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

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## ABSTRACT

Stable isotopes ( $^{13}\text{C}$  and  $^{15}\text{N}$ ) 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 ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) 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  $\delta^{13}\text{C}$  signature increased with latitude and mantle length (ML) with greater variability in the eastern stock. None of the variables could explain the variation in  $\delta^{13}\text{C}$  values for the western stock. The  $\delta^{15}\text{N}$  signature increased rapidly with ML in the eastern stock, whereas  $\delta^{15}\text{N}$  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  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  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.

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## 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 (ommatrephid) 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 ( $^{13}\text{C}$  and  $^{15}\text{N}$ ), 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  $\delta^{15}\text{N}$  ( $^{15}\text{N}/^{14}\text{N}$ ) (McCutchan et al., 2003; Vanderklift and Ponsard, 2003). Carbon isotope ratios  $\delta^{13}\text{C}$  ( $^{13}\text{C}/^{12}\text{C}$ ) reflects variability in primary production and trophic dynamics along migration routes (Rubenstein and Hobson, 2004). Stable isotope ratios ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) 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  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , 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  $\delta^{13}\text{C}$  values, which alter the absolute  $\delta^{13}\text{C}$  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  $^{13}\text{C}$  content but lower  $^{15}\text{N}$  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 ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) 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 ( $152^{\circ}\text{E}$ – $174^{\circ}\text{W}$  and  $38^{\circ}$ – $45^{\circ}\text{N}$ ) in the North Pacific Ocean from May to November in 2010 (Fig. 1). Samples were immediately frozen at  $-18^{\circ}\text{C}$  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).

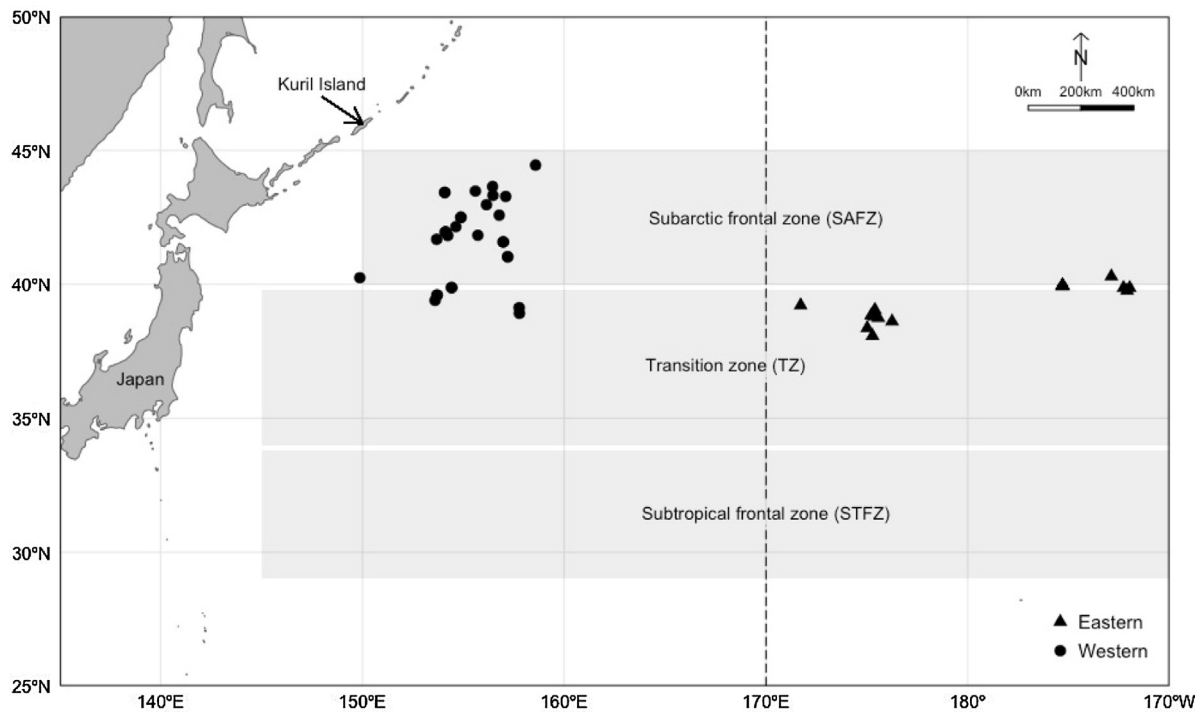
Before isotopic analysis, beaks were cleaned with MilliQ water for at least 5 min and dried in a freeze dryer (Alpha 1–4 LD Plus, Christ, Germany) for 24 h. Each beak sample was then ground to a fine powder with a Mixer Mill MM 400 (Retsch GmbH, Haan, Germany) for 1.5 min. Approximately  $1.50 \pm 0.05$  mg of each beak powder sample was then weighed in an aluminum tray using an electronic scale (BSA223S, Sartorius, Göttingen, Germany). Samples were analyzed with an ISOPRIME 100 isotope ratio mass spectrometer (Isoprime Corporation, Cheadle, UK) and a vario ISO-TOPE 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 ( $\text{N}_2$ ). USGS 24 ( $-16.049\%$  V-PDB) was used as primary standard for  $^{13}\text{C}$  and USGS 26 ( $53.7\%$  V- $\text{N}_2$ ) was used to quantify  $^{15}\text{N}$ . To assess the associated errors within and between runs, repeated analyses of internal laboratory standards ( $-26.98\%$  vPDB and  $5.96\%$  v $\text{N}_2$ ) were performed every 10 samples. The analytical precision was less than 0.1‰ both for carbon and nitrogen. The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of samples are expressed in standard notation as the following (Ruiz-Cooley et al., 2006):

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

where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the ratios of  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  in the sample and standards, respectively.  $\delta X$  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  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  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 ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) 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



**Fig. 1.** Sampling stations of *Ommastrephes bartramii*, and descriptions of Subarctic frontal zone (SAFZ), Transition zone (TZ) and Subtropical frontal zone (STFZ) in the North Pacific Ocean. Western (solid dots) and eastern (triangles) stocks represent different stock. Vertical line in 170°E (longdash) represent the geographical boundary of different stocks.

