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Stomach flushing affects survival/emigration in wild lizards: a study case with rainbow lizards (*Agama agama*) in Nigeria

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Abstract. Stomach flushing is one of the proposed techniques to study lizard diets. Apparently, it is ranged, together with direct observation and faecal analysis, as a non-harmful method for dietary studies. Some works explored the usefullness of stomach flushing, but we lack information about its effect on lizard's survival probabilities. In this paper we studied the effect of stomach flushing in an urban population of the rainbow lizard (*Agama agama*) from Calabar (Nigeria). During a period of five months of 2010, 147 lizards were noosed, sexed and individually marked. One group of lizards was stomach flushed only once, whereas the rest of lizards were not flushed. The flushed sample of lizards showed a lower survival than non-flushed lizards of all sex and age classes. In this study, the pictured diet from stomach flushing was very similar to results obtained with a faecal analysis of the same lizard population. Thus, both methods seem to be reliable to study the diet of the rainbow lizard. However, our results indicate that stomach flushing increases the probability of mortality (or at least emigration rates) in all age and sex classes, precluding its extensive use as a method to study lizard's diets.

Keywords: Africa, conservation, demography, invasive field methods.

Introduction

In the current world, with both ethic and conservation requirements asking scientists to minimize animal suffering and sacrifices during experiments, it is a priority to develop methodologies allowing robust data collection while avoiding useless loss of individuals. Because of this, ecologists typically use three 'harmless' alternative methods in order to analyze dietary habits of lizards, viz. stomach flushing (e.g., Powell and Russell, 1984; Christian et al., 1996), analysis of faecal samples (e.g., Angelici, Luiselli and Rugiero, 1997; Hawlena and Pérez-Mellado, 2009; Pérez-Mellado et al., 2011), and direct observation of lizards foraging (e.g., Sáez and Traveset, 1995). While the latter two methods are totally non-invasive, stomach flushing is apparently an invasive method.

Indeed, stomach flushing is usually practiced by inserting a narrow Teflon® tube into the esophagus of the captured lizard. The tube is twisted during insertion, pushing it slightly more when it reaches the pyloric sphincter, to pass through it. When the tube is in the stomach, water is continuously pumped to promote the vomit of stomach content (Legler, 1977; Legler and Sullivan, 1979). The mortality rate from gut flushing was reported to be over 5% in some cases (Pietruszka, 1981; Powell and Russell, 1984), with individuals that succumbed being all small lizards, and death was presumably due to excessive pumping pressure causing rupture of the gut, or directly produced by the inserted tube.

Although available literature data (and personal unpublished observations) suggest that stomach flushing may be harmful for lizards (Pietruszka, 1981; Powell and Russell, 1984), there is no experimental study available where the effects of this technique on survival of wild lizards were quantitatively assessed. In order to fill this gap, our aims in this paper are hence to explore (i) whether stomach flushing affects im-

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mediate (within one-month from treatment) and non-immediate (up to 4 months later) survival rates in lizards, and (ii) whether stomach flushing depicts the taxonomic composition of the diet differently from totally non-invasive methods as faecal pellet analysis.

As a study model, we used an urban population of rainbow lizards (*Agama agama* Linnaeus, 1758) inhabiting the city of Calabar, one of the main cities situated in the Guinea-Congolian rainforest region of south-eastern Nigeria, West Africa (White, 1983). Rainbow lizards are unthreatened at the range scale (IUCN, 2010), extremely abundant at the study area (Reid, 1986), anthropophilous, easily catchable and highly territorial (Chapman and Chapman, 1964; Harris, 1964; Yeboah, 1982; Anibaldi, Luiselli and Angelici, 1998), hence they are ideal models for a field experiment on the effects of stomach flushing on survival. In addition, they can grow relatively big (adult males may easily reach 12 cm SVL and up to 38 cm total length, see Anibaldi, Luiselli and Angelici, 1998; Chirio and LeBreton, 2007), but are still small at birth and in the first year of life (3-4 cm SVL); hence they are good for exploring the effects of stomach flushing over a wide range of lizard sizes.

Materials and methods

Study area

The field experiment was performed at an urban area (Big Quo Town) situated within the metropolitan territory of Calabar, the capital city of Cross River State (Nigeria). Lizards were studied along a transect 150 m long. The transect was a busy path. A cement wall (0.80 to 250 cm tall) bordered one side of the transect, while small grassy patches, ornamental private home gardens, and two buildings bordered the other side of the transect. The climate of the study area is equatorial, with a rainy season from April to September, a dry season from October to March, and with rather constant ambient temperatures (27-34°C) year-round.

