Using faecal samples in lizard dietary studies

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Abstract. While the use of faecal pellets is widely accepted as a primary methodological source of data for dietary studies, a recent paper advocated for the use of gut contents. This was due to the fact that faecal samples would give biased results of the diet of arthropod predators, due to a lower representation of soft-bodied prey in faecal pellets. To test this assumption, we compared the spring diet of several populations of two insular lizards from the Balearic Islands (Spain), *Podarcis lilfordi* and *Podarcis pityusensis*, using both faecal pellets and gut contents. Our results do not support the supposed bias of dietary analyses based on faecal pellets. Indeed, soft-bodied prey and particularly insect larvae are often equally represented in faecal pellets and gut contents. Alternatively, soft bodied prey are represented in different proportions in gut contents and faecal pellets, but in some cases with higher proportions being observed in the gut contents, and in other cases with higher proportions in faecal samples. We conclude that faecal pellets can be a reliable source of information for dietary studies.

Keywords: dietary methods, faecal analysis, Lacertidae, *Podarcis*.

Introduction

Dietary studies are central in the study of reptilian natural history and ecology. Moreover, they constitute the basic information needed to tackle broader questions related to the dynamics of trophic niche utilization and the predatory behaviour of animals in general (Vanhooydonck, Herrel and Van Damme, 2007). Studies on the diet are also interesting because what is eaten can affect interactions between the organism and its competitors (Sih, 1993 and references therein). Several methods are currently available in order to investigate the dietary habits of a given species. In lizards, studies are mainly carried out by analysing either stomach contents or entire digestive tracts of dead individuals (Pough et al., 2004) either wild-captured and immediately sacrificed or as part of preserved collections in museums and other science collections (Pérez-Mellado, 1989).

Three alternative methods were also additionally employed, viz. stomach flushing (Legler and Sullivan, 1979; Herrel et al., 2006), analysis of faecal samples (in the case of lacertid lizards, e.g., Angelici, Luiselli and Rugiero, 1997; Capizzi, 1999; Hawlena and Pérez-Mellado, 2009) and direct observation of lizards foraging (e.g., Sáez and Traveset, 1995). In the case of turtles, dietary studies are done principally using stomach flushing (Lima, Magnusson and Da Costa, 1997); in the case of snakes, faeces analyses and forced regurgitation are the most commonly used methods (e.g., Luiselli, 2006). Over the last few years, the analysis of the isotopic signature of the tissues of a predator has been incorporated into the series of tools used for analysing diets of free-ranging reptiles (Barrett et al., 2005; Farina et al., 2008).

Whether one or a few of these methods are actually the best in capturing the dietary habits in reptiles remains controversial. Angelici, Luiselli and Rugiero (1997) discussed the advantages and limitations of faecal analysis in dietary studies of lizards. They concluded that the best procedure to test the reliability of faecal analysis versus other methods such as stomach content is to employ the same lizard specimens or, at least, lizards belonging from

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the same populations to compare both methods. Recently, Pincheira-Donoso (2008) pointed out that faecal samples provide inaccurate estimates of lizard trophic preferences, because soft-bodied prey are destroyed during the digestive process and cannot be identified in faeces. Accordingly, insect larvae and other soft prey such as, for example, spiders, may be almost entirely absent from faeces, even if they can be important prey items (Pincheira-Donoso, 2008). However, ethical reasons argue against the sacrifice of lizards (Bekoff, 2007).

Our aim in this paper is to test the reliability of faecal analyses as a method for the study of the diet of lizards. We use two species of insular lacertid lizards from Balearic Islands, the Balearic lizard, *Podarcis lilfordi* and the Pitiusic lizard, *Podarcis pityusensis* for which, dietary data from both faecal and gut samples are available from the same populations. Therefore, we can directly test the methodology using the most careful experimental criterion of method reliability as elaborated by Angelici, Luiselli and Rugiero (1997).

Materials and methods

We surveyed the spring diet of two insular species of lizards, *Podarcis pityusensis* from Ibiza and surrounding coastal islets, and *Podarcis lilfordi* from the coastal islets of Mallorca, Menorca and the Cabrera archipelago. Nine populations from *P. lilfordi* and five populations from *P. pityusensis* were used (table 1, see Pérez-Mellado et al., 2008 and Pérez-Mellado, 2009 for more details).

