Assessing sources of variation underlying estimates of cetacean diet composition: a simulation study on analysis of harbour porpoise diet in Scottish (UK) waters

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We use a bootstrap simulation framework to evaluate the relative importance of different sources of random and systematic error when estimating diet or food consumption of cetaceans, using a data set on harbour porpoise diet in Scottish (UK) waters from 1992–2003 (N=180) as a model. We also evaluate the consequences of applying explicit weightings to individual samples and/or sub-sets ('strata') of samples. In terms of the precision of estimates of diet composition, sampling error was the most important source of error, to the extent that overall 95% confidence limits changed only very slightly when sub-sampling error and regression errors were taken into account. On the other hand, for estimates of total food consumption by the porpoise population in Scottish waters, uncertainties about population size and energetic requirements were more important than uncertainty about diet composition. In relation to the accuracy of estimates of diet composition, the study also highlighted the importance of selecting regressions appropriate to prey in the study area (as opposed to ones constructed for the same prey species in another area) and demonstrated that applying equal weighting to individual samples or sample strata can substantially alter the resulting picture of diet. Therefore, the rationale for applying such weightings needs to be carefully considered.

INTRODUCTION

The main prey species of harbour porpoises (*Phocoena phocoena*) in Scottish (UK) waters (based on 1992–2003 data) are whiting (*Merlangius merlangus*) and sandeels (Ammodytidae). Porpoise diet varied seasonally, between areas and, to a lesser extent, in relation to porpoise size, cause of death categories, and year. Some of the seasonal and regional variation in diet could be attributed to variation in prey abundance (Santos et al., 2004). In the present paper we attempt to determine the relative importance of different sources of error underlying calculations of diet composition and population food consumption in marine mammals, using Scottish harbour porpoises as an example.

Food consumption estimates for marine mammal populations are often based on simple multiplication of population size, daily food (or energy) requirements and residence time data (see, e.g. Santos et al., 2001). The total amount consumed is then assigned to different prey categories using diet composition data. Where diet data are available by season and/or sex/age/reproductive status categories, stratification of the food consumption estimates is possible. If the energy density of prey is known, total food requirements may be adjusted to take account of differences in the energy density of prey species.

In July 1994, numbers of harbour porpoises and other small cetaceans in the North Sea and adjacent waters were estimated during the Small Cetacean Abundance in the North Sea (SCANS) survey, the estimated total porpoise numbers being around 340,000 (Hammond et al., 2002). Hammond et al. (2002) also provided separate values for different sectors of the north-east Atlantic, allowing a rough estimate to be derived for 'Scottish waters'. At the time of writing, the SCANS II survey (2005) has been completed but revised estimates of porpoise population size are not yet published.

Daily food requirements of a marine mammal can be derived in several ways, often being expressed as a proportion of body weight or based on simple multiples of predicted mammalian basal metabolic rate (BMR). Lavigne et al. (1986) and Innes et al. (1987) concluded that there was no fundamental difference in energy requirements of marine and terrestrial mammals and Innes et al. (1987) derived the following equation relating food intake to body mass for adult odontocete cetaceans: $IE = 0.313 \times M^{0.66}$, where IE is the biomass ingestion rate (kg.d⁻¹) and M is body mass (kg). Applied to harbour porpoises, with body weights up to approximately 50 kg, this implies daily food consumption of around 8% of body weight—although for large whales the figure drops below 3%. However, Yasui & Gaskin (1986) suggested that daily food requirements of harbour porpoises were around 3.5% of body weight. To make use of this information, we also need to know the population structure, at least in terms of the distribution of body weights. The size-structure of the harbour porpoise population can be inferred from data on stranded animals. Ideally, daily food requirements should be expressed in terms of energy rather

than biomass, since different fish species have different (and differently varying) energy densities. However, in the absence of adequate data on variation in prey energy density, in the present paper we work with biomass.

Confidence limits for estimates of diet composition and prey consumption in marine mammals are generally regarded as difficult to evaluate due to the large number of potential sources of error involved (Santos et al., 2001). Sources of uncertainty and bias (i.e. random and systematic errors) in estimates of diet composition include limited sample sizes, sampling designs that do not adequately control for dietary heterogeneity, biases due to some prey types being less digestible and/or easier to identify than others, contamination of diet samples due to 'secondary ingestion' (e.g. inclusion in calculations of prey remains from the stomachs of fish that were eaten by a marine mammal, i.e. the 'Russian dolls' problem) and the fact that prey biomass is estimated from hard part size–prey body size relationships. In studies based on stranded animals, another problem is that the age composition of the samples will a priori more closely reflect the age-distribution of mortality, not that of the living population. Furthermore, the sample will be biased towards animals living (or at least dying) near the coast, and sick animals (Pierce & Boyle, 1991; Sekiguchi et al., 1992).

