The following supplement accompanies the article

Sources of variation in diets of harp and hooded seals estimated from quantitative fatty acid signature analysis (QFASA)

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Supplement. Evaluating QFASA model inputs

DATA SOURCES

The QFASA model uses several sources of data, each of which requires careful consideration by the researcher (Iverson et al. 2004). We evaluated the sensitivity of QFASA diet estimates to the following model inputs: prey species included (prey library), fatty acid (FA) subsets, and calibration coefficients (CC). We did this by repeatedly estimating diets for all seals (harp seals *Pagophilus groenlandicus* n = 526; hooded seals *Cystophora cristata* n = 153) with different combinations of these inputs and evaluating differences among these combinations.

MODEL INPUTS

Prey libraries

Simulations have shown that limiting the number of species in the prey library leads to fewer misclassifications and more accurate assessment of diet (Iverson et al. 2004). Therefore, we identified both a primary and secondary set of prey species to include in the QFASA model. The primary set comprised the most common prey found in stomach contents (e.g. Lawson & Stenson 1995, 1997, Lawson et al. 1995, G. B. Stenson pers. comm.) or which were known to be abundant and found at depths where harp or hooded seals are known to forage. The secondary set contained additional prey rarely found in stomachs, including some of special interest, such as Atlantic cod. In the case of harp seals, these included species such as yellowtail flounder *Limanda ferruginea*, wolfish *Anarhichas lupus* and various skates *Raja* spp. The first step was to evaluate whether the addition of these species altered the interpretation of the diet and should, thus, be retained for the final modeling procedure.

The diets, and therefore FA signatures, of fish vary with ontogeny (Budge et al. 2002). Sample size permitting, we split species into small and large size classes and evaluated differences in FA signatures by

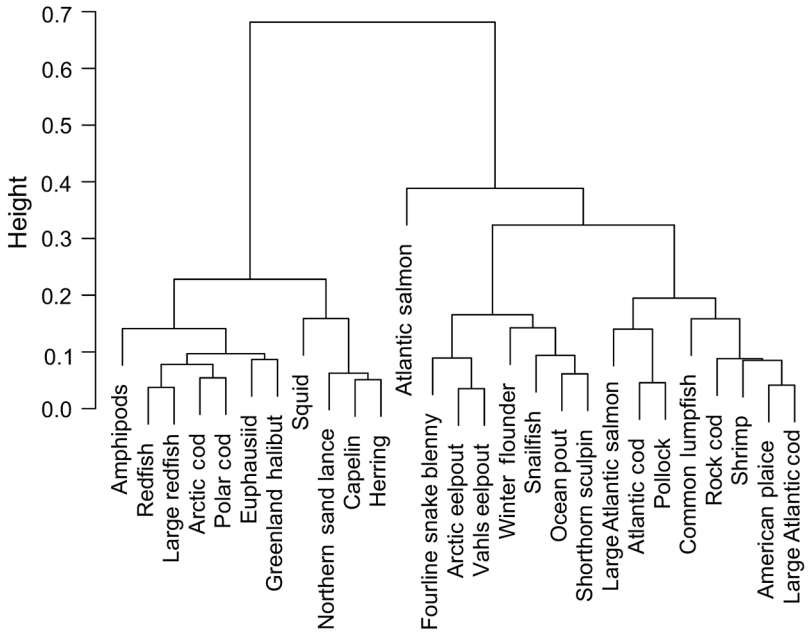

Fig. S1. *Pagophilus groenlandicus.* Hierarchical cluster analysis on the mean fatty acid signatures (primarily dietary subset) of 27 prey categories (n = 2039) for harp seal prey library. The Kulback-Liebler (KL) distance measure was used to determine how similar any 2 taxa were with respect to their fatty acid signatures. The average linkage method was used

