

## COMPARISON OF TWO TECHNIQUES FOR ASSESSING POSSUM (*TRICHOSURUS VULPECULA*) DIET FROM STOMACH CONTENTS

**Summary:** Two techniques for assessing possum (*Trichosurus vulpecula* Kerr) diet from stomach contents ("point-sampling" and "layer-separation") are described and compared. Point-sampling involves sieving stomach contents, systematically selecting fragments from the retained material then, identifying and weighing these. Layer-separation involves separation, identification, and weighing of the discrete layers apparent in most possum stomach contents. In 41 of 43 stomachs examined, we were able to separate discrete layers that nearly always comprised a single food item. To compare the two techniques both were applied to these 41 stomachs, with the point-sampling technique applied as two separate treatments using 1.4-mm and 2.0-mm sieves. There were major differences in diet composition estimates between layer-separation and point-sampling but with few differences between the two point-sampling treatments. Relative to layer-separation, point-sampling underestimated the proportions of food groups with small average fragment size and overestimated those with large fragment size. However, both techniques gave similar frequencies of occurrence for 8 of 10 food groups tested, although the apparent importance of foods based on ranking by frequency of occurrence did not accurately match the ranking based on percent composition data. Identification of material was usually easier and more complete with layer-separation than with point-sampling (i.e., there were virtually no unidentifiable stems and fibre after layer-separation). Layer-separation therefore appears likely to provide a simple technique for diet assessment in possums. Although the technique requires formal validation the existence of layers shows that there can have been little mixing (or digestion) of stomach contents, and therefore, that the layer-separation estimates cannot differ greatly from what was eaten. Techniques that involve sieving possum stomach contents appear to have serious limitations, but may be useful as a last resort when layers contain a mixture of foods, or for stomachs in which the layers are not distinguishable.

**Keywords:** brushtail possum; *Trichosurus vulpecula*; diet; sampling techniques; stomach contents.

### Introduction

Since their introduction to New Zealand in 1837, brushtail possums (*Trichosurus vulpecula* Kerr) have severely modified many indigenous forest ecosystems (Cowan, 1990a). Approximately NZ\$10 million are now spent annually controlling possums to ameliorate their adverse impacts on conservation values (J. Parkes, pers. comm.). This management effort is underpinned by ongoing research on possum impacts, of which, the quantitative analysis of possum diet forms a key part. Some early studies of possum diet in New Zealand simply recorded the presence or absence of food items in the stomachs of possums (e.g., Mason, 1958; Gilmore, 1967). However, the resulting frequency-of-occurrence data can misrepresent diet composition because some foods are eaten frequently but only in small amounts whereas others are eaten far less often but in large

quantities. More recent New Zealand studies (Fitzgerald, 1976; Warburton, 1978; Fitzgerald and Wardle, 1979; Leathwick, Hay and Fitzgerald, 1983; and Coleman, Green and Polson, 1985) have used microscopic analyses of the undigested plant cuticles in stomach contents or faecal material to identify the relative proportions of species in the diet.

Cuticle analysis, however, has some major limitations. Not all material eaten by possums has cuticles (e.g. birds eggs and flesh; Brown, Innes and Shorten, 1993), and the relative importance of fruit, flowers, and foliage is difficult to quantify (Fitzgerald, 1976; Coleman *et al.*, 1985; Cowan, 1990b). Preparation of samples for analysis is time-consuming, as material must first be blended, sieved, and chemically macerated (to separate cuticles from leaf cellular material) then washed and mounted on slides (Dunnet, Harvie and Smit, 1973; Fitzgerald, 1976). Differences between species in leaf-area-to-

weight ratios and the ability of their cuticles to survive digestion and preparation mean feeding trials are needed to derive the correction factors required to adjust the raw data (Dunnet *et al.*, 1973; Fitzgerald and Waddington, 1979; Vavra and Holechek, 1980). The inevitable random sampling error in the correction factors reduces statistical precision.

