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Improved estimates of digestion correction factors and passage rates for harbor seal (*Phoca vitulina*) prey in the northeast Atlantic

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Abstract

Diet composition in pinnipeds is widely estimated using hard prey remains recovered from feces. To estimate the size and number of prey represented in fecal samples accurately, digestion correction factors (DCFs) must be applied to measurements and counts of fish otoliths and cephalopod beaks. In this study, 101 whole prey feeding trials were conducted with six harbor seals (*Phoca vitulina*) and 18 prey species. We derived species- and grade-specific estimates of digestion coefficients (DCs) and species-specific recovery rates (RRs) to account for partial and complete digestion, respectively. Greater than 98% of otoliths were passed within three days of consumption. RRs were smallest for Atlantic salmon smolts (RR = 0.306, SE = 0.031) and increasingly larger for sandeels (RR = 0.494, SE = 0.017), flatfish (RR = 0.789, SE = 0.033), and large gadoids (RR = 0.944, SE = 0.034-1). Species-specific otolith width DCs were smallest for *Trisopterus* species (DC = 1.14, SE = 0.015) and increasingly larger for flatfish (DC = 1.27, SE = 0.045), large gadoids (DC = 1.32, SE = 0.067) and sandeels (DC = 1.57, SE = 0.035). RRs were similar to those from gray seals (*Halichoerus grypus*), but harbor seal species- and grade-specific DCs were generally smaller. Differences in partial and complete digestion rates among prey species and between seal

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species highlight the importance of applying DCFs when reconstructing diet.

Key words: digestion, digestion correction factor, digestion coefficient, recovery rate, passage rate, harbor seal, diet, prey, otolith, beak.

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The recovery of prey hard remains such as fish otoliths and cephalopod beaks from feces is widely used to estimate phocid diet (Hammond *et al.* 1994*a*, *b*; Bowen and Harrison 1996; Thompson *et al.* 1996; Tollit and Thompson 1996; Hall *et al.* 1998; Brown *et al.* 2001; Tollit *et al.* 2010; Bowen and Iverson 2013). Prey structures that are resistant to digestion can be collected from feces, regurgitate, stomachs and intestines. Fecal samples are relatively easy and quick to collect and despite providing little information about the defecating animal, they remain a valuable method for obtaining information on the diet of seal populations. The contents of a scat is typically representative of recent feeding within 12–48 h (Prime and Hammond 1987, Markussen 1993, Orr and Harvey 2001, Grellier and Hammond 2006, Phillips and Harvey 2009) and scat analysis is therefore an appropriate technique for estimating the diet of primarily coastal species such as the harbor seal (*Phoca vitulina*).

Fish otoliths and cephalopod beaks are species-specific in their shape. For pristine specimens, this allows accurate identification to species of these structures and for many species allometric relationships between otolith or beak size and fish or cephalopod size allow the size of ingested prey to be estimated accurately (Clarke 1986, Härkönen 1986, Leopold *et al.* 2001). However, when passing through the gastrointestinal

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tract of a seal, otoliths and beaks may be partially digested and thus reduced in size, and some otoliths or beaks may be completely digested. To reduce bias when reconstructing diet, digestion coefficients, and recovery rates (number correction factors) need to be applied to account for partial and complete digestion respectively (Prime and Hammond 1987; Harvey 1989; Tollit *et al.* 1997, 2004*a*, 2010; Bowen 2000; Grellier and Hammond 2006; Bowen and Iverson 2013). Failure to account for the digestion of hard prey remains using such digestion correction factors (DCFs) can lead to considerably biased estimates of diet composition and prey consumption.

Captive *in vivo* feeding trials have previously been conducted to quantify the extent of partial and complete digestion of otoliths and beaks consumed by harbor seals (Prime 1979, Silva and Neilson 1985, Cottrell *et al.* 1996, Tollit *et al.* 1997, Marcus *et al.* 1998, Phillips and Harvey 2009, Bowen and Iverson 2013). However, DCFs are limited for NE Atlantic prey species and their use when reconstructing harbor seal diet in European waters has been inconsistent; studies have used either harbor seal DCFs for a limited number of prey species (Brown *et al.* 2001, Pierce and Santos 2003), gray seal DCFs (Sharples *et al.* 2009) or no DCFs (Wilson *et al.* 2002).

The primary aims of this study were to describe DCFs for

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prey species commonly consumed by northeast Atlantic harbor seals including specifically passage rates of hard prey remains through the harbor seal gut and to obtain robust estimates of digestion coefficients and recovery rates to account for partial and complete digestion of species-specific prey hard parts. In presenting the results, we describe and recommend the least biased and most precise estimates of these quantities for use in field studies of the diet of harbor seals in this region. We also discuss aspects of the experiments of relevance and interest to their conduct and interpretation, and to future studies.

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Methods

Feeding experiments were conducted with harbor seals during March–April 2009 (one adult female) and August 2011–December 2012 (one juvenile male and four adult males) at the Sea Mammal Research Unit (SMRU), University of St Andrews (Scotland). Seals were captured in the Eden estuary, St Andrews Bay or at Ardesier, Moray Firth and housed for up to 13 mo before being released at the sites where they were caught. At SMRU, the seals were kept in ambient temperature seawater pools and fed a multi-species diet supplemented with vitamins and iron.

For the duration of the feeding experiment, seals were housed individually in an enclosure 6.20 m × 4.85 m, with access to water (a pool 3 m in diameter and 1.5 m deep) and a dry area. Overflow and outflow water passed through a 250 µm filter. The recovery rate of the system was tested using a total of 730 plastic or glass beads (2–3 mm diameter) that were randomly scattered in the pool enclosure and counted on recovery.

In total, 17 fish and one cephalopod prey species were offered to the seals (Table 1). The prey fed included those species most frequently observed in the diet of harbor seals in the UK (Pierce *et al.* 1991, Tollit and Thompson 1996, Brown and Pierce 1998, Brown *et al.* 2001, Pierce and Santos 2003). Prey were obtained commercially or through collaboration with Marine Scotland Science, Aberdeen, the Pittenweem Harbour Fishermen's Mutual Association, or Jack Wright (Fleetwood) Limited. Otoliths and beaks were fed *in situ* in whole or gutted prey (fish obtained commercially had been gutted prior to delivery). Whole prey were fed because accurate prediction of fish length and consequently the estimated proportion by weight of each species in the diet is problematic when using an experimental otolith-carrier species (Grellier and Hammond 2005). Differences in prey availability meant that different combinations of prey were offered to each individual seal.

For a minimum of 5 d prior to the start of an experiment, each seal was fed decapitated fish to clear its digestive system

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of otoliths and beaks. During experiments, seals were offered single-species meals once a day in the late afternoon. Where prey availability allowed, seals were fed the same prey species multiple times. However, multiple meals of the same species were offered only if all otoliths previously fed of that species had been recovered or if 2 d had passed with no otoliths of that species being recovered. Meal size was kept constant for individual seals but varied across individuals depending on their size. The total length of fish and mantle length of cephalopods fed were measured to the nearest 0.1 cm. The size of otoliths and beaks of the prey fed to the seals was estimated using the relationships given in Table S1.

