Testing the Accuracy of Fecal-Based Analyses in Studies of Trophic Ecology in Lizards

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Trophic niche studies are essential for evaluating ecological interactions between and within species and their evolutionary implications. For example, fundamental aspects of a wide range of hypotheses concerning population divergence, evolution of sexual dimorphism, and adaptations to fluctuating environments rely on dietary evidence. The accuracy of different methodologies used to estimate trophic specializations is therefore a fundamental issue. Under the assumption that direct observations of gastric contents provide accurate information about dietary preferences, I examined how reliably diet is reflected in fecal pellets. I conducted two main comparative tests on living lizards. First, I fed individuals of 23 species with hard- and soft-bodied organisms, and compared fecal pellets. Second, I examined prey items from natural diets represented in feces and gastric contents (stomach-flushing) in a wild population of the lizard *Liolaemus tenuis*. My results reveal that fecal samples provide inaccurate estimates of lizard trophic preferences, mainly because soft-bodied organisms are destroyed by digestive processes. Even though soft-bodied prey may be essential dietary items (as inferred from gastric analysis), these organisms may be almost entirely absent from feces. I suggest that direct gastric analyses should be the preferred method for analyzing reptilian diets.

THE study of trophic niches is a fundamental requirement for understanding different ecological and evolutionary phenomena in animals (Andersson, 1994; Schluter, 2000; Pincheira-Donoso and Núñez, 2005). Therefore, determining the accuracy of methodologies used to estimate trophic preferences is critical. For reptiles and amphibians, three primary methods of collecting data on diet have been used: examination of (1) gastric contents removed from dissected stomachs (Vitt and Zani, 1996; Vitt et al., 1997, 1998), (2) gastric contents resulting from stomach flushing (Legler and Sullivan, 1979; Bennett, 2000), and (3) fecal pellets (Rissing, 1981; Sylber, 1988; Suarez et al., 2000). Because methods 1 and 2 directly examine stomach contents, they provide the most accurate estimates of diet. Nevertheless, the accuracy of fecal samples has not been systematically investigated.

Previous studies demonstrate that reptiles and amphibians frequently feed on soft-bodied prey items (Ballinger et al., 1977; Gunzburger, 1999; Pincheira-Donoso, 2002). Indeed, soft-bodied organisms may be essential elements of the diet in a wide range of species (Hurtubia, 1973; Vitt and Zani, 1996; Pincheira-Donoso, 2005). Nevertheless, it has been suggested that these organisms are destroyed by digestive processes, being only observable in samples of actual stomach contents (Demuth and Buhlmann, 1997; Gunzburger, 1999). Therefore, the study of trophic niches based entirely or primarily on fecal samples may lead to inaccurate diet information. In the present study I investigate the potential bias that fecal-based dietary analyses may introduce. I test the hypothesis that feces do not provide accurate estimates of trophic niche, using a wide range of lizard species as study models.

MATERIALS AND METHODS

To explore accuracy of fecal-based analyses, I assessed the diets of 23 lizard species (belonging to the families Gekkonidae, Leiosauridae, and Tropiduridae; see Material Examined). I performed a comparative dietary experiment in 87 specimens kept in captivity over 3–9 weeks in independent glass boxes (40 × 50 × 35 cm; only one individual was kept per box) provided with water *ad libitum*, and fed three different known experimental diets: 1) hard-bodied prey, including adult beetles (*Tenebrio molitor*, *Eriopis conexa*, *Adalia bipunctata*, *Aegorhinus* spp., *Deromecus* spp.) and flies (*Musca domestica*); 2) partially hard-bodied prey (*Tenebrio molitor* larvae), whose skin is characterized by hard chitinous segments; 3) soft-bodied insect larvae without chitinous segments (Lepidoptera, Diptera, and Coleoptera, here identified as soft-bodied larvae). I recorded the number of prey items that each lizard ingested over a period of five to ten minutes. After that, any non-ingested prey was removed from the box.