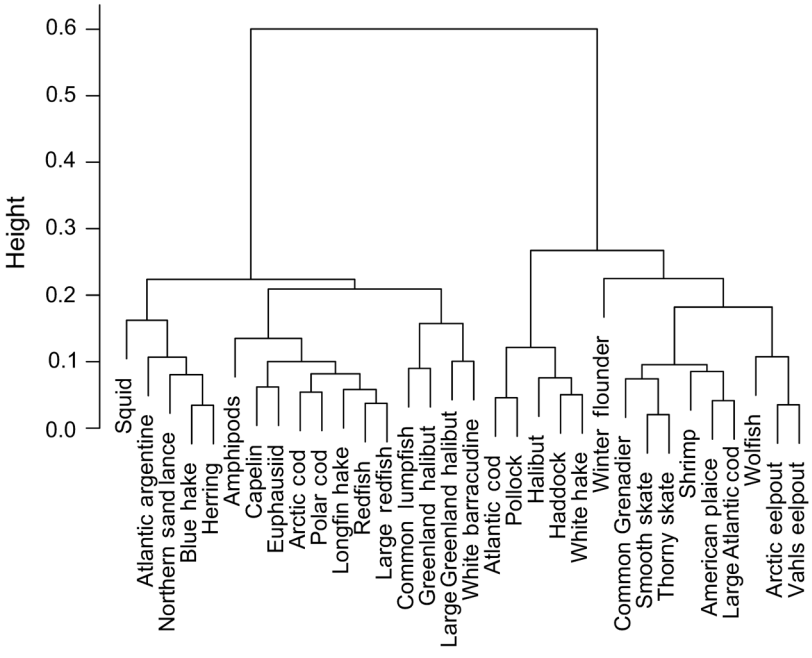

Fig. S2. *Cystophora cristata.* Hierarchical cluster analysis on the mean fatty acid signatures (primarily dietary subset) of 32 prey categories (n = 2289) for hooded seal prey library. The Kulback-Liebler (KL) distance measure was used to determine how similar any 2 taxa were with respect to their fatty acid signatures. The average linkage method was used

MANOVA. Size classes were based on either median length or a length reported in the literature at which significant changes in diet have been noted. Size classes for species were retained in subsequent modeling procedures if there were significant differences in FA simulations and they were differentiated well in cluster analysis (Figs. S1 & S2) and simulations (see 'Diet simulations' below).

FA sets

Next, we evaluated the degree to which diet estimates differed depending on whether we used 'dietary' FAs only (n = 30) or a 'primarily dietary' (n = 39) subset of FAs (Iverson et al. 2004). The primarily dietary fatty acid set includes FAs that arise from a combination of diet and biosynthesis within the predator. For example, although found in prey, levels of 14:1n-5 in predators are derived predominantly from biosynthesis, while some proportion of 22:5n-3 arises from modification of other FA (Ackman et al. 1988, Iverson 1993, Iverson et al. 1995). FAs such as 16:0, 16:1n-7, 18:0 and 18:1n-9 can arise from biosynthesis in the predator, but are also highly indicative of differences among prey species (Iverson 1993, Iverson et al. 2001). The primarily dietary FA subset accounts for 95% of total FAs, while the dietary set accounts for 51%.

Calibration coefficients

We also evaluated the effects of using different calibration coefficients on diet estimates. Calibration coefficients are necessary for the accurate estimation of diet (Iverson et al. 2004) because they account for the effects of predator metabolism on the deposition of FAs (Iverson et al. 2004). Individual FAs are deposited and/or modified in predator lipid stores in a predictable fashion (Cooper 2004, Iverson et al. 2004) such that corrections can be applied

Table S1. Calibration coefficients (CC) for fatty acids (FA) for juvenile grey seals, grey seal pups and juvenile harp seals. Data from Iverson et al. (2004)

