Stable isotope analysis of the gladius to investigate migration and trophic patterns of the neon flying squid (Ommastrephes bartramii)

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\textbf{A B S T R A C T}

The neon flying squid, Ommastrephes bartramii is broadly distributed in subtropical and temperate regions of the world’s oceans. The North Pacific population is comprised of two spawning cohorts; the autumn cohort and winter-spring cohort, which despite their apparent contiguous hatching periods, have marked differences in the mantle length between the two cohorts, indicating differences in behavior. Especially, females of the autumn cohort after remaining in the subtropical zone for spring, in the first half of the summer migrate close to the vicinity of the southern boundary of the transition area. Subsequently they migrate into the subarctic waters to feed. However, for males details are less clear and it is considered that they remain in the subtropical zone without migrating to the subarctic feeding area. However little is known about the migration strategy of O. bartramii.

Stable isotope profiles along the gladius (internal chitinous shell) have been recently analyzed and form a promising tool to back-calculate a chronological record of changes in the diet and habitat. In this study, $\delta^{13}$C and $\delta^{15}$N values were measured along the gladius to reconstruct the feeding variations and habitat of the autumn cohort during ontogeny. The results of $\delta^{13}$C values support previous studies which inferred differences in migratory behavior between males and females. These differences occurred from 260 mm mantle length.

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1. Introduction

The oceanic cephalopod, neon flying squid (Ommastrephes bartramii) is broadly distributed in temperate regions of the world's oceans (Roper et al., 1984) and has been commercially exploited in the North Pacific by Japan since 1974, and more recently by the Korean, Chinese and Taiwanese fleets. Especially the annual commercial catch of Japan amounts to 10,000–40,000 tonnes and is mainly used by the processed foods industry (Ichii et al., 2006).

The neon flying squid (O. bartramii) has a one year life span and maximum mantle length (ML) reaches 600 mm in females and 450 mm in males in the North Pacific. The fished stock is formed of 2 seasonal cohorts; the autumn cohort and the winter–spring cohort (Yatsu et al., 1997, 1998). The spawning grounds of O. bartramii in the North Pacific form south of the Japanese mainland in the area from the Ryukyu Islands to the Ogasawara Islands and in the waters off the Hawaiian Islands in the sub–tropical areas with surface temperatures ranging 21–25 °C (Bower, 1996; Ichii et al., 2004). After hatching, the planktonic stage, the paralarvae, remain for several months in the spawning area (Kato et al., 2014) prior to the start of their northern migration to the feeding area in the subarctic transition region (Ichii et al., 2004). Females of the autumn cohort remain in the subtropical zone for spring then in the first half of the summer migrate close to the vicinity of the southern boundary of the transition area. Subsequently they migrate into subarctic waters to feed. The southern migration starts in approximately September to the spawning grounds in the subtropical zone (Ichii et al., 2009). However, for males details are less clear and it is considered that they may remain in the subtropical zone without a migration to the subarctic feeding area (Yatsu et al., 1997). Individuals during the
spring northern migration and in the summer feeding grounds feed on myctophids, cephalopods and crustaceans etc., with myctophids and cephalopods being major prey items (Seki, 1993; Watanabe et al., 2004). O. bartramii are an important prey item for large predatory fish (e.g. Xiphius) and marine mammals (Seki et al., 2002). O. bartramii form a high trophic level in the food web and due to their abundance have a major impact on the structure of the food web via top-down effects on prey items and can be considered as a key species within the food web. Furthermore, O. bartramii can be considered as an indicator species reflecting the condition of the ecosystem and as such is also of significant importance for research on this ecosystem (Ichii et al., 2007).

The isotopic signature of organisms has been extensively used in ecology to establish evidence for trophic relationships within food webs and also dietary changes during ontogeny (Ruiz-Cooley et al., 2006, 2010; Parry, 2008). Changes in δ13C and δ15N values can be effective to respectively trace differences in the trophic source of food and in the trophic level of food organisms within ecosystems including oceanic squid (Cherel et al., 2009; Lorrain et al., 2011). In other isotope studies, the isotopic signature has been used for analysis of squid migration routes (Lorrain et al., 2011; Ruiz-Cooley and Gerrodette, 2012; Arguelles et al., 2012; Ruiz-Cooley et al., 2013). Especially Ruiz-Cooley et al. (2013) used a novel analysis method from both bulk tissues and individual amino acids (phenylalanine and glutamic acid) in both gladius and muscle of D. gigas captured in the Northern California Current System enabling them to detail the various migration behaviors shown by this species. This method also enables estimation of the trophic level by a comparison of the δ15N values for glutamic acid and phenylalanine (Chikaraiishi et al., 2011). The method is a powerful tool for ecological food web studies, however the requirement for specialized equipment has precluded its more generally use.

