Stable Isotope Analysis in Deep Sea Cephalopods of the Bear Seamount

Valerie Hartigan[1,2]; Mike Vecchione[1]; Michelle Staudinger[3]; Christine France[4]

[1]NMFS National Systematics Laboratory, National Museum of Natural History, Smithsonian Institution, Washington, DC; [2]Department of Marine Science, Coastal Carolina University, Conway, SC; [3]Northeast Climate Science Center, University of Massachusetts, Amherst, MA; [4]Museum Conservation Institute, Smithsonian Institution, Suitland, MD

Introduction

Deep sea cephalopods (squid and octopods) have long fascinated the imagination of human beings in both fiction and reality. Because reaching their natural habitat is difficult, scientists are finding new ways to learn about these creatures. Often, when large predatory marine mammals are beached, their stomachs are filled with cephalopod beaks, the only part of the animal that remains after digestion. Therefore, using cephalopod beaks to infer data can have broad applications. These beaks have been studied in such detail that they can be used to identify species and extrapolate animal size. With the use of new molecular technologies, cephalopod beaks can now also be analyzed to determine chemical composition using stable isotope analysis.

Analyzing the stable carbon and nitrogen ratios within these beaks can elucidate habitat use and trophic position, respectively, and help to reconstruct the food web of the cephalopods in a given community. Trophic levels can be determined from nitrogen isotopes, and carbon isotopes indicate the local source of primary production and the depth range inhabited by the sampled individual. These values can be used to determine shifts in dietary habits with growth within a species as well as determining interspecies variations.

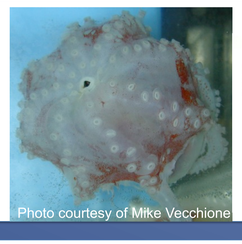


Photo courtesy of Mike Vecchione

Photo courtesy of Mike Vecchione

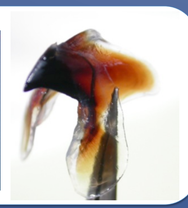


Photo courtesy of Mike Vecchione

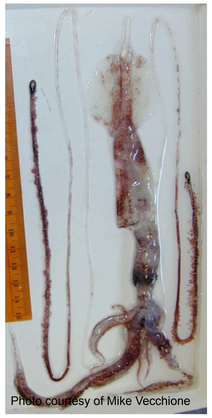


Photo courtesy of Mike Vecchione

Photos of deep sea cephalopods and their lower beaks (from top left to bottom left, then right): *Haliphron atlanticus*, *Mastigoteuthis magna, Octopoteuthis danae,* and *Chiroteuthis mega.*

Acknowledgements:
The primary author would like to thank NOAA for facilitating collection of these samples, NSF for funding the NHRE internship program, my co-authors for all of their assistance, especially Dr. Mike Vecchione, and Drs. Gene Hunt, Liz Cottrell, and Virginia Power. I would also like to thank Dr. Juli Harding for her invaluable contribution to my career and my family for their love and support.

References:
Cherel, Y., Fontaine, C., Jackson, G.D., Jackson, C.H., Richard, P., 2009. Tissue, ontogenic and sex-related differences δ13C and δ15N values of the oceanic squid *Todarodes filippovae* (Cephalopoda: Ommastrephidae). Marine Biology, 156, 699-708.
Fanelli, E., Cartes, J.E., Papiol, V., 2012. Assemblage structure and trophic ecology of deep-sea demersal cephalopods in the Balearic basin (NW Mediterranean). Marine and Freshwater Research, 63, 264-274.

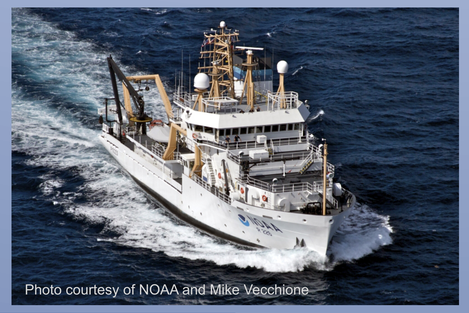


Photo courtesy of NOAA and Mike Vecchione

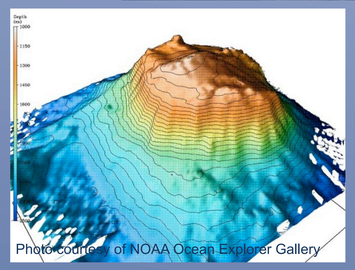


Photo courtesy of NOAA Ocean Explorer Gallery

Photo courtesy of NOAA Ship Tracker

(Top) The NOAA ship *Pisces*; (bottom left) Multibeam bathymetric profile of the Bear Seamount; (bottom right) Map of the Bear Seamount with the *Pisces* course plotted

