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Preliminary analysis of beak stable isotopes (δ^{13}C and δ^{15}N) stock variation of neon flying squid, *Ommastrephes bartramii*, in the North Pacific Ocean

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ABSTRACT

Stable isotopes (^{13}C and ^{15}N) have been confirmed as a useful tool for understanding trophic position and related dietary variation in squid. In this study, we performed isotopic analysis on *Ommastrephes bartramii* beaks to examine differences between the eastern and western stocks in the North Pacific Ocean. Isotopic values of the upper beak (UB) and the lower beak (LB) were also compared. A generalized additive model (GAM) was used to select variables that explain stock variation. Isotopic signatures (δ^{13}C and δ^{15}N) were significantly different between the two stocks whereas there was no difference in C/N ratios. All isotopic values were significantly different between the UB and LB. Trophic niche width was distinct between two stocks with little overlap. The δ^{13}C signature increased with latitude and mantle length (ML) with greater variability in the eastern stock. None of the variables could explain the variation in δ^{13}C values for the western stock. The δ^{15}N signature increased rapidly with ML in the eastern stock, whereas δ^{15}N gradually increased with latitude and ML in the western stock. The isotopic variations can be successfully explained by different migration patterns and feeding behaviors of the two stocks. The δ^{13}C and δ^{15}N values of the UB were lower than those of the LB, possibly due to variable chitin/protein ratios at different developmental stages. Future studies should account for a greater sample size and the functionality of the UB.

1. Introduction

Cephalopods, especially ommastrephid squids, play an important role in the marine food web by connecting the higher and lower trophic levels, acting as both predator and prey (Rodhouse and Nigmatullin, 1996; Navarro et al., 2013). Commercial oceanic (ommastrephid) squid landings increased drastically over the past two decades, from appropriately 2.0 million metric tons in 1990s to over 4.3 million metric tons in 2010 (FAO, 2013). Neon flying squid, *Ommastrephes bartramii*, which can reach large body sizes and yield high economic value, is widely distributed in temperate and subtropical waters of the Pacific, Indian and Atlantic Oceans, but is commercially exploited only in the North Pacific Ocean with some Asian countries (Bower and Ichii 2005; Chen et al., 2008; Jereb and Roper, 2010).

Some studies have been discussed about the population structure of *O. bartramii* based on mantle length distribution and rates of infection by helminth parasites (Murata, 1990; Bower and Margolis, 1991; Nagasawa et al., 1998; Yatsu et al., 1998). It is generally accepted that this species is believed to consist of two main cohorts, the autumn cohort that is mainly distributed east of 170°E (Eastern stock) with fishing season from May to June and the winter-spring cohort located west of 170°E (Western stock) with fishing season from July to November (Bower and Ichii, 2005). These two cohorts have been confirmed as two different stocks with major genetic differentiation, although they are overlapping geographically in some areas (Katugin, 2002; Bower and Ichii, 2005). Geographic separation of the two cohorts causes them to experience very different oceanographic conditions, which leads to asynchronous growth patterns and varied abundances (Chen and Chiu, 2003; Chen, 2010).

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Oceanic squid, tend to migrate long distances in north-south direction during their lifetime and may be exposed to different environments in each life phase (Yatsu et al., 1997; Murata and Nakamura, 1998; Ichii et al., 2004). Optimum oceanographic environment and food richness are the two main reasons cause the migration (Ichii et al., 2009). Dietary change is also closely related with the squid growth, especially adult squid (Watanabe et al., 2004, 2008). Feeding behavior tends to be the dominant factor for the migration with the body size increase in adult squid. With voracious feeding habits and a fast metabolism, squid effectively contribute to energy exchange in the marine food web (Clarke, 1996; Piatkowski et al., 2001; Boyle and Rodhouse 2008). Examining their feeding habits is important to elucidating the trophic role of squid in the marine ecosystem. Stomach content analysis is a conventional approach that directly examines feeding habits (Rodhouse and Nigmatullin, 1996; Watanabe et al., 2004; Cherel and Hobson, 2005). This time-consuming method with some obvious disadvantages was replaced by some indirect approach (Ruiz-Cooley et al., 2006; Jackson et al., 2007). Stable isotope analysis (SIA), specifically analysis of carbon and nitrogen isotopes (^{13}C and ^{15}N), has been successfully used in several studies of squid feeding habits over the last decade (Takai et al., 2000; Ruiz-Cooley et al., 2004, 2006; Lorrain et al., 2011; Argüelles et al., 2012; Miller et al., 2013). The trophic position of a species can be identified by quantifying the nitrogen isotope ratios $\delta^{15}N$ ($^{15}N/^{14}N$) (McCutchan et al., 2003; Vanderklift and Ponsard, 2003). Carbon isotope ratios $\delta^{13}C$ ($^{13}C/^{12}C$) reflects variability in primary production and trophic dynamics along migration routes (Rubenstein and Hobson, 2004). Stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C$) of animal tissues provide a record of historic diet composition (Rau et al., 1983; Hobson and Welch, 1992, 1994). Therefore, it is possible to obtain relatively long-term feeding information, which is difficult to do with conventional method (Onthank, 2013).