The pool was drained and cleaned prior to the first experimental meal and then daily within 24 h of an experimental meal being fed (average time between feeding and draining was 18:50 h). All debris were collected from the filter during draining and cleaning, and were washed through a nest of sieves of mesh sizes 2 mm, 1 mm, 600 µm, 335 µm, and 250 µm.

All prey remains were sorted and all otoliths and beaks retained. Otoliths and beaks were identified to species and counted. Broken otoliths and beaks were only counted if the widest or longest part of the otolith or the lower rostral length (LRL) of the beak was complete. Otolith length (OL) and

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width (OW) and cephalopod beak LRL were measured to the nearest 0.01 mm using digital calipers (Mitutoyo) under a binocular microscope (Kyowa optical 2D-2PL or Zeiss Stemi 2000-C). The calipers were zeroed between measurements and frequently cleaned.

Uneaten prey remains (whole prey or fish heads) were recovered from the pool daily. Lengths of undamaged fish were measured directly, whereas lengths of damaged fish were estimated from otolith measurements using regression equations (Table S2). Mean uneaten fish length was calculated from whole fish, or whole fish plus fish length estimated from either OL or OW.

For trials in which greater than 10% of prey fed was uneaten we used nonparametric bootstrap resampling to determine whether the size distribution of fish eaten was representative of the size distribution of prey fed. In each bootstrap resample, the mean length of a randomly selected sample of fish fed, equal in size to the observed percentage of uneaten fish, was calculated. 95% confidence intervals of the distribution of 1,000 bootstrapped fish lengths were calculated using the percentile method. If the observed mean length of uneaten fish, as calculated above, was out with the 95% confidence interval, the trial was discarded.

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Recovery Rates

Recovery rate (RR) was calculated as the proportion of otoliths eaten that was recovered at the end of each feeding trial. RR would be 1 if all otoliths eaten were recovered and 0 if no otoliths were recovered. The variance of recovery rate was estimated, assuming that sample proportions were approximately normally distributed, as $p(1 - p)/n$, where p is the recovery rate and n is the number of otoliths that were eaten. To estimate mean RR (with the appropriate measure of precision) for each experimental seal, each prey species, and each prey grouping, RRs were averaged and variances combined following Grellier and Hammond (2006). Results were first combined over all trials for each seal, giving each trial equal weight. Results for each seal were then combined, giving each seal equal weight, to give prey-specific RRs. Finally, results for each prey species were combined into groupings of similar prey (*e.g.*, large gadoids, flatfish, *Trisopterus* species).

Passage Rates

Cumulative daily passage rates were calculated for each prey species in each trial and combined as described above for recovery rates to give mean rates for each seal and each prey species. Prey species with similar taxonomy were grouped for presentation purposes. Cumulative daily passage rates were also

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calculated for groupings of species: large gadoids (Atlantic cod, *Gadus morhua*; haddock, *Melanogrammus aeglefinus*; hake, *Merluccius merluccius*; pollock, *Pollachius pollachius*; whiting, *Merlangius merlangus*), *Trisopterus* spp. (Norway pout, *Trisopterus esmarkii* and poor cod, *Trisopterus minutus*), all flatfish (dab, *Limanda limanda*; lemon sole, *Mirostomus kitt*; long rough dab, *Hippoglossoides platessoides*; plaice, *Pleuronectes platessa*; witch, *Glyptocephalus cynoglossus*), and all sandeels (sandeel, *Ammodytes tobianus* and greater sandeel, *Hyperoplus lanceolatus*).

Species-specific Digestion Coefficients

Digestion coefficients (mean otolith or beak size offered divided by mean otolith or beak size recovered) were calculated for fish OL and OW and squid LRL. The delta method was used to calculate the variance of each digestion coefficient (Seber 1982; Grellier and Hammond 2005, 2006). All trials from which <10 otoliths were recovered were excluded from further analyses, except for large gadoid trials because of the constraints of feeding large fish and maintaining constant meal size. The digestion coefficients from each trial were combined as described above for recovery rates to give mean values for each seal, each prey species, and each prey grouping.

Grade-specific Digestion Coefficients

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All recovered otoliths were examined and the amount by which they had been digested was classified based on external morphological features (Leopold *et al.* 2001; Fig. S1). Pristine otoliths were classified as grade 1, moderately digested otoliths as grade 2, and considerably digested as grade 3. Because of the high number of grade 3 otoliths recovered, and the high level of digestion observed in this and other studies (Tollit *et al.* 1997, Grellier and Hammond 2006), a further classification (grade 4, severely digested) was introduced. External morphological features used to classify a grade 4 otolith were: no visible sulcus or lobation or very worn surfaces (right column of Fig. S1). No attempt was made to classify the amount by which beaks had been digested.

Where ≥10 otoliths by grade were recovered from a trial, grade-specific digestion coefficients and variances were calculated and combined in the same way as for species-specific digestion coefficients. For some species the recovery of specific grades of otoliths was very low and measurements from grade 2 and grade 3 otoliths were pooled.

Grading Comparison Between Multiple Personnel

Six people were involved in grading otoliths across the course of the study. Each person had access to otolith digestion and classification reference materials (*e.g.*, Leopold *et al.*

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2001) and received individual training. Randomized checks were conducted frequently in the first 2 wk of each person's employment. Variability in otolith grading was examined across four of the six personnel at the end of the study. One hundred whiting, sandeel, plaice, and Norway pout otoliths from scats collected in the wild were graded by each person. Differences in grade assignation were determined using a least squares regression analysis with significance at the 5% level.

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RESULTS

A total of 23,313 otoliths and beaks of 18 prey species were eaten by harbor seals during 101 whole prey feeding trials. 61.4% (14,306) of otoliths and beaks were recovered from scats. 98.1% (716/730, SE = 0.51%) of beads were recovered. Loss of beads from the system was observed to be though human error.

Recovery Rates

For most prey species, there was considerable variability in recovery rates both among (interindividual variation) and within seals for prey fed to the same seal multiple times (intraindividual variability) (Fig. 1, Table 1). There was little variability for haddock, whiting, and *Trisopterus* species.

The recovery rate increased with mean undigested otolith size for each prey species within a trial up to OL = ~5 mm and

OW = ~3 mm, then varied around 1 for larger otoliths, with some lower values for the largest otoliths (Fig. 2). Note that the estimated values of RR in some trials were >1; this is discussed below.

Passage Rates

The majority of otoliths passed were recovered by the second day of feeding (*i.e.*, within 40 h; Table 2), regardless of whether final recovery rates were high, medium or low (Table 1).

Species-specific Digestion Coefficients

Digestion coefficients varied among individual prey species (Table 3). Inter- and intra-seal variability in digestion coefficients is shown in Figure 3. Digestion coefficients calculated using OL were different from those calculated using OW. Overall, cross-trial differences were small, but the range was wider for some species than others. The relationship between digestion coefficient and mean OW or mean OL of prey offered was not significant (OL: adj. R^2= −0.00065, inverse-variance weighted regression: intercept = 1.1396; slope = 0.0035; P = 0.333 and OW: adj. R^2= 0.0154, inverse-variance weighted regression: intercept = 1.134; slope = 0.0152; P = 0.125). Note that the estimated digestion coefficient values in some trials were <1; this is discussed below.

