Analysing Faecal Samples of Ragworm Predators: Not Just a Matter of Counting Mandibles

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Short notes

Analysing faecal samples of Ragworm predators: not just a matter of counting mandibles

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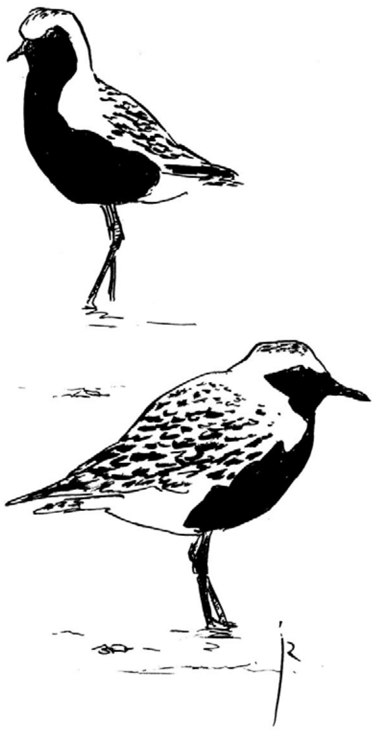

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Ragworms *Hediste diversicolor* are an important prey item in the diet of many estuary birds. The standard method for estimating the number of Ragworms in faeces or pellets is simply dividing the counted number of Ragworm mandibles by two, which can easily lead to an underestimation of the real number of Ragworms present. Here, I present two alternative methods, which avoids bias in estimates of the number of Ragworms in samples. I compared the three methods by applying them to faecal samples of Grey Plover *Pluvialis squatarola* and Redshank *Tringa totanus* from the Tagus estuary, Portugal. The alternative methods yielded significantly higher estimates of the number of Ragworms present in each sample, a difference that can have repercussions on the outcome of dietary studies of Ragworm predators. The pros and cons of each method are discussed with regard to the use of faeces and pellets for ecological research, and the situation in which each method could be applied is discussed.

Key words: diet composition, diet studies, Grey Plover, polychaetes, Redshank, waders

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Introduction

The Ragworm *Hediste diversicolor* is a common estuarine macroinvertebrate, distributed throughout the European Atlantic shorelines, from the Baltic and North Sea to the western Mediterranean (Hayward & Ryland 1995). This polychaete is a common prey for many waders (Goss-Custard *et al.* 1977, Moreira 1994, Moreira 1996, Rippe & Dierschke 1997, Scheiffarth 2001).

Many studies use faeces, pellets and stomach content to investigate the diet of wild animals (e.g. Hartley 1948, Duffy & Jackson 1986, Pierce & Boyle 1991). The prey items ingested can be identified through indigestible structures, like the bones of vertebrates (Kloskowski *et al.* 2000), the exoskeleton of arthropods (Jiguet 2002), the shells of bivalves (Dekinga & Piersma 1993) or the chaetae and mandibles of polychaetes (Zwarts & Wanink 1993).

The mandibles of Ragworms are easily found in faeces and pellets, and can give useful information on the number and size of the ingested individuals (e.g. Zwarts & Wanink 1993, Masero *et al.* 1999, Scheiffarth 2001). Since mandibles are paired structures, the most commonly used method to quantify the minimum number of

Ragworms is simply dividing the total number of mandibles by two (Worrall 1984, Rippe & Dierschke 1997, Lopes *et al.* 1998, Scheiffarth 2001). However, this will cause a systematic underestimation, as mandibles will get lost during digestion, and possibly also during the collection and analysis of faeces and pellets.

Here I examine two alternative methods that may yield better estimates of the minimum number of Ragworms in faeces or pellets. For the first method (hereafter called 'maximum of one side'), mandibles must be divided into right and left mandibles, which can easily be evaluated by the curvature of the mandible and the position of the teeth (mandibles are always curved inwards and only have teeth on the upper margin). The minimum number of Ragworms corresponds with the maximum number of mandibles of one of the sides. The second alternative method (hereafter called 'paired by sizes') is more time consuming, but should lead to better estimates. For this analysis, all mandibles must be measured, after which the mandibles are grouped in pairs with one right and one left mandible of similar size. The minimum number of Ragworms is the sum of mandible pairs and unpaired mandibles.

Methods

In order to evaluate the different methods, a set of bird faeces samples was analysed, and the resulting estimates of the number of Ragworms present in the faeces was compared. Faeces of Grey Plover *Pluvialis squatarola* (n = 67) and Redshank *Tringa totanus* (n = 56) were collected in an intertidal area of the Tagus estuary in Portugal. Since Ragworms are known to be an important prey item for these birds at this location (Moreira 1996), a large number of mandibles were expected in the faeces.