FA	Grey CC	Pup CC	Harp CC
14:00	0.86	0.95	0.94
16:00	0.74	0.83	0.63
16:1n-7	1.52	1.30	1.62
16:2n-6	0.76	0.81	0.74
16:2n-4	1.50	0.89	0.95
16:3n-6	0.86	1.00	1.12
17:00	1.40	0.78	0.91
16:3n-4	0.68	0.98	0.87
16:4n-1	0.59	0.97	0.77
18:00	0.84	0.64	0.79
18:1n-9	3.46	1.15	2.79
18:1n-7	1.41	1.04	1.44
18:1n-5	1.04	0.99	1.00
18:2n-6	2.02	1.04	1.57
18:2n-4	0.98	0.94	0.86
18:3n-6	1.08	0.78	0.94
18:3n-4	2.32	1.01	2.59
18:3n-3	2.27	1.07	1.48
18:3n-1	0.95	0.88	0.95
18:4n-3	0.96	0.96	0.99
18:4n-1	1.10	1.01	1.39
20:1n-11	3.42	0.97	2.84
20:1n-9	0.81	0.91	1.00
20:1n-7	0.71	0.82	1.06
20:2n-6	1.65	1.02	1.39
20:3n-6	1.07	0.91	1.00
20:4n-6	0.82	0.92	1.04
20:3n-3	1.16	0.98	0.98
20:4n-3	2.11	1.00	1.50
20:5n-3	0.65	0.82	0.80
22:1n-11	0.20	0.47	0.35
22:1n-9	0.27	0.49	0.59
22:1n-7	0.18	0.90	0.26
21:5n-3	1.37	1.02	1.45
22:4n-6	1.00	1.03	1.00
22:5n-6	1.04	0.96	0.76
22:4n-3	2.58	1.01	1.55
22:5n-3	4.64	1.09	3.91
22:6n-3	1.11	1.00	0.94

to account for this effect of metabolism. Calibration coefficients (CC) have been determined for a number of different phocid seals and seabird species through feeding experiments on captive individuals (e.g. Iverson et al. 2004, 2007).

Although the effect of metabolism on individual FAs is similar across diverse species (i.e. FAs that are consistently higher or lower in predator than prey; Iverson et al. 2004, 2006, 2007), the magnitude of CC for individual FAs can vary, which will affect overall diet estimates. Therefore, we evaluated different CC as well as combinations of these different sets on diet estimates. CC derived from juvenile harp seals (n = 5), juvenile grey seals (n = 8) and grey seal pups (n = 17) (from Iverson et al. 2004; our Table S1) are based on relatively small sample sizes, therefore, we used combinations of these three species values to generate new sets of average CC (see 'Results—Calibration coefficients' below).

Diet simulations

Diet simulations (Iverson et al. 2004) were conducted using each prey library to evaluate how well the QFASA model could differentiate individual prey species or size categories within prey species. Prey species occupying similar dietary niches may have similar FA signatures (i.e. Budge et al. 2002). However, species of fish and invertebrates are typically well-differentiated by their FA signatures (Budge et al. 2002) likely due in part to the fact that despite similarities, diets are rarely identical among species. Diets of seals often span multiple trophic levels where both fish and their prey, or even the prey of those prey, are consumed. For example, harp seals are known to feed on redfish, capelin and amphipods (e.g. Lawson & Stenson 1995, 1997, Lawson et al. 1995). This may pose an analytical problem in the application of a technique which traces the assimilated portions of unmodified fatty acids and attempts to find the closest statistical match between multiple

Table S2. Species composition of specified diets from the harp seal prey library and mean estimated diets of pseudo-seals over the 1000 simulation runs for each of the 4 diets with noise set at 10%

Diet	Species	Specified diet	Estimate	SD
1	Amphipods	0.09	0.08	0.04
	Arctic cod	0.225	0.18	0.06
	Capelin	0.225	0.22	0.096
	Euphausiids	0.09	0.09	0.063
	Herring	0.18	0.15	0.071
	Northern sand lance	0.09	0.11	0.069
2	Amphipods	0.135	0.11	0.041
	Arctic cod	0.27	0.23	0.071
	Atlantic cod	0.09	0.06	0.073
	Capelin	0.225	0.18	0.072
	Northern sand lance	0.045	0.06	0.054
	Rock cod	0.045	0.03	0.027
	Shorthorn sculpin	0.09	0.04	0.037
3	Large Atlantic cod	0.045	0.02	0.01
	Capelin	0.09	0.09	0.071
	Herring	0.495	0.47	0.071
	Northern sand lance	0.045	0.04	0.042
	Large redfish	0.225	0.19	0.058
4	American plaice	0.225	0.19	0.081
	Large Atlantic cod	0.225	0.12	0.078
	Greenland halibut	0.225	0.20	0.052
	Winter flounder	0.225	0.22	0.052

Table S3. Species composition of specified diets from the hooded seal prey library and mean estimated diets of pseudo-seals over the 1000 simulation runs with noise set at 10%