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Grade Specific Digestion Coefficients

Of all otoliths recovered, 1.4% were classified as grade 1 (pristine), 5.0% as grade 2 (moderately digested), 27.8% as grade 3 (considerably digested), and 65.9% as grade 4 (severely digested). Because pristine otoliths have, by definition, not been affected by digestion the grade 1-specific digestion coefficient was fixed at 1.00. For Atlantic cod, haddock, and all large gadoids, measurements from grade 2 and 3 otoliths were pooled (Table 4).

As for the species-specific digestion coefficients, grade specific digestion coefficients varied among individual prey species and digestion coefficients calculated using OL were different from those calculated using OW. Standard errors were relatively small for almost all species. There was no overlap of 95% confidence intervals for grade 3 and 4 digestion coefficients for the same species; however, confidence intervals for grades 2 and 3 typically overlapped.

Grading Comparison Between Multiple Personnel

There were significant differences among personnel in the grading of Norway pout, sandeel, and whiting otoliths but not in the grading of plaice otoliths (Table S3A). In this analysis, person 1's estimates (the most experienced team member) are represented in the intercept and is the standard against which

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the others are compared. In three species (Norway pout, sandeel, and whiting) all the coefficient values were positive (Table S3B) and significant ($P \leq 0.05$) for whiting and Norway pout. This indicates that person 1 tended to assign lower grades. The greatest difference in grading was between person 1 and person 2 (the person with the least experience). The least difference was between person 1 and person 3, who was the next most experienced grader.

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Discussion

We quantified the passage, recovery, and digestion of otoliths and beaks of typical prey of northeast Atlantic harbor seals and generated correction factors for estimating prey numbers and size. Such estimates improve accuracy in the assessment of diet composition, prey biomass and total consumption and inform understanding of foraging behavior, fisheries and marine predator overlap (Beverton 1985; Hammond *et al.* 1994*a*, *b*; Phillips and Harvey 2009; Ringrose 1993; Laake *et al.* 2002; Tollit *et al.* 1997, 2004*a*, *b*; Zeppelin *et al.* 2004). These estimated quantities can be used to minimize bias and maximize precision in studies of harbor seal diet in the NE Atlantic. Notwithstanding this, several aspects of our experiments and results require further consideration.

Experimental Anomalies

This study produced a number of experimental anomalies that warrant closer examination.

Recovery rates >1 were calculated for a few individual trials: cod (*n* = 2), whiting (*n* = 2), haddock (*n* = 1), Norway pout (*n* = 1) and poor cod (*n* = 2). Recovery rates >1 should, of course, be impossible. They likely occurred because some recovered otoliths had been in the stomachs of fish that were fed in the trials; so-called secondary prey/ingestion. The majority of the fish fed were not gutted in order to mimic ingestion of prey in the wild. This resulted in mean recovery rates slightly >1 for Norway pout, poor cod, and haddock (Table 1).

It is possible that secondary ingestion may have positively biased estimated recovery rates. Applying positively biased recovery rates in a field study would under-correct for complete digestion. However, the impact on estimated diet composition is difficult to predict because it would depend on the amounts of different prey species in the diet and the relative positive bias in species-specific recovery rates.

For a small number of prey species, some grade- or species-specific digestion coefficients were <1 (Table 3, 4) which should, of course, be impossible. For species-specific digestion coefficients, this occurred only for pollock and red gurnard,

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which are minor prey species in the diet of harbor seals in the NE Atlantic (*e.g.*, Wilson 2014). For grade-specific digestion coefficients, this occurred only for sandeels and *Trisopterus* spp. There are a number of possible explanations for these anomalous results.

First, all otoliths and beaks may not have been correctly graded or measured. Multiple personnel were involved in the grading and measuring of otoliths/beaks in this study and the grading of otoliths is to some extent subjective. Differences were evident in grading categorization among personnel and this was particularly dependent on experience level (see below). Some measurement error could have occurred but there is no reason to think that this could have led to a tendency for digestion coefficients to be biased in this way.

Second, the need to use regression equations to establish the otolith sizes of the fish fed in the experimental trials introduced estimation error. This error should be symmetrical but if, by chance, otoliths that were smaller than predicted by the regression were digested less than average, the resulting estimated digestion coefficient could be >1 simply as a result of estimation error.

Third, smaller otoliths may be eroded and completely digested at greater rates than larger otoliths. Although we

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show, on average, that larger otoliths do not have significantly larger digestion coefficients than smaller otoliths, this may still be a contributing factor to some estimated digestion coefficients being >1. Harvey (1989) suggested that otoliths that are small, thin or encased in a thinner cranium or optic capsule may be more susceptible to complete digestion. If smaller otoliths did have a higher probability of being completely digested in our experimental trials, the back-calculated mean undigested size of otoliths remaining would be larger than the mean size fed which could lead to negative bias in digestion coefficient estimation. There was variation in the size of prey fed in each trial so this may have occurred to some extent in our experiments.

Applying negatively biased digestion coefficients in a field study would undercorrect for partial digestion. However, as is the case for recovery rates, the impact on estimated diet composition is difficult to predict because it would depend on the amounts of different prey species in the diet and the relative negative bias in species-specific digestion coefficients.

Feeding Behavior of Seals

Feeding method has been shown to affect otolith digestion in captive gray seals, *Halichoerus grypus* (Grellier and Hammond

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2005) so otoliths/beaks were fed *in situ* in whole or gutted prey in this study. The seals used in the experiments were generally willing to eat a varied diet; however, some individuals were more selective in their feeding choices than others. The way in which seals consumed prey in the experiments varied depending on prey size. Small prey (<25 cm) were typically ingested underwater while larger prey were brought to the surface and some very large (>65 cm) prey were left untouched by the seals. Some large prey (Atlantic salmon, Atlantic cod and flatfish) were ripped into small pieces before ingestion and seals were observed to struggle without success to consume whole the heads of large prey (Atlantic salmon and cod). Some heads were torn into pieces during consumption and otoliths possibly lost or crushed. In the wild this could also lead to some otoliths not being consumed. The nonconsumption of very large prey and the breaking up of long or wide prey during feeding is likely a morphological limitation linked to mouth-gape size or, as in odontocetes, the size of the pharynx limiting the largest size of prey that can be consumed whole (MacLeod *et al.* 2007).

If the heads of some large prey are not consumed or are broken up in the wild, otoliths will be lost resulting in bias in estimates of diet composition and prey consumption. The "all structure" technique is a prey identification method that can be

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used to partially correct for such bias. By using all structures in the scat prey can be identified that previously would not have been counted and can aid in improving recovery rates (Olesiuk *et al.* 1990, Cottrell *et al.* 1996, Brown and Pierce 1998, Cottrell and Trites 2002, Phillips and Harvey 2009, Gibble and Harvey 2015). However, it is challenging to incorporate this information within a robust quantitative analysis of the size and number of otoliths and beaks recovered from scats.

Differences in wild and captive seals' food intake rate, meal composition, and activity state, and consequent effects on digestion rates are to be expected (Prime and Hammond 1987, Pierce *et al.* 1991, Bowen 2000, Tollit *et al*. 2004*a*, Casper *et al*. 2006, Phillips and Harvey 2009). In this study only single species meals were fed to seals and rates of consumption and activity were not measured. Further work to explore how some of these complexities could be taken into account are desirable.