The limitations of faecal cuticle analysis prompted us to explore alternative techniques for analysing possum stomach contents as part of a study of possum forage consumption in broadleaved-conifer forest in the central North Island. Initially we adapted a macroscopic sorting technique widely used to assess deer diet. This involved sieving stomach contents then systematically selecting (point-sampling), identifying, drying, and weighing a subset of the retained fragments (Nugent, 1983). Because possums chew their food more finely than ruminants, we used smaller sieves than the 4.0-mm or 5.6-mm sieves routinely used in deer diet studies. The fragments retained by these smaller sieves were still large enough to be identified under low-power magnification, avoiding the need to separate and identify leaf cuticles. Several other recent New Zealand possum diet studies have also used this technique (e.g., Owen and Norton, 1995; Parkes and Thomson, 1995). Although this technique at first seemed to provide useful indications of diet composition, it became apparent that the differential ability of foods to pass through the sieve was an important bias. Subsequently, we investigated physical separation of unsieved stomach material into its component parts (layer-separation). Because possums in forested habitats are predominantly arboreal, they tend to focus on one food at a time (presumably the tree they happen to be in). Initial inspection of stomach contents revealed that they usually contained bands or layers of material each comprised of single food items. This evidence that the material from different feeding sessions remains discrete and identifiable provides reasonable proof that there can be little mixing of stomach contents. With care and practice the discrete layers within stomachs can usually be separated, identified, and weighed, thus providing an alternative and potentially useful technique for assessing possum diet.

This paper compares data from layer-separation and two point-sampling treatments of the same 41 possum stomachs, to assess the potential of the approaches for analysis of diet composition, and, assuming that layer-separation provides the least biased estimate of stomach content, quantify the biases, if any, of sieving stomach contents through 1-2 mm sieves.

## Methods

### Collection and selection of stomachs

Possum stomachs were obtained as part of a sample of several hundred possums poisoned with cyanide paste in a 4 km<sup>2</sup> area of broadleaved-conifer forest in the headwaters of the Waihaha catchment, Pureora Conservation Park, central North Island, New Zealand (175°44'E, 38°45'S). Sampling was spread across 1 year by taking eight samples of 19-51 stomachs collected about 6 weeks apart. Stomachs were removed intact from each possum and frozen. Later, 43 randomly selected stomachs (five or six per sample) weighing more than 100 g were used to compare the two techniques for diet analysis.

### Description of techniques

*Layer-separation:* Stomachs were thawed and weighed. For all but one of the 43 stomachs, the contents were firm enough to maintain their shape when the stomach wall was removed by cutting along the length of the greater curvature (Fig. 1). Discrete separable layers apparently containing fragments of single food items were recognisable in 41 of these samples, but the layers tended to become less distinct in the region close to the exit to the duodenum. Material in this region was therefore discarded before analysis (see Fig. 1). The layers were then separated using tweezers and a spatula or spoon. Frequent checking was required to confirm identity of some layers as they were being separated because adjacent layers were sometimes very similar in appearance and texture. Colour could also vary

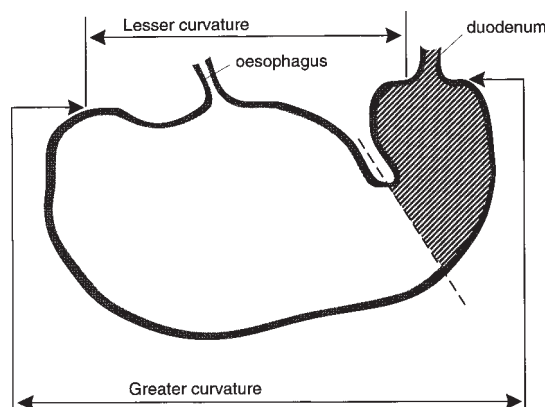


Figure 1: Lateral view of a possum stomach, showing the line of greater curvature along which the stomach wall is cut and peeled back. The shaded portion of the stomach contents is then discarded.

within a layer comprising a single food type. Food items were identified, usually under a low powered (20X) stereoscopic microscope, using characteristics such as colour, texture, shape, leaf margin and venation patterns, hairs, and scales.

Once identification and separation of layers was complete, the wet weight of each separated food item was measured. A small sub-sample (approx. 10%) was then taken from each item, weighed, dried at 70<sup>o</sup> C for 24 h (to attain constant weight), and reweighed. Where layer-separation is used as a stand-alone technique, all of the material from each layer can be dried and weighed (rather than subsampled as in this trial). Total dry weight of each food item was then calculated using the wet-to-dry-weight ratio for the subsamples. Minor food items (< 2 g total wet weight) were not subsampled and dried. Instead, the total dry weights for these items were calculated using the wet-to-dry-weight ratio for all other items in the same stomach. The dry weight of each food item was then expressed as a percentage of the estimated dry weight of all the material analysed from that stomach.