Protocol

The field experiment was carried out in January, February, March, April, and May, 2010. From 2nd to 21th January, a sample of 147 lizards (44 males, 68 females, 35 subadults) was noosed, sexed, measured to snout-vent length (SVL), and individually marked by toe-clipping before being released. Part of this sample (22 males, 34 females, and 18 subadults) was stomach flushed only once, whereas the rest of the sample was not flushed. The study transect was re-explored for seven consecutive days in each survey, from hr 0730 to 1700 (Lagos time), in February (20th-26th) March (21st-27th), April (21st-27th) and May (22nd-28th), and all lizards encountered were recaptured. Individuals which were encountered for the first time in these other survey periods were also marked, but were excluded from our analyses and not processed for stomach flushing. They will be considered in ongoing demographic projects on this lizard population. In order to avoid multiple capture of a same individual within each survey period, we painted on the lizard's back with a white number done with non-toxic paint. This latter marking technique allowed easy individual identification of the marked lizards from distance without need of further recaptures, at least within each survey period. The lizards were then released to the field.

Identifiable food items were obtained from a total of 69 lizards (93.2% of flushed sample). Food items collected by stomach flushing were later analyzed in the lab with standard methodology (Legler and Sullivan, 1979), that is, identifying prey remains, under a binocular microscope, to order level. In addition, a total of 311 fecal pellets were collected from the wall; since rainbow lizard's faeces are by far larger than those of smaller sympatric scincids of the genus *Trachylepis*, these pellets were unambiguously identified to species level due to their size. However, the individuality of the lizards producing the various pellets was not known. In order to make comparisons with contents of stomachs flushed, we randomly selected a sample of 69 pellets among the totals collected. These pellets were also analyzed in the lab with standard methods (Pérez-Mellado et al., 2011).

Statistical analyses

As the basic assumptions underlying a "closed population" scenario, i.e., no significant deaths, birth and/or migration (Jolly, 1965, 1982), could not be fulfilled, we used the Jolly-Seber open population option. In our case, the probability that a lizard alive at the moment of release in the ith sample will survive till the time of capture in the $i+1$th sample is:

$$\Phi_i = M_{i+1}/\hat{M}_i - r_i - n_i,$$

M_i = estimated total number of marked specimens in the population on year i, r_i = total number of marked specimens recaptured on year i, n_i = total number captured on year i.

We divided our lizard samples by treatment (stomach flushed versus control) and by population category (males, females, subadults). We defined 'immediate survival' the survival rates calculated one month after treatment, i.e., in the February 2010 survey. We defined 'overall survival' the survival rates calculated four months after treatment, i.e., in the May 2010 survey. Survival rate estimates were always <1.0, confirming the lack of sampling biases such as loss or to a failure to recognise marked individuals. The estimated survival rate can be converted to a 'loss rate' y (the effect of death and migration) as follows:

$$\hat{\gamma}_i = 1 - \hat{\Phi}_i.$$

More details on these algorithms are given in Simply Tagging (2007).

Differences in catchability among individuals were assessed by the zero-truncated Poisson test (Gurmu, 1991), and temporal variability in capture probabilities by Leslie's test for equal catchability (Carothers, 1971).

In dietary analyses, we counted the number of stomachs flushed or faecal pellets containing a given prey item and not the numbers of food items recorded in total. In addition, we pooled males, females, and subadults in the stomach flushing dataset because otherwise direct comparisons with faecal pellet dataset would have been impossible (given that the identity of the lizard individual producing a given pellet was unknown). For calculating the similarity in taxonomic diet composition between stomach flushing and faecal pellet datasets, we calculated the niche overlap index of Pianka (1986), with values ranging from 0 (no similarity) to 1 (total identity).

Survival/catchability analyses were performed using the software program Simply Tagging [version 1.31] (see Henderson and Seaby, 2002). Apart from survival/catchability analyses, all other statistical analyses were performed with Statistica [version 6.0]. Parametric tests were used only after verifying data normality and homoscedasticity (Zar, 1984). Median differences in survival between flushed and control individuals were tested by Mann-Whitney U-test, and differences in the observed numbers of insect larvae (soft-bodied prey items) between stomachs flushed and faecal pellets were tested by observed-versus-expected χ^2 test. All tests were two-tailed, with α set at 5%.