Passage Rates

In this study the majority of otoliths and beaks were passed within 2–3 d. Harbor seal diet composition estimated using scat analysis is thus likely to be representative of the true diet of this species which has average foraging trip durations ranging of a few days. Studies in the United Kingdom have found average trip lengths of harbor seals to vary from 1 d

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in the Thames estuary, southeast England to 4.5 d in the Moray Firth, northeast Scotland (Sharples *et al.* 2012). Foraging trip duration should be explored in other studies to investigate the likely representativeness of diet inferred from scat analysis.

Recovery Rates

Otoliths greater than 5 mm in length and 3 mm in width were recovered at consistently high rates. However, for smaller sized otoliths there was a marked decline in otolith recovery (Fig. 2). These relationships are driven by species-specific differences in complete digestion of otoliths and, as expected, recovery rates were greater for prey species with relatively large, robust otoliths. This is consistent with other studies, which show that large otoliths are less likely to be completely digested (Tollit *et al.* 1997, 2003; Grellier and Hammond 2005, 2006). There is the potential for considerable bias in estimated diet composition if species-specific differences in recovery rates are not used, in particular, the importance of small fish is likely to be underestimated (Bowen 2000).

Digestion Coefficients

In agreement with other studies (Murie and Lavigne 1986, Tollit *et al.* 1997, Grellier and Hammond 2006), we found that the amount by which an otolith is digested is species-specific. Experimental trials to explore variation in digestion

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coefficients as a function of prey size were not conducted. However, by feeding prey of a size range representative of that which harbor seals have been found to eat in the wild (Sharples et *al.* 2009, Wilson 2014), we have incorporated prey size variability into our estimated digestion coefficients. Furthermore, we found no significant relationship between digestion coefficient and otolith size.

The estimated coefficients of variation of species- and grade-specific digestion coefficients were smaller for OW than for OL in almost all cases (Table 3, 4). Using otolith width to correct the size of otoliths recovered from scats collected in the wild would therefore tend to result in more precise estimates. One notable exception is that Atlantic cod has a markedly smaller CV for OL than for OW (Tables 3, 4) and overall results show that otolith length is the better measurement for cod, as also found for gray seals (Hammond and Grellier 2006).

For herring, estimated fish size was sensitive to the choice of DC. When seals were fed herring during the trials they tended to vomit or to show symptoms of diarrhea; this may be a reason why the results for herring were inconsistent. A comparison of digestion coefficients for harbor seals shows that the species-specific DC generated by Tollit *et al.* (1997) provide the most realistic estimates of fish size and we

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recommend that this value should be used in diet estimation analysis and not the estimates from our experiments.

Grade-specific Digestion Coefficients

External morphological features to grade the degree of digestion of otoliths and the application of grade-specific digestion coefficients have previously been used to improve estimates of prey biomass (Tollit *et al.* 1997, 2004*a*; Grellier and Hammond 2006). The use of grade-specific DCs has two advantages. First, differences in digestion rates are expected between wild and captive seals (Prime and Hammond 1987, Pierce *et al.* 1991, Bowen 2000, Tollit *et al*. 2004*a*, Casper *et al*. 2006, Phillips and Harvey 2009) and application of grade-specific DCs reduces bias introduced as a result of differences in activity, meal size/composition, *etc*. Second, grade-specific DCs improve precision in estimating fish size and thus in estimates of diet composition.

However, there are also potential disadvantages. Grading of otoliths is partially subjective, so some variation among graders is to be expected even with the consistent levels of training, access to the same reference materials and the collaborative work atmosphere implemented in our study. Perhaps unsurprisingly, we found technical experience to be a particular source of variation. In situations where multiple personnel are

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grading and measuring otoliths in a diet project we recommend that the batches of otoliths they process are chosen randomly from experiments and also from scats collected from haul-out sites. This will avoid a single individual working on material from a single region and/or season and diffuse any individual grading/measuring variability and possible bias.

The effects of variation in digestion grading in otoliths have not previously been explored but a limited comparison of the use of species-specific *vs*. grade-specific digestion coefficients revealed only minor differences in estimates of diet composition for NE Atlantic harbor seals (Wilson 2014). Nevertheless, reducing bias and increasing precision are important, especially if results are used to inform management (*e.g.*, Hammond and Wilson 2016; Wilson and Hammond 2016). Using grade-specific DCs does achieve this, even if there is some variability among graders and the effects may be relatively small, and we recommend their use in diet studies based on scat analysis.

Cross-study Comparisons

Recovery rates in this study were comparable to those for gray seals (Grellier and Hammond 2005) but were consistently higher than those reported by Tollit *et al*. (1997) for harbor seals. Our mean species-specific digestion coefficients were similar overall compared to those of Tollit *et al.* (1997). However, our grade-specific digestion coefficients were smaller than those previously reported for both harbor and gray seals (Tollit *et al.* 1997, Grellier and Hammond 2006). Feeding method may account for differences in harbor seal studies. Grellier and Hammond (2005) showed that otoliths were more digested in experiments where seals were presented otoliths or heads inside a "carrier" species, resulting in greater digestion coefficients than those from *in situ* experiments. Despite some prey species-specific differences, the passage rates we report are comparable with those from studies of gray seals (Grellier and Hammond 2006) and Pacific harbor seals (Phillips and Harvey 2009). Differences in physiology and food processing strategies, *e.g.*, delayed digestion (Sparling *et al.* 2007) between these species may account for differences in rates of otolith erosion.

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For Peer Review

Supporting Information

The following supporting information is available for this article online at http://

Table S1. Regressions used to infer the size of otoliths and beaks of the prey items fed to seals.

Table S2. Regressions used to infer the size of otoliths and beaks of the prey items fed to seals.

Table S3. Summary of the linear model results for examining variation in the grading of otoliths across laboratory personnel. Table S3A shows the analysis of variance. Table S3B shows the coefficient estimates and their significance (*).

Figure S1. Images in the left column of pristine (grade 1, upper image), moderately digested (grade 2, lower left image) and considerably digested (grade 3, lower right image) otoliths and in the right column severely digested (grade 4) otoliths. Such images were used as a guide to classify otoliths by the level of digestion. Images of grade 1, 2, and 3 otoliths were taken from Leopold *et al*. (2001). Dab, whiting and, Norway pout have been presented as examples from the main prey groups fed.

For Peer Review

Figure 1. Feeding trial recovery rates for (A) large gadoids, (B) flatfish, and (C) other species showing intra- and inter-seal variability. Each symbol represents a different seal.

Figure 2. Recovery rate plotted against mean undigested otolith length (A) and width (B). Each point represents the recovery rate of a prey species from a single trial.

Figure 3. Inter- and intra-seal variation in digestion coefficients for each trial. Each symbol represents a different seal. Species-specific digestion coefficients by individual feeding trial are displayed for (A) large gadoid otolith length, (B) flatfish otolith length, (C) other species otolith length, (d) large gadoid otolith width, (e) flatfish otolith width, (f) other species otolith width.

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For Peer Review

[4305]-39

Table 1. Details of the experimental prey consumed and recovered. Mean RR is the prey-specific recovery rate; the proportion of otoliths/ beaks eaten that was recovered. RRs and variances were averaged over all trials for each seal and then across seal giving each trial and then each seal equal weight. A value of 1 means that all otoliths/beaks eaten were recovered. Where RR > 1 (haddock, poor cod, Norway pout, all *Triscopterus* spp.) a RR value of 1 should be used in estimates of diet of wild pinnipeds as RR > 1 is an experimental anomaly (see Discussion). NCF is the number correction factor that was calculated as the inverse of the recovery rate (Bowen 2000).