**Table 1**

Summary information of sampled eastern stock of *Ommastrephes bartramii* in North Pacific Ocean (max: maximum; min: minimum; ave: average; std: standard deviation).

| ID  | Catch Date<br>(yy/mm/dd) | Coordinate        | ML(mm) | Sex | Maturity<br>stage | Upper beak          |                     |                               | Lower beak          |                     |                               |
|-----|--------------------------|-------------------|--------|-----|-------------------|---------------------|---------------------|-------------------------------|---------------------|---------------------|-------------------------------|
|     |                          |                   |        |     |                   | $^{13}\text{C}$ (‰) | $^{15}\text{N}$ (‰) | $^{13}\text{C}/^{15}\text{N}$ | $^{13}\text{C}$ (‰) | $^{15}\text{N}$ (‰) | $^{13}\text{C}/^{15}\text{N}$ |
| 2   | 2010/6/9                 | 172°17'W, 39°52'N | 454    | ♀   | 3                 | −18.22              | 9.06                | 3.79                          | −17.71              | 9.59                | 3.26                          |
| 75  | 2010/5/30                | 175°34'E, 38°45'N | 285    | ♀   | 1                 | −18.82              | 6.52                | 4.09                          | −18.39              | 6.76                | 3.48                          |
| 103 | 2010/6/20                | 175°18'W, 39°57'N | 253    | ♂   | 2                 | −18.82              | 5.28                | 4.26                          | −18.72              | 6.24                | 3.58                          |
| 136 | 2010/6/20                | 175°18'W, 39°57'N | 244    | ♀   | 1                 | −18.70              | 5.95                | 4.37                          | −18.61              | 6.17                | 3.62                          |
| 139 | 2010/6/20                | 175°18'W, 39°57'N | 248    | ♂   | 1                 | −18.67              | 7.07                | 4.14                          | −18.32              | 7.35                | 3.45                          |
| 161 | 2010/5/18                | 175°22'E, 38°49'N | 403    | ♀   | 2                 | −18.32              | 8.32                | 4.28                          | −17.88              | 9.35                | 3.41                          |
| 164 | 2010/5/18                | 175°22'E, 38°49'N | 363    | ♀   | 2                 | −18.06              | 8.65                | 4.10                          | −17.70              | 9.07                | 3.51                          |
| 165 | 2010/5/18                | 175°22'E, 38°49'N | 293    | ♀   | 1                 | −18.90              | 6.70                | 4.34                          | −18.58              | 6.55                | 3.61                          |
| 179 | 2010/5/24                | 175°24'E, 39°03'N | 346    | ♀   | 2                 | −18.18              | 7.82                | 4.07                          | −17.93              | 8.75                | 3.40                          |
| 182 | 2010/5/16                | 176°15'E, 38°37'N | 418    | ♀   | 2                 | −18.51              | 7.66                | 4.09                          | −18.13              | 8.37                | 3.20                          |
| 194 | 2010/6/10                | 171°58'W, 39°52'N | 446    | ♀   | 3                 | −18.43              | 8.21                | 3.99                          | −17.92              | 8.58                | 3.34                          |
| 218 | 2010/5/18                | 175°22'E, 38°49'N | 435    | ♀   | 2                 | −18.10              | 8.12                | 4.01                          | −17.74              | 8.72                | 3.29                          |
| 223 | 2010/5/18                | 175°22'E, 38°49'N | 208    | ♀   | 1                 | −18.40              | 4.81                | 4.10                          | −17.97              | 5.41                | 3.54                          |
| 270 | 2010/5/10                | 171°43'E, 39°13'N | 473    | ♀   | 3                 | −18.01              | 10.31               | 3.26                          | −17.13              | 10.35               | 3.08                          |
| 274 | 2010/5/21                | 175°14'E, 38°51'N | 325    | ♀   | 2                 | −18.35              | 7.79                | 4.18                          | −17.85              | 8.31                | 3.48                          |
| 281 | 2010/5/21                | 175°14'E, 38°51'N | 461    | ♀   | 3                 | −17.84              | 9.03                | 3.94                          | −17.68              | 9.13                | 3.30                          |
| 291 | 2010/5/19                | 175°17'E, 38°50'N | 305    | ♀   | 2                 | −18.62              | 6.56                | 3.26                          | −17.95              | 6.72                | 3.11                          |
| 346 | 2010/6/17                | 172°53'W, 40°18'N | 376    | ♀   | 2                 | −18.27              | 8.52                | 3.99                          | −17.92              | 9.29                | 3.37                          |
| 385 | 2010/5/27                | 170°01'E, 38°22'N | 352    | ♀   | 2                 | −18.14              | 7.82                | 4.22                          | −17.91              | 8.13                | 3.58                          |
| 404 | 2010/6/8                 | 172°06'W, 39°46'N | 319    | ♀   | 1                 | −18.83              | 5.86                | 4.30                          | −18.31              | 6.85                | 3.11                          |
| 415 | 2010/6/20                | 175°18'W, 39°57'N | 250    | ♂   | 1                 | −18.57              | 5.70                | 4.13                          | −18.15              | 6.88                | 3.05                          |
| 417 | 2010/6/25                | 176°28'W, 39°47'N | 255    | ♂   | 1                 | −18.82              | 5.39                | 4.24                          | −18.55              | 5.87                | 3.47                          |
| 418 | 2010/6/20                | 175°18'W, 39°57'N | 265    | ♀   | 1                 | −18.81              | 6.39                | 3.22                          | −18.28              | 6.67                | 3.10                          |
| 421 | 2010/6/26                | 175°38'W, 39°37'N | 244    | ♂   | 1                 | −18.39              | 5.65                | 4.22                          | −18.35              | 6.52                | 3.47                          |
| 424 | 2010/6/20                | 175°18'W, 39°57'N | 220    | ♂   | 1                 | −18.29              | 5.83                | 4.08                          | −18.26              | 6.52                | 3.50                          |
| 428 | 2010/6/20                | 175°18'W, 39°50'N | 212    | ♂   | 2                 | −18.70              | 4.65                | 3.99                          | −18.38              | 5.58                | 3.31                          |
| 430 | 2010/6/27                | 176°48'W, 39°57'N | 232    | ♂   | 1                 | −18.86              | 5.05                | 4.18                          | −18.81              | 6.03                | 3.62                          |
| 432 | 2010/6/20                | 175°18'W, 39°47'N | 270    | ♂   | 2                 | −18.80              | 5.42                | 4.06                          | −18.58              | 6.04                | 3.41                          |
| 441 | 2010/6/29                | 176°28'W, 39°57'N | 230    | ♂   | 2                 | −18.79              | 5.57                | 4.14                          | −18.50              | 5.44                | 3.53                          |
| 448 | 2010/6/20                | 175°18'W, 39°57'N | 253    | ♀   | 1                 | −18.65              | 6.46                | 3.19                          | −18.34              | 6.49                | 3.11                          |
| max | –                        | –                 | 473    | –   | –                 | −17.84              | 10.31               | 4.37                          | −17.13              | 10.35               | 3.62                          |
| min | –                        | –                 | 208    | –   | –                 | −18.90              | 4.65                | 3.19                          | −18.81              | 5.41                | 3.05                          |
| ave | –                        | –                 | 315    | –   | –                 | −18.50              | 6.87                | 4.01                          | −18.15              | 7.39                | 3.38                          |
| std | –                        | –                 | 84     | –   | –                 | 0.30                | 1.48                | 0.33                          | 0.38                | 1.43                | 0.18                          |

**Table 2**  
Summary information of sampled western stock of *Ommastrephes bartramii* in North Pacific Ocean (max: maximum; min: minimum; ave: average; std: standard deviation).