Different tissues have been analyzed for isotopic signatures $\delta^{15}N$ and $\delta^{13}C$, the most common of which is muscle due to its easy availability (Ruiz-Cooley et al., 2004, 2006; Stowasser et al., 2006; Parry, 2008; Cherel et al., 2009a, 2009b; Hunsicker et al., 2010; Argüelles et al., 2012). Unlike archival tissues, muscle is continuously regenerating with fast cell metabolism and protein synthesis (Cherel et al., 2009b; Moltschaniwskyj and Carter, 2010), so historic nutrient records are not retained (Onthank, 2013). Additionally, muscle has high lipid content yielding negative $\delta^{13}C$ values, which alter the absolute $\delta^{13}C$ value and confound interpretation of foraging behavior dynamics (DeNiro and Epstein, 1977; Focken and Becker, 1998; Logan et al., 2008; Ruiz-Cooley et al., 2011). As a main feeding organ, the beak has very low lipid content and it has been verified that the beak has a slightly higher ^{13}C content but lower ^{15}N content compared to lipid-free muscle (Cherel and Hobson, 2005; Hobson and Cherel, 2006; Cherel et al., 2009b). The growth of beak is deposited with continuous layers of cells (Raya et al., 2010), so different parts of beak can represent different life stages (Cherel et al., 2009b; Guerra et al., 2010). Thus, beak is confirmed as a useful archival tissues that can reflect long-term ecological and life history information to examine habitat and trophic variation (Cherel and Hobson, 2005; Hobson and Cherel, 2006; Ruiz-Cooley et al., 2006; Hunsicker et al., 2010; Ruiz-Cooley et al., 2013; Xavier et al., 2014).

In this study, we used quantitative beak stable isotopic data ($\delta^{13}C$ and $\delta^{15}N$) to estimate the different trophic level of *O. bartramii* between two stocks with pertinent dietary-induced migration patterns. We also used a generalized additive model (GAM) to analyze the main factors influencing isotopic variation in different life stages. The isotopic difference between upper and lower beaks was also analyzed. This study improves our understanding of the stock variation pattern in feeding behavior and tests different migration routes of two *O. bartramii* stocks in the North Pacific Ocean.

2. Materials and methods

A total of 60 random squid samples were obtained from the Chinese commercial jigging vessel *F/V Jinhai 827* operated on two main fishing grounds (152°E–174°W and 38°–45°N) in the North Pacific Ocean from May to November in 2010 (Fig. 1). Samples were immediately frozen at −18 °C for laboratory work. Samples from different areas were treated as different stocks based on age back-calculation analysis, in which eastern stock and western stock belong to autumn cohort and winter-spring cohort, respectively (Tables 1 and 2) (Jin et al., 2015; Fang et al., 2016).

Dorsal mantle length (ML) was measured to the nearest 1 mm in the laboratory. The maturity stage was also determined according to Lipinski and Underhill (1995). The upper (UB) and lower beaks (LB) were dissected from the buccal mass, washed with fresh water and stored in 75% ethyl alcohol, which does not change the isotopic composition of tissues and prevents beaks from dehydrating (Hobson et al., 1997).

Before isotopic analysis, beaks were cleaned with MilliQ water for at least 5 min and dried in a freeze dryer (Alpha 1-4 LD Plus, Christ, Germany) for 24 h. Each beak sample was then ground to a fine powder with a Mixer Mill MM 400 (Retsch GmbH, Haan, Germany) for 1.5 min. Approximately 1.50 ± 0.05 mg of each beak powder sample was then weighed in an aluminum tray using an electronic scale (BSA223S, Sartorious, Göttingen, Germany). Samples were analyzed with an ISOPRIME 100 isotope ratio mass spectrometer (Isoprime Corporation, Cheadle, UK) and a vario ISOTOPE cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) in the Key Laboratory of Sustainable Exploitation of Oceanic Fisheries Resources, Ministry of Education, at Shanghai Ocean University.

The standard reference material for carbon was Vienna Pee Dee Belemnite (V-PDB) and for nitrogen was atmospheric nitrogen (N_2). USGS 24 (−16.049‰ V-PDB) was used as primary standard for ^{13}C and USGS 26 (53.7‰ V-N_2) was used to quantify ^{15}N. To assess the associated errors within and between runs, repeated analyses of internal laboratory standards (−26.98‰ vPDB and 5.96‰ vN_2) were performed every 10 samples. The analytical precision was less than 0.1‰ both for carbon and nitrogen. The $\delta^{13}C$ and $\delta^{15}N$ of samples are expressed in standard notation as the following (Ruiz-Cooley et al., 2006):

$$\delta X(‰) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 1000$$

where R_{sample} and R_{standard} are the ratios of $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in the sample and standards, respectively. δX is the measure of the ratio of heavy to light isotope ratio in the sample.

All the isotopic values had constant variance and conformed to normal distribution. We used an independent two-group *t*-test to analyze the $\delta^{13}C$ and $\delta^{15}N$ variation between the two stocks. Differences of isotopic values between upper and lower beaks were also evaluated with a paired *t*-test. Convex hull of carbon–nitrogen biplot was also constructed to evaluate the trophic niche width for two stocks. Variable geographic ranges of the two stocks may cause isotopic variation, so the distance to the shelf break (DSB) was calculated for each sample. We used R to calculate DSB with function "dist2Line" in the package of "geosphere", which calculates the minimum distance between a specific point to the nearest continental shelf border along a great circle (shortest distance on a sphere) (Hijmans, 2014). It is possible that the relationships between two dependent variables ($\delta^{13}C$ and $\delta^{15}N$) and three independent variables (latitude (LAT), ML, DSB) are nonlinear and multivariate (Argüelles et al., 2012), thus generalized additive models (GAM) were used to analyze isotopic variation in different situation (Wood, 2006; Argüelles et al., 2012). The best fitted model