		Length (cm)		No. of otoliths/ beaks		Mean			No. of	
Common name	Scientific name	Min	Max	eaten	recovered	RR	SE	NCF	seals	trials
Dab	*Limanda limanda*	10.2	33	585	415	0.755	0.04	1.379	3	5
Lemon sole	*Microstomus kitt*	15.6	32.1	210	83	0.474	0.06	2.44	2	3
Long rough dab	*Hippoglossoides platessoides*	8.6	23.7	438	386	0.887	0.02	1.133	2	2
Plaice	*Pleuronectes platessa*	13.9	36.4	492	403	0.854	0.04	1.219	6	9
Witch	*Glyptocephalus cynoglossus*	24.6	43.8	68	66	0.976	0.02	1.025	2	2
All flatfish		8.6	43.8	1,793	1,353	0.789	0.03	1.439	6	21
Atlantic cod	*Gadus morhua*	13	60.9	232	211	0.881	0.09	1.204	3	11
Haddock	*Melanogrammus aeglefinus*	11.5	40.6	486	485	1.005	0	0.995	3	9
Hake	*Merluccius merluccius*	45.1	54.1	26	23	0.893	0.06	1.136	1	2
Pollock	*Pollachius pollachius*	43.6	55.2	8	8	1	0	1	1	1
Whiting	*Merlangius merlangus*	11.5	36.7	1,229	1,180	0.94	0.03	1.071	6	14
All large gadoids		11.5	60.9	1,981	1,907	0.944	0.03	1.081	6	37

For Peer Review

[4305]-40

Greater sandeel	*Hyperoplus lanceolatus*	18.3	33.4	544	266	0.6	0.02	2.421	2	2
Sandeel	*Ammodytes tobianus*	7.5	22.1	13,235	5,692	0.389	0.01	3.704	5	10
All sandeels		7.5	33.4	13,779	5,958	0.494	0.02	3.062	5	12
Norway pout	*Trysopterus esmarkii*	9.3	19.9	3,440	3,477	1.026	0	0.98	6	8
Poor cod	*Trysopterus minutus*	7.8	23.7	1,171	1,186	1.008	0	0.993	5	7
Trisopterus spp.		7.8	23.7	4,611	4,663	1.017	0	0.986	6	15
Herring	*Clupea harengus*	18.8	29.8	377	140	0.428	0.07	2.697	4	8
Red gurnard	*Chelidonichthys cuculus*	21.6	35.2	82	47	0.58	0.08	1.741	1	2
Salmon smolt	*Salmo salar*	13.8	18.9	448	137	0.306	0.03	3.31	2	2
Squid	*Loligo forbesii*	60	272	117	98	0.837	0.102	1.233	3	3

For Peer Review

[4305]-41

Table 2. Percentage of the total number of otoliths and beaks recovered, calculated per day. The approximate number of hours after feeding is 16 h for day 1 then +24 h for each subsequent day.

	Day													
Prey	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Flatfish	67.6	87	98.6	99.2	99.5	99.5	99.8	99.8	99.9	100	100	100	100	100
Large gadoids	73.2	96.2	99.7	100	100	100	100	100	100	100	100	100	100	100
Sandeels	46.2	91.7	99.6	99.7	99.8	99.9	99.9	100	100	100	100	100	100	100
Trisopterus spp.	47.5	92.2	99.5	100	100	100	100	100	100	100	100	100	100	100
All fish	56.1	92.1	98.8	99.7	99.9	99.9	100	100	100	100	100	100	100	100
Squid	56.7	79.5	81.6	81.6	81.6	82.3	82.3	82.3	99	99	99	99	99	100

For Peer Review

[4305]-42

Table 3: Species-specific digestion coefficients (DC) for harbor seals. Where DC < 1 (OL; Pollock, red gurnard) a DC value of 1 should be used in estimates of diet of wild pinnipeds as DC < 1 is an experimental anomaly (see Discussion).

				Number of		
Prey species	DC	SE	CV	Seals	Trials	Otoliths recovered
Otolith length						
Dab	1.28	0.035	0.028	3	5	383
Lemon sole	1.22	0.112	0.092	2	3	57
Long rough dab	1.18	0.023	0.02	2	2	367
Plaice	1.17	0.048	0.041	6	9	358
Witch	1.09	0.033	0.03	2	2	61
All flatfish	1.19	0.05	0.042	6	21	1226
Atlantic cod	1.24	0.066	0.053	3	11	150
Haddock	1.17	0.038	0.032	3	9	376
Hake	1.93	0.172	0.089	1	2	14
Pollock	0.98	0.028	0.028	1	1	5
Whiting	1.69	0.09	0.053	5	12	537
All large gadoids	1.4	0.079	0.056	6	35	1082
Greater sandeel	1.61	0.048	0.03	2	2	213
Sandeel	1.28	0.02	0.016	5	10	5097
All sandeels	1.45	0.034	0.024	5	12	5310
Norway pout	1.18	0.013	0.011	6	8	3364

For Peer Review

Poor cod	1.17	0.018	0.016	5	7	1138
Trisopterus spp.	1.17	0.016	0.013	6	15	4502
Herring	1.16	0.051	0.044	4	8	87
Red gurnard	0.99	0.034	0.034	1	2	30
Salmon smolt	1.27	0.037	0.029	2	2	112
Squid (lower	1.12	0.053	0.041	3	3	98
Otolith width						
Dab	1.35	0.035	0.026	3	5	414
Lemon sole	1.32	0.081	0.062	2	3	80
Long rough dab	1.22	0.024	0.019	2	2	385
Plaice	1.18	0.041	0.035	6	9	395
All flatfish	1.27	0.045	0.036	6	21	1340
Atlantic cod	1.23	0.063	0.051	3	11	210
Haddock	1.23	0.024	0.02	3	9	485
Hake	1.8	0.144	0.08	1	2	23
Pollock	1.1	0.071	0.065	1	1	8
Whiting	1.25	0.033	0.027	6	14	1180
All large gadoids	1.32	0.067	0.051	6	37	1906
Greater sandeel	1.75	0.049	0.028	2	2	266
Sandeel	1.4	0.022	0.015	5	10	5687
All sandeels	1.57	0.035	0.023	5	12	5953
Norway pout	1.13	0.012	0.011	6	8	3476
Poor cod	1.14	0.018	0.016	5	7	1186
Trisopterus spp.	1.14	0.015	0.013	6	15	4662
Herring	1.3	0.058	0.044	4	8	139

For Peer Review

[4305]-44

Red gurnard	1.04	0.037	0.036	1	2	42
Salmon smolt	1.24	0.033	0.026	2	2	136

For Peer Review

[4305]-45

Table 4. Grade-specific digestion coefficients (DC) for harbor seals. Where DC < 1 (OL and OW Grade 2; sandeel, all sandeel, poor cod, Norway pout, all *Trisopterus* spp. grade 2) a DC value of 1 should be used in estimates of diet of wild pinnipeds as DC < 1 is an experimental anomaly (see Discussion).