Results

Survival

The summary of the individual histories for the lizards studied here is presented in table 1. Overall (i.e., pooling males, females and subadults), the flushed sample retained a lower survival than the control sample after four months (table 2). In males, stomach-flushed individuals had a significantly lower survival than untreated individuals (table 2). Indeed, whilst 50% of the untreated individuals were still present in the wall in May, only 13.6% of the flushed individuals were recaptured in May. The same trend also appeared for females (44.1% versus 17.6% still found in May) and for juveniles (respectively 17.6% and 5.6%). Control adults of both sexes exhibited similar mean

Table 1. Summary of the capture histories for rainbow lizards at the study area.

Sex	Treatment	January	February	March	April	May
Male	control	1	0	0	0	0
Male	control	1	1	1	1	0
Male	control	1	0	1	0	0
Male	control	1	1	1	1	1
Male	control	1	0	0	0	0
Male	control	1	0	0	0	0
Male	control	1	1	1	1	1
Male	control	1	1	1	1	1
Male	control	1	1	1	1	1
Male	control	1	1	1	1	1
Male	control	1	1	0	0	0
Male	control	1	0	1	1	1
Male	control	1	0	0	0	0
Male	control	1	1	0	1	1
Male	control	1	0	1	1	1
Male	control	1	1	1	1	1
Male	control	1	1	1	0	1
Male	control	1	1	1	0	0
Male	control	1	1	0	0	0
Male	control	1	1	0	1	1
Male	control	1	0	0	0	0
Male	control	1	0	0	0	0
Male	flushed	1	1	1	0	0
Male	flushed	1	0	0	0	0
Male	flushed	1	0	0	0	0
Male	flushed	1	1	1	1	0
Male	flushed	1	1	0	0	0
Male	flushed	1	1	0	0	0
Male	flushed	1	0	1	1	1
Male	flushed	1	0	0	0	0
Male	flushed	1	1	0	0	0
Male	flushed	1	0	0	0	0
Male	flushed	1	0	0	0	0
Male	flushed	1	0	0	0	0
Male	flushed	1	1	1	1	1
Male	flushed	1	0	0	0	0
Male	flushed	1	0	0	0	0
Male	flushed	1	1	0	0	0
Male	flushed	1	0	0	0	0
Male	flushed	1	0	0	0	0
Male	flushed	1	1	1	1	1
Male	flushed	1	1	0	0	0
Male	flushed	1	1	0	0	0
Male	flushed	1	0	0	0	0
Female	control	1	1	1	1	1
Female	control	1	1	1	1	1
Female	control	1	1	1	1	0
Female	control	1	0	0	0	0
Female	control	1	1	1	1	1
Female	control	1	0	1	1	1
Female	control	1	1	0	0	0
Female	control	1	1	1	1	1
Female	control	1	1	1	1	0
Female	control	1	1	1	0	0
Female	control	1	0	0	0	0

Table 1. (Continued).

Sex	Treatment	January	February	March	April	May
Female	control	1	0	0	0	0
Female	control	1	1	1	1	1
Female	control	1	1	1	1	1
Female	control	1	1	1	1	1
Female	control	1	1	1	1	1
Female	control	1	1	1	1	0
Female	control	1	1	1	1	1
Female	control	1	1	0	0	0
Female	control	1	1	1	1	1
Female	control	1	0	0	0	0
Female	control	1	1	1	1	1
Female	control	1	0	0	0	0
Female	control	1	1	1	1	1
Female	control	1	1	0	0	0
Female	control	1	1	1	1	1
Female	control	1	1	1	1	1
Female	control	1	0	1	0	0
Female	control	1	1	0	0	0
Female	control	1	0	0	0	0
Female	control	1	0	0	0	0
Female	control	1	1	1	1	0
Female	control	1	1	0	0	0
Female	control	1	1	0	0	0
Female	flushed	1	1	0	0	0
Female	flushed	1	1	1	1	1
Female	flushed	1	1	0	0	0
Female	flushed	1	0	0	0	0
Female	flushed	1	0	0	0	0
Female	flushed	1	0	1	1	1
Female	flushed	1	1	1	1	1
Female	flushed	1	0	0	0	0
Female	flushed	1	0	0	0	0
Female	flushed	1	1	1	1	1
Female	flushed	1	0	0	0	0
Female	flushed	1	0	0	0	0
Female	flushed	1	1	0	0	0
Female	flushed	1	0	0	0	0
Female	flushed	1	1	1	1	1
Female	flushed	1	1	0	0	0
Female	flushed	1	0	0	0	0
Female	flushed	1	0	0	0	0
Female	flushed	1	0	0	0	0
Female	flushed	1	1	1	0	0
Female	flushed	1	0	0	0	0
Female	flushed	1	1	0	0	0
Female	flushed	1	0	0	0	0
Female	flushed	1	0	0	0	0
Female	flushed	1	1	0	0	0
Female	flushed	1	1	0	0	0
Female	flushed	1	0	0	0	0
Female	flushed	1	0	0	0	0
Female	flushed	1	1	1	1	0
Female	flushed	1	0	0	0	0
Female	flushed	1	0	0	0	0
Female	flushed	1	1	1	1	1
Female	flushed	1	1	0	0	0