In practice some of these errors can be eliminated by good experimental design, some can be evaluated and others remain unquantifiable. An important development over the last 15 years has been the application of computer simulations to estimate the errors that arise in the collection and analysis of samples and to estimate confidence limits, specifically Monte Carlo procedures and the bootstrap (e.g. Reynolds & Aebischer, 1991; Punt et al., 1992; Efron & Tibshirani, 1993; Hammond & Rothery, 1996; Shelton et al., 1997; Warren et al., 1997; Tirasin & Joergensen, 1999). Reynolds & Aebisher (1991) used a bootstrap procedure to estimate confidence limits for fox diet composition. Since then, bootstrapping has been used to estimate confidence limits for diet and/or food consumption of several marine mammal species, including seals (Hammond & Rothery, 1996; Shelton et al., 1997; Boyd, 2002) and sperm whales (Santos et al., 2001). Hammond & Rothery (1996) explored the consequences, for precision and accuracy respectively, of random and systematic errors in the various parameters that are used to estimate seal diet composition based on faecal analysis. They showed that digestion error (i.e. digestive reduction in the size of hard parts that are then measured to estimate original prey size) was the most important source of error. The present paper does not attempt to carry out a similarly exhaustive sensitivity analysis. Rather, as far as possible, we have examined the consequences of known errors associated with an existing data set on porpoise diet. We were unable to quantify digestion error: stomach contents have been subjected to varying degrees of digestive erosion so that digestion coefficients derived for fish otoliths in seal faeces (e.g. Tollit et al., 1997) are not appropriate. A solution to this is to 'grade' prey remains according to the state of digestion and then use 'grade-specific' digestion coefficients derived from *in vivo* or *in vitro* experiments. Otoliths in the present data set had not been graded, nor are all the required digestion coefficients available.

One important source of error, inherent in the reconstruction of prey biomass from measurements on hard remains such as otoliths, arises from natural intraspecific variation in the body weight of animals with hard parts (e.g. otoliths) of a given size. Most publications that provide parameters for regressions relating prey hard part size to prey body length or weight provide no information on the associated errors. 'Regression' error can be simulated in several ways, e.g. by simulating variability in regression coefficients (Hammond & Rothery, 1996) or residual values. In the present paper we simulate variation in residuals.

A related issue is that the estimated 'regression' error may depend on sample size, i.e. how many fish were measured to derive the regression. Furthermore the result will probably depend on whether one or both otoliths of each fish were used to construct the regression: in the latter case there is an element of pseudo-replication (not all otoliths represent independent samples)—although, since diet samples will often contain both otoliths of the same fish, it is arguably also more realistic. Also, estimation of fish weight is often a two-stage process, using regressions of fish length on otolith length, followed by application of regressions of fish weight on fish length. Clearly this may introduce additional errors (see Casteel, 1976).

Relationships between the linear dimensions of prey hard parts (e.g. otolith length) and prey body weight are usually curvilinear (e.g. $W=aL^b$ where W is the body weight, L is the otolith length, and a and b are constants). In fitting a linear regression to log-transformed data on otolith size and body weight, we implicitly assume that the errors around the straight line are normally distributed, i.e. at every otolith size, untransformed body weight follows a log-normal distribution. The mean of a set of log-normally distributed Y values is not the same as the expected Y derived from the straight line fitted to log-transformed values. This bias in expected Y is usually ignored but, as noted by Beauchamp & Olson (1973) and Hammond & Rothery (1996), can be corrected by multiplying each weight estimate by $e^{v/2}$ (or $10^{v/2.\log_e(10)}$ if using base-10 logarithms)—where v is the variance about the relevant regression line.

Another issue that is rarely considered, when deriving overall diet composition, is the relative weighting applied to data from different individual stomachs. Very commonly, estimated prey biomass is summed across all stomachs and the importance of each prey category is then expressed as a percentage. This implicitly weights the contribution of each stomach in proportion to the estimated total biomass of prey present therein. An alternative would be to explicitly assign all stomachs an equal weighting.

In the present paper, we use published data on porpoise diet, energy requirements and population size, coupled with unpublished estimates of average body weight, to answer several related questions:

1. What biomass of fish do harbour porpoises in Scottish waters consume annually?

2. How certain can we be of diet composition and food consumption estimates, and which sources of error have most effect on the precision and accuracy of estimates?

3. Are the estimates robust to variation in sample stratification and the weighting assigned to individual samples?

MATERIALS AND METHODS
The diet data set

Data on porpoise diet were taken from Santos et al. (2004), also incorporating more recent data up to May 2003. The data arise from stomach contents of stranded (and, occasionally, by-caught) porpoises from the Scottish coast, collected since 1992 by the Scottish Agricultural College (SAC) Veterinary Science Division. Excluding any animals whose stomachs contained only prey categories for which biomass could not be estimated (e.g. unidentified fish otoliths) this gave a basic sample set of 180 animals.

Methodology for prey identification and estimation of prey hard part size was described in Santos et al. (2004). For each individual porpoise, the importance of each prey category was expressed as total (reconstructed) prey weight. For the present paper, new estimates were made of prey weight, based on a newly compiled set of regressions (see below). For comparison we also recalculated the overall diet based on the set of regressions used by Santos et al. (2004).

Reconstructing prey weights

Using original data held by the authors or supplied by Edward Brown (University of Aberdeen), Martin Collins (British Antarctic Survey), Tero Härkönen (Swedish Museum of Natural History), John Hislop (FRS Marine Laboratory) and Mario Rasero (Instituto Español de Oceanografía), regressions for the main fish and cephalopod prey of porpoises were recalculated to derive error estimates. A list of the parameters of these regressions is given in Appendix 1. Distributions of residuals for linear regressions fitted to log-transformed data were generally very close to normal and generally had similar standard deviations (in the range 0.065 to 0.150). For some species we had no access to original data and therefore used regressions from Leopold et al. (2001) or Härkönen (1996) for fish and from Clarke (1986) for cephalopods. Leopold et al. (2001) provide regression relationships in the form $FW = (a \times OL)^b$ rather than the more usual $FW = a \times OL^b$ and estimated parameter values using weighted non-linear regression, so their error terms are not directly comparable. In these cases we assumed a standard deviation value of 0.12 for the residuals. We also derived new regressions for groups of species, by combining raw data from several species (see Appendix 1). These combined regressions usually, as would be expected, had slightly higher residual standard deviations than single species regressions.

