

# On the application of stomach-flushing to Namib Desert lizards

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## 1 INTRODUCTION

Dietary investigations of reptiles generally involve the collection and sacrifice of individuals within a population. This practice provides information on the population diet and, perhaps, inter-individual variation, but precludes information on within-individual variability since each lizard can yield only one datum point. Such dietary studies are incompatible with demographic studies of the same populations. In cases where both dietary and demographic data are required, faecal analyses may be possible for some species (e.g., *Agama agama*, Harris 1964), however, the general approach has been to collect individuals for dietary data from areas nearby the principal study area (e.g., Dunham 1983). This approach requires assumptions about both the similarity of the food base between areas and about individual feeding habits in those areas. In extremely patchy habitats these assumptions may not be valid, thus limiting the usefulness of dietary data. Nonetheless, even this approach may not be suitable for studies of rare or endangered species (e.g., Powell & Russell 1984).

Stomach-flushing has been described as a feasible method of obtaining diet samples from turtles, lizards and anurans (Legler 1977, Legler & Sullivan 1979). It has been evaluated for several North American lizard species with respect to the acquisition of diet samples, the accuracy with which the effectiveness of an individual flushing event can be assessed, and post-flushing survival (Pietruszka 1981). This analysis demonstrated the general efficacy of the technique but also indicated that inherent species differences could significantly influence its effectiveness. The present study was undertaken, therefore, to assess the suitability of stomach-flushing for dietary analysis of Namib Desert lizards.

## ABSTRACT

A stomach-flushing technique used on North American turtles, lizards and anurans is examined with respect to seven species of Namib Desert lizards and is evaluated in detail for several of these. The procedure reliably provides stomach contents for subsequent analysis. Variation in body size can influence its effectiveness in some species, although the influence is minimal. Mortality from the procedure is low (2.5%, overall).

