Fish species identified in bearded seal diet using stomach contents and fecal DNA

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Introduction

The diet of marine mammals can be assessed using various techniques, but each has limitations. Therefore, a combination of these techniques will likely yield the best dietary information. In this study, we use the combination of stomach contents and prey DNA molecules extracted from feces to identify fish consumed by bearded seals (*Erignathus barbatus*).

- When using stomach contents alone, complete digestion of some prey and retention of others varies, which can yield a biased and incomplete interpretation of prey composition.
- Otoliths from sculpin (*Myoxocephalus* spp., *Gymnocanthus* spp.) and snailfish (*Liparis* spp.) are often found in the stomachs of bearded seals collected in Alaska (Quakenbush et al. 2011), but it is unknown what species they represent.
- DNA molecules present in the gut and feces can be isolated and processed to identify prey species. This gene-based approach can identify important prey items that were previously undetected or unidentifiable using hard parts from stomach contents.


Photo: Michael Cameron, NOAA

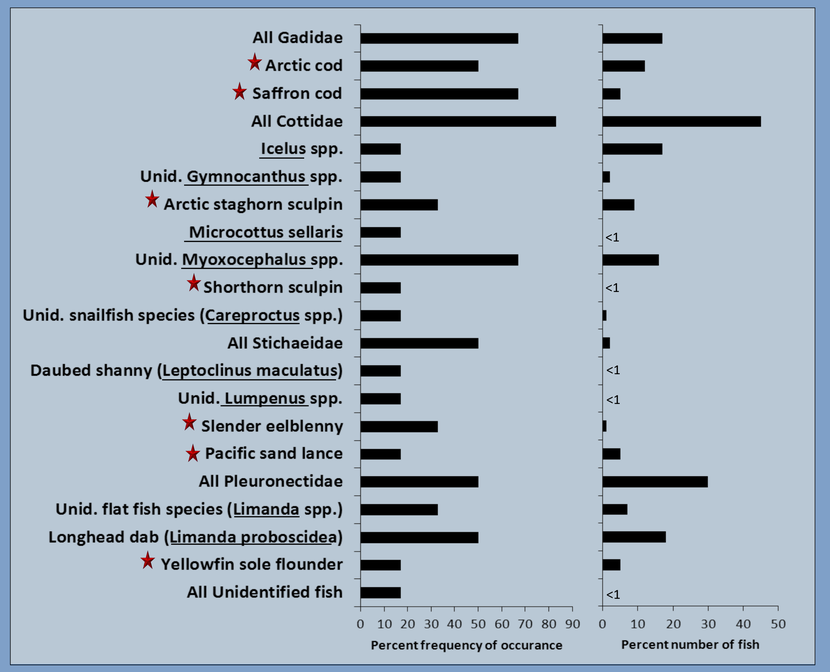
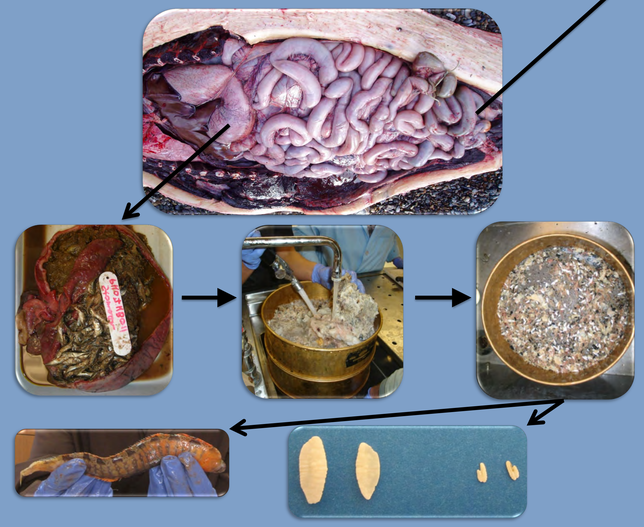


Figure 1. *Percent frequency of occurrence and number of fish identified from 6 bearded seal stomachs, one stomach contained no fish. A minimum of 347 fish were identified in these stomachs. ★indicates fish included in the DNA reference library.*

Methods

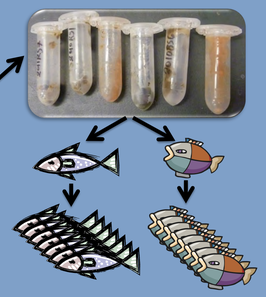
Stomachs and intestinal content were collected from 7 (3 female, 4 male) adult (>5 years) bearded seals harvested for subsistence use in the spring near Point Hope and Barrow, Alaska in 2008 and 2010. Samples were frozen at -20^{o}C until analyzed.