Methods

- In Fall 2012, the NOAA ship *Pisces* collected individuals representing 54 species of cephalopods using midwater trawling at the Bear Seamount
- Beaks from 26 of these species were removed and preserved in ethanol
- The lower beak from each individual was described, photographed, rinsed with distilled water and then placed in a drying oven for 24-48 hours
- Upon removal from the oven, beaks were homogenized using a mortar and pestle and the resulting powder was placed into vials
- Sample powder was weighed into tin cups on a microbalance to a target weight of 0.6-0.8 mg and the tin cups containing the powder were crushed
- Acetanalide B, urea, and atropine were used as test standards
- Samples and standards were then analyzed using a Costech Instruments Elemental Combustion System, a Thermo Scientific Conflo IV unit, and a Thermo Scientific Delta V Advantage Isotope Ratio Mass Spectrometer
- Data were corrected to reference gas samples of N_2 and CO_2 and were then linearly corrected to international standards of atmospheric N_2 and Pee Dee Belemnite.

(Left): Elemental Analyzer and Mass Spectrometer used for stable isotope analysis

(Right): Squid species used in preliminary results (from top to bottom): *Ornithoteuthis antillarum, Abraliopsis morisii, Sthenoteuthis pteropus,* and *Taonius pavo*

Photo courtesy of Mike Vecchione

Photo courtesy of Mike Vecchione

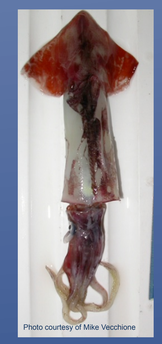
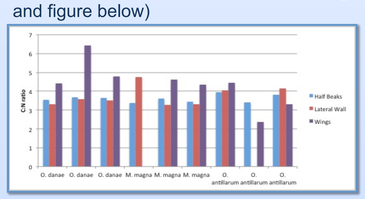


Photo courtesy of Mike Vecchione

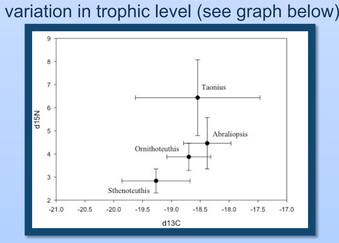
Photo courtesy of Mike Vecchione

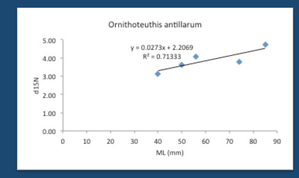
Results

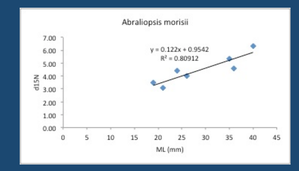
- Preliminary results showed that different parts of the beaks have different elemental compositions
- The wings of juvenile and subadult beaks typically have a much higher carbon to nitrogen ratio than other portions of the beak (see graph and figure below)

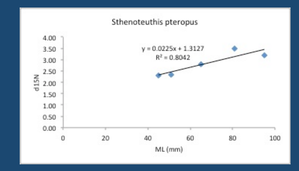


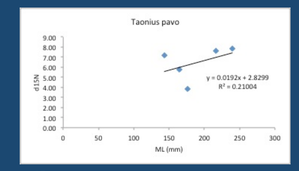
- $\delta^{15}N$ values increase as a function of mantle length (see graphs on right)
- Preliminary bi-plots suggest interspecies variation in trophic level (see graph below)











$\delta^{15}N$ plotted against mantle length for (top to bottom) *Ornithoteuthis antillarum, Abraliopsis morisii, Sthenoteuthis pteropus,* and *Taonius pavo*

Discussion

The C:N ratios of the different subsections of the lower beaks show that, in juvenile and subadult beaks, the newly formed and transparent portions, particularly the wings, have a relatively low nitrogen signal due to dilution by a large amount of carbon. The wings, as the most newly formed region of the beak, are enriched in carbon-13 relative to the other portions of the beak (Cherel et al., 2009). In the smallest beaks, it was necessary to use the whole beak to obtain an adequate sample size for analysis. Comparing young beaks to older beaks that have been subsampled or are more fully darkened could be inaccurate because of differences in the level of chitin and the increased carbon signature in younger beaks. This is an important result to establish future methods for stable isotope analysis in cephalopod beaks.

The increasing $\delta^{15}N$ with increasing mantle length indicates that, in these four species, trophic level does increase with growth. Trophic level discriminations are defined by a difference of 2.75‰ in $\delta^{15}N$ (Fanelli et al., 2012). *Taonius pavo* shows the largest difference of 3.95‰ from the smallest squid measured to the largest, indicating an approximate increase of 1.4 trophic levels. *Abraliopsis morisii* showed an increase of approximately 1.2 trophic levels from the smallest measured squid to the largest. *Sthenoteuthis pteropus* and *Ornithoteuthis antillarum* remained within the same trophic level throughout the growth stages evaluated. The preliminary bi-plot indicates that, based on known depth ranges for these species, trophic level increases with depth. Additional investigation is needed to verify that this remains true for other species as well. More cephalopod samples, as well as baseline samples for this community, are currently being processed to further establish trophic relationships.