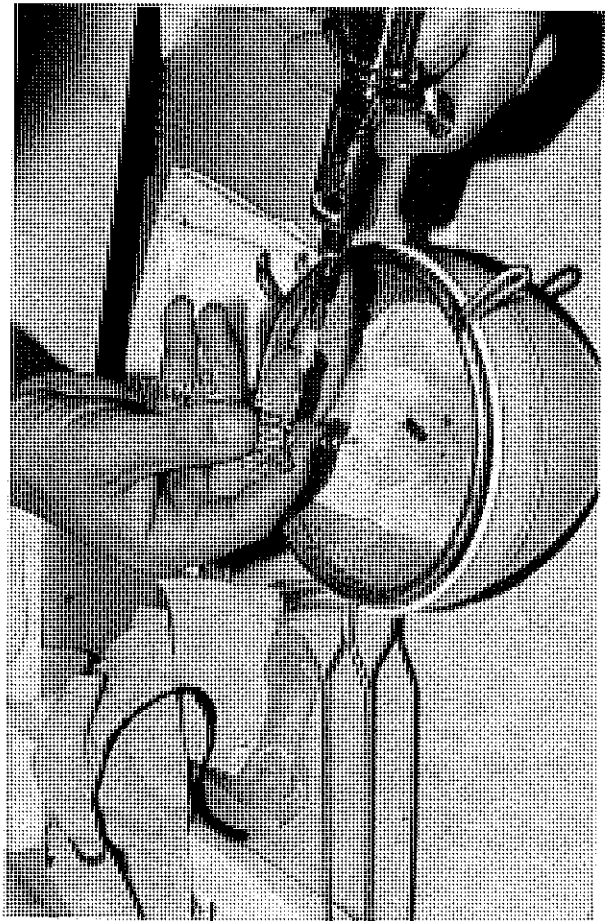
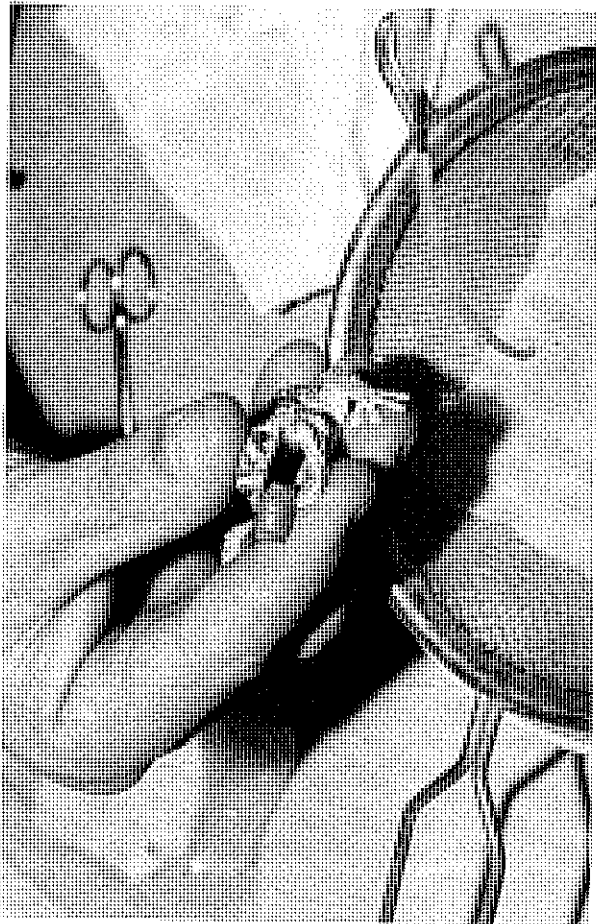
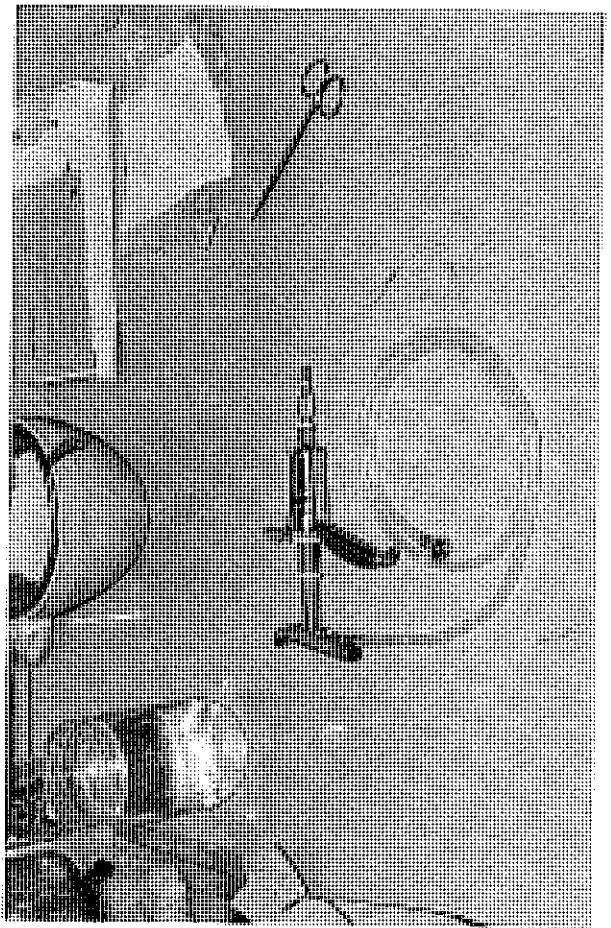


PLATE I: Stomach-flushing sequence and apparatus. (a)- rubber prosthesis holding mouth open; (b)- insertion of stomach cannula; (c)- recovery of food bolus; (d)- stomach-flushing equipment.