Prey species	Grade	DC	SE	CV	Number of seals	Number of trials	No. of otoliths recovered
Otolith length							
Dab	2	1.09	0.052	0.048	1	2	28
	3	1.18	0.033	0.028	3	5	143
	4	1.45	0.075	0.052	3	4	205
Lemon sole	3	1.12	0.076	0.068	1	1	16
	4	1.45	0.137	0.095	2	3	37
Long rough dab	3	1.07	0.019	0.018	2	2	246
	4	1.48	0.047	0.032	2	2	119
Plaice	2	1.03	0.019	0.019	1	1	27
	3	1.02	0.052	0.051	2	3	85
	4	1.32	0.07	0.053	3	3	94
Witch	3	1	0.032	0.032	1	1	13
	4	1.1	0.036	0.032	2	2	46
All flatfish	2	1.06	0.036	0.034	2	3	55
	3	1.08	0.042	0.039	3	12	503
	4	1.36	0.073	0.054	3	14	501
Atlantic cod	2+3	1.15	0.053	0.046	3	7	30

For Peer Review

[4305]-46

	4	1.31	0.046	0.035	3	9	115
Haddock	2+3	1.05	0.033	0.031	3	6	25
	4	1.21	0.023	0.019	3	8	351
Hake	4	1.93	0.134	0.07	1	2	14
Whiting	2	1.07	0.034	0.031	2	2	15
	3	1.12	0.018	0.016	2	3	39
	4	1.39	0.033	0.023	2	6	403
All large gadoids	2+3	1.1	0.043	0.039	3	13	55
	4	1.46	0.059	0.04	3	25	883
Greater sandeel	4	1.68	0.043	0.026	2	2	199
Sandeel	2	0.93	0.02	0.022	2	4	344
	3	1.02	0.032	0.031	4	7	1,275
	4	1.4	0.026	0.018	4	8	2,526
All sandeels	2	0.93	0.02	0.022	2	4	344
	3	1.02	0.032	0.031	4	7	1,275
	4	1.54	0.034	0.022	4	10	2,725
Norway pout	2	0.91	0.018	0.02	2	3	60
	3	1.01	0.018	0.018	3	4	915
	4	1.22	0.011	0.009	3	4	1,609
Poor cod	2	0.99	0.045	0.045	1	1	11
	3	1.11	0.024	0.022	2	3	135
	4	1.23	0.021	0.018	3	4	748
Trisopterus spp.	2	0.95	0.031	0.033	2	4	71
	3	1.06	0.021	0.02	3	7	1,050
	4	1.22	0.016	0.013	3	8	2,357
Red gurnard	3	1.01	0.034	0.034	1	2	23
Salmon smolt	3	1.12	0.022	0.02	2	2	35
	4	1.37	0.05	0.036	2	2	73

For Peer Review

[4305]-47

Otolith width							
Dab	2	1.14	0.045	0.04	1	2	30
	3	1.23	0.031	0.026	3	5	148
	4	1.53	0.06	0.039	3	4	229
Lemon sole	3	1.13	0.07	0.062	1	1	16
	4	1.49	0.116	0.077	2	3	55
Long rough dab	3	1.1	0.02	0.018	2	2	251
	4	1.54	0.047	0.031	2	2	132
Plaice	2	1.03	0.014	0.014	1	1	27
	3	1.08	0.046	0.043	2	3	94
	4	1.29	0.074	0.057	3	3	100
All flatfish	2	1.09	0.03	0.027	2	3	57
	3	1.14	0.042	0.037	3	12	523
	4	1.46	0.074	0.051	3	14	566
Atlantic cod	2+3	1.16	0.059	0.051	3	7	34
	4	1.32	0.068	0.052	3	10	169
Haddock	2+3	1.07	0.035	0.033	3	6	40
	4	1.25	0.023	0.018	3	9	445
Hake	4	1.8	0.144	0.08	1	2	23
Whiting	2	1.02	0.017	0.016	3	4	29
	3	1.03	0.011	0.011	3	4	89
	4	1.22	0.021	0.017	3	8	791
All large gadoids	2+3	1.12	0.047	0.042	3	13	74
	4	1.39	0.061	0.044	3	30	1,431
Greater sandeel	4	1.82	0.047	0.026	2	2	252
Sandeel	2	0.95	0.021	0.022	2	4	359

For Peer Review

[4305]-48

	3	1.07	0.035	0.033	4	7	1,375
	4	1.54	0.028	0.018	4	8	2,914
All sandeels	2	0.95	0.021	0.022	2	4	359
	3	1.11	0.06	0.054	4	9	1,387
	4	1.68	0.038	0.022	4	10	3,166
Norway pout	2	0.9	0.019	0.022	2	3	61
	3	0.98	0.014	0.014	3	4	944
	4	1.16	0.01	0.009	3	4	1,636
Poor cod	2	0.97	0.043	0.045	1	1	11
	3	1.09	0.023	0.021	2	3	141
	4	1.19	0.021	0.018	3	4	773
Trisopterus spp.	2	0.93	0.031	0.034	2	4	72
	3	1.03	0.018	0.018	3	7	1,085
	4	1.18	0.016	0.013	3	8	2,409
Herring	3	1.28	0.038	0.03	1	1	18
Red gurnard	3	1.02	0.029	0.028	1	2	25
	4	1.22	0.064	0.052	1	1	10
Salmon smolt	3	1.1	0.025	0.023	2	2	37
	4	1.34	0.046	0.034	2	2	95

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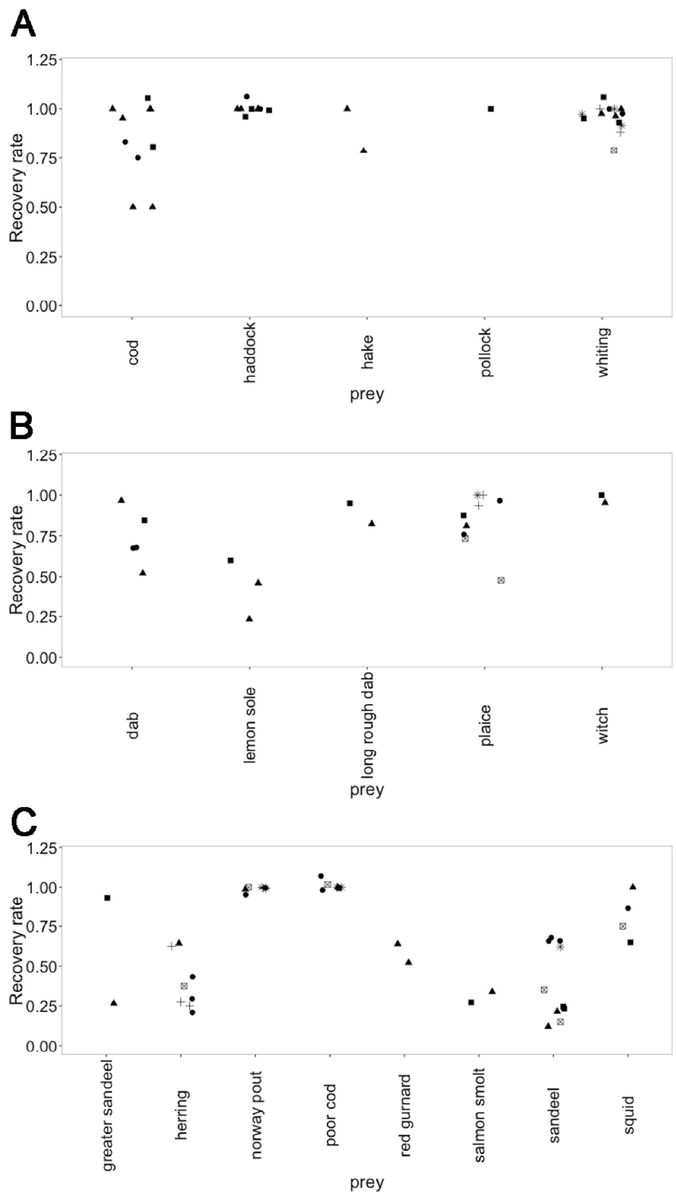