Table 1. (Continued).

Sex	Treatment	January	February	March	April	May
Female	flushed	1	0	0	0	0
Subadult	control	1	1	0	0	0
Subadult	control	1	0	0	0	0
Subadult	control	1	0	0	0	0
Subadult	control	1	1	1	0	0
Subadult	control	1	0	0	0	0
Subadult	control	1	0	0	0	0
Subadult	control	1	1	1	1	1
Subadult	control	1	0	0	0	0
Subadult	control	1	1	0	1	1
Subadult	control	1	1	1	0	0
Subadult	control	1	0	0	0	0
Subadult	control	1	0	1	0	0
Subadult	control	1	0	1	0	0
Subadult	control	1	1	1	1	1
Subadult	control	1	1	0	0	0
Subadult	control	1	0	0	0	0
Subadult	control	1	0	0	0	0
Subadult	flushed	1	0	0	0	0
Subadult	flushed	1	0	0	0	0
Subadult	flushed	1	0	0	0	0
Subadult	flushed	1	0	0	0	0
Subadult	flushed	1	1	0	0	0
Subadult	flushed	1	0	0	0	0
Subadult	flushed	1	1	1	0	0
Subadult	flushed	1	1	0	0	0
Subadult	flushed	1	0	0	0	0
Subadult	flushed	1	0	0	0	0
Subadult	flushed	1	0	1	1	1
Subadult	flushed	1	1	0	0	0
Subadult	flushed	1	0	0	0	0
Subadult	flushed	1	1	0	0	0
Subadult	flushed	1	0	1	0	0
Subadult	flushed	1	1	1	1	0
Subadult	flushed	1	0	0	0	0
Subadult	flushed	1	0	0	0	0

survival (>0.82), higher than mean survival of control subadults (0.609). Flushed females had slightly higher mean survival than flushed males (about 0.61 versus 0.59), and their survival was higher than that of flushed subadults (0.5). Overall, despite the relatively small sample size, there were marginally significant median differences in survival between flushed and control individuals (Mann-Whitney U-test, $U = 18.5$, exact $P = 0.05$). Temporal differences in catchability did not occur among survey periods (in all cases, $P > 0.05$ at Leslie's χ^2 test, table 2), whereas individual differences in catchability were significant only for females (zero

Table 2. Survival, temporal and individual catchability, in rainbow lizards at the study area.

	Mean survival	Median survival	SD survival	SE survival	Temporal catchability	Individual catchability	n
Total sample flushed	0.577	0.548	0.175	0.101	$\chi^2 = 7.00$; $P = 0.637$	$\chi^2 = 10.03$; $P = 0.018$	74
Total sample control	0.777	0.792	0.033	0.019	$\chi^2 = 18.41$; $P = 0.915$	$\chi^2 = 49.35$; $P < 0.0001$	73
Males flushed	0.589	0.568	0.2	0.046	$\chi^2 = 1.99$; $P = 0.368$	$\chi^2 = 4.59$; $P = 0.100$	22
Males control	0.82	0.84	0.061	0.05	$\chi^2 = 3.91$; $P = 0.950$	$\chi^2 = 6.1$; $P = 0.107$	22
Females flushed	0.615	0.504	0.226	0.024	$\chi^2 = 5.00$; $P = 0.416$	$\chi^2 = 10.5$; $P = 0.0059$	34
Females control	0.826	0.813	0.073	0.014	$\chi^2 = 13.99$; $P = 0.45$	$\chi^2 = 10.13$; $P = 0.017$	34
Subadults flushed	0.5	0.5	0.167	0.15	not defined	$\chi^2 = 1.62$; $P = 0.202$	18
Subadults control	0.609	0.576	0.294	0.164	$\chi^2 = 2.00$; $P = 0.367$	$\chi^2 = 2.74$; $P = 0.253$	17