| ID   | Catch date<br>(yy/mm/dd) | Coordinate        | ML(mm) | Sex | Maturity<br>stage | Upper beak          |                     |                               | Lower beak          |                     |                               |
|------|--------------------------|-------------------|--------|-----|-------------------|---------------------|---------------------|-------------------------------|---------------------|---------------------|-------------------------------|
|      |                          |                   |        |     |                   | $^{13}\text{C}$ (‰) | $^{15}\text{N}$ (‰) | $^{13}\text{C}/^{15}\text{N}$ | $^{13}\text{C}$ (‰) | $^{15}\text{N}$ (‰) | $^{13}\text{C}/^{15}\text{N}$ |
| 9    | 2010/7/9                 | 157°47'E, 38°55'N | 187    | ♀   | 1                 | −17.34              | 7.86                | 4.02                          | −17.43              | 8.00                | 3.45                          |
| 156  | 2010/9/13                | 156°27'E, 43°39'N | 285    | ♀   | 2                 | −17.53              | 9.51                | 4.09                          | −17.29              | 10.22               | 3.25                          |
| 234  | 2010/8/2                 | 157°12'E, 41°02'N | 178    | ♂   | 1                 | −18.14              | 7.35                | 4.08                          | −18.11              | 7.59                | 3.54                          |
| 237  | 2010/8/2                 | 157°12'E, 41°02'N | 163    | ♂   | 1                 | −18.11              | 8.17                | 3.63                          | −18.06              | 8.62                | 3.20                          |
| 324  | 2010/11/13               | 149°52'E, 40°15'N | 341    | ♂   | 3                 | −17.92              | 8.95                | 3.96                          | −17.76              | 8.98                | 3.33                          |
| 520  | 2010/10/3                | 153°41'E, 41°41'N | 293    | ♂   | 2                 | −18.35              | 8.96                | 3.93                          | −17.97              | 9.60                | 3.37                          |
| 536  | 2010/8/10                | 156°59'E, 41°35'N | 304    | ♂   | 3                 | −17.86              | 9.08                | 4.02                          | −17.59              | 9.30                | 3.34                          |
| 541  | 2010/8/10                | 156°59'E, 41°35'N | 215    | ♂   | 1                 | −18.09              | 8.35                | 3.99                          | −18.11              | 8.69                | 3.52                          |
| 654  | 2010/7/19                | 134°25'E, 39°53'N | 211    | ♀   | 2                 | −17.85              | 8.19                | 4.16                          | −17.89              | 8.50                | 3.59                          |
| 683  | 2010/7/19                | 134°25'E, 39°53'N | 194    | ♀   | 2                 | −17.99              | 7.51                | 4.15                          | −17.73              | 8.39                | 3.40                          |
| 854  | 2010/8/15                | 154°14'E, 41°49'N | 274    | ♂   | 3                 | −17.75              | 8.34                | 4.19                          | −17.60              | 8.63                | 3.46                          |
| 963  | 2010/10/23               | 154°38'E, 42°09'N | 374    | ♀   | 2                 | −18.55              | 8.79                | 4.10                          | −18.23              | 9.12                | 3.40                          |
| 1004 | 2010/9/22                | 154°05'E, 43°26'N | 361    | ♀   | 2                 | −17.89              | 8.87                | 4.00                          | −17.63              | 9.50                | 3.33                          |
| 1009 | 2010/9/22                | 154°05'E, 43°26'N | 381    | ♀   | 2                 | −17.82              | 10.11               | 3.19                          | −17.57              | 9.72                | 3.07                          |
| 1046 | 2010/10/21               | 155°43'E, 41°50'N | 351    | ♀   | 2                 | −18.32              | 9.05                | 3.90                          | −17.78              | 9.49                | 3.07                          |
| 1101 | 2010/8/17                | 155°36'E, 43°29'N | 247    | ♀   | 2                 | −18.08              | 9.27                | 4.02                          | −17.92              | 9.46                | 3.37                          |
| 1132 | 2010/10/10               | 156°09'E, 42°58'N | 326    | ♀   | 2                 | −18.06              | 9.79                | 3.81                          | −17.82              | 9.65                | 3.28                          |
| 1150 | 2010/7/20                | 153°42'E, 39°36'N | 201    | ♀   | 2                 | −18.34              | 7.07                | 3.56                          | −17.95              | 7.31                | 3.30                          |
| 1167 | 2010/7/20                | 153°42'E, 39°36'N | 207    | ♂   | 1                 | −18.28              | 8.38                | 3.94                          | −18.35              | 8.54                | 3.36                          |
| 1298 | 2010/10/16               | 157°06'E, 43°17'N | 242    | ♂   | 1                 | −17.93              | 8.94                | 3.89                          | −17.66              | 9.51                | 3.33                          |
| 1329 | 2010/9/11                | 156°28'E, 43°19'N | 267    | ♀   | 2                 | −18.12              | 9.62                | 3.73                          | −17.97              | 9.72                | 3.20                          |
| 1425 | 2010/8/7                 | 154°53'E, 42°30'N | 313    | ♀   | 2                 | −17.84              | 8.52                | 3.31                          | −17.53              | 8.92                | 3.11                          |
| 1440 | 2010/8/7                 | 154°53'E, 42°30'N | 256    | ♂   | 2                 | −17.79              | 8.88                | 3.75                          | −17.41              | 8.82                | 3.35                          |
| 1679 | 2010/10/12               | 156°47'E, 42°35'N | 236    | ♀   | 2                 | −18.04              | 8.96                | 4.03                          | −17.85              | 9.26                | 3.54                          |
| 1703 | 2010/7/6                 | 157°46'E, 39°08'N | 225    | ♂   | 1                 | −18.15              | 8.37                | 4.14                          | −17.89              | 9.16                | 3.41                          |
| 1817 | 2010/9/19                | 158°35'E, 44°27'N | 261    | ♂   | 2                 | −18.17              | 9.22                | 4.07                          | −17.98              | 9.49                | 3.21                          |
| 1854 | 2010/11/5                | 154°07'E, 41°57'N | 421    | ♀   | 3                 | −18.52              | 9.05                | 4.06                          | −18.17              | 9.69                | 3.23                          |
| 1855 | 2010/11/5                | 154°07'E, 41°57'N | 405    | ♀   | 3                 | −18.15              | 10.22               | 3.70                          | −17.72              | 10.13               | 3.18                          |
| 1874 | 2010/11/5                | 154°07'E, 41°57'N | 281    | ♂   | 3                 | −18.10              | 9.64                | 3.98                          | −17.89              | 9.41                | 3.39                          |
| 1878 | 2010/7/13                | 153°36'E, 39°24'N | 231    | ♀   | 2                 | −17.99              | 8.53                | 3.72                          | −17.63              | 8.49                | 3.38                          |
| max  | –                        | –                 | 421    | –   | –                 | −17.34              | 10.22               | 4.19                          | −17.29              | 10.22               | 3.59                          |
| min  | –                        | –                 | 163    | –   | –                 | −18.55              | 7.07                | 3.19                          | −18.35              | 7.31                | 3.07                          |
| ave  | –                        | –                 | 274    | –   | –                 | −18.04              | 8.79                | 3.90                          | −17.82              | 9.06                | 3.33                          |
| std  | –                        | –                 | 71     | –   | –                 | 0.26                | 0.76                | 0.24                          | 0.26                | 0.69                | 0.13                          |

was the one with the lowest AIC (Akaike information criterion) value (Akaike, 1974). Relationships were developed for the upper and lower beaks and isotopic values ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) were compared between stocks.