To ensure a simple and standardized estimation procedure for prey weight, we avoided the use of 2-stage estimation. The usual argument for adopting 2-stage estimation is that the otolith length–fish length relationship is relatively consistent while the length–weight relationship varies seasonally—and monthly length–weight relationships are available for many commercially fished species (e.g. Coull et al., 1989). One problem with the latter regressions though is that most contain an *ad hoc* adjustment to take account of the fact that weight was measured for gutted fish.

Food consumption by harbour porpoise populations

A crude estimate of the annual consumption of main prey (*I*, tonnes) by the harbour porpoise population in Scottish waters was made using the following simple equation:

$$I = N \times Pi \times F \times 365$$

where N is the harbour porpoise population size, based on data in Hammond et al. (2002), Pi is the proportion by weight of prey species i in the diet and F is the average weight of food eaten daily by a harbour porpoise.

We assumed that diet data for Scottish porpoises could be applied to the east coast of Britain, west northern North Sea and waters off Shetland and Orkney (SCANS survey blocks C, D and J respectively), giving a population size of 78,418. The coefficient of variation (CV) for the population estimate differed between areas, the median CV for the three survey blocks in question being 0.25. The approximate standard deviation for the overall population estimate, for use in calculating confidence limits (see below), is thus 0.25×78418=19605.

The weight of food eaten daily by a harbour porpoise was calculated as 3.5% of body weight (Yasui & Gaskin, 1986), using the average body weight for harbour porpoises of known weight stranded in the study area. The standard deviation for this estimate is used in calculation of confidence limits (see below).

Confidence limits for diet and population consumption

Approximate confidence limits for diet composition, based on quantifiable uncertainty in specific parameters, were derived using bootstrap methods. The basic bootstrap procedure was written in Microsoft QBasic®. In addition to purpose-written routines, the simulation incorporates a standard shell-sort routine. In the simplest version of the simulation, when a sample is selected, all the associated prey weights are added to the overall diet. Overall diet is represented as an array, each element of which represents a different prey category. When *n* samples have been taken, weights for each prey category are expressed as percentages of the all-categories total and the results stored. After 1000 runs, the median and 95% confidence limits are calculated separately for each category by sorting the 1000 importance measures for that category, and identifying the median, 26th and 975th value in the sorted sequence (see Santos et al., 2001, for further details). This routine was adapted to incorporate one or more of the following sources of random error:

(a) sampling error, i.e. the uncertainty resulting from sampling only *n* stomachs from the population. We use re-sampling with replacement. One of the stomachs is chosen at random from the *n* available, the data are extracted, and the stomach is replaced in the pool of available stomachs. This is carried out *n* times.

(b) Sub-sampling error, i.e. the uncertainty arising from measuring only a sub-sample of otoliths when very large numbers were present in the stomach. The procedure used is analogous to that described above.

(c) Regression error, which was simulated by drawing residual values at random from a normal distribution with zero mean and known standard deviation (using a previously generated set of 10,000 random values from a standard normal distribution). When regression error was not included, all estimated fish weights were corrected for bias by multiplying each individual weight estimate by $10^{v/2.\log e(10)}$ where
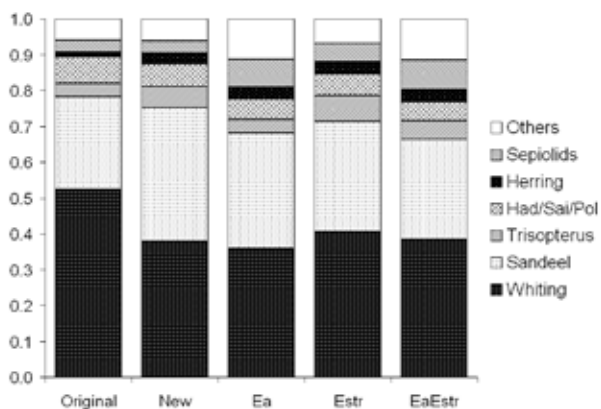
Figure 1. Overall diet composition for harbour porpoises in Scottish (UK) waters. Contributions by different prey categories are expressed in terms of proportion by weight (Had/Sai/Pol, haddock, saithe and pollack). Data treatments were as follows: Original, using the original set of regressions used by Santos et al. (2004); New, using regressions compiled for the present study and listed in Appendix 1; these regressions are also used for treatments 3 to 5; Ea, weighting all samples equally; Estr, stratifying data by season and weighting all seasons equally; EaEstr, stratifying data by season, weighting all samples equally within seasons and then weighting all seasons equally.

v is the variance about the relevant regression line. [When regression error is included, there is no bias.]