*Point-sampling treatments:* Separated food items (excluding the dried subsamples) for each stomach were then recombined, thoroughly mixed, and divided into halves. The halves were sieved separately using different sized sieves (1.4 and 2.0 mm) by gently floating the stomach contents through the sieve in a sink full of water. Any large clumps of material were carefully broken up by hand. For each sieve, the material retained was placed in a tray containing a grid of 100 sampling points. A thin (5-10 mm) layer of water was added and the material stirred to distribute the retained fragments evenly. In a modification of the point-sampling approach used by Chamrad and Box (1964) for deer diet assessment, the fragment nearest each of the 100 sampling points was then removed for identification. This sampling intensity gives imprecise estimates of the composition of individual samples (e.g., assuming the sample composition estimates are binomially distributed (Stevens, 1977), a component making up 20% of 100 identifications will have 95% confidence limits of approximately 40% (i.e.,  $\pm 8\%$ ) of the estimate (from Table F in Box, Hunter and Hunter, 1978)). It was chosen for the original possum diet study because it is generally more cost effective to analyse many samples imprecisely than to measure a few very precisely (Puglisi, Liscinsky and Hooper 1978). Food fragments were identified using a low-powered (20X) stereoscopic microscope, then dried and weighed, and the dry weights were expressed as a percentage of the total weight for the 100 fragments for each sieve size.

## Data analysis

With the exception of some herbaceous, fungal, invertebrate, and fibrous material, all food items were identified to genus or species. To simplify analysis, individual food items were pooled into 11 main food groups. One group, 'ground-level foliage', comprises a wide range of mainly herbaceous plants including herbs, ferns, and small woody seedlings. This grouping was chosen because when possums feed on the ground they often consume a range of foods in quick succession forming a stomach food layer comprising a mixture of items which cannot be readily separated by the layer-separation technique. The relative importance of each group in the annual diet was then estimated by averaging percent dry weight across the 41 samples for each of the three "treatments" (layer-separation, and point-sampling using the 1.4-mm and 2.0-mm sieves). The resulting mean percentages of annual diet were compared using repeated-measures ANOVA. For food groups showing significant differences between the three treatments, paired comparisons between treatments were also made, again using repeated-measures ANOVA. Frequencies of occurrence (the percentage of stomachs in which a food group was identified) were calculated for each food group, and differences between treatments were tested using Cochran's Q-test.

## Results

### Percentage composition of diet

Overall, the apparent relative importance of the 11 food groups in the diet differed substantially between the two techniques (Fig. 2). Although all three treatments identified totara (*Podocarpus hallii*) as the most important food, the point-sampling treatments overestimated ground-level foliage and large fruits in relation to the percentage estimated by layer-separation, introduced unidentifiable stems and fibre as a low-ranking but common component of diet, and virtually eliminated flowers from the ranking.

For both point-sampling treatments, the grouping made up of unidentifiable stems and fibre (fragments of leaf veins, midribs, petioles, and stems without leaf lamina) comprised 3% of annual diet (Fig. 2) and up to 29% of individual samples. The mean percentage of unidentified stem and fibre did not differ between the point-sampling treatments. With the layer-separation technique, however, any stem or fibre was attributed to the food item comprising the layer concerned, so that no stem or fibre was unidentified.

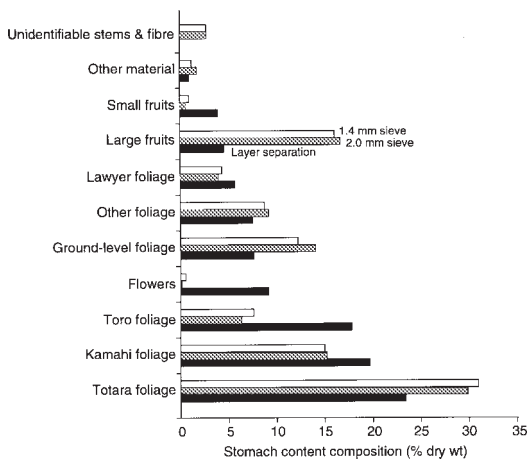


Figure 2: Estimated mean percentage composition of stomach contents from point-sampling following sieving with a 1.4-mm sieve and a 2.0-mm sieve, and from layer-separation.