### 3. Results

#### 3.1. Isotopic variation between two stocks

Isotopic values of western stock were higher than those of eastern stock (Tables 1 and 2). The independent two-group *t*-test results indicated that ML was significantly different between the two geographical stocks ( $P < 0.05$ ; Table 3). Isotopic values were also significantly different between stocks ( $P < 0.05$ ; Table 3). There was no difference between the C/N ratio of the UB and LB ( $P > 0.05$ ; Table 3). Isotopic values of UB were also lower than those of LB (Tables 1 and 2) and these values were all significantly different between the two stocks ( $P < 0.05$ ; Table 3).

The considerable overlapped trophic niche width of two stocks was observed from the biplot (Fig. 2A). Western stock had more concentrated trophic niche width with higher  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (Fig. 2A). In the eastern stock, the trophic niche width was changed substantially from low  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  to high  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (Fig. 2A). This transformation was also accompanied with the ML increase.

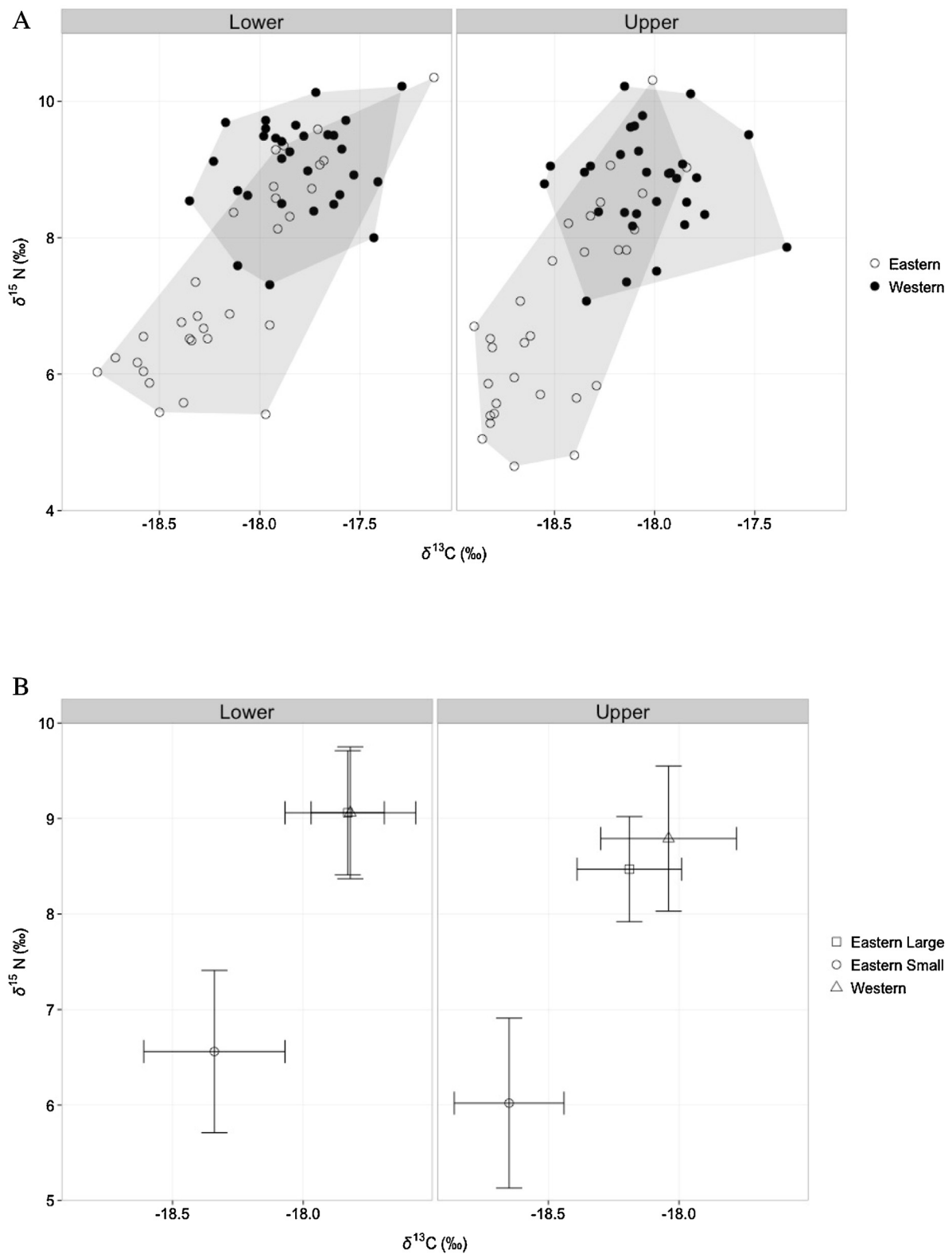
Considering the disparity of trophic niche width of eastern stock for different body size, two subgroups were established to evaluate the variation of eastern stock in different sizes. It is obvious that eastern large squids (ML > 350 mm, mean ML 418.1 mm) have similar trophic level (mean  $\pm$  SD Upper  $\delta^{13}\text{C}$ :  $-18.2 \pm 0.2$ ,  $\delta^{15}\text{N}$ :  $8.5 \pm 0.5$ ;

**Table 3**  
Analysis of isotopic values of western and eastern North Pacific Ocean *Ommastrephes bartramii* stocks (*t*-test results).

| Group     | Variable | df | <i>t</i> | <i>P</i>           |
|-----------|----------|----|----------|--------------------|
| Stock     | ML       | 58 | 2.01     | 0.05               |
|           | CU       | 58 | −6.30    | <0.01              |
|           | NU       | 58 | −6.31    | <0.01              |
|           | C/NU     | 58 | 1.38     | 0.17 <sup>ns</sup> |
|           | CL       | 58 | −4.02    | <0.01              |
|           | NL       | 58 | −5.78    | <0.01              |
|           | C/NL     | 58 | 1.08     | 0.28 <sup>ns</sup> |
| UB and LB | CE       | 29 | −9.89    | <0.01              |
|           | NE       | 29 | −7.71    | <0.01              |
|           | C/NE     | 29 | 14.10    | <0.01              |
|           | CW       | 29 | −7.83    | <0.01              |
|           | NW       | 29 | −4.97    | <0.01              |
|           | C/NW     | 29 | 17.00    | <0.01              |

Abbreviations: ML = Mantle length, CU = Carbon isotope value of upper beak, NU = Nitrogen isotope value of upper beak, C/NU = Carbon/Nitrogen value of upper beak, CL = Carbon isotope value of lower beak, NL = Nitrogen isotope value of lower beak, C/NL = Carbon/Nitrogen value of lower beak, CE = Carbon isotope value of eastern stock, NE = Nitrogen isotope value of eastern stock, C/NE = Carbon/Nitrogen value of eastern stock, CW = Carbon isotope value of western stock, NW = Carbon isotope value of western stock, C/NW = Carbon/Nitrogen value of western stock.  
ns = non-significant.