To assess the degree of bias introduced by fecal-based estimates under field conditions, I compared diet composition in a wild population of the iguanian species *Liolaemus tenuis* as inferred from fecal pellets and stomach flushing of a sample of 36 individuals collected during January 2004 in Curanilahue (37°28′S, 73°20′W), Bio Bio Region of Chile. The total *L. tenuis* sample consisted of 15 adult males and 21 adult females (see Pincheira-Donoso and Núñez, 2005 for details on adult body size). Male and female samples were randomly divided into two equal subsets. In 18 individuals (males: snout–vent length [SVL] = 55.3 ± 2.8 mm, $n = 7$; females: SVL = 54.9 ± 2.7 mm, $n = 11$), gastric contents were studied by stomach flushing in the field (Legler and Sullivan, 1979; Bennett, 2000). The remaining 18 specimens (males: SVL = 55.0 ± 3.7 mm, $n = 8$; females: SVL = 54.2 ± 3.6 mm, $n = 10$) were kept in small plastic field boxes (see above) to collect natural fecal samples, as detailed above. To evaluate frequency of insect larvae within the natural diet of additional *Liolaemus* species, gastric contents from dissected stomachs of *L. lemniscatus* ($n = 15$) and *L. schroederi* ($n = 15$)
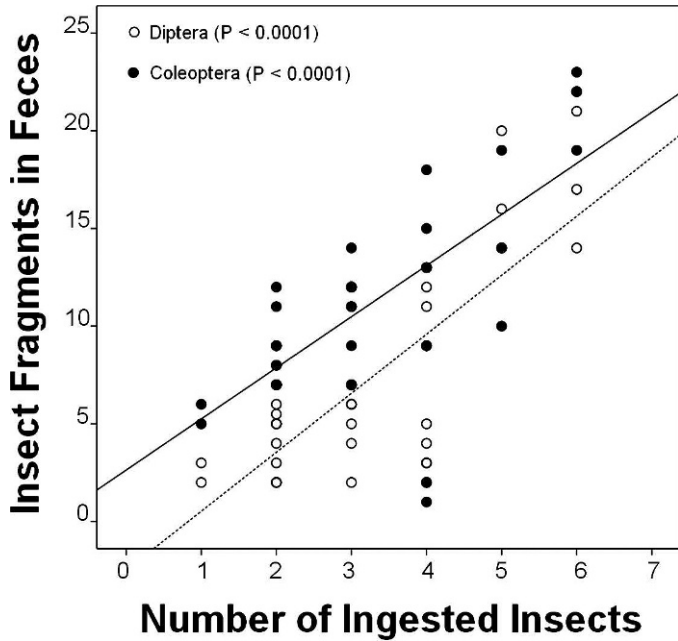
Fig. 1. Relationship of prey fragments identified in the fecal samples to the number of ingested coleopterans ($R^2 = 0.486$, $P < 0.0001$) and dipterans ($R^2 = 0.579$, $P < 0.0001$) by different lizard species.

Fig. 2. Relationship of partially hard- (PHBL, $R^2 = 0.004$, $P = 0.668$) and soft-bodied larvae (SBL, $R^2 = 0.014$, $P = 0.296$) fragments identified in the fecal samples to the number of ingested larvae by different lizard species.

were also analyzed. Samples of both species are housed in the herpetological collection of the Museo Nacional de Historia Natural de Chile (MNHNC). Institutional abbreviation as listed in Leviton et al. (1985).

Dietary contents were spread on Petri dishes with water, identified to order, and later counted and measured in width and length using digital callipers to the nearest 0.01 mm. For analyses, ants (Formicidae) were separate from hymenopterans (Pianka, 1986; Vitt et al., 1997). Prey identification followed the criteria of Peña (1996), and was supported by reference collections housed in the National Museum of Natural History of Chile. Individual prey volumes were estimated as regular ellipsoids following the model: volume $= 4/3 \, \pi \, (w_t/2)^2 \, (L/2)$, where w_t is the total prey width, and L is the total prey length (Vitt and Zani, 1996; Vitt et al., 1997). The final value of the total width (w_t) was obtained by standardizing the whole width values obtained across the prey body length. For individual lizards, numeric and volumetric percentages of each prey category were calculated to obtain species means.

