

Trophic variation with length in two ommastrephid squids, *Ommastrephes bartramii* and *Sthenoteuthis oualaniensis*

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Abstract From 1998 to 2001 a total of 200 *Ommastrephes bartramii* (27 paralarvae) and 170 *Sthenoteuthis oualaniensis* (14 paralarvae) were sampled from the Central North Pacific. One group of non-paralarval *O. bartramii* ($n = 30$) was sampled from farther northwest in 1996. The $\delta^{15}\text{N}$ of mantle muscle of non-paralarval *O. bartramii* ($\bar{x} = 12.4\text{‰}$) was significantly greater than that of non-paralarval *S. oualaniensis* ($\bar{x} = 8.1\text{‰}$) ($P < 0.001$). The $\delta^{15}\text{N}$ of whole paralarvae of *O. bartramii* ($\bar{x} = 6.4\text{‰}$) was not significantly different than paralarvae of *S. oualaniensis* ($\bar{x} = 6.1\text{‰}$) ($P = 0.528$). There was no significant difference between the mantle muscle $\delta^{15}\text{N}$ values of male ($n = 95$, $\bar{x} = 13.3\text{‰}$) and female ($n = 18$, $\bar{x} = 12.9\text{‰}$) *O. bartramii* greater than 300 mm mantle length (ML) ($P = 0.15$). There was also no significant difference between the mantle muscle $\delta^{15}\text{N}$ values of male ($n = 15$, $\bar{x} = 7.2\text{‰}$) and female ($n = 26$, $\bar{x} = 7.3\text{‰}$) *S. oualaniensis* in the same size range ($P = 0.41$). Overall there was a distinct logistic increase in $\delta^{15}\text{N}$ with mantle length for *O. bartramii*, whereas *S. oualaniensis* showed an exponential increase in $\delta^{15}\text{N}$ with mantle length that was stronger within individual years than with all samples combined. In general, adult *O. bartramii* are more than a trophic level above *S. oualaniensis* (4.3‰, 1.3 TLs). Because of the nature of the sampling protocol, this study could not separate spatial and temporal effects on the $\delta^{15}\text{N}$ signals from each squid species. This study demonstrates the ability of stable isotope analyses to

differentiate trophic levels between squid species as well as track trophic changes across size ranges from paralarvae to adults. Additional research is needed to validate these trophic changes across size within individuals.

Introduction

Stable isotopes of nitrogen have proven to be a valuable tool in numerous studies on food webs and trophic relationships (Hobson and Welch 1992; Kling et al. 1992; Hobson et al. 1994). In the ocean a substantial portion of these studies have been devoted to investigating the phytoplankton/bacterial level (Hoch et al. 1992; Waser et al. 1998a, b), or, at higher trophic levels in coastal and near shore environments (Thomas and Cahoon 1993; Fantle et al. 1999; Montcreif and Sullivan 2001). Less work has been done on higher trophic levels in open ocean ecosystems (Sholto-Douglas et al. 1991; Rau et al., 1992; Kline et al. 1998; Takai et al. 2000). Studies on trophic relationships in general have shown body size to be one of the more important biological factors in structuring aquatic food webs and directing the flow of energy from the smallest organisms to the largest (Borgmann 1987; Peters et al. 1996; France et al. 1998). Ecosystem wide trophic enrichments of $\delta^{15}\text{N}$ with increasing size across taxa have been documented in several marine ecosystems (Sholto-Douglas et al. 1991; France et al. 1998; Kline 1997).

Recent studies on the isotopic signatures of cephalopods have revealed a great deal about cephalopod trophic structures (Kl-Cooley et al. 2004, 2006; Cherel and Hobson 2005) and stable isotopic signatures of cephalopods and their predators. This study aims to focus on two ecologically important pelagic squids in the Central North

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Pacific, *Sthenoteuthis oualaniensis* and *Ommastrephes bartramii*.

In the present study body size is examined in relation to trophic enrichment of $\delta^{15}\text{N}$ in two oceanic squids *O. bartramii* and *S. oualaniensis*. This researcher hypothesized that a rapid increase in $\delta^{15}\text{N}$ with mantle length should occur in these species as they grow in size from paralarvae to full-grown adult predators. In addition, based on previous stomach contents analyses by Parry (2006) *O. bartramii* adults were hypothesized to reach a higher trophic level (represented by higher $\delta^{15}\text{N}$ mantle muscle values) than *S. oualaniensis* at their respective maximum sizes collected.