Populations were selected because we had data from the diet of adult individuals from the same spring period (April and May months), from different years, with samples of faecal pellets and gut contents. Populations included in this study represent a wide range of different ecological conditions, from very small coastal islets to large islands such as Colom in the case of *P. lilfordi*, and Formentera in *P. pityusensis* (Pérez-Mellado et al., 2008; Pérez-Mellado, 2009). We included the analysis of 242 gut contents (from specimens captured in 1988, see Pérez-Mellado, 1989) and 1312 faecal pellets from *P. lilfordi* and 95 guts and 208 faecal pellets from *P. pityusensis* (table 1). In the case of gut contents of *P. lilfordi*, the results given here were already published (Pérez-Mellado, 1989), while gastric contents of *P. pityusensis* were analyzed from specimens stored at the herpetological collection of Alexander Koenig Museum, Bonn, Germany. These samples were collected in 1930. Faecal samples from both species were collected during spring 2006 at each population. In the case of gut contents, lizards were immediately euthanized after collection, in order to stop the digestive process (Pérez-Mellado, 1989). Faeces were obtained directly from the field and taken by hand or entomological forceps and individually stored in Eppendorf vials.

Prey remains were identified to order or family level always by the same observer (VPM). Then, from faecal and

Table 1. Populations of *P. lilfordi* and *P. pityusensis* under study. We give the number of digestive tracts and faecal pellets analyzed for each population as well as the probabilities of the Fisher exact test. Fisher test A was applied to frequencies of insect larvae in digestive tracts and faecal pellets (figs 1 and 3) and Fisher test B to frequencies of soft-bodied prey in digestive tracts and faecal pellets (figs 2 and 4, see more details in the text). In larger islands, as Colom and Formentera, faecal pellets were collected at same locations as individual digestive tracts (the southwestern beach in Colom and Trocadors peninsula in Formentera).

Species	Population	No. of digestive tracts	No. of faecal pellets	Fisher test A	Fisher test B
P. lilfordi	Aire	44	246	1.879×10^{-6}	2.2×10^{-16}
"	Colom	32	229	0.001253	7.182×10^{-9}
"	Addaia gran	28	326	1.989×10^{-13}	8.054×10^{-8}
"	Addaia petita	27	25	0.2744	7.243×10^{-5}
"	Sargantana	43	79	0.004637	0.05526
"	Rovells	9	41	0.4066	0.4709
"	Porros	5	114	1	0.008857
"	Sanitja	40	180	0.02439	0.5142
"	Bledas	14	72	2.124×10^{-5}	0.1143
P. pityusensis	Bleda Plana	39	10	0.593	0.08592
"	Espardell	17	68	1	0.06446
"	Gastavi	12	23	0.002828	1.414×10^{-6}
"	Penjats	14	23		
"	Formentera	13	84	0.3860	0.5351

gut samples and in both species under study, we calculated the proportion of insect larvae, as well as the proportion of a group of selected soft-bodied prey, including insect larvae, Araneae, Opilionida, Pseudoscorpionida, Diptera, and Lepidoptera. Frequencies of larvae and the pooled frequency of the whole group of soft-bodied prey were compared with frequencies of the remaining prey items using a Fisher's exact test, because of its greater accuracy with frequencies lower than 5 (McKillup, 2005). The average percentage of larvae and the average percentage of the group of soft-bodied prey were compared among faecal and gut samples with one-way ANOVAs. When underlying assumptions for ANOVA were violated, especially the homogeneity of variances checked with Levene's test, we employed Welch's ANOVA (Zar, 1999). Statistical calculations were done with JMP and R base packages (R Developmental Core Team, 2009). In all cases, statistical tests were two-tailed and alpha was set at 5%. Means are presented $\pm$ 1 Standard Error (S.E.).

Results

In *P. lilfordi*, we detected significant differences in the frequencies of soft/hard prey in five populations: Aire, Colom, Addaia gran, Addaia petita and Porros. However, in three of them, Aire,

Addaia petita and Porros, a significantly higher proportion of soft prey was present in faecal samples (table 1). For insect larvae, we obtained significant differences in six populations, from which, on Aire Island the proportion of larvae was higher in faecal samples. No differences were detected in Rovells, Porros and Addaia petita (table 1 and figs 1 and 2). In fact, at Porros islet, insect larvae were absent from gut contents.

