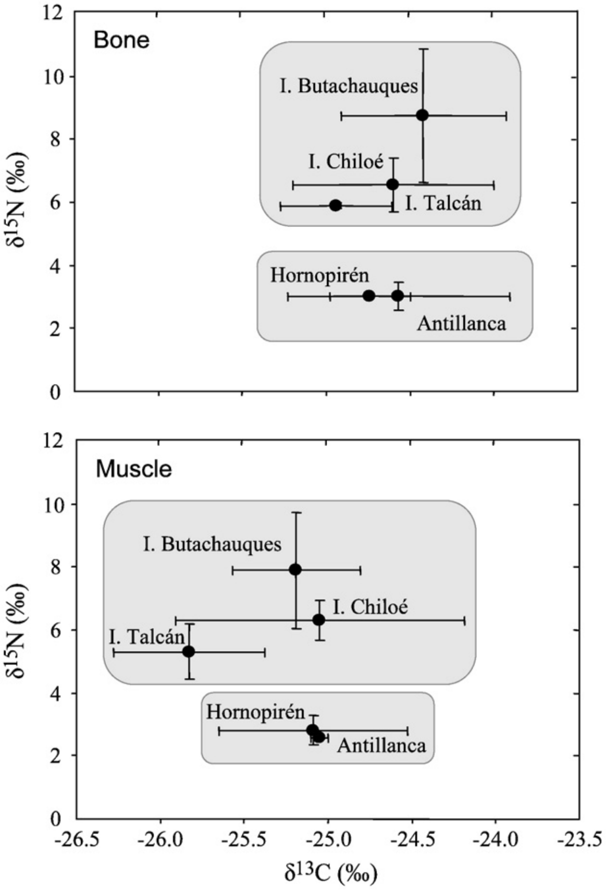

Fig. 2. Mean (± SE) carbon and nitrogen isotopic ratios of bone collagen and muscle of *Liolaemus pictus* from mainland and island localities from Sourthern Chile.

4. Discussion

4.1. Dietary analysis

The diet of *L. pictus* was characterized by the consumption of animal prey, seeds, and fruit (19 prey types) which characterizes the diet of an opportunistic predator (Rocha et al., 2004). Ortiz (1974) reported that *L. p. pictus* from the mainland feed on dipterans, homopterans, arachnids, hymenopterans, and coleopterans, yet Willson et al. (1995) and Willson et al. (1996) indicated that *L. p. chiloensis* from Chiloé Island have a frugivorous diet. Furthermore, Olesen and Valido (2003) suggested that island lizards tend to eat fruits. Our results indicate that the diet of the mainland and island populations incorporate fruit, even though vegetable diets have been described previously as of low importance in *Liolaemus* lizards (Fuentes and di Castri, 1975; Vidal and Labra, 2008). On the other hand, *Liolaemus* lizards have been described as specialized predators of arthropods (e.g., *L. fuscus*, *L. lemniscatus*, *L. tenuis*; Hurtubia, 1973). The behavior of consuming a vast array of prey types, including both relatively sedentary (e.g., larvae) and highly mobile preys (e.g., spiders, orthopterans), has also been found for some *Liolaemus* species from Chile (see Fuentes, 1981; Vidal and Labra, 2008; and references therein). The variation in the consumption of invertebrates and fruit by *L. pictus* is remarkable. In fact, the taxonomic origin of animals and plants consumed appears to be different between mainland and island localities. While lizards consumed a higher number of invertebrates than fruit, individuals from Talcan Island consumed more fruit than the other populations. Urbina and Zúñiga (1977) indicated that this insular population (assigned to *L. p. talcanensis* subspecies), also consumes grasses, which suggests that the trophic spectrum of *L. pictus* could be wider. Since we do not have data on prey availability at each locality, we do not know if this differential consumption of invertebrates and fruit reflects selectivity of this prey type in mainland and island populations.

The large difference in nitrogen signatures among individuals and populations seems to indicate a variation of the relative position in the food web. However, this large variation among populations seems to be attributable, in part, to the great difference in the δ^{15}N of producers in each location (Table 2). In fact, the calculation of the tropic level revealed that island populations are near to the third position, i.e., secondary consumers, whereas in mainland populations the tropic level is near four, i.e., tertiary consumers. Thus, our results suggest that the relative positions differ between island and mainland populations and that mainland lizards tend to consume more animal prey and island lizards consume more fruit. This type of intraspecific variation in enrichment values has been found for seasonal changes of diet (Darimont and Reimchen, 2002; Vizzini and Mazzola, 2003; Dalerum and Angerbjörn, 2005) and changes in the assemblage of prey items in diet (Beaudoin et al., 1999). In this sense, we hypothesize that the differences observed in the isotopic composition between localities reflect differences in resource use. These changes in lizard diets could reflect the high flexibility of resource use at each site along the distribution. These results are also consistent with the variation found in the stomach content, i.e., Talcan population have more fruit in the gut and lower values of tropic level than the remainder populations.