Diet	Species	Specified diet	Estimate	SD
1	Amphipods	0.18	0.16	0.047
	Atlantic argentine	0.18	0.17	0.039
	Capelin	0.18	0.12	0.068
	Longfin hake	0.09	0.08	0.076
	Large redfish	0.27	0.28	0.107
2	Arctic cod	0.225	0.143	0.073
	Atlantic argentine	0.225	0.218	0.05
	Blue hake	0.135	0.068	0.051
	Euphausiid	0.135	0.119	0.076
	White barracudine	0.18	0.145	0.057
3	Atlantic argentine	0.225	0.226	0.041
	Capelin	0.225	0.191	0.06
	Herring	0.225	0.211	0.062
	Large redfish	0.225	0.194	0.066
4	Amphipods	0.18	0.167	0.069
	Large Greenland halibut	0.225	0.143	0.059
	Longfin hake	0.27	0.249	0.109
	Large redfish	0.225	0.224	0.105
5	Atlantic argentine	0.225	0.217	0.042
	Capelin	0.225	0.179	0.072
	Northern sand lance	0.225	0.22	0.067
	Winter flounder	0.225	0.208	0.041

potential prey and the predator. Thus, from the previous example, one might overestimate the proportion of redfish in the diet at the expense of capelin and amphipods because these in turn form the basis of redfish diets. Therefore, we used simulation to evaluate the ability of QFASA to differentiate specific prey, both within and between prey species and to allow us to understand potential prey substitutions.

Detailed procedures for the simulations are provided in Iverson et al. (2004). To conduct the simulations, the prey base was randomly split into 2 subsets; a simulation and an estimation subset. The simulation set was sampled in the proportions specified by the simulated diet (see below) to construct a 'pseudo-seal' signature with additional random prey added in to create 10% noise. Subsequently, the diets of 'pseudo seals' were estimated using the other 50% of prey to evaluate how well the true diet was estimated by QFASA.

We constructed 4 to 5 diets for simulations for both harp and hooded seal prey bases containing 4 to 7 prey species (Tables S2 & S3). Some of these were meant to represent the diets of free-ranging seals found in the literature, as well as our initial results from QFASA. Others represented difficult or 'worst case' estimation scenarios as some prey were more similar to one another than to all other species in the FA database. We used hierarchical cluster analysis to determine the relative similarity of prey species' signatures. For example, in harp seal prey simulations, Diet 1 and Diet 4 represented pelagic- and benthic-species, respectively, while Diets 2 and 3 were mixtures of pelagic and benthic species (Table S2). Noise was meant to represent incidental consumption of prey species that were not included in the assumed diet. As the noise was set at 10% for these simulations, accurate estimation would give a total of 10% other prey.

RESULTS

Prey libraries

Following our assessment of differences in prey FA signatures (Tucker 2007), we used the primary prey library comprised of 2039 individual prey representing 24 species to estimate the diets of harp seals (Fig. S1) and the primary prey library comprised of 2289 individuals representing 29 species (Fig. S2) to estimate the diets of hooded seals.

FA sets

We estimated diets for all seals using both dietary and primarily dietary FA sets. This was done for the different CCs and results were similar. Using the harp seal prey library, relative to the primarily dietary FA set, the dietary subset underestimated proportions of amphipods, arctic cod, capelin and northern sand lance *Ammodytes dubius*, and overestimated redfish and euphausiids (Fig. S3). Amphipods, arctic cod, sand lance and capelin are all known to be important dietary items of harps based on stomach contents (e.g. Lawson & Stenson 1995, 1997, Lawson et al. 1995). Furthermore, redfish are known to be predators of capelin, sand lance and arctic cod, while euphausiids are prey of capelin, sand lance and arctic cod (Scott & Scott 1988, Froese & Pauly 2007). Thus, the misclassifications were logical and it would appear that the primarily dietary FA subset provides a more accurate assessment of diet, presumably because more information is used to discriminate species. These primarily dietary FAs are clearly abundant in this ecosystem and are considered important trophic indicators (Budge et al. 2002, Iverson et al. 2004). In the hooded seals the main components of diet were similar for both the primarily dietary and dietary FA sets. We used the results from the primarily dietary FA set.