To calculate confidence limits for population food consumption we introduced further steps into the original routine, multiplying the proportional importance of each prey category in the diet by daily food requirements and by population size. Instead of storing prey category importance as **%** weight, we stored the estimated total amount eaten (tonnes). Variation in daily food requirements was simulated by assuming that this parameter was normally distributed, using available values for mean and standard deviation:

Simulated daily food consumption = Mean+z×Standard deviation

where z is a standard normal variable, values of which were chosen at random from a standard normal distribution (using the previously generated set of 10,000 random values, see above).
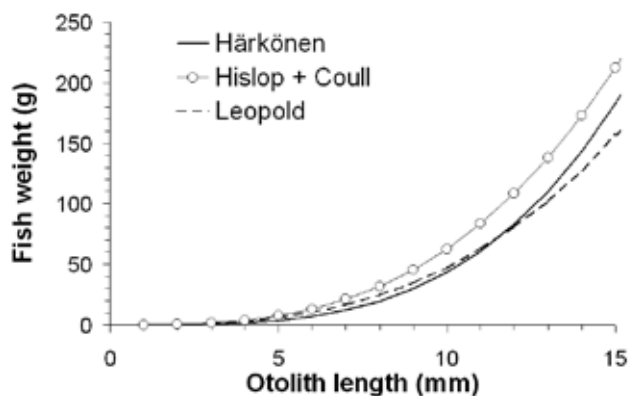

Figure 2. Visual comparison of three regression relationships, for whiting body weight against otolith length. Sources are: Härkönen (1986), Leopold et al. (2001), J.R.G. Hislop (FRS Marine Laboratory, unpublished data) and Coull et al. (1989).
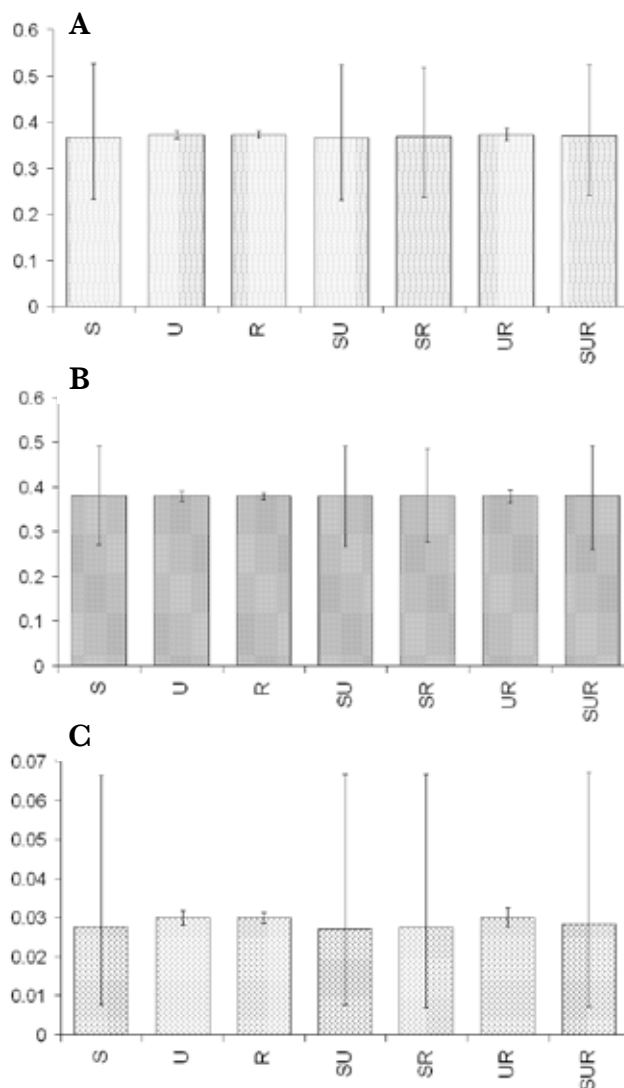
Figure 3. Proportion by weight of three prey categories in porpoise diet: median and 95**%** confidence limits for estimates, taking into account three different sources of error and combinations thereof (S, sampling error; U, sub-sampling error; R, regression error). (A) Sandeels; (B) whiting; (C) herring.

The population size estimates in Hammond et al. (2002) follow a log-normal distribution. To simulate errors in population size we first generated 10,000 random values from a log-normal distribution whose values had the appropriate mean and standard deviation. Population size could then be sampled at random from this set of values.

Stratification and sample weighting — effects on accuracy

In additional simulations we evaluated the consequences of alternative choices for sample stratification and sample weighting. Normally, no explicit 'weighting' is applied to data from different individual stomach samples. Thus, when calculating the overall diet, the contribution of each stomach is proportional to the total estimated reconstructed weight of the prey contained therein. As an alternative, each non-empty sample was assigned an equal weighting by rescaling all prey weights to sum to 1.0 for each stomach.