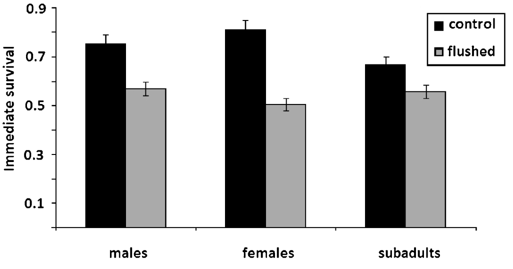

Figure 1. Immediate survival (i.e., survival calculated one month after flushing, with standard error) in rainbow lizards at the study area.

truncated poisson test, at least $P < 0.02$ at χ^2 test, table 2).

Immediate survival was also consistently lower in flushed than in control individuals, either considering males, females and subadults pooled (immediate survivals for flushed and control samples respectively 0.548 and 0.740) or considering these categories of individuals separately (fig. 1).

Diets

Taxonomic diet composition of rainbow lizards, as pictured from both stomach flushing and faeces analysis, is presented in table 3. Adult beetles and ants were the main prey items according to both methods, but 13 prey categories were recovered by stomach flushing versus 11 by faecal pellets. Nonetheless, the two categories which were not found in faecal pellets (Opilionida and Chilopoda) were found in only a single stomach each (table 3). The number of insect larvae (soft-bodied prey items) was similar by stomach flushing ($n = 11$) and by faecal pellets ($n = 7$)

Table 3. Taxonomic diet composition of rainbow lizards at the study area, based on both stomach flushing and faeces analyses. Note that numbers here refer to number of stomachs (or pellets) containing a given prey item and not numbers of food items in total.

Food items	Stomachs flushed	Faeces
Araneidae	4	6
Opilionidae	1	0
Chilopoda	1	0
Miriapoda	2	3
Insecta		
Coleoptera adults	31	34
Coleoptera larvae	5	4
Lepidoptera adults	3	2
Lepidoptera larvae	6	3
Vespoidea	4	1
Apoidea	4	4
Formicoidea	11	14
Dermaptera	7	3
Blattodea	9	8

($\chi^2 = 0.889$, df $= 1$, $P = 0.346$). Overall, similarity in terms of taxonomic diet composition by two methods was very high ($O_{\text{Pianka}} = 0.9802$).

Discussion

Our study showed that both the immediate survival and the overall survival of rainbow lizards differed clearly between treatment and control, with stomach flushing treatment causing a reduced survival in all categories analysed (males, females, subadults). Of course, we could not be sure that all non-recaptured individuals died, as some of them may have emigrated. In either case, it is evident that stomach flushing treatment remarkably affected the tested individuals either increasing their likelihood to die or forcing them to emigrate, possibly due to stress. We think that, because *Agama agama* has a social system with strong dominance/hierarchy categories (e.g., dominant and subordinate males) (e.g., Harris, 1964), injuries (even if not remarkably serious) caused by stomach flushing may reduce dominance of previously dominant individuals, which can lose their social status and be relegated or forced to migrate. In any case, emigration is also associated to increased mortality (for instance due to predation, intraspecific aggression, reduction of efficiency of food searching in unfamiliar habitats etc; see, for example, Christian and Tracy, 1981; Clobert et al., 1994 and references therein). Hence, systematic use of stomach flushing may produce an important extent of disturbance to a natural population of lizards to be accepted as a reliable field technique.