Lower  $\delta^{13}\text{C}$ :  $-17.8 \pm 0.1$ ,  $\delta^{15}\text{N}$ :  $9.1 \pm 0.6$ ) with those of western stock (mean ML 274.4 mm; Upper  $\delta^{13}\text{C}$ :  $-18.0 \pm 0.3$ ,  $\delta^{15}\text{N}$ :  $8.8 \pm 0.8$ ; Lower  $\delta^{13}\text{C}$ :  $-17.8 \pm 0.2$ ,  $\delta^{15}\text{N}$ :  $9.1 \pm 0.7$ ) (Fig. 2B). The eastern small squids (ML < 350 mm, mean ML 262.8 mm) presented a very distinct trophic level with a low value (Upper  $\delta^{13}\text{C}$ :  $-18.6 \pm 0.2$ ;  $\delta^{15}\text{N}$ :  $6.0 \pm 0.9$ ; Lower  $\delta^{13}\text{C}$ :  $-18.3 \pm 0.3$ ;  $\delta^{15}\text{N}$ :  $6.5 \pm 0.8$ ) compared to large sized groups (Fig. 2B).

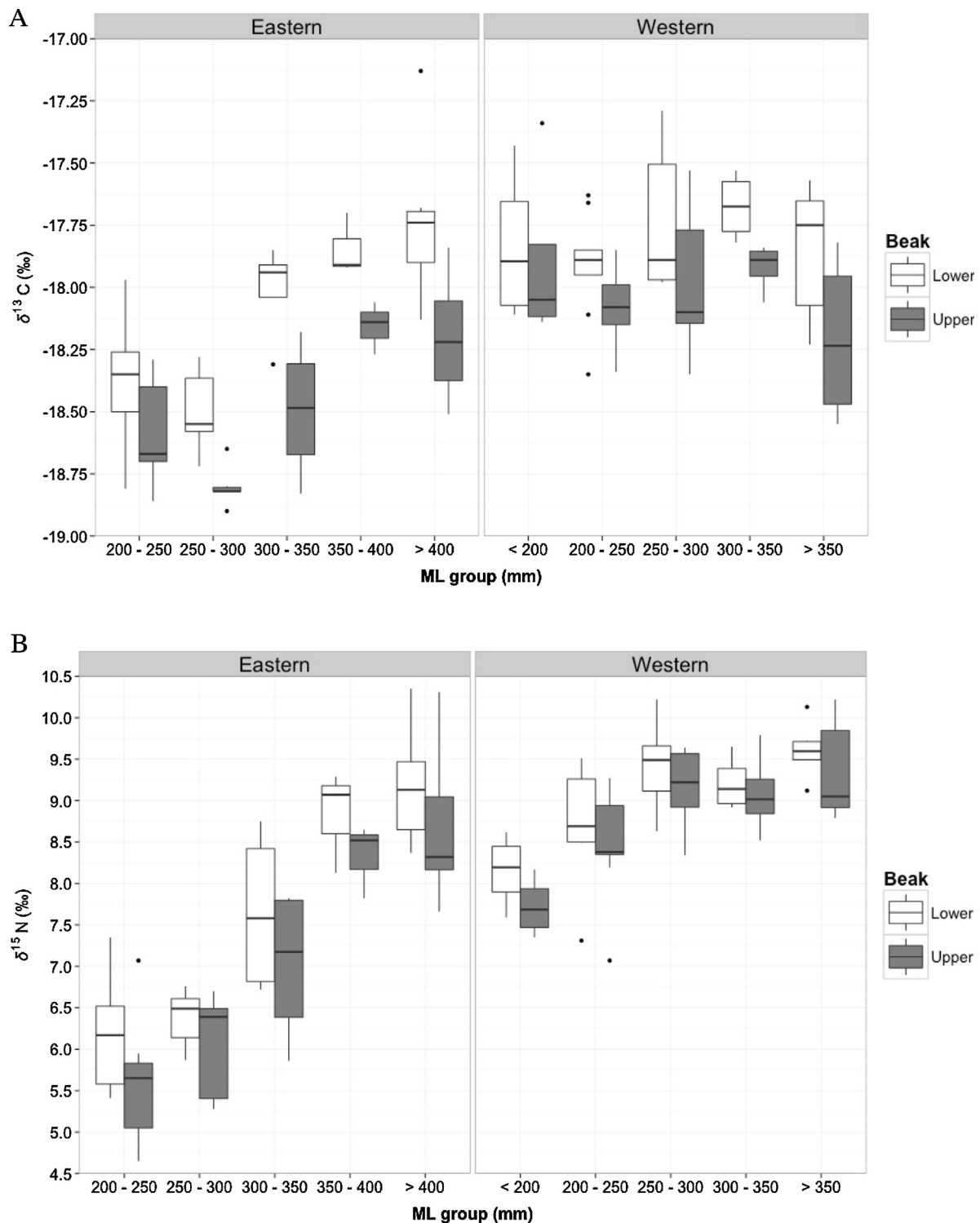


**Fig. 2.** Biplot of convex hull carbon-nitrogen isotopes (A) and mean  $\pm$  standard deviation isotopic values (B) of Western and Eastern stocks for upper and lower beaks. Eastern large individuals mean ML > 350 mm; Eastern small individuals mean ML < 350 mm.

### 3.2. Isotopic variation among different ML groups

The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values varied between ML groups (Fig. 3). For a given stock, the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values increased with ML by 0.5–0.8‰ for  $\delta^{13}\text{C}$  and 3–3.5‰ for  $\delta^{15}\text{N}$  in the eastern stock (Fig. 3).

In the western stock,  $\delta^{15}\text{N}$  values also changed by 1.5–2‰ with increasing ML, but  $\delta^{13}\text{C}$  values changed little between ML groups (Fig. 3). The isotopic values in LB were slightly higher than those in UB (Fig. 3).



**Fig. 3.** Isotopic variation ( $\delta^{13}\text{C}$  (A) and  $\delta^{15}\text{N}$  (B)) of Western and Eastern stocks for upper and lower beaks. Stick in boxplot means the range of isotopic values, upper counter means the 25th quartile, lower counter means the 75th quartile, solid line in the box mean the medium value.

$\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values increased rapidly from ML 250–300 mm to 350–400 mm in the eastern stock (Fig. 3). Isotope values plateaued in ML 350–400 mm and >400 mm for the eastern stock. In the western stock, there was a gradual increase in  $\delta^{15}\text{N}$  values from ML >200 mm to 250–300 mm, whereas values leveled off in ML 300–350 mm and >350 mm (Fig. 3).