## 2 MATERIALS AND METHODS

Between 8 November 1984 and 22 August 1985 120 lizards of seven species (*Aporosaura anchietae*, *Angolosaurus skoogi*, *Mabuya capensis*, *Meroles cuneirostris*, *M. suborbitalis*, *Pachydactylus laevigatus*, *Rhoptropus afer*) were captured and subjected to stomach-flushing either at Gobabeb, Namibia, or at a study site on the Unjab River in the northern Namib Desert. Results from each flush were coded numerically as either "complete", "incomplete" or "stomach empty". Stomach contents were labelled and preserved in 70% ethanol. Any harmful effects due to stomach-flushing were noted. Lizards were then maintained individually in cloth bags for 12-24 h to check for latent effects. Eighty-one lizards were subsequently autopsied; each stomach was examined and assigned a subjective numerical classification as to its degree of fullness (empty, partially full,  $<1/2$ ; full; distended, Pietruszka 1981). Effectiveness of stomach-flushing was analysed statistically using the methods of Neter & Wasserman (1974) and Sokal & Rohlf (1981).

### 2.1 Stomach-flushing Procedure

The method used in this study is similar to that described by Legler (1977) and Legler & Sullivan (1979). However, it differs in several details that result in less trauma to individual lizards and so it will be described fully here. The principle behind stomach-flushing is that, given a stream of water of appropriate intensity, a food bolus located in the stomach can be dislodged by displacement and forced out through the esophagus and mouth without damage to the lizard. The apparatus used in the procedure consists of a syringe to which is attached a two-way valve and filling tube, allowing for continuous filling from a small container of water, and a stomach cannula. Legler & Sullivan (1979) describe an inexpensive apparatus using disposable plastic syringes. However, the two-way valve mentioned by these authors has become excessively expensive (price ca. \$36.00(US) in August 1984). An excellent alternative which is locally available is the Vetmatic Continuous Syringe (5 ml barrel) which currently sells for under R30.00. The stomach cannula used in this study consisted of ca. 75 mm of catheter tubing (1.7 mm O.D.) attached to a shortened 18-gauge syringe needle. Although Legler & Sullivan (1979) used a variety of cannula sizes in their application, I have found this unnecessary for lizards ranging from 1.0 g body weight to lizards in excess of 120 g.

During flushing, the lizard is held in one hand and its mouth is gently opened by inserting a small plastic ruler between the jaws and applying steady downward pressure to the lower mandible. Once open, the mouth is held in that position by placing a small rubber stopper (in this study a 3-7 mm diameter cylindrical pencil eraser) at the angle of the jaw so as not to block the opening to the esophagus. This is held in place by

pressure applied to the jaws with the thumb and forefinger (Plate 1a). The remaining three fingers are used to support the lizard's body and palpate the stomach. The stomach cannula is then moistened with water and inserted into the stomach through the esophagus (Plate 1b). During insertion a small amount of water may be injected into the stomach for lubrication but this is often unnecessary. With slightly built species the cannula can be felt (by palpation) as it is inserted into the stomach. This, coupled with an increased resistance once the stomach has been entered, serves to indicate that the cannula is in place. Subsequently, a stream of water is injected into the stomach. At the same time the cannula is momentarily withdrawn about 5 mm to reduce the likelihood of forcing water past the pylorus. This sequence is continued until the food bolus passes through the esophagus and out of the mouth (Plate 1c). The bolus is caught in a square of cotton gauze held by a metal sieve. A complete flush can be assessed by palpation of the stomach and by the emergence of a small amount of mostly digested material after retrieval of the main bolus. Although it may be fragmented, in many cases the bolus is retrieved intact, covered by a mucous film, and ends in a mass of digested material. Diet samples obtained in this way are then labelled and stored for later analysis.

The above procedure differs considerably from that of Legler & Sullivan (1979) in that their method requires propping the mouth open through the use of plastic rings that must be removed once the bolus reaches the mouth. The food is then removed with forceps and the cannula reinserted as many times as necessary to recover the stomach contents. The necessity for continued reinsertion of the cannula during flushing increases the likelihood of passing it through the pyloric sphincter and injecting water into the intestine with death usually following (Legler & Sullivan 1979, Pietruszka 1981). With a rubber stopper properly in place there is very often no need of using forceps at all to remove stomach contents; forceps were generally only used to remove large food items from the mouth when it was felt that further increases in water pressure would injure the lizard.