RESULTS

Experiment.—In the fecal samples from specimens fed with hard-bodied insects, a high frequency of coleopteran and dipteran fragments was observed, including heads, wings, legs, abdominal segments, thorax, and several complete and almost undamaged specimens (i.e., *Eriopis conexa*, *Deromecus* spp.). Many fragments allowed identification of specimens to the level of species (i.e., *Tenebrio molitor*, *Eriopis conexa*). Bivariate regression analyses revealed that the number of coleopteran ($R^2 = 0.486$, $F_{1,29} = 27.4$, $P < 0.0001$) and dipteran ($R^2 = 0.579$, $F_{1,32} = 43.9$, $P < 0.0001$) fragments found in the fecal pellets are significantly predicted by the number of ingested individuals of these hard-bodied insects (Fig. 1).
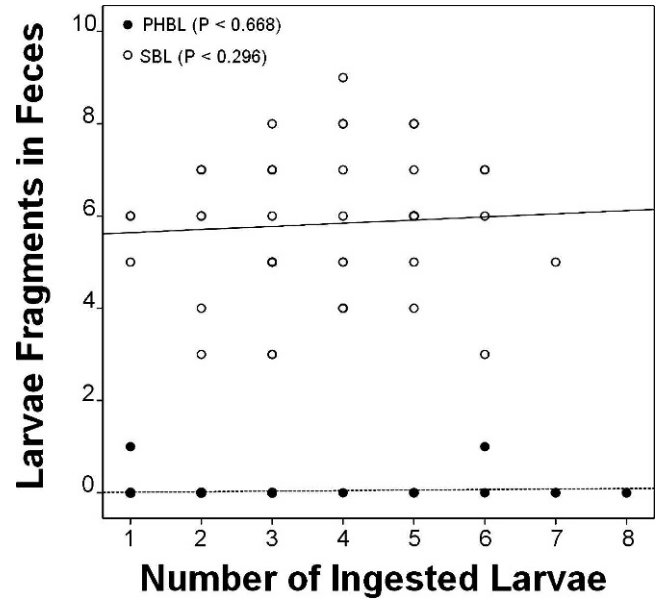
Small fragments of partially hard-bodied larvae (*Tenebrio molitor*) were observed in the 44 fecal pellets of lizards fed this type of diet (frequency = 100%). However, the number of larvae ingested do not predict the number of fragments observed in fecal pellets ($R^2 = 0.004$, $F_{1,43} = 0.187$, $P = 0.668$; Fig. 2).

No clear evidence of soft-bodied larvae fragments were observed in feces (78 pellets) from individuals fed this type of diet. Small hard fragments similar to cephalic capsules of larvae (see Peña, 1996) were observed in two fecal pellets (2.7%). However, in 97.4% (76 pellets) of the fecal pellets examined, no soft-bodied larvae fragments were observed. Bivariate regression analyses revealed that the number of ingested soft-bodied larvae do not predict the number of fragments found in fecal pellets from lizards fed soft-bodied prey ($R^2 = 0.014$, $F_{1,76} = 1.11$, $P = 0.296$; Fig. 2).