Materials and methods

Details of collection (jigging) of non-paralarval squids are described in Parry (2006). During cruises aboard the FTS HOKUSEI MARU from 1998 to 2001 samples were taken from captured squids for stable isotope analyses (Fig. 1). Thirty *O. bartramii* were collected from a single station by colleagues aboard the FTS HOKUSEI MARU in 1996 from farther northwest than all the other samples (41°N latitude and 155°E longitude) and were not shown in Fig. 1. Paralarval squids were collected using a 1 m² ring plankton net with a 333 μm mesh with a plastic cod end attached to the tail end of the net. Each plankton tow was approximately 25 min long. The plankton net was lowered to roughly 40 m (estimated from wire angle and amount of cable) depth and was towed for 5 min. The net was then raised in 10 m increments every 5 min until it was just

below the surface at which point it was towed for another 5 min.

A total of 190 *O. bartramii* (27 paralarvae) and 170 *S. oualaniensis* (14 paralarvae) were sampled from 1998 to 2001 (Table 1).

After a squid was caught, weighed, and had its stomach removed, a roughly 2 cm² segment of muscle was cut away from the anterior mantle, in an area between the gladius and the mantle locking-cartilage, using metal forceps and dissection scissors. Forceps and scissors were cleaned with 100% methanol and rinsed with distilled water between samples. Care was taken to only sample muscle without attached cartilage or other non-muscular tissue (the dorsal photophore in *S. oualaniensis* was specifically avoided). The muscle samples were placed in 20 ml glass scintillation vials that were capped, labeled and immediately frozen at -20°C. For analysis in the lab the muscle sample was thawed and removed from the vial and a thin (3–4 mm thick) section of the muscle sample was cut away using dissection scissors and the edges were trimmed, leaving a section of muscle which had not been exposed to any outside materials. The sectioned muscle sample was then placed into a clean 20 ml glass scintillation vial and dried at 60°C overnight. The dried piece was then ground into a fine powder using a Wig-L-Bug dental amalgamater (Crescent Dental MFG. Co, Model 5AR). A sub-sample of the resulting powder was then weighed between 300 and 1,000 μg on a microbalance and placed into foil boats that were crushed into cubes to seal the material inside. This weight range was calculated so that sufficient N was obtained for analysis considering the C:N ratios of the samples used. For almost all paralarvae, whole animals

Fig. 1 Map showing sampling stations for all years except the northern station in 1996 (41°N latitude and 155°E longitude)

