Armhook Squid Diet Analysis using eDNA Metabarcoding

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Introduction

- Sports fishermen **report more squid** catches, making Armhook squid (*Berryteuthis magister*) a **species of interest** and have **requested biological assessments**.
- Questions are raised as to what *B. magister* eat.
- *B. magister* **diet analysis has not been attempted** before **in the SE Alaska** region.
- **eDNA metabarcoding methods** allow for **thorough diet analysis** for organisms that primarily **digest prey during consumption.**

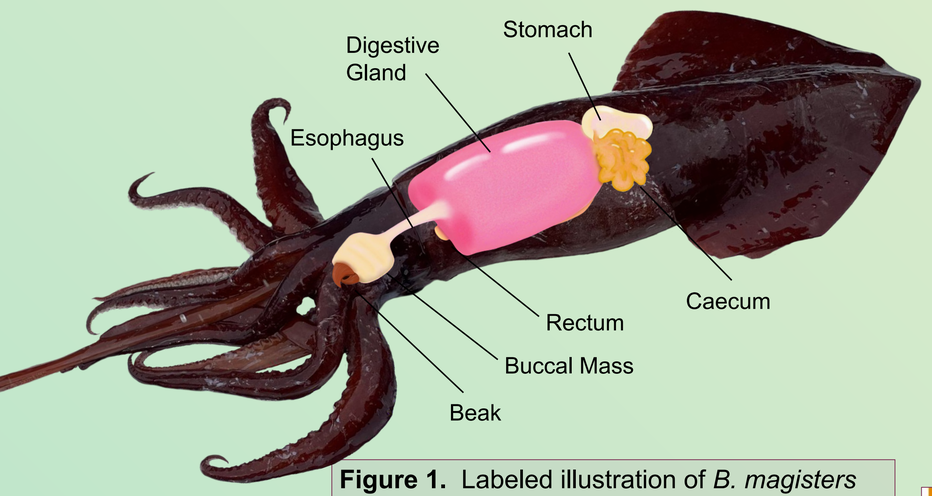


Figure 1. Labeled illustration of *B. magisters* digestive system. Illustrated by C. Springer

Hypotheses

Null: eDNA methods and analyses of *Berryteuthis magister* digested contents provide sufficient evidence to determine if they eat species of osteichthyes.

Alternative: eDNA methods and analyses of *B. magister's* digested contents are not sufficient to resolve if osteichthyes are apart of their diet.

Methods

- Stomach contents (N=81) were preserved in Longmire solution.
- DNA was extracted with a Qiagen Stool Kit.
- Performed PCRs with MiFish primers (Miya et al., 2015), Quantified DNA
- Samples were run on a Illumina MiSeq.
- Amplicon Sequence Variants(ASVs) were analyzed to identify taxa.
- Data was processed and visualized in R studios.

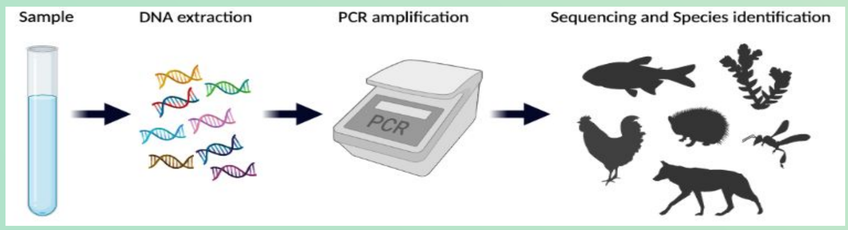


Figure 2. Illustrated process of methods. Created with BioRender.com

Results

- DNA was identified in 54 out of 81 stomachs.
- Little to no contamination confirmed with negatives.
- 14 taxa were identified.
- Top 7 Taxa:

Clupea pallasii

~46 cm

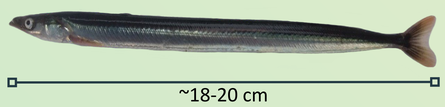
Citharichthys stigmaeus

~15-20 cm

Gadidae

~69-86 cm

Ammodytes spp.

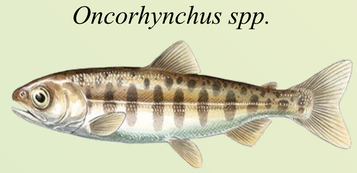


~18-20 cm

Stenobrachius spp.

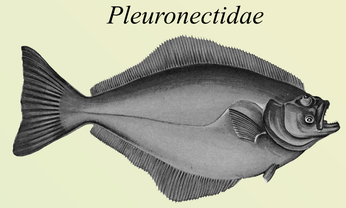
~20 cm

Oncorhynchus spp.



~46-62 cm

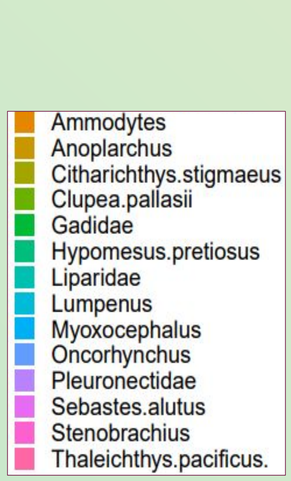
Pleuronectidae



~ 6 - 244 cm

Table 1. 14 identified taxa from *B. magister* stomach contents, with cumulative reads, mean and st. dev.

taxon <chr>	n_squid <int>	total_reads <int>	mean <dbl>	st.dev <dbl>
Citharichthys.stigmaeus	34	39038	1148	1507
Clupea.pallasii	12	41857	3488	7296
Gadidae	9	10878	1209	1962
Ammodytes	6	2295	382	548
Oncorhynchus	5	4327	865	1165
Pleuronectidae	5	4505	901	1606
Stenobrachius	5	2465	493	496
Lumpenus	3	4097	1366	2358
Sebastes.alutus	2	2340	1170	997
Anoplarchus	1	381	381	NA
Hypomesus.pretiosus	1	249	249	NA
Liparidae	1	174	174	NA
Myoxocephalus	1	865	865	NA
Thaleichthys.pacificus.	1	292	292	NA



Note: Taxa were identified to the lowest identifiable taxonomical unit.

Figure 3. Reads of taxa (upper row) and proportion of taxa (lower row) in individual squid stomachs caught in April, June, July, and August of 2022.

Conclusions

- Results support the Null hypothesis.
- The diet of *B. magister* could be classified as opportunistic.
- Prey size ranges from 6 - 70 centimeters.
- Few species in each stomach indicates they eat one organism at a time.

Future Steps

- Repeat this study with invertebrate primers and compare ratios between invertebrate and vertebrate diet contents.
- Research the diet contents when extracted from other portions of the digestive system.
- How often do *B. magister* eat, what is their digestion rate?

Acknowledgements

- Research reported on this poster was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under Award Number P20GM103395. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.
- NOAA ABL Genetics Lab for providing assistance, time and space for me to work!
- UAS Navarro Lab
- Shelter Island Lodge for providing squid!

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