The estimated mean percentage of annual diet differed significantly between the three treatments for six of the other 10 food groups (Fig. 2; Table 1). For five, subsequent pairwise comparison revealed significant differences between the layer-separation technique and both point-sampling treatments. For kamaha (*Weinmannia racemosa*) foliage the difference was significant only at the  $p < 0.1$  level. In contrast, both point-sampling treatments produced statistically similar results for five of these six food groups (Fig. 2; Table 1).

The differences between point-sampling and layer-separation were most extreme for flowers

(Fig. 2), because most flower fragments in this study (mainly the primordial buds of bush lawyer (*Rubus cissoides*)) passed easily through the sieves. For one stomach, layer-separation assessed flowers as 76.6% of the content, but point-sampling failed to identify this material. Point-sampling therefore underestimated (relative to layer-separation) the use of flowers. Use of fruits that were finely chewed (small-fruit food group) may also be underestimated by point-sampling (Fig. 2) for the same reason, but the difference between estimates was not significant, probably reflecting the small sample size (only four stomachs contained this food). In contrast, point-sampling estimates of consumption of large fruits were greater than for layer-separation (Fig. 2). This food group consisted of fruit that were usually swallowed whole or in large fragments, or fruit whose pulp and seeds often formed sticky aggregations that seldom passed through either sieve. In the most extreme example, *Coprosma tenuifolia* fruit comprised 3.0% of the stomach contents assessed by layer-separation, but 83.5% of the material retained in the 2.0-mm sieve and 91.0% in the 1.4-mm sieve.

Estimated use of foliage also differed between techniques (Fig. 2). The relative importance of ground-level foliage was overestimated and toro (*Myrsine salicina*) foliage was underestimated by point-sampling compared with layer-separation. These differences reflect differing average fragment size of these two food groups. Soft herbaceous foliage (ground-level foliage) was usually coarsely chewed and typically contained many fragments of approximately 1 cm in length, most of which were therefore retained by the sieves. In contrast the more leathery foliage of toro was always finely chewed with few fragments longer than about 2 mm, so most

Table 1: Repeated measures ANOVA results for the three assessment treatments for each food group, and  $p$  values for pairwise comparisons of food groups with significant three-way comparisons. Three-treatment comparison results are not given for "Stems and fibre" as this food group did not occur when using layer-separation.

Food group	Three-treatment comparison		Pairwise comparisons ( $p$ values only)		
	$F$	$p$	Layer vs. 2mm sieve	Layer vs. 1.4mm sieve	Sieve, 2.0mm vs. 1.4mm
Totara foliage	7.4	0.001	<0.001	<0.001	NS
Kamaha foliage	3.1	<0.05	<0.1	<0.1	NS
Toro Foliage	17.1	<0.001	<0.001	<0.001	NS
Flowers	7.0	<0.01	<0.05	<0.05	NS
Ground foliage	10.9	<0.001	<0.01	<0.01	0.042
Other leaves	0.7	NS	-	-	-
Lawyer foliage	1.6	NS	-	-	-
Large fruit	10.6	<0.001	<0.01	<0.01	NS
Small fruit	2.1	NS	-	-	-
Other material	1.1	NS	-	-	-
Stems and fibre	-	-	<0.01	<0.01	NS

passed through the sieves. Similarly the totara foliage eaten consisted mainly of soft, coarsely chewed terminal buds or long fibrous pieces of cuticle from older leaves that tended to be disproportionately retained by the sieves and, therefore, comprised a greater proportion of sieve-based than layer-separation estimates of diet.

### Frequency of occurrence

The mean number of food layers present in stomachs was 4.2 (range: 2-8). Of the food items known to be present in each sample (i.e., detected using at least one of the treatments), the total number missed by any one of the treatments was similar (Table 2). Food items missed by the layer-separation technique were generally small components of the stomach contents, and also tended to be food items whose use was overestimated by point-sampling. The largest single food item missed by layer-separation (invertebrates) comprised <10% of the stomach contents estimated by point-sampling (averaged across both sieve sizes). In contrast, food groups comprising up to 77% of an individual stomach contents as assessed by layer-separation were occasionally missed entirely by one or both the point-sampling treatments.