The faeces were scrapped from the sediment and kept frozen until further analysis. Each faeces was sieved through a 63-μm mesh and examined for mandibles under a stereoscope. All mandibles were identified as right or left and measured using an eye-piece graticule (Zwarts & Esselink 1989).

Mandibles were dissected from a reference collection of Ragworms collected in the same estuary (n = 1188) to calculate the natural variation in the sizes between right and left mandibles from the same individual. This natural variation was used to set a threshold to the size difference allowed when pairing mandibles found in a faecal sample.

Since the diets of Redshanks and Grey Plovers in the Tagus estuary are similar (Moreira 1996), data from both species were pooled. All faecal samples without mandibles (44%) were excluded from the analysis as the estimates yielded by the different methods would be the same. The minimum number of Ragworms per faeces was estimated using the three competing methods, and the results were compared using Wilcoxon matched pairs tests. All results are presented as mean ± SE.

Results

In the reference collection, the average difference between the two mandibles from the same individual was 2.0% ± 0.06 (n = 1188; range 0–6.1%), expressed as percentage of the size of the smaller mandible. I decided that a maximum difference of 5% was acceptable when pairing mandibles from the faecal samples, which should be considered a good decision threshold as out the 1188 Ragworms from the reference collection, only 21 individuals showed a difference between mandibles larger that 5%.

As expected, the estimated number of Ragworms per faeces was higher using the 'paired by sizes' approach (5.6 ± 0.84, n = 69), than with the 'total divided by two' (3.8 ± 0.71, n = 69). The 'maximum of one side' technique gave an intermediate estimate (4.6 ± 0.75, n = 69). The estimates obtained by each of the methods were significantly different (Table 1). It was observed that the 'maximum of one side' method gave estimates that were different from our highest estimate in over 60% of the samples, and the 'total divided by two' failed to give the highest estimate in over three quarters of the samples (Table 1). In fact, the 'total divided by two' gave, on average, an estimate of 1.65 Ragworms per faeces less than the best estimate, i.e. an underestimate by about 30%.

Table 1. Comparisons between three methods for estimating the number of Ragworms in faeces ($n = 69$). Given are average differences in the estimated number of worms per faeces in pairwise comparisons (with associated Wilcoxon test), and the percentage of samples in which methods gave different results. PS: paired by sizes; MS: maximum of one side; N/2: total divided by two.

	Average difference (Ragworms per faeces)	Wilcoxon pairs test		% different results
		Z	*P*	
PS vs. N/2	1.65±0.19	7.17	<0.001	75.4
MS vs. N/2	0.85±0.12	6.57	<0.001	53.6
PS vs. MS	0.94±0.14	5.65	<0.001	60.9

Discussion

The most common approach for estimating the minimum number of Ragworms in faeces yielded significantly lower estimates than the two other methods here described. The 'paired by sizes' approach would give the best possible estimate, since it discriminates between mandibles originating from the same individual and unique mandibles that cannot be paired with any other mandible in the sample. This method may result in an underestimate when the two mandibles of an individual prey are lost, and/or pairs are incorrectly matched. The 'paired by sizes' method is considerably more time consuming than the other methods since it involves measuring every mandible in the sample.

Of course, one can question the validity of these approaches. It is possible that some of the mandibles are expelled through regurgitates. In the present study we were unable to find any pellets, so probably most remains were being excreted through faeces, but in areas where birds produce more pellets this can bias the analysis. This is a general methodological flaw of using faecal samples, however, the methods here described can also be applied to pellet samples.

Another important factor that may flaw results obtained by the presented methods is the mixing of preys within the intestinal tract as from the time a prey is ingested until its remains are excreted, additional prey are consumed. Thus, the two mandibles of one individual worm may not be excreted in the same faeces, potentially resulting in an overestimate of the number of worms. However, excretion of mandibles seemed to occur in waves as some of the faeces contained many more (up to 79) than others. Counting mandibles from the same individual in two separate faeces seems therefore less likely but appropriate feeding trials are required to test this idea.