To explore the consequence of sample stratification, we divided the data into four seasonal (quarterly) groups.
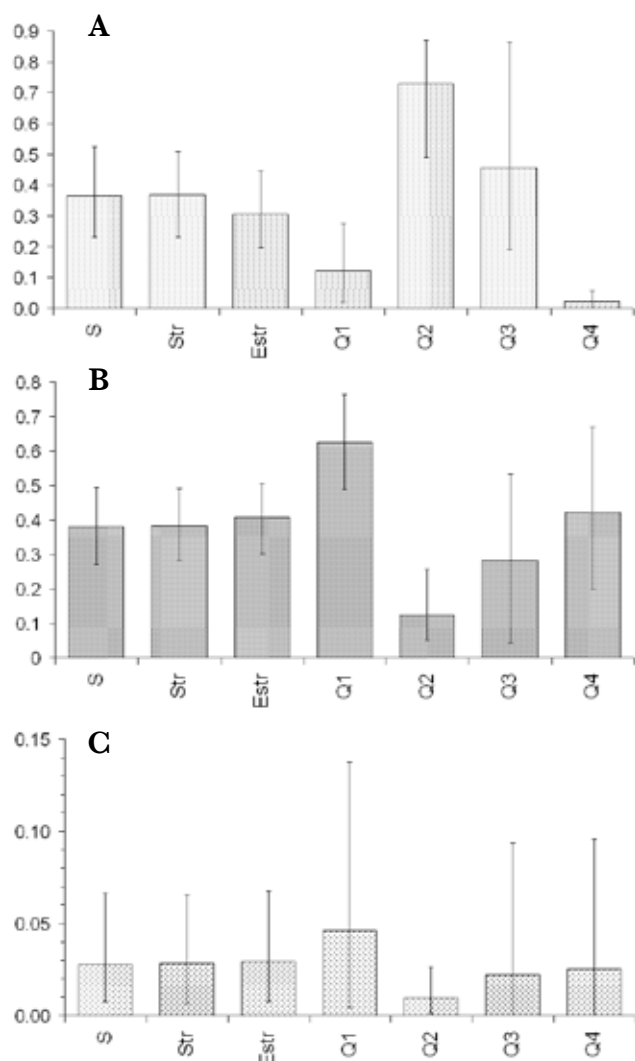
Figure 4. Proportion by weight of three prey categories in porpoise diet: median and 95% confidence limits for estimates based on different data stratification options. In all cases, estimates take into account sampling error (S, sampling error only; Str, with stratification; Estr, with stratification and equally weighted strata; Q1–Q4, separate estimates for each quarter of the year). (A) Sandeels; (B) whiting; (C) herring.
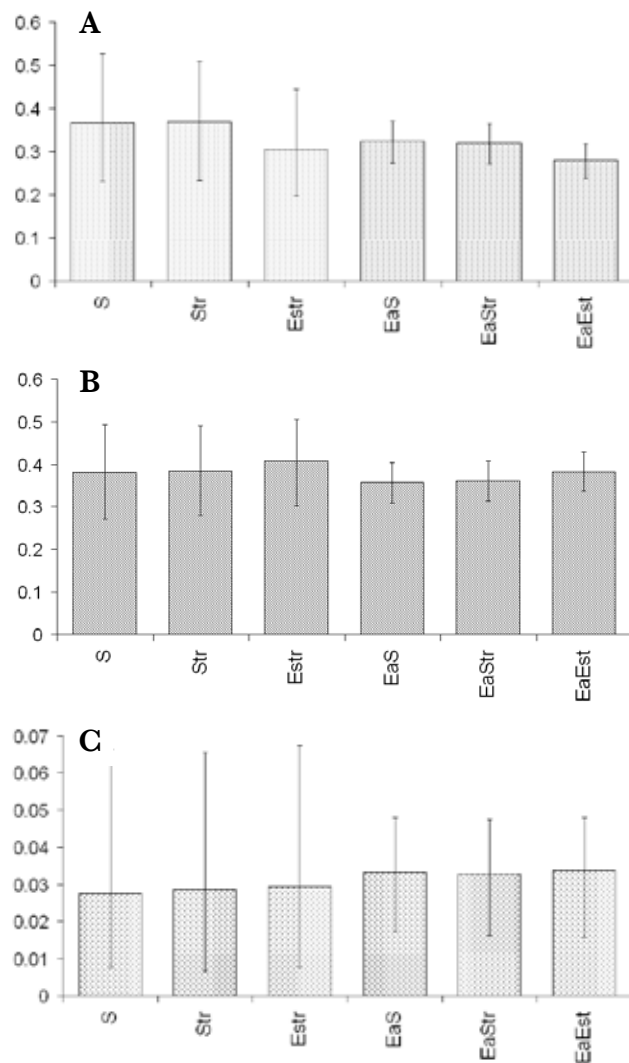

Figure 5. Proportion by weight of three prey categories in porpoise diet: median and 95% confidence limits for estimates based on different sample weighting options. In all cases, estimates take into account sampling error (S, sampling error only; Str, with stratification; Estr, with stratification and equally weighted strata; Ea, equally weighted samples). (A) Sandeels; (B) whiting; (C) herring.

Santos et al. (2004) showed that seasonal variation was one of the strongest patterns evident in the diet data. Confidence limits (for sampling error) were then estimated for the overall diet and for the summed diet from the four quarterly strata. In the latter cases, resampling with replacement was set to occur only within strata, ensuring consistent representation of each stratum in the overall diet. Another hidden bias in the overall diet calculation is that the numbers of stomachs collected in each quarter was not the same. We therefore repeated the simulations with data from each quarter being assigned equal weighting and, finally, again with each sample being assigned equal weighting within seasons and then each season being assigned equal weighting.

RESULTS

Overall diet composition

Application of the newly compiled set of regressions (Appendix 1) resulted in an estimated overall diet that was

similar to that resulting from using the set of regressions from Santos et al. (2004), but in which sandeels increased in importance (from 25.8% to 37.2% of total prey biomass) relative to whiting (which dropped from 52.4% to 37.9% by weight of the diet, see Figure 1). A comparison of the different regression equations available for whiting suggested that use of Härkönen's (1986) or Leopold et al.'s (2001) regressions, for whiting body weight on otolith length, would result in substantially smaller body weight estimates than the combination of regressions for whiting in Scottish waters, from J.R.G. Hislop (unpublished data; fish length from otolith length) and Coull et al. (1989; fish weight from fish length), that was used by Santos et al. (2004) (Figure 2).