For most food groups, estimated frequency of occurrence did not differ between the techniques (Table 2). The exceptions were unidentifiable stems and fibre, flowers, and ground-level foliage. For unidentifiable stems and fibre, frequency-of-occurrence values were not statistically compared because this grouping was not distinguished by the layer-separation technique. For flowers and ground-level foliage, the differences in values between point-sampling and layer-separation were less

marked than those identified for the percentage composition. Consequently, overall dietary importance based on ranked frequencies of occurrence did not vary greatly between techniques (ignoring unidentifiable stem and fibre), indicating that frequency-of-occurrence data were somewhat less sensitive to the technique used. However, the apparent relative dietary importance based on frequencies of occurrence differed from that based on percentage composition (Fig. 2; Table 2), most notably by the elevation of ground-level foliage to the top of the rankings for both point-sampling treatments.

## Discussion

Because the two techniques produced different estimates of diet, we need to address the question of which approach is likely to be the more accurate. As we did not test the two techniques against a known diet we cannot be certain whether either technique provided an accurate estimate of actual diet. Our presumption that layer-separation is more accurate than point-sampling therefore depends on our untested inference that the existence of single-food layers means there can be very little mixing of stomach contents. The best evidence that single-food layers exist are the layers containing flowers and fruit because of their markedly contrasting colour and texture compared with foliage. These are easily distinguished, and the complete absence of foliar material was obvious, usually regardless of their position in the stomach. The layer-separation technique was capable of detecting mixtures of food items within single layers, and frequently did so, but usually only within ground-level-foliage layers. The

Table 2: Number and frequency of occurrence (%) in 41 possum stomachs for ten food groups using three assessment treatments. Cochran's  $Q$ -test statistic and  $p$  values are given for the comparison of the three treatments for each food group. An eleventh food group ("Stems and fibre") was only identified with the point-sampling treatments and is not shown. The total number of food groups known to be present but not detected by a particular treatment ("Missed items") is also shown, and expressed as a percentage of the total number of food groups detected.

Food group	Layer separation n (%)	2.0 mm sieve n (%)	1.4 mm sieve n (%)	$C^2$	$p$
Totara foliage	25 (61)	25 (61)	24 (59)	2.0	NS
Toro foliage	23 (56)	22 (54)	24 (59)	3.0	NS
Kamahahi foliage	23 (56)	23 (56)	23 (56)	0.0	NS
Ground foliage	19 (46)	29 (71)	24 (59)	13.6	<0.01
Other leaves	17 (41)	17 (41)	18 (44)	2.0	NS
Large fruit	15 (37)	14 (34)	13 (32)	2.0	NS
Other material	15 (37)	12 (29)	13 (32)	1.4	NS
Lawyer foliage	8 (20)	11 (27)	11 (27)	3.6	NS
Flowers	9 (22)	5 (12)	6 (15)	6.5	<0.05
Small fruit	4 (10)	3 (7)	3 (3)	2.0	NS
Missed items	20 (13)	17 (11)	19 (12)	3.0	NS

lack of mixing of stomach contents that we infer is consistent with possums being hind gut fermenters (Hume, 1982) with relatively little digestion taking place within the stomach.

Differences in estimates of diet composition between the two techniques appeared to be readily explained in terms of average fragment size of food items. Although not measured, it was obvious that some food items were consistently comprised of larger fragments than other items, resulting in a differential ability of food items to pass through the sieves used. Food items comprised of large fragments were overestimated by point-sampling and those comprised of small fragments underestimated compared with layer-separation.

If, as we presume, mixing of stomach contents does not occur, and given that the marked discrepancies between the two techniques can be explained as deficiencies with point-sampling, we consider the layer-separation estimates of percentage composition are likely to provide the least biased measures of actual stomach contents, at least for the portion of the stomach we analysed. Validation of the layer-separation technique could be achieved by comparing estimates of diet composition obtained from this technique with a known diet fed to captive possum.

Compared to layer-separation point-sampling produced more biased estimates of stomach content composition for almost half of the food groups present in this study. One important food group (flowers) was almost completely missed, while the importance of another (large fruits) was greatly overestimated. The presence of unidentifiable stems and fibre as a dietary component in point-sampling estimates will have added further bias, although this would, on average, have been small (c. 3%). Theoretically, the estimates for identified groups could have been adjusted upward accordingly by this factor, except that the proportion of unidentifiable fragments usually differs between species (Havstad and Donart, 1978; Barker, 1986; Norbury, 1988).