## 3 RESULTS

To adequately assess the usefulness of this technique requires information on (1) post-flushing survival, (2) how efficiently stomach contents may be obtained from particular species, and (3) the accuracy with which an investigator can assess the effectiveness of a specific flushing event (Pietruszka 1981). Since lizards are flushed independently of one another, each outcome can be classified as either successful or unsuccessful relative to several categories of possible stomach-flushing results. Data of this type were used to calculate, in all species with sufficient sample sizes, binomial estimates of success (Hollander & Wolfe 1973) for several categories of stomach-flushing

results: the probabilities of 1) obtaining the entire stomach contents from non-empty stomachs, 2) obtaining all or most of the stomach contents from non-empty stomachs, 3) obtaining at least some of the contents from non-empty stomachs, 4) accurately assessing an individual stomach-flushing event (i.e., complete, entire contents obtained; incomplete, but most of the contents obtained; stomach empty) and 5) survival after flushing.

As with a study of North American lizards (Pietruszka 1981), the present analysis indicates that the overall effectiveness of stomach-flushing is quite good (Table 1). One nearly always covers something from non-empty stomachs and there is a high probability of obtaining all or most of the stomach contents. Also as in North American lizards, the effectiveness of particular flushing events can be assessed (by palpation and the visual characteristics of the food bolus) with a high degree of accuracy. Finally, the survival of lizard species exposed to flushing is high (97.5% overall).

In contrast with the earlier study of North American lizards, there was no statistically significant heterogeneity among those lizard species for which I had adequate sample sizes (*A. anchietae*, *M. cuneirostris*, *R. afer*) for any category of stomach-flushing results (G-tests,  $P > 0.5$ , all categories). This may be because none of the species for which I have adequate sample sizes appears to exhibit food gorging as does *Cnemidophorus tigris* in the Great Basin Desert of the United States. In that species gorging significantly lowered the effectiveness of stomach-flushing (Pietruszka 1981).

### 3.1 Influence of body size

An inverse relationship between stomach-flushing success and body size has been suggested for turtles (Le-

gler 1977). The presence of a similar relationship was demonstrated among several species of North American lizards (Pietruszka 1981); however, this had only a minor effect on the overall effectiveness of the technique for these species and was, in fact, produced by attributes inherent to one of the species used in the analysis. In the present study, the body sizes (snout-vent length, SVL) of lizards stomach-flushed ranged from 32.7 mm (*Aporosaura*) to 155.0 mm (*Angolosaurus*). For three species there were sufficient sample sizes over a range of body sizes to examine whether body size might influence individual results. Linear regressions of success on lizard body size (using indicator variables, Neter & Wasserman 1974) were used to determine whether variation in size altered stomach-flushing effectiveness. In *Aporosaura* (SVL range: 32.7-49.9 mm) these analyses revealed no significant relationships between body size and any category of stomach-flushing result, although for category 1 (all contents obtained) the trend towards increased effectiveness at larger body sizes was suggestive ( $n=19$ ,  $r=0.3084$ ,  $0.05 < P < 0.10$ ).

For *Meroles cuneirostris* (SVL range: 34.5-58.0 mm) significant positive relationships were apparent between body size and three categories of stomach-flushing results: (1) category 1, all contents obtained ( $n=28$ ,  $r=0.5407$ ,  $P < 0.005$ ), (2) category 2, all or most contents obtained ( $n=28$ ,  $r=0.6182$ ,  $P < 0.001$ ), and (3) assessment accuracy ( $n=28$ ,  $r=0.6228$ ,  $P < 0.001$ ). In the first case 29.24% of the variation in stomach-flushing results could be explained by differences in body size; in the latter two cases 38.22% of the variation in results was thus explained. Thus for *M. cuneirostris* one obtains more of the stomach contents and can more reliably assess them with lizards of large size.

The sample of *Rhotropus afer* comprised a narrow range of body sizes (43.3-53.5 mm SVL). Nonetheless,

TABLE 1: Probabilities ( $p \pm SD$ ) of stomach-flushing success.<sup>1</sup> Sample sizes for the total number of lizards flushed/autopsied in parentheses.