### 3.3. GAMs selection and simulation

ML was identified as the single most important variable in all GAMs (Table 4). There was no difference between UB and LB in both the stocks for the variables identified as significant in the GAMs (Table 4). The GAM models explained from 23.8% to 91.6% of the variation in  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values (Table 5).

**Table 4**The AIC values for the selection of General Additive Model (GAM) for *Ommastrephes bartramii* isotopic analysis.

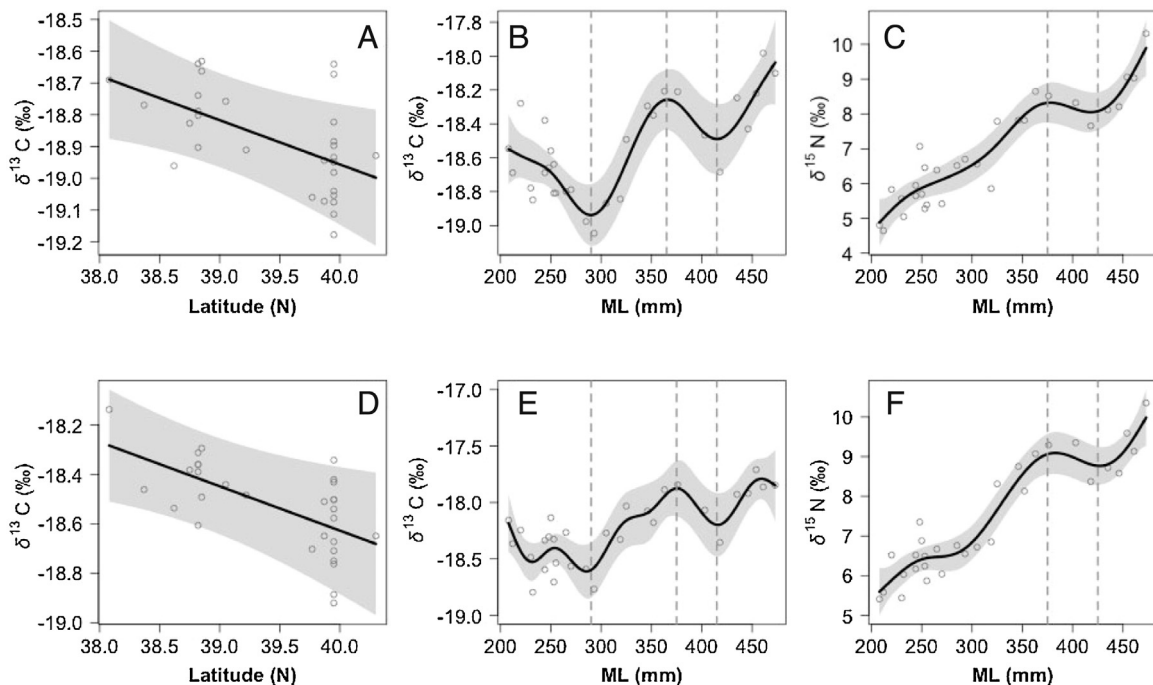
| RV                    | AO | LAT     | DSB    | ML      | LAT + DSB | LAT + ML | DSB + ML | ALL     |
|-----------------------|----|---------|--------|---------|-----------|----------|----------|---------|
| $\delta^{13}\text{C}$ | UE | 10.983  | 6.334  | -14.325 | 7.669     | -19.932  | -12.378  | -19.823 |
|                       | LE | 14.924  | 9.068  | -6.809  | 7.730     | -11.827  | -4.819   | -6.257  |
|                       | UW | 8.076   | 6.213  | 7.705   | 7.636     | 8.617    | 3.134    | 5.027   |
|                       | LW | 6.610   | 5.317  | 5.751   | 7.194     | 6.881    | 5.200    | 7.150   |
| $\delta^{15}\text{N}$ | UE | 102.491 | 96.104 | 55.705  | 95.754    | 57.623   | 57.304   | 58.836  |
|                       | LE | 100.444 | 94.158 | 46.486  | 95.518    | 48.115   | 47.486   | 49.243  |
|                       | UW | 55.235  | 52.596 | 46.658  | 52.819    | 44.287   | 49.110   | 46.031  |
|                       | LW | 47.909  | 48.668 | 42.425  | 46.922    | 37.307   | 44.511   | 37.700  |

Abbreviations: RV = Response variable, AO = Analyzed object, LAT = Latitude, DSB = Distance to shelf break, ML = Mantle length, ALL = All the three independent variables, UE = Upper beak of eastern stock, LE = Lower beak of eastern stock, UW = Upper beak of western stock, LW = Lower beak of western stock. Underlines are the lowest AIC values which the best model chosen.

**Table 5**Statistical outputs of GAMs in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for *Ommastrephes bartramii*.

| Variable              | Sample part | Source | e.d.f | F     | P                  | %explained |
|-----------------------|-------------|--------|-------|-------|--------------------|------------|
| $\delta^{13}\text{C}$ | UE          | LAT    | 1.00  | 5.07  | 0.03               | 81.6       |
|                       |             | ML     | 6.28  | 9.99  | <0.01              |            |
|                       | LE          | LAT    | 1.00  | 5.50  | 0.029              | 83.0       |
|                       |             | ML     | 8.25  | 7.19  | <0.01              |            |
| $\delta^{15}\text{N}$ | UW          | ML     | 1.51  | 1.70  | 0.20 <sup>ns</sup> | 32.8       |
|                       | LW          | ML     | 1.83  | 0.87  | 0.44 <sup>ns</sup> | 23.8       |
|                       | UE          | ML     | 2.16  | 69.82 | <0.01              | 84.7       |
|                       | LE          | ML     | 5.76  | 36.33 | <0.01              | 91.6       |
|                       | UW          | LAT    | 1.00  | 9.11  | <0.01              | 66.1       |
|                       |             | ML     | 4.30  | 8.24  | <0.01              |            |
|                       | LW          | LAT    | 1.00  | 10.82 | <0.01              | 70.4       |
|                       |             | ML     | 3.38  | 6.35  | <0.01              |            |