208x370mm (300 x 300 DPI)

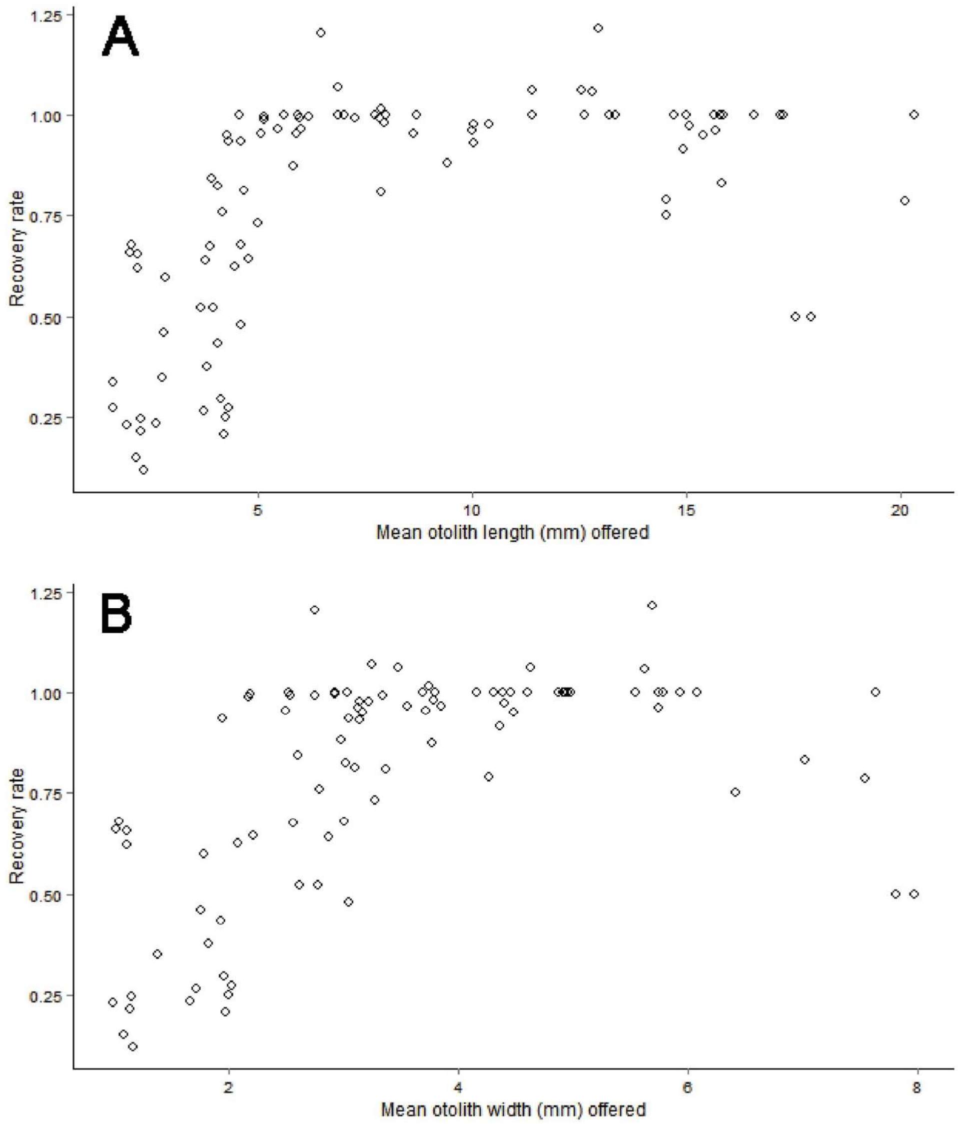

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Table S1. Regressions used to infer the size of otoliths and beaks of the prey items fed to seals.

Species	OL regression	r^2	*n*	OW regression	r^2	*n*	Source
Atlantic cod	OL = 0.266 FL + 2.306	0.93	518	OW = 0.122 FL + 0.811	0.96	547	1
Haddock	OL = 0.383 FL + 1.560	0.97	450	OW = 0.137 FL + 0.703	0.96	469	1
Whiting	OL = 0.564 FL − 0.198	0.98	559	OW = 0.142 FL + 0.55	0.96	637	1
Hake	OL = 0.365 FL + 1.991	0.98	60	OW = 0.131 FL + 1.046	0.96	62	1
Pollack	OL = 0.243 FL + 2.551	0.97	294	OW = 0.097 FL + 1.066	0.96	304	1
Norway pout	OL = 0.436 FL + 0.028	0.98	257	OW = 0.186 FL + 0.002	0.98	257	1
Poor cod	OL = 0.362 FL + 1.718	0.95	267	OW = 0.178 FL + 0.731	0.93	275	1
Sandeel	OL = 0.185 FL − 0.056	0.93	332	OW = 0.085 FL + 0.079	0.91	337	1
Greater sandeel	OL = 0.141 FL + 0.510	0.96	399	OW = 0.057 FL + 0.409	0.95	410	1
Atlantic herring	OL = 0.154 FL + 0.386	0.96	514	OW = 0.061 FL + 0.472	0.93	541	1
European plaice	OL = 0.203 FL + 0.486	0.99	752	OW = 0.119 FL + 0.641	0.97	787	1
Common dab	OL = 0.179 FL + 0.734	0.97	508	OW = 0.107 FL + 0.699	0.95	513	1
Lemon sole	OL = 0.091 FL + 0.624	0.87	240	OW = 0.059 FL + 0.356	0.89	240	1
Long rough dab	OL = 0.213 FL + 0.477	0.95	322	OW = 0.137 FL + 0.730	0.91	338	1
Witch	OL = 0.114 FL + 1.602	0.89	81	—	—	—	2
Atlantic salmon	OL = 0.024 FL + 1.715	0.03	49	OW = 0.013 FL + 1.047	0.01	49	3
Gurnard[a]	OL = 0.111 FL + 0.726	0.94	735	OW = 0.079 FL + 0.697	0.90	741	1
Squid (lower rostral length)	LRL = 0.0099 ML + 0.807	0.85	518	—	—	—	4

Note: Otolith length (OL), otolith width (OW) and lower rostral length (LRL) were measured in mm; fish length (FL) and squid mantle length (ML) were measured in cm. Source data provided by: (1) M. Leopod (Wageningen-IMARES, PO Box 167, Landsdiep 4, NRL-1797 SZ Den Hoorn, Texel, The Netherlands), (2) T. Härkönen (Swedish Museum of Natural History, Box 50007, 104 05 Stockholm, Sweden), (3) C. Sievers and L. J. Wilson (Sea Mammal Research Unit, Scottish Oceans Institute, University of St. Andrews, East Sands, KY16 8LB, U.K.) and (4) M. B. Santos (Instituto Español de Oceanografía, Centro Oceanográfico de Vigo, Spain) and G. J. Pierce (University of Aberdeen, Oceanlab, Newburgh, Aberdeenshire, AB41 6AA, U.K.). Sources 1 and 2 are summarized in Leopold *et al.* (2001) and Härkönen (1986), respectively. Sources 3 and 4 are unpublished data (December 2012).