Interestingly, the negative effect of stomach flushing was stronger in adult lizards than in subadults. In these latter individuals, indeed, treatment and control groups showed more similar survival rates (either immediate survival or in overall) than in adults. This is counterintuitive, since it was predicted that smaller and more delicate individuals would have suffered higher survival risks (Powell and Russell, 1984). Several reasons may explain this counterintuitive pattern. Firstly, a lower detectability of juveniles compared to adults may have partially biased the results (catchability is currently lower in juveniles, see, for example, Krebs, 1989) thus 'flattening' the actual differences in survival rates between treatment and control subadults. As an alternative, it is possible that large lizards are more stressed than smaller individuals because of their body vigour, forcing the experimenters to be less delicate when handling them for flushing their stomachs. It is also possible that the stronger pressure exerted with probing tube to open the pyloric sphincter in adult individuals, could increase the probability of rupturing gut walls (pers. obs. in other lizard species as *Podarcis hispanica* and *Podarcis carbonelli*). In this respect, adult males and adult females showed consistent and clear trends towards a strongly decreased survival (and possibly increased emigration) with treatment.

Our study also revealed that the diet composition of rainbow lizards was pictured in a very similar way (>98% identity) either employing stomach flushing or simply collecting faeces from the soil. This mirrors previous results showing that faeces analysis provides reliable data for analysis of diet composition in lizards, similar in terms of quality to data gathered from stomach dissection (Angelici, Luiselli and Rugiero, 1997; Pérez-Mellado et al., 2011). Both methods revealed that rainbow lizards are insectivorous, with a preponderance for adult beetles and, to a lesser extent, ants. A similar preference for beetles was also observed in conspecifics from Malindi, Kenya (Anibaldi, Luiselli and Angelici, 1998). Apart from the cases in which pellets are collected from the soil (as in the present case), in studies of adaptive radiation faeces can be also employed, providing that they are obtained directly from lizards. Thus, in this way, experimenters can have with the faecal sample, information on the sex, age and other characteristics of lizards, and hence faecal pellets can provide the same additional information about the animal producing the pellet than a stomach flushing sample or a gut content. Concerning the soft-bodied prey and their supposedly underestimation in analyses of faeces (Pincheira-Donoso, 2008), a previous paper (Pérez-Mellado et al., 2011) treats specifically this issue, showing no risk of any significant bias between faeces and stomachs analyses.

Legler (1977) and Legler and Sullivan (1979) proposed the use of stomach flushing as a reliable technique to obtain food samples from tortoises, anurans and lizards. In spite of this, only a minority of studies of lizards' diet employed this method. Pietruszka (1981) applied the method with apparently good results to five lizard species and only found seven individuals that died as a direct result of stomach flushing.

Unfortunately, specific mortality was higher for smaller lizards, 8.7% of lizards in the case of *Uta stansburiana* (Pietruszka, 1981). We tested this method in several species of European lacertid lizards, including *Iberolacerta cyreni*, *Podarcis carbonelli*, *Podarcis hispanica*, *Podarcis muralis*, *Psammodromus algirus* and *Psammodromus hispanicus* (Pérez-Mellado, 1983 and unpublished results). Our results were discouraging. In two of these species: *P. carbonelli* and *P. hispanica*, we obtained the whole stomach content from 25 individuals of each species (Pérez-Mellado, 1983), but lizards were clearly damaged by the flushing, even if the Teflon tube was gently introduced in the gut. Observed damages included the rupture of gut's walls, a severe impairement of lizard's locomotion, that were unable to escape after release (100% of captured lizards) and the rapid death of, at least, three individuals. The method was abandoned for this reason. In addition, the *post mortem* inspection of stomachs and intestines of lizards revealed that in at least 30% of cases from *P.muralis*, *Psammodromus algirus* and *Psammodromus hispanicus*, some prey remains, soft and hard-bodied, were retained in the stomach after flushing (Pérez-Mellado, unpublished results).

Hence, in conclusion, we strongly suggest avoiding the use of stomach flushing as a tool for obtaining food items from wild populations of lizards, as it may produce much increased mortality rates and over-emigration (due to stress and excessive disturbance with lizard handling and processing). It is still possible that stomach flushing may be less dangerous with species different from rainbow lizards, for instance with species showing less sophisticated social systems (see Chapman and Chapman, 1964; Harris, 1964; Yeboah, 1982; Anibaldi, Luiselli and Angelici, 1998). However, as a precautionary rule, it is better to avoid the use of this method without prior species-specific evaluation of correlated costs for lizard survivals. Instead, we suggest the use of faecal analysis as a routine tool for dietary studies of lizards (Pérez-Mellado et al., 2011).

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