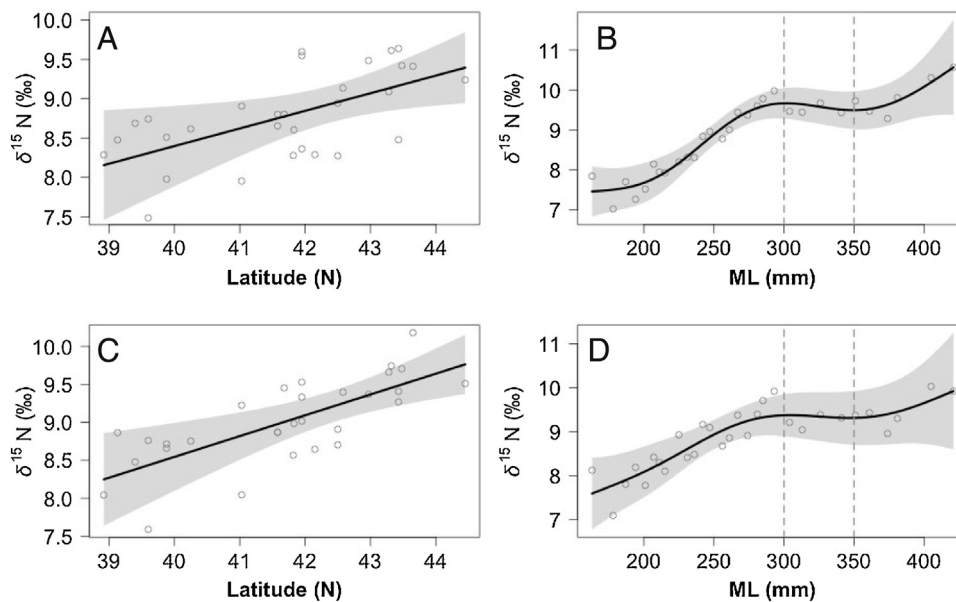
Abbreviations: IV = Isotopic variable, RV = Response variable, LAT = Latitude, DSB = Distance to shelf break, ML = Mantle length, ALL = All the three independent variables, UE = Upper beak of eastern stock, LE = Lower beak of eastern stock, UW = Upper beak of western stock, LW = Lower beak of western stock; s = non-significant.



**Fig. 4.** General Additive Models (GAMs) for significant variables of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the eastern stocks for upper (A–C) and upper (D–F) beaks. Black solid line is the mean value, grey filled area is the 95% confident interval.

In the eastern stock, latitude and ML were the two main effects for  $\delta^{13}\text{C}$  values of UB and LB (Fig. 4). The  $\delta^{13}\text{C}$  values decreased with latitude and increased with ML with little variability (Fig. 4). ML was also the only variable that had significant effects on  $\delta^{15}\text{N}$  values and  $\delta^{15}\text{N}$  increased with ML (Fig. 4C and F). An isotopic plateau could be seen between ML 350 and 400 mm (Fig. 4).

In the western stock, all of the three isotope variables failed to explain  $\delta^{13}\text{C}$  values in the GAMs for both UB and LB (Table 5). The  $\delta^{15}\text{N}$  values increased linearly with latitude. A similar result was observed for  $\delta^{15}\text{N}$  with  $\delta^{15}\text{N}$  values gradually increasing with ML and then reaching a plateau between ML 300 and 350 mm (Fig. 5).



**Fig. 5.** General Additive Models (GAMs) for significant variables of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the western stocks for upper (A–B) and lower (C–D) beaks. Black solid line is the mean value, and grey filled area is the 95% confidence interval.

#### 4. Discussion

Intraspecific variation is usually affected by both biotic factors (e.g., intrinsic genetic structure) and abiotic factors (e.g., environmental conditions) (Pech et al., 2004; Sandoval-Castellanos et al., 2010). Variation between stocks may be due to asynchronous growth, disparity in migration routes and differences in diet composition, which has been shown in other ommastrephid squids (Crespi-Abril and Baron, 2012; Liu et al., 2013; Arbuckle and Wormuth, 2014). Trophic niche width of the two stocks was separate except eastern large individuals (Fig. 2). Different feeding habitat of the two areas is the main reason to impact this disparity. Watanabe et al. (2008) indicated that winter-spring cohort mainly prey micronektonic squid (*Watasenia scintillans*) and Japanese anchovy (*Engraulis japonicus*) when stay in the subarctic frontal zone (SAFZ) and prepare southward migration during the late autumn and early spring (Fig. 1). The migration of prey species was also influenced by the variation of Kuroshio–Oyashio (Takahashi et al., 2001). Adults of autumn cohort mainly fed on the transitional-water myctophid (*Symbolophorus californiensis*), squid (*Onychoteuthis borealijaponica*) and subtropical water myctophids (*Ceratoscopelus warmingii*), squid (*Gonatus berryi* and *Berryteuthis anonychus*) (Watanabe et al., 2004), which has similar trophic level with the preys for western stock. However, juveniles fed on zooplankton, planktonic crustaceans (Euphausiids and amphipods) actively (Watanabe et al., 2004), which has lower trophic level than fish and squid. And this latitude is also the suitable habitat for the male squids of eastern stock always staying in the spawning/nursery ground (subtropical frontal zone, STFZ). This is the main reason why the feeding niche width distinct with little overlap for two stocks, as well as the different size for eastern stock (Fig. 2).

Marine phytoplankton  $\delta^{13}\text{C}$  decreases from the equator poleward at different rates between the southern and northern hemispheres (Rau et al., 1982; Takai et al., 2000). Lower  $\delta^{13}\text{C}$  values at higher latitudes in the eastern stock suggest that variation of phytoplankton  $\delta^{13}\text{C}$  could be reflected in squid muscle (Fig. 3A, Takai et al., 2000). Latitudinal variation in the western stock failed to explain  $\delta^{13}\text{C}$  variability, even though the western stock has a greater latitudinal range than the eastern stock (Table 1). The  $\delta^{13}\text{C}$  signature of the western stock may also be affected by proximity

to shore, since the western stock is closer to the shelf break than the eastern stock (Fig. 1). There seem to be more factors involved in  $\delta^{13}\text{C}$  dynamics of the western stock, which should be considered in future analysis.

ML is another factor influencing  $\delta^{13}\text{C}$  values (Fig. 3A). Oceanic squid often experience multiple environments along large-scale migration throughout their lifetime. All of the individuals less than 300 mm ML are males in this study. Females and males share the same habitat until they reach ~25 cm ML. During gametogenesis, females in the autumn cohort migrate north to the transition zone (TZ) and then to the SAFZ, while males remain in the spawning/nursery ground (STFZ) (Fig. 1) (Ichii et al., 2009). Once mature, females migrate south and meet males for breeding in the STFZ (Chen and Chiu, 2003; Ichii et al., 2009). This explains the sharp decrease of  $\delta^{13}\text{C}$  in small squid and rapid increase of  $\delta^{13}\text{C}$  in large squid, due to higher Chl-a concentration at the TZ and SAFZ compared to the less productive STFZ (Seki et al., 2002; Ichii et al., 2004). Increasing  $\delta^{13}\text{C}$  values of squid at ML 350–400 mm and over 400 mm can be explained by the autumn cohort squid of this size swimming south to the STFZ, thus experiencing lower Chl-a concentration and decreasing lower  $\delta^{13}\text{C}$  levels throughout their migration route (Figs. 3A, 4B, E).