The differential ability of foods to pass through the sieves used in this trial indicate that they were too large to determine the composition of possum stomach contents accurately. Theoretically the problems associated with our point-sampling technique could be overcome by reducing fragments to a uniform size, and using a smaller sieve size. However, the smallest fragments retained by the 1.4-mm sieve were close to the minimum practical size that could be identified without using high-powered microscopy and time-consuming cuticle analysis. Furthermore, Barker (1986) found that even after grinding to homogenise fragment size, species

differed in the proportions retained during sieving. Therefore, point-sampling appears of limited value as a stand-alone technique for accurately quantifying possum diet, except perhaps where most of the important foods are of similar fragment size and texture.

Several other studies have shown that biased estimates of herbivore diet can be obtained by using inappropriate sieve mesh sizes. Fibrous plant material was grossly overestimated in caribou diets when using sieves larger than 1 mm (Beregrud and Russell, 1964; Scotter, 1966). Significant differences in the proportions of various grass parts retained in 1 and 2 mm sieves were reported by Owaga (1978) for several antelope species. For deer rumen content assessment there is little difference in the composition of material retained in sieves up to c. 6 mm, but larger sieves produce markedly biased results (Harlow and Hooper, 1971; Eastman, 1974; McCaffery, Tranetki and Piechura, 1974; Nugent, 1983).

The layer-separation technique has some major advantages over other diet assessment techniques used for possums. There is no need to adjust estimates for differences in cuticle area-to-weight ratios for different foods, or for differences in the ability of foods to survive the preparation process. Neither is there a need to correct for differential digestion (as is required with faecal cuticle analysis and other microhistological techniques; Dunnet *et al.*, 1973; Fitzgerald and Waddington, 1979; Vavra and Holechek, 1980). The technique can be used to quantify consumption of flowers and fruit, and produces data directly comparable with that for foliage. Previous researchers have had to present data for flowers or fruit in a non-comparable form (Coleman *et al.*, 1985; Cowan, 1990b). Identification of items is typically more straight forward than with any other technique (stomach or faecal) because individual fragments do not have to be identified in isolation from other fragments of the same food item. As a result little material is unidentifiable.

The layer-separation technique is not without flaws. Its most important limitation is that not all stomachs can be analysed. Although only 5% of the stomachs used in this study did not contain discrete food layers, it is likely that a far greater proportion of nearly empty stomachs (those < 100 g wet weight) cannot be analysed. This makes the use of fast-acting poisons (e.g., cyanide paste) or kill traps preferable to live trapping as a method of collecting possums, because stomachs of possums killed at the time of capture are likely to contain more material than those from possum killed several hours after capture.

Another problem with layer-separation is that not all material within each stomach can be separated into individual foods. Firstly, layers tend to become mixed as they near the exit to the duodenum, so that this material had to be discarded for this study. It is uncertain whether this introduced or increased any biases. Mixtures of food items are sometimes found within single layers, particularly when possums feed on the ground. While the ability to separate ground- and canopy-level feeding is a potentially useful advantage for the technique, diet studies will usually require the composition of such mixed layers to be somehow quantified. Possible solutions are to simply estimate by eye the relative proportion of species within the mixture, or, more objectively, to sieve the mixture and quantify its composition. If the ground-feeding layers are small and the different species within the ground-feeding mixtures are of similar texture and size, as in this study, any biases are unlikely to be great.

Some difficulty can be experienced in ensuring that layers are accurately separated particularly as the number of layers within the stomach increase. Stomachs in this study had a mean of 4.2 layers, and most could be sorted by an experienced observer in 0.5-2 hours. Where stomach contents are more varied considerably more time may be required.

In using stomach contents to estimate diet, we assume that possums consume the various foods in a random order. If they do not, then our sampling will bias estimates of diet composition, because most possums are caught well before the night ends. However, we know of no evidence indicating systematic feeding sequencing in possums. A similar potential bias arises from possums being caught at ground level, so the last foods eaten tend to be ground foods. Consequently, these may be overestimated in any analysis based on stomach contents of ground-killed possums.

Despite the problems discussed, layer-separation appears to be a potentially useful technique for assessing possum diet, although its inferred ability to produce estimates of diet composition that are less biased than those from point-sampling has yet to be formally validated. It provides comparable data on the relative importance of flowers, fruit, and foliage, and, potentially, on the relative importance of ground level and canopy plants as food sources. In contrast, sieving of stomach contents with a >1.4-mm sieve is likely to produce more biased estimates, particularly for some fruits and flowers, unless all ingested foods are of similar fragment size and texture. However, the point-sampling technique described here could be used to overcome the main deficiency in the layer-separation technique, the inability to separate the mixtures that occur in some layers.

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