Fecal samples versus gastric contents in **Liolaemus tenuis.**— Examination of fecal pellet contents of *Liolaemus tenuis* ($n = 18$; 24 pellets) revealed spiders (cephalothoraxes and legs), coleopterans, dipterans, hymenopterans, ants, homopterans, and hemipterans. No clear evidence of soft-bodied items was detected (Fig. 3). The presence of soft-bodied larvae was suspected in only one fecal pellet, in which a small fragment similar to a cephalic capsule (Peña, 1996) was found (Fig. 3). The same diversity of hard-bodied prey items found in the feces (spiders, coleopterans, dipterans, hymenopterans, ants, homopterans, and hemipterans) was recorded in gastric contents obtained through the technique of stomach flushing. Nevertheless, this latter procedure revealed soft-bodied larvae as one of the two most important items in the natural diet of *L. tenuis* in the study area (Curanilahue; see above). Specifically, the volumetric results of the stomach-flushing analysis showed soft-bodied larvae as the most important dietary item (25.6%, 0.04% in feces), followed by coleopterans (22.2%, 21.1% in feces), dipterans (21.6%, 19.9% in feces), and hymenopterans (20.9%, 23.3% in feces; Fig. 3). Numerically, coleopterans were observed as
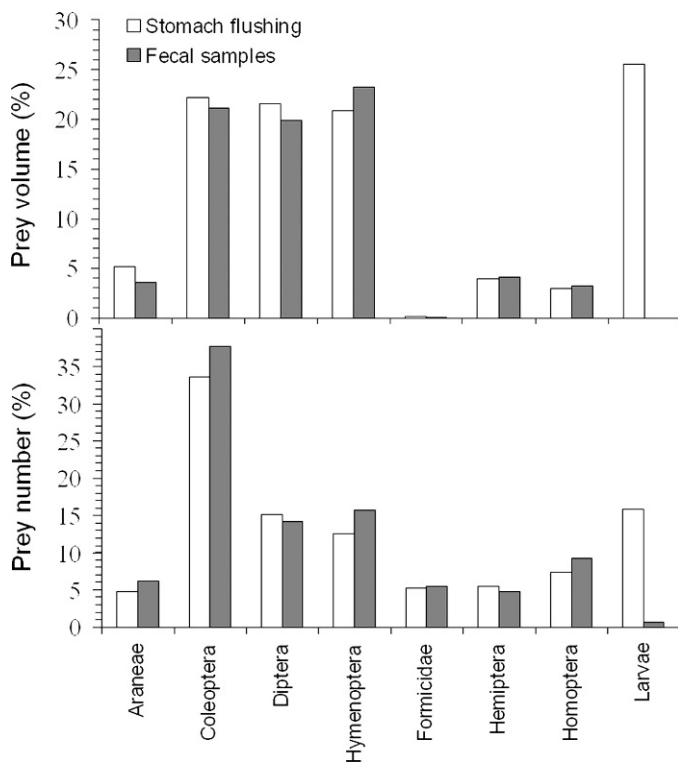
Fig. 3. Analysis of the volumetric and numeric relative proportions of the natural diet of *Liolaemus tenuis* from the locality of Curanilahue, Chile, observed from gastric contents (stomach flushing) and fecal samples.

the main prey item (33.6%, 37.6% in feces), followed by soft-bodied larvae (15.9%, 0.7% in feces), dipterans (15.1%, 14.2% in feces), and hymenopterans (12.5%, 15.7% in feces; Fig. 3).

The additional analyses conducted on two other *Liolaemus* species showed that soft-bodied insect larvae are important constituents of the natural diets of *L. lemniscatus* (*n* = 15) and *L. schroederi* (*n* = 15). In the former, soft-bodied insect larvae comprised 17.5% volumetrically and 6.6% numerically of the diet, and were the second most important items by volume among a total of 17 items identified. In *L. schroederi*, the volumetric proportion of soft-bodied larvae was 11.6%, while the numeric proportion was 2.7%, being the fourth most important dietary item by volume among a total of fourteen items identified for this species.