We failed to detect any significant difference between the proportion of larvae and soft/hard prey from guts or faecal samples of *P. pityusensis* (table 1) in four populations. Only in the case of Gastaví, did we detect significant differences in the proportion of larvae and soft-bodied prey, but in both cases, with higher proportions corresponding to faecal samples (table 1 and figs 3 and 4). On the islet of Gastaví islet, larvae were absent from gut contents. At Penjats islet, we
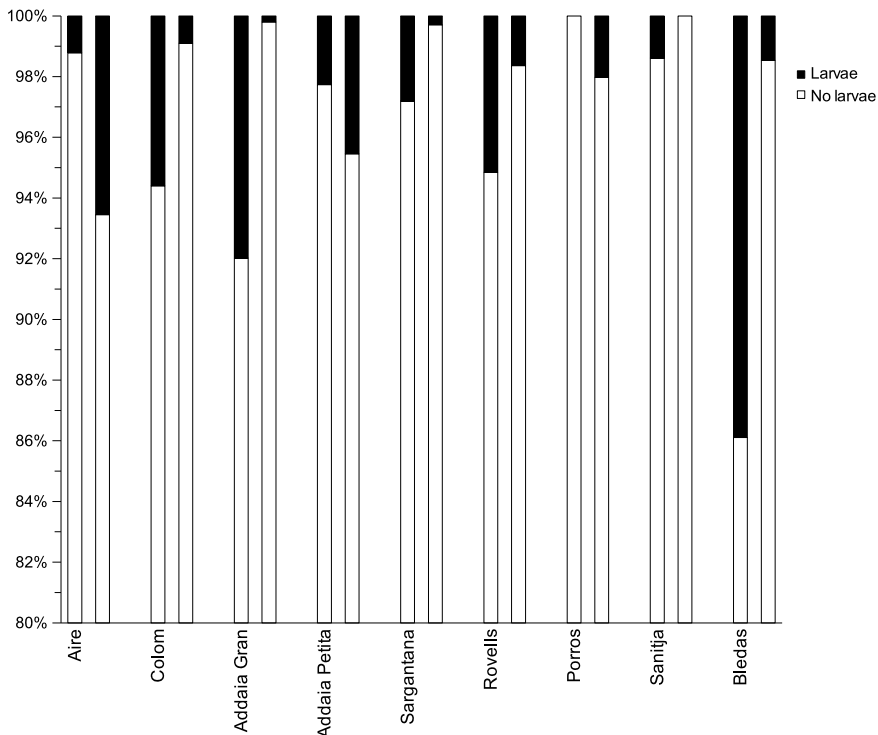

Figure 1. Percentage of gut contents (left) and faecal pellets (right) with and without insect larvae in insular populations of *P. lilfordi* under study.
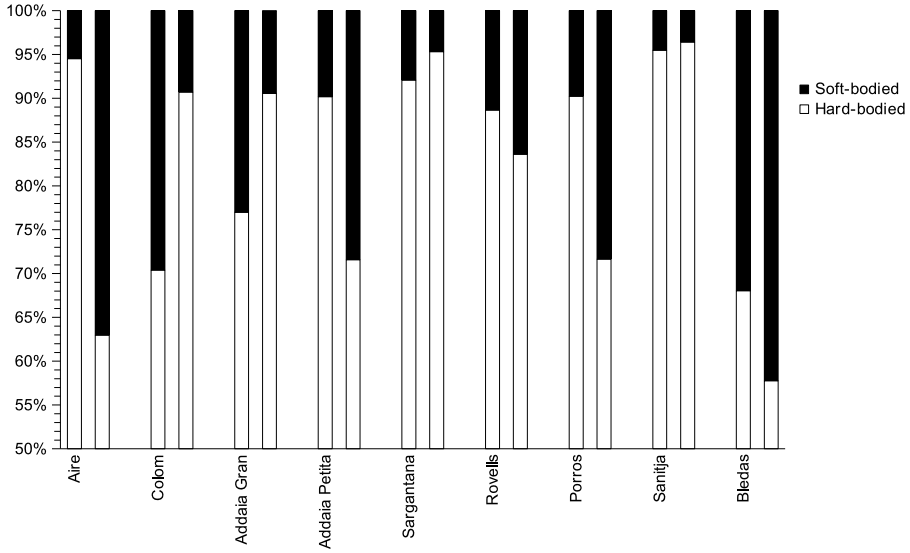
Figure 2. Percentage of gut contents (left) and faecal pellets (right) with soft-bodied vs hard-bodied prey in insular populations of *P. lilfordi* under study.
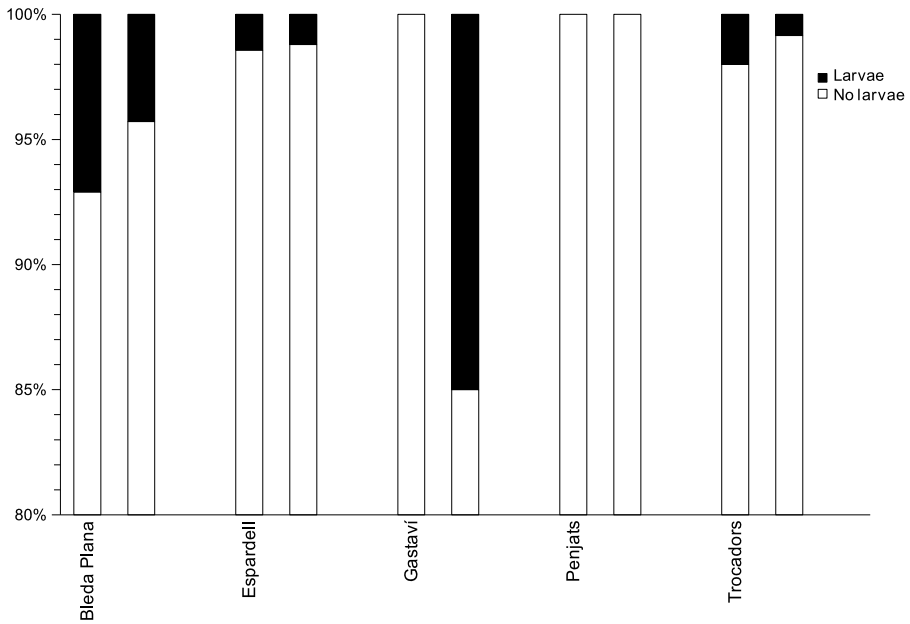