In this study, we examine the potential to use samples of internal chitinous shell, the gladius (Hunt and Nixon, 1981), to reconstruct ontogenetic changes of habitat and feeding habits and any differences between males and females of the autumn cohort of O. bartramii.

2. Materials and methods

2.1. Sample collection

Sampling was carried out using individual squid sampled on the Kaiyo-maru of the Fisheries Agency during December of 2013. Table 1 shows the Sample ID, collection date, location and biological characteristics. All samples were individually sampled by jigging. The maximum hook depth was set at 150 m depth. The fishing survey was carried out at each sampling station between sunset and sunrise, and carried out for 1 h. All specimens (N = 9) were measured on board for mantle length (ML), sexed, and were assessed reproductive maturity. Reproductive maturity was assessed on the basis of the maturity stages 1–3 proposed by Watanabe et al. (2008). The gladius of each sample was stored at –20 °C until return to the land laboratory.

2.2. Stable isotope analysis

Morphologically the gladius (pen) can be differentiated into 3 sections, the prostracum, the conus and the rostrum (Bizkov and Arkhipkin, 1997). Due to the clarity of the daily rings, the prostracum section of the pen has been frequently used in aging studies (Perez et al., 1996; Bizkov and Arkhipkin, 1997).

Samples of the prostracum (Fig. 1) were cleaned with distilled water and KimWipes (Kimberly–Clark) and sectioned into 20 mm samples using acetone washed scissors. After each section of the prostracum was individually freeze-dried, it was pulverized in a mortar into a powder. Then to delipify, chloroform–methanol (2:1) was added and after 10 min of being centrifuged at 800 G the supernatant was removed. This process was repeated 3 times. After delipifying samples were dried in an oven at 80 °C for 1 h. Each powdered sample was then placed in a tin container (Thermo Scientific Co.) using an acetone washed spatula. The encapsulated tin container samples were then analyzed by mass spectrometry (Thermo Scientific Co., Delta VSM Isotope Ratio Mass Spectrometer). Isotope ratios of samples are reported as δ13C or δ15N values:

\[ \delta^{13}C = \frac{(R_{\text{sample}}/R_{\text{standard}}) - 1}{} \times 1000 \]

where the Rsample and Rstandard are the 13C / 12C and 15N / 14N ratios of measured samples and standards, respectively. Results are presented in ‰ notation with respect to Pee Dee Belemnite (PDB) scale for δ13C and atmospheric N2 (Air) scale for δ15N.

2.3. Oceanographic data

To describe the sampling site, we used sea surface data. The water mass structure has an important role in the formation of spawning grounds. Sea surface temperatures were derived using the NOAA Optimum Interpolation Sea Surface Temperature Ver. 2 climate dataset (http://www.esrl.noaa.gov/psd/data/gridded/data.noaa.oisst.v2.html, accessed 1 March 2013). The optimum interpolation sea surface temperature (SST) analysis is produced weekly on a one-degree grid. The analysis uses in situ and satellite SST’s plus SST’s simulated by sea-ice cover. Before the analysis is computed, the satellite data are adjusted for biases using the method of Reynolds (1988) and Reynolds and Marsico (1993).

For drawing and interpolation of environmental data we used the Generic Mapping Tools (Wessel and Smith, 1998) provided by Hawaii University.

2.4. Statistical model

To test differences between males and female, statistical models were applied. The models were built by generalized linear model (GLM). The GLM was used to generate a robust predictive equation. Two explanatory variables were included in the analysis using a GLM (Eqs. (2) and (3)), as follows:

\[ \delta^{13}C = \alpha_0 + \alpha_1 ML + \alpha_2 \text{Sex} \]  

(2)

\[ \delta^{13}N = \alpha_0 + \alpha_1 ML + \alpha_2 \text{Sex} \]  

(3)

The factor Sex is a categorical variable. ML indicates mantle length. The selection of variables was also based on the Akaike information criterion (AIC; Akaike, 1974). The finite sample corrected AIC was used in model selection.