Nevertheless, the tropic level calculated from the δ^{15}N of muscle was not correlated with dietary determinations by analyzing gut contents. This fact could be explained by the results of gut content and isotopic determinations; they have a different temporal resolution. Whereas diet samples represent a snapshot of the recent consumption, muscle and bone collagen reflect the integration of dietary inputs over longer time periods (Martínez del Rio et al., 2009). Thus, the isotopic composition of a tissue is the result of the integration of isotopic inputs over time, and the time window of isotopic incorporation into a tissue depends on the turnover rate of the tissue's constituent elements (Tieszen et al., 1983). In reptiles, the isotopic composition of tissues, such as muscle, may reflect an integration of several weeks or months (Seminoff et al., 2007; Fisk et al., 2009), which does not reflect the temporal scale at which dietary switches may occur. Thus, the absence of any significant correlation between the calculated trophic level and the proportion of invertebrates among populations seems to suggest that different populations probably feed on different prey types throughout the year. Furthermore, among island populations of *L. pictus* there is a similar feeding ecology, suggesting that environmental conditions may be more similar among than between island and mainland localities.

4.2. Intestinal morphology and physiology

Several studies have described the intestinal morphology and diet in a wide range of lizard species (Greene, 1982; Schwenk, 2000; Herrel et al., 2004). These studies suggest that the length of the small intestine tends to be negatively associated with the amount of animal prey in the diet. However, we did not detect a clear association between intestinal morphology and diet across the mainland and island populations of *L. pictus*. Mainland populations, which consume a small amount of fruits, such as *Nertera granadensis*, have relatively longer small intestines than the island populations that consume more diverse fruits and plants (e.g., *Galium hypocarpium*, *Gaultheria sp.*). Moreover, the correlation analysis revealed no association between the dietary habits (from the stomach content and isotope analisys) nor any of the morphological variables. It has been reported that the structure of an individual's intestine may change in response to diet of vertebrates (Sabat and Bozinovic, 2000; Starck and Beese, 2002; Elliott and Bellwood, 2003; Castañeda et al., 2006). However, the primary vegetable factor in the diet of *Liolaemus* lizards is fleshy fruit, which probably has relatively high digestibilities (Valido and Nogales, 2003). Thus, no specific anatomical modifications would be expected, as is the case in true herbivorous lizards (Iverson, 1982; Cooper and Vitt, 2002).

Levels of many digestive enzymes in vertebrates appear to be modulated reversibly according to the levels of dietary substrates (Stevens and Hume, 1995). In addition, the overall ability of a lizard to extract energy from food depends on the activity of digestive enzymes (Andrews and Asato, 1977; Beaupre et al., 1993). In *L. pictus*, the differences in dietary incorporation seem to be uncoupled by biochemical features of the small intestine. Indeed, in insular populations we observed an increase of aminopeptidase activity. In this sense, island individuals appear to be more frugivores, have a shorter small intestine and have higher APN activity than mainland individuals, suggesting that gut morphology and biochemistry is well suited to efficiently hydrolyze and breakdown nutrients from animal tissues. The significant, and counterintuitive, association between the trophic level of populations and the APN activities also support these results. Unfortunately, there are no studies regarding the lizard's ability to change gut biochemistry in response to dietary substrates. Recently, Iglesias et al. (2009) demostrated that the activity of APN and disaccharidases did not differ between different populations of *Lophognathus temporalis*. However, these authors found that *L. temporalis* showed significant seasonal changes in their total enzyme activities, because of a larger intestinal mass in the wet season. This seasonal effect in gut morphology and physiology seems to be coupled with food intake, as has been reported for other lizards (Christel et al., 2007; Naya et al., 2009). Thus, differences in gut physiology could be the result of differences in food consumption rather that differences in the specific substrates in the intestinal lumen. This is a hypothesis that should be tested in the future.

Surprisingly, APN activity was near one order of magnitude lower than maltase activity. This noticeable difference was already reported in other species of the same genus, *L. nigroviridis* (Naya et al., 2009). Judging by the relative importance (RI) of animal taxa in the diets of *L. pictus*, *Liolaemus* lizards would exhibit higher activity of APN than carbohydrases as has been reported recently in the insectivorous lizard, *Lophognathus temporalis* (Iglesias et al., 2009). However, this was not the case. The enzymatic activity is not the only variable that influences the efficiency by which animals hydrolyze and assimilate nutrients, which demonstrates that comparisons between the activities of different enzymes in the same individuals or populations may be not be appropriate to predict the foraging preferences and digestive abilities. As pointed out by Karasov (1996), differences in retention time of food also play a significant role in the assimilation of dietary substrates (Karasov, 1996). It is possible that even when the enzymatic activities for a particular substrate are low, animals might still efficiently break down substrates if the retention time is sufficiently low (Afik et al., 1995).

In summary, we found that the different populations of *L. pictus* from the island and mainland do consume at different levels in the food web and probably incorporate different proportions of animals and plants into their diets. In this sense, the presence of few predators and competitors on islands, climatic differences, and the role of colonization events of island populations may often explain the observed divergence in morphology, physiology, diet, and genetic variability between island and mainland populations (Irschick et al., 1997; Clegg and Owens, 2002; Calsbeek and Smith, 2003; Castilla et al., 2008). In addition, it has been suggested that the dietary divergence is the main promoter of the island–mainland change (Clegg and Owens, 2002). However, our results suggest that changes in prey type are not always paralleled with changes in the physiology of the gut at an inter-population level, supporting the conventional wisdom (see Feder et al., 1987) that asserts that an animal's first response to a selective pressure is behavioral. This kind of dietary divergence between mainland and island lizards have been recently reported in the genus *Podarcis* (Castilla et al., 2008) which are able to adapt to new food sources when colonizing novel habitats. Thus, the rapid changes in feeding behavior seem to be a common feature of lizards, allowing them to expand their tropic niche when resources are available.

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