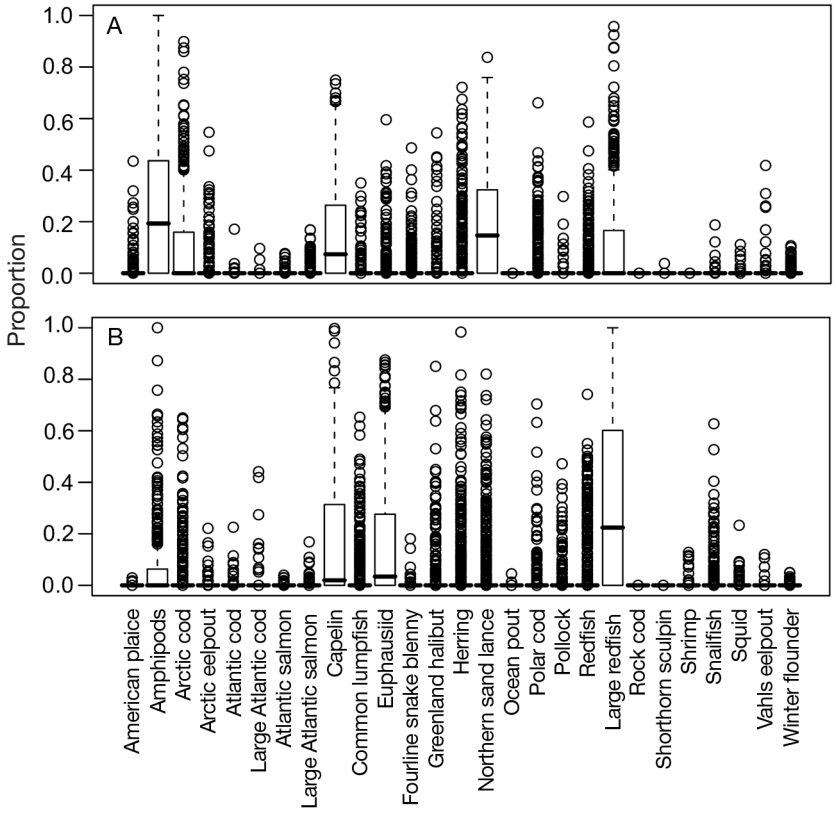

Fig. A3. *Pagophilus groenlandicus.* Average diet estimates for harp seals (n = 526) using primary prey library, grey-harp-pup CCs and the (A) primarily dietary FA set, or (B) dietary FA set. Dots represent outliers defined as being any value greater or less than 1.5 times the interquartile range (75th percentile–25th percentile) above the 75th (or below the 25th) percentile

Calibration coefficients

There were no large differences in diet estimated from the 3 sets of CC or combinations of those sets. In fact, the species composition of the diet estimates for harp seals were all similar with amphipods, artic cod, capelin, sand lance and redfish as main components (Fig. S4). As we had no *a priori* reason to select one calibration set over the other in the final modeling, we averaged the diet estimates derived using the harp CC, the average of grey and harp CCs, and the average of the grey, harp and pup CCs to incorporate that source of uncertainty in the standard errors. The harp seal set is species specific; however, sample size is small, thus, including the grey seal set (a related phocid species) likely incorporates more of the potential variation. The pup calibration set is perhaps the best defined (Iverson et al. 2004) since a sub-sample of milk was measured in conjunction with the mother–pup pairs providing a direct link with ingested food and fat deposits in the 'predator'. These also represent the scenario of ingesting a high fat diet. Although there are no species-specific CC for hooded seals, the main components of diet were similar for the different sets of CC. Although proportions varied (Fig. S5), we averaged diet estimates using the grey-harp mean CCs and grey-harp-pup mean CC.

Diet simulations

The model estimated the true diet (comprised of 90% specified prey, 10% noise) well with the major species in the diet distinguished from others in each of the prey databases with between 71 and 83% of the specified diet returned (Tables S2 & S3). Nevertheless, there was some misidentification (7 to 20%) of the diet to other prey types in addition to the expected 10% noise. Misclassifications involved closely related species, as illustrated in the cluster analysis (Figs. S1 & S2). For example, in harp seal Diet 1, 10% of the arctic cod signature was proportioned to polar cod *Arctogadus glacialis* and redfish. Atlantic cod was typically misclassified by a different size of Atlantic cod, or American plaice *Hippoglossoides platessoides* and shrimp *Pandalus* sp. Diet 2 was the most complex diet with a mixture of pelagic and benthic species and performed the least well. All specified diet items were underestimated and allocated to a mix of other related species (predominantly polar cod, euphausiids, shrimp, squid and winter flounder).