[a] The gurnard regression was developed across measurements from both red and grey gurnard species.

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Table S2. Regressions used to infer the size of otoliths and beaks of the prey items fed to seals.

Species	OL regression	r^2	*n*	OW regression	r^2	*n*
Atlantic cod	FL = 3.49 OL – 6.64	0.88	268	FL = 7.84 OW – 5.51	0.86	275
Haddock	FL = 2.53 OL – 3.27	0.90	236	FL = 6.99 OW – 4.00	0.90	240
Whiting	FL = 1.73 OL + 0.81	0.79	303	FL = 6.74 OW – 2.97	0.86	315
Poor cod	FL = 2.61 OL – 3.84	0.96	144	FL = 5.22 OW – 2.98	0.94	144
Sandeel	FL = 5.00 OL + 1.16	0.86	170	FL = 10.92 OW	—	172
Dab	FL = 5.43 OL – 3.49	0.88	261	FL = 8.88 OW – 5.40	0.9	261
Plaice	FL = 4.85 OL – 2.07	0.76	405	FL = 8.15 OW – 4.70	0.79	405

Note: Otolith length (OL), otolith width (OW) were measured in mm; fish length (FL) was measured in cm.

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Table S3. Summary of the linear model results for examining variation in the grading of otoliths across laboratory personnel. (A) shows the analysis of variance, (B) shows the coefficient estimates and their significance (*).

(A)

Species	df	Sum sq	Mean sq	*F*	*P*
Norway pout	3	5.01	1.67	5.49	<0.05*
Sandeel	3	2.9	0.97	3.75	<0.05*
Plaice	3	0.49	0.16	1.45	0.22
Whiting	3	3.06	1.02	3.89	<0.05*

(B)

	Estimate	SE	*t*	*P*
Norway Pout				
Intercept	3.49	0.06	63.29	
Person 2	0.31	0.78	3.98	<0.05*
Person 3	0.17	0.78	2.18	<0.05*
Person 4	0.21	0.78	2.69	<0.05*
Sandeel				
Intercept	3.53	0.05	69.52	
Person 2	0.23	0.07	3.2	<0.05*
Person 3	0.16	0.07	2.23	<0.05*
Person 4	0.09	0.07	1.25	0.21
Plaice				
Intercept	3.89	0.03	116.7	
Person 2	– 0.04	0.05	– 0.84	0.40
Person 3	– 0.06	0.05	– 1.27	0.20
Person 4	0.03	0.05	0.64	0.53
Whiting				
Intercept	3.61	0.05	70.48	
Person 2	0.21	0.07	2.9	<0.05*
Person 3	0.18	0.07	2.49	<0.05*
Person 4	0.21	0.07	2.9	<0.05*

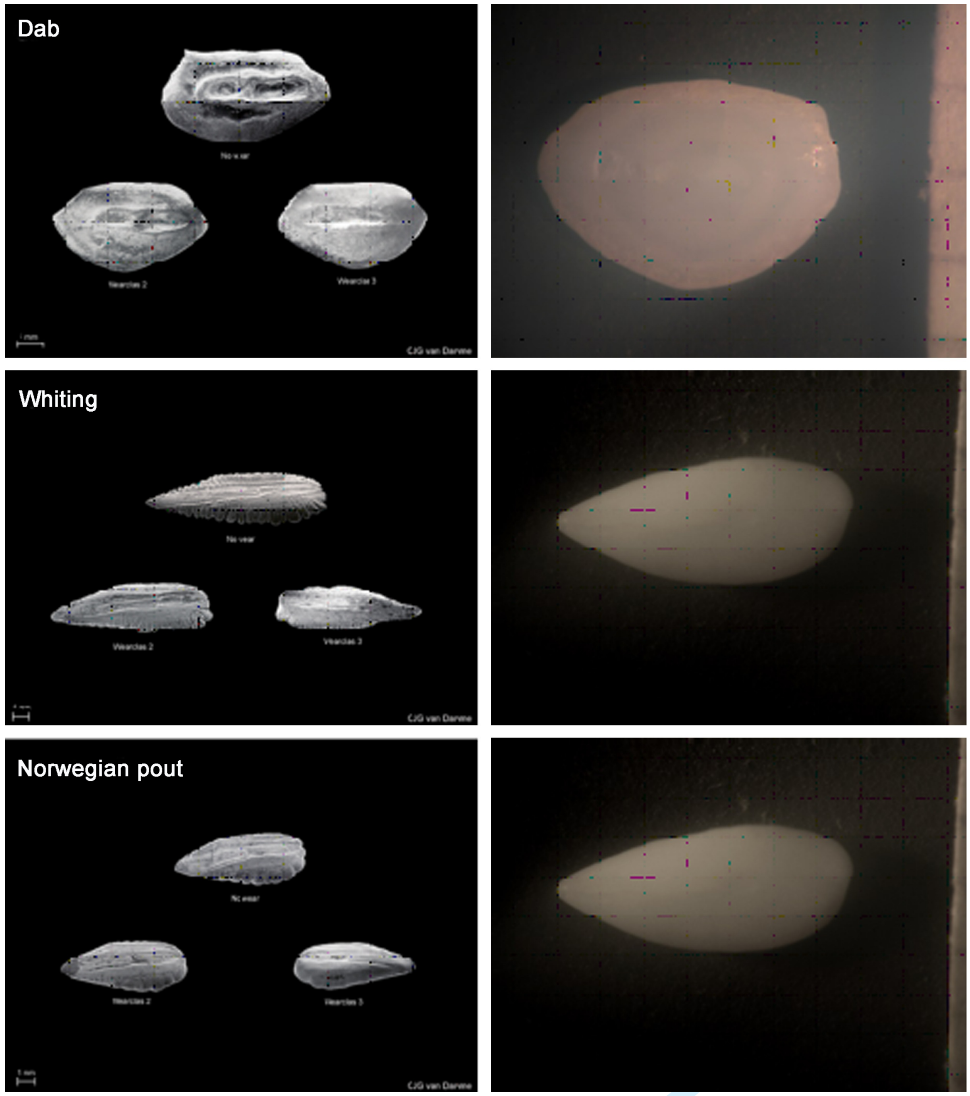

Figure S1. Images in the left column of pristine (grade 1, upper image), moderately digested (grade 2, lower left image) and considerably digested (grade 3, lower right image) otoliths and in the right column severely digested (grade 4) otoliths. Such images were used as a guide to classify otoliths by the level of digestion. Images of grade 1, 2, and 3 otoliths were taken from Leopold *et al.* (2001). Dab, whiting and, Norway pout have been presented as examples from the main prey groups fed.