Effects on precision of different sources of random error

Of the three main sources of error explored, sampling error is more important than either sub-sampling or regression error, resulting in much wider 95% confidence
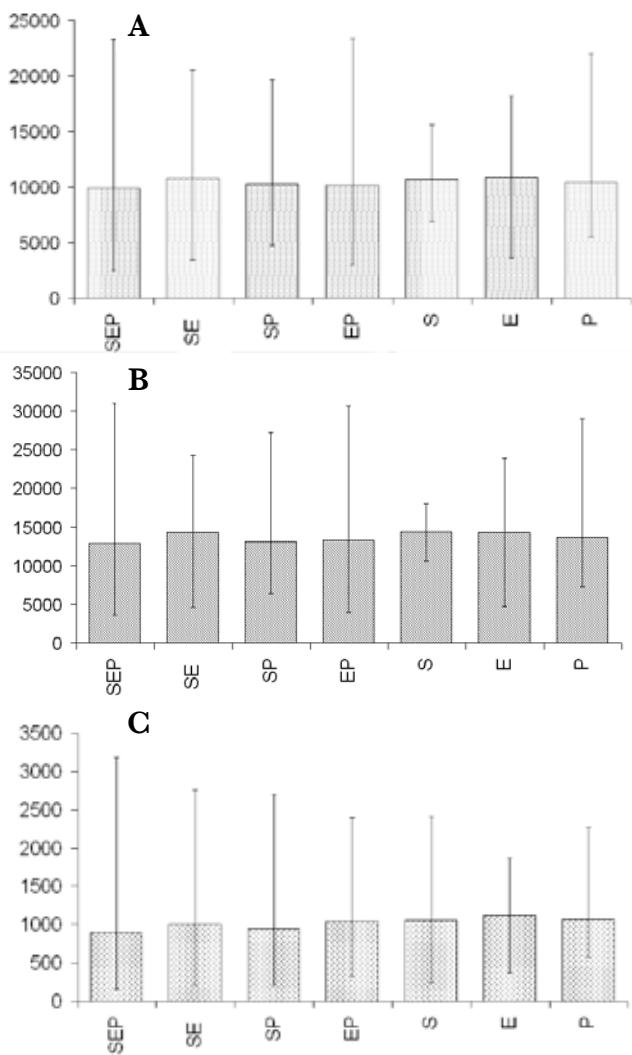
Figure 6. Estimated annual consumption (tonnes) by porpoises in Scottish waters for three prey categories in porpoise diet: median and 95% confidence limits for estimates based on different sources of error: S, sampling error for diet composition; E, uncertainty about daily energy consumption (based on observed variation in body weight); P, uncertainty in population size estimates. (A) Sandeels; (B) whiting; (C) herring.

limits for the importance of whiting, sandeels and herring (Figure 3A–C). For whiting, the 95% confidence limits due to sampling error represent approximately ±30% of the median value of proportion (by weight) in the diet, and for sandeels ±40% of the median value. For herring, which is much less important in the diet, the upper 95% confidence limit is well over twice the median value. These results were repeatable, as seen from comparison of results from repeat runs of the simulation (not illustrated). As expected, the most variable median values and widest confidence limits (relative to median importance) are seen for the least important of the three prey categories, namely herring. It may also be noted that 95% confidence limits obtained by considering only sampling error are not increased by adding sub-sampling and regression error.

Sample stratification and sample weighting

Imposing stratification on the procedure for calculating confidence limits (i.e. ensuring consistent representation of

data from each season) resulted in a very slight reduction of confidence limits for the importance of the three prey categories considered here (sandeels, whiting, herring; see Figure 4). Equal weighting of data from each season had little effect on confidence limits but resulted in median importance (in terms of % weight) of sandeels in the diet falling from 37% to 31%; conversely whiting importance shifted upwards from 38% to 40%. The greater sensitivity of the sandeel data to application of equal weighting of strata is expected given that sandeel importance differed more between the seasons than did the importance of whiting (Figure 4).

Superimposing equal weighting of samples on a version of the simulation which takes account of sampling error, with or without seasonal stratification, leads to a shift in median importance of each prey category and a marked reduction in confidence limits. The two most important prey categories (whiting and sandeel) decreased slightly in importance when samples were weighted equally, whereas herring increased slightly in importance (Figure 5). The effect on estimated overall diet composition is shown in the last three bars of the histogram in Figure 1: the minor prey categories tended to increase in importance when samples were weighted equally.

Food consumption by harbour porpoises

The estimated average weight of food eaten daily per harbour porpoise (calculated as 3.5% of porpoise weight) was 1.23 kg (standard deviation=0.41 kg, N=75). Using this figure, together with an estimated population size of 78,418 (see above) and assuming that the overall diet composition (Figure 1, histogram bar 2) can be applied to all animals on each day of the year, annual totals for amounts eaten were derived and are presented in Figure 6. Taking into account sampling error (for diet calculation) and uncertainty in porpoise body weight and population size, for sandeels and whiting the upper 95% confidence limit of the consumption estimate is more than twice the median, while for herring it is almost four times the median value. As expected, given the assumption of an underlying log-normal distribution, confidence limits related to population size are asymmetric. It is evident that, given the presently available datasets, uncertainty in the average weight of a porpoise and uncertainty about population size are more important than uncertainty about diet composition in terms of their effects on precision of the estimates. Indeed, for the more common prey (sandeels and whiting), once variability in population size and porpoise weight have been taken into account, including sampling error results in little change in confidence limits.