Species	Category				
	(1)	(2)	(3)	(4)	(5)
	Stomach contents obtained			Observer assessment accuracy	Survival
all	all/most	some			
<i>Aporosaura anchietae</i> (31/28)	.789 ± .10*	.789 ± .10*	.789 ± .10*	.786 ± .08	.936 ± .04
<i>Meroles cuneirostris</i> (33/28)	.929 ± .05	.929 ± .05	1.000	.933 ± .07	1.000
<i>Pachydactylus laevigatus</i> (5/1)	1.000	1.000	1.000	1.000	1.000
<i>Rhotropus afer</i> (37/15)	.867 ± .09	.867 ± .09	.933 ± .07	.800 ± .11	.973 ± .03
<i>Meroles suborbitalis</i> (2/0)	—	—	—	—	1.000
<i>Mabuya capensis</i> (2/0)	—	—	—	—	1.000
<i>Angolosaurus skoogi</i> (10/9)	.667 ± .33*	.667 ± .33*	1.000*	.778 ± .15	1.000
Overall (118/81)	.864 ± .04*	.879 ± .04*	.942 ± .03*	.852 ± .04	.975 ± .01

1 -  $p = B/n$ , where B = number of successful trials, n = sample size; SD =  $[p(1-p)/n - 1] .5$

\* - Calculations are based on smaller sample sizes due the presence of lizards with empty stomachs: *A. anchietae* - 19; *A. skoogi* - 3; overall - 66.

several patterns of potential body size effects were apparent. First, a marginally significant negative relationship was evident between body size and post-flushing survival ( $n=37$ ,  $r=-0.2753$ ,  $P=0.048$ ). Although this suggests greater mortality from stomach-flushing with larger lizards, it must be noted that only 1 of 37 *Rhoptropus* died as a result of the procedure. Thus, this statistical result may be biologically trivial. There was also a suggestive trend between body size and the likelihood of obtaining all or most of the stomach contents from non-empty stomachs (category 1). Here also the relationship was negative but was nonsignificant ( $n=15$ ,  $r=-0.3506$ ,  $0.05 < P < 0.10$ ). In this case body size can explain only about 12.3% of the variation in stomach-flushing success.

Looking at the general relationship between body size and stomach-flushing success across species, only one category of results showed any relation to lizard body size. There was a highly significant positive trend between body size and the likelihood of obtaining at least some of the contents of non-empty stomachs (category 3:  $n=66$ ,  $r=0.2988$ ,  $P < 0.01$ ). However, this explains only 8.93% of the variation in stomach-flush results. In general, body size appears to have little influence on the acquisition of diet samples, although it may be more important for some species than for others.

### 3.2 Weight gain from stomach-flushing

Previous studies have indicated a slight weight gain in lizards subjected to stomach-flushing as a result of water retention in the gut (Legler & Sullivan 1979; Pietruszka 1981). On average all species in the present study showed a positive weight gain as a result of the stomach-flushing procedure, although this amounted to only a small percentage of pre-flushing body weight (Table 2). In *Aporosaura* the average weight gain due to water retention from stomach-flushing (Table 2) was considerably less than the 10.8% average increase

observed by Louw & Holm (1972) under conditions of voluntary water consumption after prolonged dehydration. This suggests that for some Namib species, at least, the amount of water introduced into the digestive tract during flushing may be well within physiological tolerance limits. The degree to which water is retained in a lizard's gut (with a resultant weight gain) appears to depend on the size of the food bolus and the ease with which it is dislodged during flushing. When food was retrieved in a well consolidated bolus it was often obtained easily and with little weight gain by the lizard (and in some cases a weight loss). On the other hand, when the bolus was fragmented, there was always an increase in weight after flushing. Lizards with empty stomachs at the time of flushing tended to have the largest increases in body weight although this was not always the case.