Faced with the decision of which method should be used when analysing faeces or pellets, one must take several factors into consideration. Although very informative about the diet, faeces are a limited source of information. Soft-bodied prey will generally fail to be detected (Santos *et al.* 2005), and different prey types may be processed at different rates by the digestive organs (Rosenberg & Cooper 1990). Also, some prey remains are more frequently expelled through pellets, so the use of faeces or pellets can lead to different results (Sanchez *et al.* 2005). This means that neither faeces nor pellets would provide a good estimation of prey intake rates and may also fail to provide accurate data on diet composition (Pierce & Boyle 1991), unless proper feeding trials are performed on captive birds under controlled diets. However, faeces and pellets can provide useful information and are therefore frequently used in diet studies (e.g. Scheiffarth 2001, Ausden *et al.* 2003, Sanchez *et al.* 2005). Based on the estimated number of individual prey in a sample, information can be gathered on the relative importance of each prey type in the diet (Worrall 1984, Scheiffarth 2001). The sizes of ingested preys can also be derived from the sizes of the prey remains found in faeces, giving information on preferred size classes and on prey biomass (Pierce & Boyle 1991, Rippe & Dierschke 1997). Accordingly, if one resorts to the use of faeces and pellets, the best option is to try and use methods that minimize some of the limitations of these methods, like the two alternative methods presented in this paper.

One can ask how biologically significant a difference of 0.85–1.65 Ragworm per faeces is. In the most extreme case, when comparing the traditional 'total divided by two' method with the 'paired by sizes' method, the more conservative approach might underestimate the number of Ragworms in the faeces by 30%. In temperate estuaries, like the Tagus, the diet of waders like Grey Plover, Redshank, Bar-tailed Godwit *Limosa lapponica*, or Dunlin *Calidris alpina* may be composed of Ragworms for over 85% (Moreira 1996, Lourenço *et al.* 2005). Such an underestimation of the number of Ragworms in the faeces of these birds can therefore seriously bias studies on their foraging ecology.

If one aims to compare the ingested number of Ragworms on a temporal or geographical scale than the most appropriate method seems the 'maximum of one side'. On the basis of our results, this approach gives a better estimate than the 'total divided by two' method without much more effort. Errors are expected to be similar in samples from different seasons or localities, leading to more or less standardised comparisons. Conversely, if a study aims to estimate the relative importance of several prey types in a diet or the numeric importance of each Ragworm size class, then the 'paired by sizes' method should be preferred.

Dietary studies are essential for the understanding of ecological processes within and between species and so will always be an important aspect of ecological research. The methodological problems discussed here, emphasize the need for further experimental studies to improve the way in which indirect methods, like faecal analysis, can provide us with sound information on the diet of wild animals.

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SAMENVATTING

De zeeduizendpoot *Hediste diversicolor* is een belangrijke prooi voor veel wormetende vogels van estuaria en andere gematigde getijdengebieden. Het aardige van zeeduizendpoten is dat hun relatief grote en sterke kaken in braakballen en feces van de vogels kunnen worden teruggevonden. In veel voedselecologische studies wordt het aantal kaken per braakbal of feces geteld om het aandeel van de zeeduizendpoot in het voedsel te schatten en de grootte van de kaken gemeten om de grootte van de gegeten prooien te bepalen. De meest toegepaste methode om het aantal gegeten wormen te bepalen is eenvoudigweg het aantal gevonden kaken door twee te delen. Dit kan echter gemakkelijk tot een onderschatting van het aantal wormen leiden. De auteur stelt twee alternatieve methodes voor die tot nauwkeurigere schattingen kunnen leiden: (1) het onderscheiden van linker- en rechterkaken en dan het grootste aantal van die twee aanhouden als de beste benadering van het aantal geconsumeerde wormen, (2) het 'linken' van de linker- en rechterkaken aan elkaar, waarna het aantal paren en de overgebleven ongepaarde kaken worden opgeteld. In een door de auteur verzamelde referentiecollectie had 95% van de wormen een verschil tussen de linker- en rechterkaak van minder dan 5%. Hij stelt daarom voor 5% grootteverschil als grenswaarde voor het vormen van een paar aan te houden. De drie methoden werden vergeleken aan de hand van feces van de Zilverplevier *Pluvialis squatarola* en van de Tureluur *Tringa totanus* verzameld in het estuarium van de Taag in Portugal. De geteste methoden leidden tot beduidend verschillende ramingen van het aantal zeeduizendpoten per feces, waarbij de methode van het 'linken' van linker- en rechterkaken het hoogste scoorde. Het leverde een 32% hogere schatting op dan het totaal aantal kaken te delen door twee en een 18% hogere uitslag dan het maximale aantal linker- of rechterkaken aan te houden. Het gevonden verschil suggereert dat het de moeite waard kan zijn om voor voedselstudies waarbij een nauwkeurige schatting van het aantal gegeten zeeduizendpoten van belang is, het arbeidsintensieve 'linken' van linker- en rechterkaken toe te passen. Voor studies naar bijvoorbeeld seizoen- of locatieafhankelijke patronen in het aantal geconsumeerde zeeduizendpoten zou met de andere twee methodes kunnen worden volstaan, aangenomen dat de afwijking in schatting niet afhankelijk is van de plaats of het seizoen dat de monsters zijn verzameld. (YIV)

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