DISCUSSION

Selection of appropriate regression equations

This study has focused mainly on the importance of different sources of random error, yet one of the most striking results relates to potential bias in estimating diet composition. The marked shift in apparent importance of whiting and sandeels when basing calculations on an alternative set of published regressions (as compared to that used by Santos et al., 2004) is not particularly surprising but it highlights the

importance of selecting regressions that relate to fish in the study area. For data on whiting otolith lengths, application of Härkönen's (1986) or Leopold et al.'s (2001) regressions leads to substantially smaller weight estimates than the combination of regressions from J.R.G. Hislop (unpublished data) and Coull et al. (1989). Hislop and Coull et al. both worked on fish in Scottish waters while the other studies were based on fish collected in the southern North Sea. Note that we consider the set of regressions used by Santos et al. (2004) to have been the most appropriate and the results on overall diet presented here are not therefore intended to supersede those in Santos et al. (2004).

Random errors and precision of dietary estimates

Of the different random errors affecting the precision of estimates of diet composition, the effect of sampling error outweighed those of sub-sampling error and regression error to the extent that 95% estimates based on sampling error alone were rather similar to those arising when all three sources of error were taken into account. An obvious caveat is that this result is based on a particular sample size (180 stomachs, collected over 12 years), a particular sub-sampling protocol (measuring not less than 30 otoliths or beaks of a given prey species when more than 30 were present) and, indeed, a particular cetacean species (porpoise), which has a relatively narrow diet. If sample size were very much higher, and associated confidence limits correspondingly narrower, other sources of error, e.g. regression error, would become relatively more important, as shown by Hammond & Rothery (1996). However, when sampling of diet is based on opportunistic use of strandings and/or by-catches it is unlikely that (say) an order of magnitude increase in sample size could be achieved in a reasonable time frame—and directed lethal sampling from small cetaceans is very unlikely to be justified or desirable.

Sample stratification

Clearly it is important to identify 'strata' in the sampled population that differ in their diets, and to ensure that they are all adequately (but not necessarily equally) represented. Thus, if no explicit weighting is applied to individual temporal strata, an ad hoc summary of diet is likely to be 'biased' towards time periods or population segments associated with a high rate of strandings. Whether equal weighting of data from each season is justified depends on whether the porpoise population is present in equal numbers in the study area all year round. Although there are no published data on seasonal migrations of porpoises in the North Sea, such migrations are recorded elsewhere, e.g. the Baltic Sea (Koschinski, 2002).

Some bias is inescapable when relying on opportunistic sampling. The drawbacks of using stranded specimens in dietary analysis have been extensively discussed elsewhere (e.g. Pierce & Boyle, 1991; Sekiguchi et al., 1992). Sample composition depends on many factors. Fishery by-catches will tend to provide samples of 'healthy' individuals (Kuiken et al., 1994), although stomach contents may be biased towards the target species of the fishery and associated species (Waring et al., 1990). Bottlenose dolphins had killed over half of the porpoises from which stomach contents were

obtained in the present study, and these were mainly small porpoises. Ross & Wilson (1996) found that the majority of porpoises killed by bottlenose dolphins were 100 to 140 cm in length, which corresponds to juvenile or pre-pubertal animals between 1 and 3 years of age.

Weighting of data from individual stomach samples

Equal weighting of stomach contents from different individuals clearly substantially altered the overall picture of diet, as well as resulting in narrower confidence limits. Ultimately whether weighting is justified depends on the purpose for which the diet composition estimate is being prepared. Probably the strongest argument against applying any explicit weighting is that meals will naturally vary in size and animals will inevitably have died at different stages of the feeding cycle and therefore have more or less full stomachs. An alternative to equal weighting, which is sometimes used, is to consider only stomach contents containing fresh prey remains, i.e. effectively ignoring stomachs containing only traces of food remains. In harbour porpoises in the present study this would not have been an option: very few stomachs contained any fresh prey remains.

Finally, the issue of sample weighting is simply a variation on the general problem of whether one should use the mean of ratios or the ratio of means (P. Hammond, personal communication). Another example is the estimation of prey profitability in optimal foraging studies: the average ratio of energy value and handling time vs the ratio of summed energy value and summed handling time (see Stephens & Krebs, 1986; Pierce et al., 1993). In the context of calculating the proportion of the diet represented by each prey species, how much these two calculations differ depends on how prey are distributed across stomachs.

Food consumption

Confidence limits on consumption estimates were extremely wide. The most important result, however, is that uncertainty around the diet estimate was lower than that associated with energetic demands and population size (ignoring for the moment that we also do not have a good idea about average food requirements—and acknowledging that the assumption that food requirements are a fixed percentage of body weight is a very crude approximation). In other words, to improve the precision of the consumption estimates, efforts are needed to generate more precise estimates of population size and energy requirements. In fact, our assumptions about variation in food requirements, in particular that porpoise body weight is a random normal variable, were rather unrealistic. A life table could be constructed from strandings data to provide an indication of the proportion of the population in each age- or size-class, and bootstrapping could be used to generate confidence limits for these proportions. Since different prey species differ widely in energy density (e.g. sandeels are around twice as energy dense as whiting: Murray & Burt, 1977), ideally calculations should be in terms of energy content rather than biomass. Furthermore, variation in fish energy density, including seasonal and size-related variation, and seasonal variation in porpoise energy demands need to be considered.