### 3.3 Mortality

In the present study, the vast majority of lizards stomach-flushed recovered from the procedure almost immediately. Only three of the 120 lizards subjected to stomach-flushing died as a direct result of the procedure, corresponding to an overall mortality of 2.5%. Among the species to which the procedure was applied, only those that subjectively appeared to be the most delicate suffered any mortality: *Aporosaura*, 6.4%; *Rhoptropus*, 2.7%. In the cases where death did occur, signs of severe distress similar to those noted for North American lizards (Pietruszka 1981: gasping, loss of righting response) were apparent within several minutes after flushing. In all cases mortality was the result of forcing the water stream past the pylorus and filling the intestine with water, which resulted in an immediate and irreversible inflation of the body and subsequent death. Death from stomach-flushing can also result from rupture of the stomach or esophageal wall, although this is realized principally in species with a gorging type feeding behaviour (Pietruszka 1981).

TABLE 2: Weight gain (g) as a result of stomach-flushing. Values are averages; SD in ( ).

Species	N	weight before	weight after	Percent change
<i>Aporosaura anchietae</i>	27	2.5 (1.0)	2.6 (1.0)	+ 5.03 (4.71)
<i>Meroles cuneirostris</i>	33	4.8 (1.5)	4.9 (1.5)	+ 2.69 (4.47)
<i>Pachydactylus laevigatus</i>	2	15.4 (4.0)	16.3 (4.2)	+ 5.51 (0.06)
<i>Rhoptropus afer</i>	36	3.5 (0.6)	3.7 (0.4)	+ 8.17 (12.76)
<i>Meroles suborbitalis</i>	2	6.5 (1.9)	7.1 (2.5)	+ 6.66 (6.69)
<i>Mabuya capensis</i>	2	3.5 (0.1)	3.6 (0.1)	+ 4.32 (1.96)
<i>Angolosaurus skoogi</i>	10	44.5 (28.2)	47.9 (30.5)	+ 6.17 (5.37)
Overall	112	-	-	+ 5.47 (8.36)



The potential for delayed mortality from stomach-flushing appears minimal for at least one of the species investigated. Subsequent work with individually marked *M. cuneirostris* (now) > 20 have been stomach-flushed), revealed no alteration of normal behaviour patterns over days to weeks, indicating the absence of latent effects from the procedure. Similar long term responses are expected for other Namib Desert species.

#### 4 DISCUSSION

The results of the use of stomach-flushing on Namib Desert lizards generally agree with those conducted with North American species. Stomach-flushing represents an excellent non-destructive way of obtaining stomach samples for dietary analysis in lizard populations. An apparent difference of the present study from earlier work, however, is the greater potential among Namib species for lizard body size to affect stomach-flushing efficiency, with reduced efficiency in very small individuals. While for most species this influence appears to be small, it must, nevertheless, be considered in the analysis and interpretation of dietary data derived from stomach-flushing. To a large degree, however, such body size effects may be overcome by practicing the method on a particular species (Pietruszka 1981). It is, therefore, wise for researchers using the technique to familiarize themselves with it on fairly large lizards before applying it to smaller individuals.

The stomach-flushing procedure used in the present study is similar to that described by Legler & Sullivan (1979). The principal difference is associated with the prosthesis used to hold the mouth open during flushing. Legler & Sullivan's plastic rings, through which the stomach cannula was inserted, appear to be cumbersome and potentially injurious to lizards as they require additional manipulation of the lizard besides the actual flushing. This additional manipulation (forceps removal of food, cannula reinsertions) undoubtedly increases the trauma to lizards and may well have been responsible for the "slight to extreme lethargy" in stomach-flushed individuals noted by these authors. Because of this, Legler & Sullivan's variation of the stomach-flushing technique cannot be highly recommended.

As stomach-flushing is being applied to more and more species of lizards, the generality of its applicability is becoming apparent. The technique can be used in the field by a single worker and large numbers of lizards can be processed in a relatively short time. Based upon the lizard species used in this study, stomach-flushing should be widely applicable to Namib Desert species. Because of the high reliability and low mortality associated with the technique and hence the low impact on natural populations,

stomach-flushing is the preferred method of obtaining dietary information for many species, especially where characterization of population diets is the study goal. The fact that diet samples can be obtained repeatedly from the same individuals through time (Legler & Sullivan 1979; Pietruszka 1981) allows for the investigation of individual feeding preferences and thus adds an important dimension to studies of feeding biology in lizards.

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