Figure 3. Percentage of gut contents (left) and faecal pellets (right) with and without insect larvae in insular populations of *P. pityusensis* under study.

did not detect insect larvae in guts nor faecal pellets.

If we consider the whole set of samples from each lizard species under study, we can compare the average percentage of larvae and other soft-bodied prey from faecal and gut samples. In *P. lilfordi*, we did not find any significant difference between the average percentage of insect larvae from faecal and gut samples (one-way ANOVA, $F_{1,16} = 2.43$, $P = 0.14$). A similar
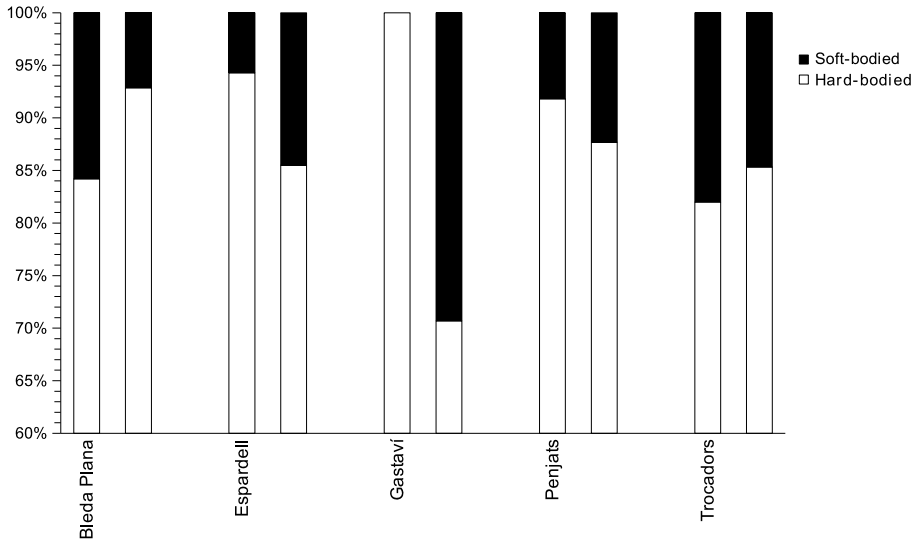
Figure 4. Percentage of gut contents (left) and faecal pellets (right) with soft-bodied vs hard-bodied prey in insular populations of *P. pityusensis* under study.

result was observed for *P. pityusensis* ($F_{1,8} = 0.17$, $P = 0.69$). For the remaining soft-bodied prey, results were similar: indeed, no significant differences in both species were detected in the average percentage of Pseudoscorpionida, Opilionida, Araneae, Diptera, and Lepidoptera between gut contents and faecal pellets. The exception was the average percentage of Araneae in *P. lilfordi* samples (heterogeneous variances, Welch's one-way ANOVA, $F_{1,16} = 5.35$, $P = 0.0401$), but with a higher percentage of spiders detected in faecal pellets (in gut contents, $x = 3.66 \pm 1.54$, in faecal pellets, $x = 8.71 \pm 1.54$).