Various ***Fish species*** were collected in the Chukchi Sea and used to create a prey DNA reference library.

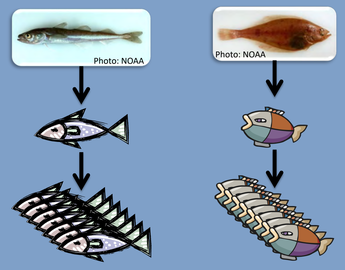


Stomach contents were rinsed in two sieves and prey items were identified to the lowest possible taxonomic level (Figure 1). Percent frequency of occurrence and percent number were calculated for all fish taxa.

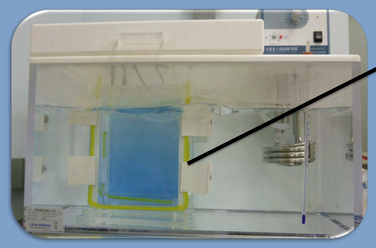
- Frequency of occurrence = $\frac{\text{\# of stomachs containing fish a taxon}}{\text{total \# of stomachs containing fish}}$
- Percent number = $\frac{\text{\# of fish in a taxon}}{\text{total \# of fish identified}}$



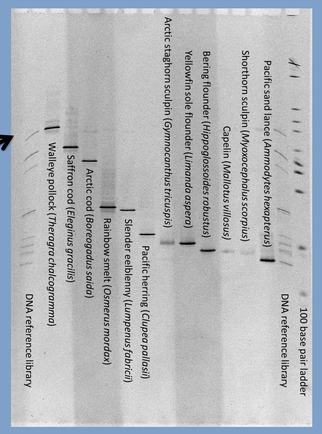
*A sample of **feces** was removed from the lower colon of the seals and DNA was extracted using QIAGEN DNA stool kits. From this, DNA the 16S Fish gene was targeted and amplified using protocols described by Tollit et al. 2009.*



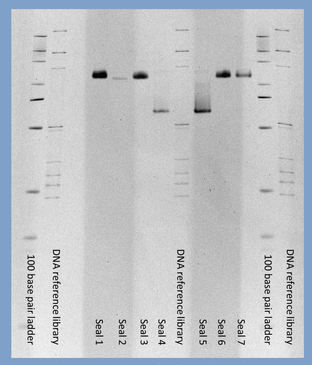
***Fish DNA** was extracted from fish muscle using a modified QIAGEN Puregene protocol. Then the 16S Fish gene was targeted and amplified using the same protocol as described for fecal samples.*



*Using **denaturing gradient gel electrophoresis (DGGE),** the PCR products for both fecal and fish samples were separated on a gradient from 35 to 60% denaturants. This technique can separate DNA sequences with as little as one base pair difference.*



DGGE gel with fish PCR products. Each of the 12 fish species is clearly separated on the gel. Fish were combined to make the DNA reference library.



***DGGE gel with fecal PCR** products and fish reference library.*

Results

- At least twelve species of fish were detected in the stomachs of bearded seals in this study.
- The DGGE successfully separated the fish in the reference library, and two different DNA bands were detected in the fecal DGGE gel. However, the bands detected in the feces did not match any of the fish in the reference library.

Conclusions

We identified many fish from the DNA reference library in stomach contents; however, we did not detect the DNA from any of these species in feces. Although one seal had no fish in its stomach, it had DNA in its feces, which we suspect was fish DNA. Digestion and gut passage rates likely influenced the presence of prey items in the feces.

Future steps:

- Determine DNA sequences from the fecal amplifications to identify species.
- Include more known prey species in the DNA reference prey library.
- Homogenize feces before subsampling to see if the number of fish identified increases.
- Modify the fecal amplification or DNA extraction procedures to see if more species are detected.
- Evaluate the cost benefits of this DNA approach vs. developing technologies.

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Tollit, D. J. , et al. 2009. Development and application of DNA techniques for validating and improving pinniped diet estimates. Ecological Applications. 19(4). 889-905.
Quakenbush, L., et al. 2011. Biology of the Bearded Seal (*Erignathus Barbatus*) in Alaska, 1961-2009. Final Report to NMFS.