The results from our simulations suggest that QFASA can distinguish prey species in our library appropriately, with misallocations of 7 to 20% to other species. For diets that were specifically chosen to have species with similar signatures, an increasingly large percentage was attributed to other prey while fits were better and more consistent when diet items were more easily distinguished. In cases were the estimates had higher errors, misallocations in simulations were generally small and to closely related species and/or similar with respect to functional patterns of feeding. Thus, the dominant prey delineated by QFASA are likely robust, as demonstrated in controlled feeding experiments (Iverson et al. 2004, 2007).

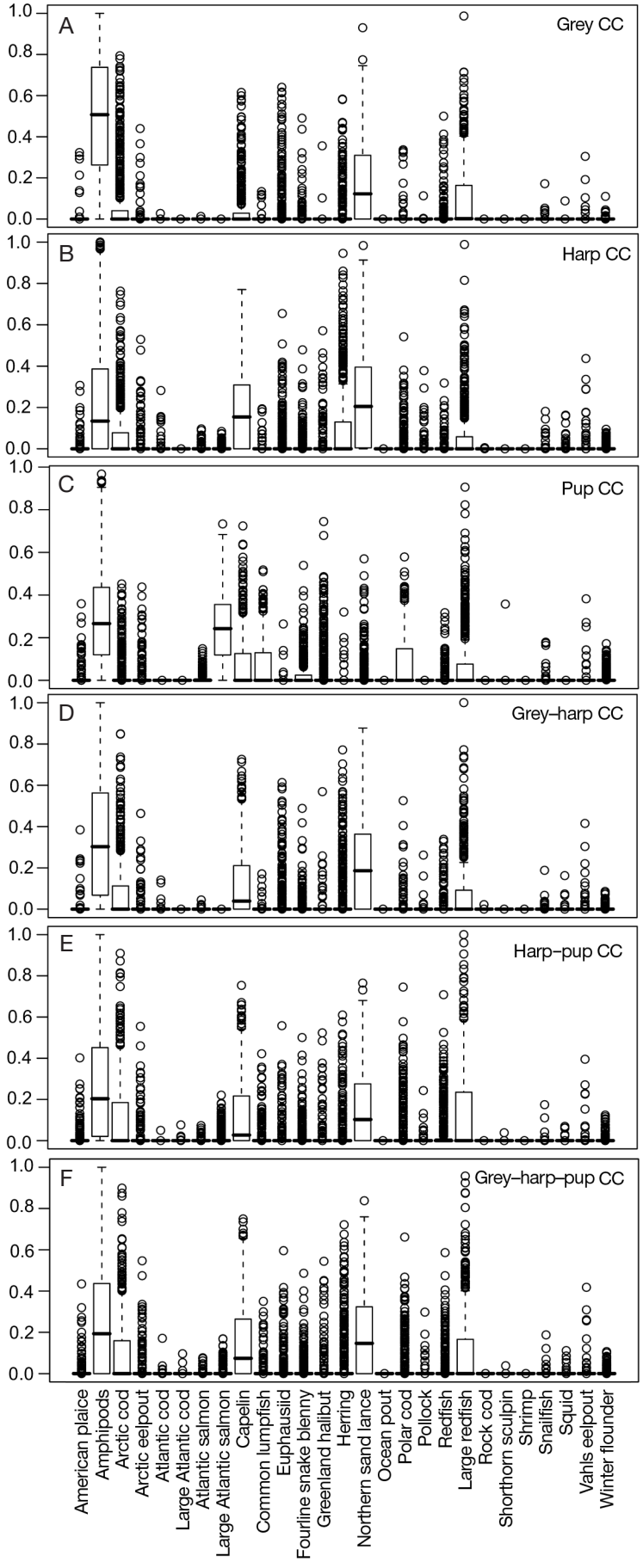

Fig. S4. *Pagophilus groenlandicus*. Mean diet estimates of harp seals (n = 526) using grey, harp, pup CCs or combinations of these. Dots represent outliers defined as being any value greater or less than 1.5 times the interquartile range (75th percentile–25th percentile) above the 75th (or below the 25th) percentile