Discussion

The principal conclusion that can be derived from the present study is that our results do not support the statement made by Pincheira-Donoso (2008) regarding detection probabilities of soft-bodied prey in faecal pellets. We found a large variety of scenarios, from populations where larvae or other soft-bodied prey were more frequent in gut contents than in faecal pellets, to those with the opposite situation. In no case could we demonstrate that faecal pellets systematically contained less soft-bodied prey

than gut contents. During our survey, we did not detect any group exclusively based gut contents, while some prey types were only present on faecal pellets, as in the case of Opilionida, only detected in fecal pellets of *P. lilfordi*. A limitation of our study is that faecal pellets and gut contents were obtained at the same populations and during the same season, but in different years (see Methods). We suspect that the observed differences in prey composition between faecal pellets and gut contents could be due to differences in prey availability in different years. Yet, it is unlikely that prey availability differences among years affect the detectability of soft-bodied prey in either faecal or gut content samples. Our data thus confirm the conclusions of Angelici, Luiselli and Rugiero (1997), who suggested that faecal pellets can used for dietary studies of lizards. Our results show that faecal samples retain a large amount of soft-bodied prey remains. Yet, the identification of prey remains is somewhat less easy in faecal samples compared to stomach contents (Angelici, Luiselli and Rugiero, 1997), but is possible with some training and experience (see also, Hódar, 1996, 1997).

It is obvious that volumetric calculations cannot be done with most of the fragments included in faecal samples. But, at least for small lizards under 80 mm SVL, prey remains from digestive tracts, even if we focus the analysis only to stomach contents, are also frequently fragmented and not useful for direct measurements (Angelici, Luiselli and Rugiero, 1997). Only whole prey items could be useful for volumetric calculations, and whole items are not common in digestive tracts of small lacertid lizards (Pérez-Mellado, 1989; Pérez-Mellado and Corti, 1993 and personal observations). Thus, with small lacertid lizards, we encounter similar problems for volumetric calculations with prey remains from faecal and gut samples. An alternative method, at least for some prey items, is the calculation of total prey size with the measurement of particular anatomical pieces (see, for example, Calver and Wooller, 1982; Hódar, 1996, 1997) and the use of regression equations to estimate prey body sizes or biomass (Sage, 1982).

Working with protected species and frequently endangered populations, as it is the case of several insular populations of the genus *Podarcis* (Pérez-Mellado and Corti, 1993) or large Canarian lizards, as *Gallotia simonyi* (Pérez-Mellado et al., 1999), the use of faecal pellets was mandatory. Soft-bodied prey recovered in these studies (Pérez-Mellado and Corti, 1993; Pérez-Mellado et al., 1999) and detected in a similar way as identified in gut contents (Pérez-Mellado, 1989).

The study of diets remains a very interesting topic in lizard ecology and a complementary tool to understand foraging behaviour of lizards, and the interactions of lizards with plants and other organisms. Dietary studies of poorly known species are badly needed but, in our opinion, researchers need to improve their identification skills of prey remains from faecal samples and need to include direct observations of foraging and prey capture as a complement, rather than returning to the classical analysis of digestive tracts. Conservation issues preclude the use of this method in strictly protected lizards, and ethics does the same in the case of more common species. Sacrifice of a free-ranging animal on scientific grounds is allowed only when there is no other way of obtaining data, but in this case lizard biologists certainly have alternative methods. Indeed, we can obtain a reliable description of a lizard diet with the analysis of a sample of faecal pellets, providing the knowledge of which elements of different prey types are identifiable. Angelici, Luiselli and Rugiero (1997) concluded that faecal analysis gives a reliable picture of the diet of *Lacerta bilineata*, fully comparable with the diet obtained with stomach dissection of lizards. Davies (1976) showed also that faecal analyses gave estimates of the diet of avian insectivores similar to those obtained with emetic techniques (see also, Calver and Wooller, 1982). Rosenberg and Cooper (1990) reached a similar conclusion, also applicable to several insectivorous lizards (see, for example, Hódar and Pleguezuelos, 1999 and references therein). In addition, the use of faecal pellets can give us a good picture of interactions of lizards with other organisms, as it is the case in lizard pollination syndromes and seed dispersal (see, for example, Valido and Nogales, 1994).

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