Arguably the least tractable of the three sources of error is probably that associated with population size. Surveying cetaceans is expensive and time-consuming. Projects like the SCANS survey, that use the best available methodology, are unlikely to be practical more than once a decade (SCANS II took place 11 years after SCANS I).

It was not possible to quantify all sources of error for our estimates of diet composition and food consumption. In particular we have not explored the issue of otolith digestion. While several studies have estimated loss and size reduction of otoliths during passage through pinniped digestive tracts (e.g. Tollit et al., 1997), there have been few attempts to apply such methodology to cetacean stomach contents. The scope for experimental studies on cetaceans is limited and *in vitro* digestion studies have been of limited value. In some cases, the option of basing dietary estimates only on fresh prey remains is a viable solution, although few fresh remains were found in porpoise stomachs by Santos et al. (2004). Grading of otoliths from stomach contents according to state of digestion, coupled with *in vitro* calibration, offers a possible way forward.

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Appendix 1. *Regression parameters used for the calculation of regression error, based on equations of the form* $W = a \times H^b$, *where* W = *body weight (g),* H = *hard part size (mm),* a *and* b *are the regression coefficients, SD is the standard deviation of the residuals and N is the sample size. Whenever SD was unknown, a value of 0.12 was assigned. Hard part measurements used were otolith length (OL), otolith width (OW), lower rostral length (LRL), lower hood length (LHL), upper rostral length (URL) and upper hood length (UHL). Data sources were: Edward Brown, unpublished data (B), Clarke (1986) (Cl), Martin Collins, unpublished data (Co), Härkönen (1986) (H), Leopold et al. (2001) (L), Graham Pierce, unpublished data (P) and Mario Rasero, unpublished data (R).*

Prey category	Measurement	log a	b	SD	N	Source	Comment
3/5-bearded rockling	OL	−0.03343	3.81	0.12	166	L	5-bearded rockling
4-bearded rockling	OL	−0.64129	4.14	0.12	201	L	
Alloteuthis	LRL	0.868589	2.75	0.12	116	Cl	
Blue whiting	OL	−1.8353	3.51	0.12	670	L	
Blue whiting	OW	−0.40028	3.91	0.12	71	L	
Cod	OL	−2.1067	4.45257	0.1081	153	B	
Cod	OW	−0.18584	3.98698	0.09023	153	B	
Eledone	LHL	1.4549	1.922	0.1108	33	P	
Gadidae	OL	−1.10761	3.04075	0.16365	491	B+H	Haddock, cod, whiting, 2 *Pollachius* spp., 3 *Trisopterus* spp.; all fish <100 g
Gadidae	OW	0.12777	2.91889	0.15145	464	B+H	Haddock, cod, whiting, 2 *Pollachius* spp., 3 *Trisopterus* spp.; all fish <100 g
Gobiidae	OW	−0.77552	5.3687	0.1355	11	H	Sand goby
Greater sandeel	OL	0.05268	2.20542	0.1261	101	B	Sandeels
Had/Sai/Pol	OL	−1.43089	3.62575	0.21234	669	B+H	Haddock, 2 *Pollachius* spp.
Had/Sai/Pol	OW	0.0127	3.86834	0.2578	599	B	Haddock, saithe
Haddock	OL	−1.7046	3.70958	0.10466	321	B	
Haddock	OW	−0.14034	3.79377	0.1317	326	B	
Herring	OW	1.50133	2.6202	0.08061	186	B	
Herring/sprat	OW	1.01759	4.33306	0.10621	239	B+H	
Ling	OL	−0.7029	3.6208	0.0893	72	B	
Loligo	LRL	1.06819	2.93792	0.14983	332	P	
Mackerel	OL	0.6763	3.1295	0.1223	112	B	
Mackerel	OW	1.89566	4.0466	0.12197	184	B	
Ommastrephidae	LRL	0.21714	3.01183	0.09468	309	Co+R	*Todarodes, Todaropsis*
Ommastrephidae	URL	0.04338	3.0834	0.1126	309	Co+R	*Todarodes, Todaropsis*
Rossia	LHL	0.946762	1.65	0.12	38	Cl	
Sandeel	OL	0.05268	2.20542	0.1261	101	B	
Sandeel	OW	0.71342	2.47447	0.1149	101	B	
Scad	OL	−0.5183	2.98	0.12	236	L	
Sepietta	LHL	0.673156	0.74	0.12	67	Cl	
Sepiola atlantica	LHL	0.173716	0.35	0.12	69	Cl	
Sepiolid	LHL	0.423438	0.545	0.12	136	Cl	*Sepiola, Sepietta*
Sepiolid	UHL	−0.19216	0.35	0.12	(136)	Cl+P	Based on UHL/LHL ratio in *Sepiola atlantica*
Sprat	OW	0.9883	4.6953	0.1039	53	H	
Trisopterus	OL	−1.51543	3.58017	0.10572	257	B+H	*T. esmarkii, T. minutus*
Trisopterus	OW	−0.03785	3.15732	0.10794	225	B	*T. esmarkii, T. minutus*
Whiting	OL	−1.89907	3.5375	0.0908	55	H	
Whiting	OW	−0.11965	3.73317	0.12715	358	B	