ML had no effect on  $\delta^{13}\text{C}$  variation of the western stock. The  $\delta^{13}\text{C}$  values of the western stock were higher than eastern stock values for every ML group (Fig. 3A). The winter-spring cohort (western stock in this study) inhabits an area with relatively low Chl-a concentration in their early life stages and then migrates north to an area of high productivity (Ichii et al., 2009). Thus, this stock tends to consist of small-sized individuals. The earlier that these squid mature, the longer they inhabit the more productive area. DSB is also a potential factor contributing to  $\delta^{13}\text{C}$  differences between stocks, although it was not significant in the GAM. A larger sample size is needed to explore its effect in future analysis.

Nitrogen isotopes ( $^{15}\text{N}$ ) have been shown to reflect latitudinal variation in phytoplankton isotopes (Wada and Hattori, 1990). Maximum nitrate concentrations are easily reached at relatively low levels compared with other nutrient saturation concentrations, although more nitrate rises to the euphotic layer by vertical water mixing at high latitudes (Takai et al., 2000). The majority of squid from the western stock in this study were caught in the nitrate-rich

water off Kuril Island where significant isotope fractionation results in the low  $\delta^{15}\text{N}$  values of this area (Fig. 1, Takai et al., 2000). Isotope fractionation is also reduced by fast utilization of inorganic nitrogen compounds, so the phytoplankton often has lower  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  at higher latitudes than at lower latitudes. However, the negative relationship between latitude and isotopic variation is weak and may deteriorate with trophic variation, which differed by 1.2‰ in  $\delta^{15}\text{N}$  compared to 0.05‰ in  $\delta^{13}\text{C}$  among size groups (Fig. 3B). The diet of the winter-spring cohort changes from zooplankton to fish and squid as they grow along their northern migration (Watanabe et al., 2004). This may explain why  $\delta^{15}\text{N}$  values increased with latitude in the western stock. The autumn cohort also migrates north while maturing into adult squid (Ichii et al., 2009). The eastern stock may have similar  $\delta^{15}\text{N}$  variation, but it is possible that latitude was not significant in this analysis due to low sample size.

The  $\delta^{15}\text{N}$  signature tends to increase abruptly with ML in the eastern stock (Figs. 3 B, 4 C, F). The rate of  $\delta^{15}\text{N}$  increase is about 1‰ between ML groups except for between ML groups D and E (ML 350–400 mm and ML > 400 mm) (Fig. 3B). The overall  $\delta^{15}\text{N}$  difference was approximately 3‰, which represents nearly a trophic level (Mingawa and Wada, 1986; Petersen and Fry, 1987). During the northern migration of autumn cohort females from STFZ to SAFZ, the main prey items are fish and squid based on stomach content analyses (Watanabe et al., 2004; Bower and Ichii 2005). Meanwhile, males still living in the STFZ prey on lower trophic species such as euphausiids and amphipods. Therefore, sexual differences and mantle size variation in feeding behavior lead to trophic variation between size classes. This may also explain similar patterns in the western stock. A  $\delta^{15}\text{N}$  plateau was also observed in both stocks at different MLs, which is discussed in a previous study of the same species caught off Hawaii (Parry, 2008). The leveling off of  $\delta^{15}\text{N}$  values is observed in squid undergoing reproductive maturation. In this phase, somatic growth yields to reproductive growth, resulting in less nitrogen incorporated into other tissues (Parry, 2008). This pattern results from squid reproductive maturation and energy partitioning rather than migration dynamics.

Interestingly, an increasing trend continued after the plateau in  $\delta^{15}\text{N}$  values for both stocks (Figs. 4 and 5). Parry (2008) analyzed the muscle of *O. bartramii* in waters off Hawaii and observed  $\delta^{15}\text{N}$  values did not change after 350–400 mm ML. This suggested that *O. bartramii* reached peak (maybe maximum)  $\delta^{15}\text{N}$  concentrations when values became constant (Parry, 2008). In this study we analyzed beaks rather than muscle and reported low  $\delta^{15}\text{N}$  values in the beak, which mainly consists of chitin, compared to 3–4‰  $\delta^{15}\text{N}$  in muscle (Schimmelmann and DeNiro, 1986; Hobson and Cherel, 2006; Cherel et al., 2009b). Mean maximum  $\delta^{15}\text{N}$  values of muscle are nearly 15–16‰ (Parry, 2008), whereas mean maximum  $\delta^{15}\text{N}$  values of the beak are about 11–12‰. Chitin tends to have lower  $\delta^{15}\text{N}$  content than protein content, since protein is the main chemical structure in the pigmented part of the beak (Miserez et al., 2008). Beak pigmentation is related to the maturation process and occurs over a very short period of the squid life cycle (García, 2003). The maturation process is accompanied by protein enrichment due to rapid beak pigmentation, leading to the  $\delta^{15}\text{N}$  values in larger squid reaching a potential threshold.

We also compared the isotopic signatures between UB and LB in this study.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were significantly different between UB and LB and the UB exhibited lower values than the LB in both stocks (Fig. 3). The opposite scenario was observed in a previous study (Hobson and Cherel, 2006). Differences in chitin content between the UB and LB may be the most important factor influencing isotopic signatures. The UB size is larger than the LB's, so smaller individuals have more chitin structure resulting in a higher proportion of enriched  $\delta^{15}\text{N}$  pigmented parts in the LB than the UB. This is reversed for larger squid with a darker, more pigmented UB. Early beak development may also have an impact on the

isotopic signature in the UB and LB (Boletzky, 2007; Uchikawa et al., 2009). While the beak is an effective archival tissue to explore isotopic variation representing different feeding habitats and trophic levels, the LB is the best structure for such analysis (Cherel and Hobson, 2005; Hobson and Cherel, 2006; Ruiz-Cooley et al., 2006; Cherel et al., 2009b). In order to study the rare or deep-sea species, undigested beaks from stomachs of larger fish or marine mammals is the only material for the related investigation. The UB and LB ratio has been found to vary for some species (Xavier et al., 2011). Therefore, the UB should also be analyzed to evaluate functionality in order to comprehensively understand squid ecology.

In conclusion, we analyzed  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in the beaks of two *O. bartramii* stocks, and compared isotopic values between the UB and LB. The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were significantly different between the two stocks except for C/N and UB and LB significantly differed in all isotopic signatures. Both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values increased drastically with size in the eastern stock, while  $\delta^{15}\text{N}$  only slightly increased and  $\delta^{13}\text{C}$  varied minimally in the western stock. Trophic niche width was distinct between two stocks with little overlap. GAMs selected latitude and ML as significant variables in explaining  $\delta^{13}\text{C}$  variation, whereas ML was the only significant factor for  $\delta^{15}\text{N}$  in the eastern stock. In the western stock, none of the variables explained the  $\delta^{13}\text{C}$  variation, while latitude and ML were both significant for  $\delta^{15}\text{N}$ .  $\delta^{15}\text{N}$  leveled off in both stocks, which may relate to squid consuming the highest trophic levels of prey after reaching reproductive maturity. Stable isotope values of the UB were less than those of the LB, possibly due to different proportions of chitin between the UB and LB. Future studies might focus on increasing sample size, analyzing different structures and identifying the function of the UB.

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