Furthermore we observed that males and females were caught at the same place up to around 260 mm ML (Sakai et al., 2010).
Table 1
Ommastrephes bartramii Sample ID, collection date, location and biological characteristics.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Collection date</th>
<th>Location</th>
<th>Mantle length (mm)</th>
<th>Body weight (g)</th>
<th>Sex</th>
<th>Maturity stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>705-1</td>
<td>6 Dec 2013</td>
<td>28°30′N; 170°00′W</td>
<td>559</td>
<td>5480</td>
<td>Female</td>
<td>3</td>
</tr>
<tr>
<td>705-2</td>
<td>6 Dec 2013</td>
<td>28°30′N; 170°00′W</td>
<td>383</td>
<td>1286</td>
<td>Male</td>
<td>3</td>
</tr>
<tr>
<td>803-1</td>
<td>8 Dec 2013</td>
<td>28°00′N; 166°59′W</td>
<td>520</td>
<td>4144</td>
<td>Female</td>
<td>3</td>
</tr>
<tr>
<td>803-3</td>
<td>8 Dec 2013</td>
<td>28°00′N; 166°59′W</td>
<td>354</td>
<td>1396</td>
<td>Male</td>
<td>3</td>
</tr>
<tr>
<td>805-1</td>
<td>9 Dec 2013</td>
<td>29°52′N; 166°59′W</td>
<td>552</td>
<td>5034</td>
<td>Female</td>
<td>3</td>
</tr>
<tr>
<td>805-2</td>
<td>9 Dec 2013</td>
<td>29°52′N; 166°59′W</td>
<td>352</td>
<td>1436</td>
<td>Male</td>
<td>3</td>
</tr>
<tr>
<td>604MCN-1</td>
<td>2 Dec 2013</td>
<td>30°00′N; 172°55′W</td>
<td>510</td>
<td>4640</td>
<td>Female</td>
<td>3</td>
</tr>
<tr>
<td>604MCN-2</td>
<td>2 Dec 2013</td>
<td>30°00′N; 172°55′W</td>
<td>372</td>
<td>1434</td>
<td>Male</td>
<td>3</td>
</tr>
<tr>
<td>604MCN-3</td>
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<td>30°00′N; 172°55′W</td>
<td>348</td>
<td>1239</td>
<td>Male</td>
<td>3</td>
</tr>
</tbody>
</table>

For animals larger than 260 mm ML by the stepwise variable selection (Table 2). The effects of Sex were not significant and removed for the most parsimonious model for animals up to 260 mm ML and larger.

For the relationship between δ¹³C values and ML of males and females Fig. 4 shows there was no difference up to a ML size of approximately 260 mm ML, with values ranging between −19.5 and −18.5‰. After a size of 260 mm ML the patterns between males and females differ, for males values range −19.0 to −18.3‰ indicating a slight increase, while for females a decline was observed with values at the end point ranging from −20.3 to −18.7‰. In the GLM analysis, the models were improved from the full models (AIC: −45.07 for animals up to 160 mm ML and 31.43 for animals above 260 mm ML) to the most parsimonious models with the lowest AIC (−46.46 for animals up to 260 mm ML and 31.43 for animals larger than 260 mm ML) by the stepwise variable selection. The effects of Sex were not significant and removed for the most parsimonious model for animals up to 260 mm ML. Whereas, Sex and ML were taken into the model for animals larger than 260 mm ML as significant variables.

In Fig. 5 we show CN plots for animals up to 260 mm ML (Fig. 5a) and for sizes above 260 mm ML (Fig. 5b). The δ¹⁵N fluctuation range of animals up to 260 mm ML was larger than that of animals larger than 260 mm ML (F-test, p < 0.05). Based on these differences in patterns, we suggest that the mantle length of 260 mm forms a boundary indicating a change in migration and feeding behavior as discussed below.

3. Results

The sea surface temperature for the sampling points and the monthly averaged temperatures for each sampling point were compared (Fig. 2). The surface water temperatures of sampling points where mature individuals were collected were all in the range of 22.5–24.5 °C.