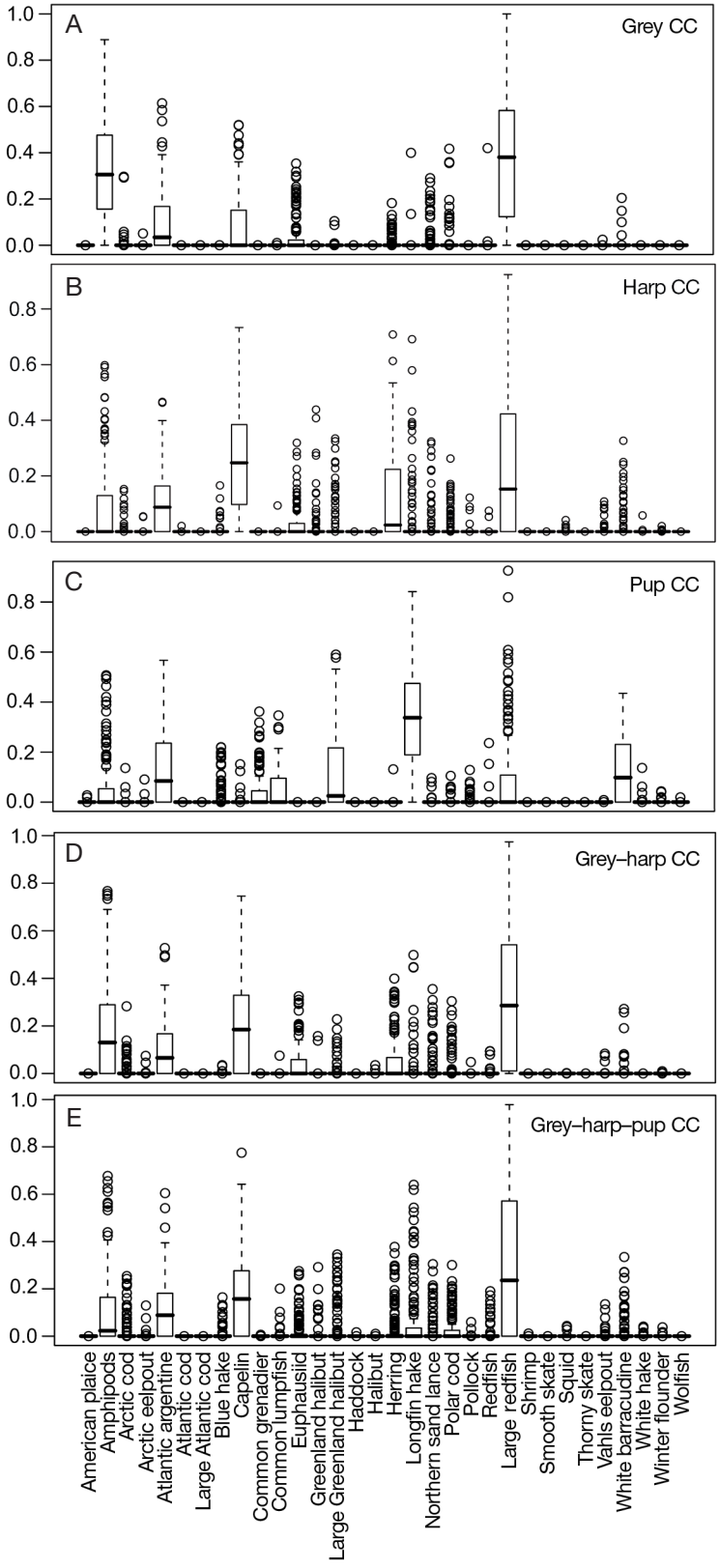

Fig. S5. *Cystophora cristata*. Mean diet estimates of hooded seals (n = 153) using grey, harp, pup CCs or combinations of those. Dots represent outliers defined as being any value greater or less than 1.5 times the interquartile range (75th percentile–25th percentile) above the 75th (or below the 25th) percentile

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