DISCUSSION

My results provide robust evidence to reject the null hypothesis that feces offer accurate estimates of trophic niche in lizards. Studies of feeding ecology in these animals based entirely or primarily on fecal samples may result in significantly misleading conclusions. Direct analyses of gastric contents (i.e., removed stomach, stomach flushing) appear to be necessary to accurately describe diets. This study revealed that fecal samples retain identifiable remains of hard-bodied prey (Fig. 1), but do not retain identifiable parts of soft-bodied prey items (Fig. 2), as a consequence of digestion (Demuth and Buhlmann, 1997). Although these observations were performed exclusively on lizards, it is likely that similar conclusions can be extended to dietary studies on other animals that feed on soft-bodied organisms. Indeed, previous analyses of salamander (Gunzburger, 1999) and turtle diets (Demuth and Buhlmann, 1997) suggest that

digestive processes may destroy soft-bodied prey items, affecting the accuracy of results based on fecal pellets. It is worth noting that, since I pooled data across taxa, species-specific effects are obscured. Hence, although these general findings demonstrate that fecal pellets are insufficient sources of information for studies of trophic ecology, it is likely that variation in the value of fecal contents exists between species.

Previous dietary studies in lizards (Avila-Pires, 1995; Vitt and de Carvalho, 1995; Vitt et al., 1997) have shown that soft-bodied prey items may represent important fractions of natural diets of these reptiles. Similarly, my results reveal that soft-bodied larvae represent the most important volumetric item in the natural diet of *L. tenuis* estimated from stomach flushing (Fig. 3), and the second and the fourth most important items in the natural diets of *L. lemniscatus* and *L. schroederi*, respectively. Remarkably, when analyzing fecal pellets of *L. tenuis* obtained from a sample with similar characteristics in the number of individuals, body size per sex, sex ratio, and collection data (see Materials and Methods), soft-bodied items were almost entirely absent.

In addition, the dry condition of fecal pellets can substantially alter the natural volumetric proportions of prey items. For example, in fecal samples from lizards fed partially soft-bodied prey, mealworms (*Tenebrio molitor* larvae) were identified on the basis of some skin fragments, but estimations of their volumetric contribution to the diet was not possible. Also, in the case of spiders, only legs and cephalothoraxes were found in feces, whereas abdomens, the most voluminous body part of these animals (Roberts, 2001; Hillyard, 2004), were observed in all gastric contents.

In conclusion, the study of fecal samples represents a weak estimation of diets in lizard species feeding on soft-bodied prey items, such as insect larvae and spiders. In the same way, other soft-bodied items, such as Aphididae (Homoptera), annelids, shell-less gastropods, and anuran larvae are expected to be poorly estimated from feces (Mattison, 1992; Gunzburger, 1999; Arnold, 2002). Consequently, studies of dietary ecology based on fecal pellets should also incorporate gastric samples obtained from removed stomachs or from the stomach flushing methods. Stomach flushing, however, appears as the preferred method for future diet studies because it is highly accurate, allows the study of large samples without sacrificing individuals, and can be conducted in a short period of time without keeping specimens in the lab (Legler and Sullivan, 1979; Bennett, 2000).

MATERIAL EXAMINED

Family Gekkonidae: *Homonota gaudichaudii* (*n* = 6), *Phyllodactylus gerrhopygus* (*n* = 10); Family Leiosauridae: *Diplolaemus bibronii* (*n* = 4), *D. leopardinus* (*n* = 2), *Pristidactylus torquatus* (*n* = 9); Family Tropiduridae: *Liolaemus alticolor* (*n* = 7), *L. andinus* (*n* = 9), *L. araucaniensis* (*n* = 6), *L. chiliensis* (*n* = 10), *L. chillanensis* (*n* = 7), *L. cyanogaster* (*n* = 8), *L. elongatus* (*n* = 6), *L. fuscus* (*n* = 9), *L. jamesi* (*n* = 6), *L. lemniscatus* (*n* = 24; 15 specimens housed in MNHNC, numbered as MNHNC-DPD 00037–00051), *L. monticola* (*n* = 9), *L. nitidus* (*n* = 9), *L. pictus* (*n* = 8), *L. pleopholis* (*n* = 5), *L. schroederi* (*n* = 20, 15 specimens housed in MNHNC, numbered as MNHNC-DPD 00052–00066), *L. tenuis* (*n* = 44), *L. zapallarensis* (*n* = 7), *Phrynosaura reichei* (*n* = 4).

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