Subsequently the gladius measured from the tip of the conus as the gladius length (GL) showed a strong correlation with the mantle length (r² = 0.976, ML = 0.944 GL + 29.412). From this result, based on the gladius length, the mantle length at the time of each point of the gladius development can be back-calculated.

Fig. 3 shows δ¹⁵N values of the MLs. δ¹⁵N values of animals up to a size of approximately 260 mm ML showed a gradually increase. However the variation was large during this stage of growth and values of δ¹⁵N varied between 5 and 11‰. In all males (blue lines in Fig. 3), the δ¹⁵N value declined after reaching a size of 260 mm ML. By comparing the final values (end point for each individual) between individuals sampled at the same sampling station (Fig. 3), the δ¹⁵N values are extremely close between male and female samples.

In the GLM analysis, the models were improved from the full models (AIC: 209.39 for animals up to 260 mm ML and 226.53 for animals larger than 260 mm ML) to the most parsimonious models with the lowest AIC (207.4 for animals up to 260 mm ML and 224.92 for animals larger than 260 mm ML) by the stepwise variable selection (Table 2). The effects of Sex were not significant and removed for the most parsimonious model for animals up to 260 mm ML and larger.

4. Discussion

From the environmental analysis of the sampling stations, the surface waters were in the range of 22.5–24.5 °C and also from evidence that the sampled individuals were mature for this annual species, we suggest that the samples were collected prior to spawning in the spawning grounds. As O. bartramii dies after the spawning period (DFO, 1999), the gladii of these samples can be used to estimate values of δ¹⁵N and δ¹³C during the whole life cycle.

In this research, for sizes of up to 260 mm ML, the regression line of CN plot was steep (δ¹⁵N = 1.43 δ¹³C + 35.54, r² = 0.29, p < 0.05) indicating an inclusion in the diet of higher trophic level organisms based on the broad spread of values for δ¹⁵N but for δ¹³C the change in values was small. On the other hand, for sizes larger than 260 mm ML the regression line of CN plot was less steep (δ¹⁵N = 2.09 δ¹³C + 47.87, r² = 0.08, p < 0.05) with δ¹⁵N showing a more moderate variation compared to up to 260 mm ML sizes but δ¹³C showing a greater range of values.

In the relationship between δ¹³C values and ML for males and females there was no difference up to a ML size of approximately 260 mm ML. On the other hand, after a size of 260 mm ML the patterns between males and females differ. In marine ecosystems, δ¹³C values vary greatly with latitude and/or over inshore/offshore gradients (Lorain et al., 2011). Aita et al. (2011) based on isotope mapping showed that there are differences in δ¹³C values

Therefore two GLMs were carried out separately for animals up to 260 mm ML and animals larger than 260 mm ML by using the statistical software R, version 2.15.3 (http://cran.r-project.org/, accessed 1 March 2013).

![Fig. 2. Horizontal distribution of sea surface temperature and sampling points. Stars indicate sampling points.](image-url)
among different areas in the North Pacific. From these results, δ¹³C can be considered as an indicator of their habitat. Therefore, this also allows an examination of differences, as in the current research between males and females at the size approximating 260 mm ML, where the values for δ¹³C differ suggesting the potential phenomenon that the habitat area differed between sexes from approximately this ML stage and subsequently the migration pattern differs between sexes. Until now, the phenomenon of a difference in male and female migration behavior has been inferred based on fisheries catch data (Yatsu et al., 1997). However independent supporting evidence has not been available. Our isotope
analysis provides further evidence of this phenomenon and new knowledge of the size at the start of the migration.

In summary, the CN plot (Fig. 5) showed there were differences between males and females of the autumn cohort. In the case of males, they are considered to forage in a narrow latitudinal area during their life span. On the other hand, females up to 260 mm ML also forage in the same area. However from 260 mm ML females start to migrate northwards and forage over a more extensive latitudinal area.

In future research, by using evidence from oxygen isotope analysis and amino acid analysis, we anticipate the history of migration can be analyzed in closer relation to the marine environment and
thus enable a more detailed assessment of the migration behavior. Furthermore, we anticipate that differences in migration strategy of this species can be determined by comparing samples from the autumn cohort and winter–spring cohort.

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