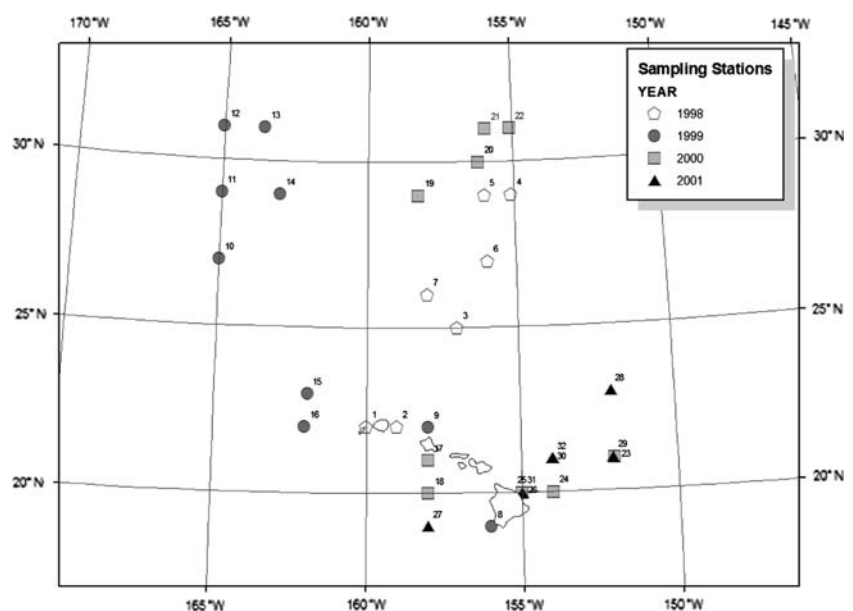


Table 1 The station numbers and years for each squid category collected

Station ID	Year	Ob ♂	Ob ♀	ObP	ObU	So ♂	So ♀	SoP
1	1998	–	–	–	–	–	7	–
2	1998	–	–	–	–	1	4	–
3	1998	–	–	7	–	2	3	–
4	1998	5	1	–	1	–	–	–
5	1998	5	–	–	–	–	–	–
6	1998	20	–	–	3	–	10	–
7	1998	14	–	–	–	–	5	–
8	1999	–	–	–	–	–	9	–
9	1999	–	–	2	–	–	–	1
10	1999	8	–	2	1	–	8	–
11	1999	3	–	3	1	–	6	–
12	1999	1	2	–	2	–	–	–
13	1999	17	6	–	–	–	–	–
14	1999	11	4	–	–	–	10	–
15	1999	4	1	–	–	–	4	–
16	1999	–	–	–	–	–	9	–
17	2000	–	–	–	–	–	–	2
18	2000	–	–	1	–	–	–	5
19	2000	1	–	–	–	–	9	–
20	2000	1	–	–	–	–	1	–
21	2000	3	–	–	–	–	–	–
22	2000	4	1	–	–	–	–	–
23	2000	–	–	2	–	2	6	4
24	2000	–	–	3	–	1	4	–
25	2000	–	–	4	–	1	8	2
26	2000	–	–	3	–	3	2	–
27	2001	–	–	–	–	–	1	–
28	2001	–	2	–	–	2	4	–
29	2001	16	–	–	–	–	6	–
30	2001	3	2	–	–	–	–	–
31	2001	–	–	–	–	–	20	–
32	2001	–	–	–	–	4	6	–
North station	1996	18	12	–	–	–	–	–
Total		134	31	27	8	16	140	14

Ob, *Ommastrephes bartramii*; So, *Sthenoteuthis oualaniensis*; ♂, male; ♀, female; P, paralarvae; and U, unknown sex

were analyzed because their small size excluded sampling specific muscle tissue. One paralarvae of *O. bartramii* was large enough (5 mm mantle length) for separation of mantle and head/viscera for a “one off” comparison of mantle and head/viscera.

All samples, which were investigated for ^{15}N , were analyzed using the system standardized by the Isotope Biogeochemistry Laboratory of the Marine Geology &

Geophysics Division of the University of Hawaii at Manoa. This system consisted of a Thermoquest/CE Instruments Automated Elemental Analyzer (model 1110 NC 2500) interfaced to a Finnigan Mat Delta-S stable isotope ratio mass spectrometer via a Finnigan MAT ConFlo II Elemental Analyzer (model 1110 NC 2500) using a Finnigan MAT ConFlo II (Continuous Flow) interface. Results were calculated using the standard δ notation relative to atmospheric N_2 (Air) for $\delta^{15}\text{N}$.

Ciclosanone was used as a reference material to monitor analytical behavior of the analyzer, and a total of 229 analyses of the standard were performed. Standards were analyzed at the beginning and end of each sampling session as well as internally (for every 5–7 samples a standard was analyzed). For $\delta^{15}\text{N}$ the mean and standard deviation were -4.8 and 0.2% . The minimum and maximum $\delta^{15}\text{N}$ values obtained for ciclosanone in this study were -4.0 and -5.4% .

The standardized δ notation is used to report all values for stable isotope ratios. The δ notation is expressed in the following relationship:

$$\delta X = \left[\left(R_{\text{sample}} / R_{\text{standard}} \right) - 1 \right] \times 1000$$

X is ^{15}N and R_{sample} is the ratio of the heavy to light isotope $^{15}\text{N}/^{14}\text{N}$ in the sample. R_{standard} is the ratio of heavy to light isotope or $^{15}\text{N}/^{14}\text{N}$ in atmospheric nitrogen.

Non-paralarval squids were analyzed separately from paralarval squids for basic information on mean $\delta^{15}\text{N}$ values. The mean size (ML) and $\delta^{15}\text{N}$ values for non-paralarval squids of each species were compared between years using ANOVA; however the northern *O. bartramii* samples were excluded from these comparisons because of their narrow size range and the obvious effects of size on $\delta^{15}\text{N}$. Because of the observed effect of size (ML) on $\delta^{15}\text{N}$, *O. bartramii* of ML > 300 mm (the size where $\delta^{15}\text{N}$ seemed to plateau) were used for gender comparisons. *O. bartramii* of ML > 300 mm were compared with females using a two-sample t test. Because of the observed effect of size (ML) on $\delta^{15}\text{N}$, and that most females were larger than males, only *S. oualaniensis* of ML < 161 mm (the largest size male collected) were used for gender comparisons. The $\delta^{15}\text{N}$ values for *O. bartramii* and *S. oualaniensis* were plotted against mantle length, and model regressions were fitted to the data. Regressions were plotted for all *S. oualaniensis* combined, as well as within individual years because a fairly continuous size representation was obtained through most years. Regressions were only plotted for all *O. bartramii* combined because of the lack of smaller sized squids during most years. For species comparisons the $\delta^{15}\text{N}$ values of each species (paralarval and non-paralarval) were compared to each other using two sample t tests.

Results

O. bartramii

The mean $\delta^{15}\text{N}$ of mantle muscle of all non-paralarval *O. bartramii* was 12.4‰ ($n = 165$, $\text{SD} = 1.9\%$). The mean $\delta^{15}\text{N}$ of all but one of the whole paralarval *O. bartramii* was 6.4‰ ($n = 26$, $\text{SD} = 1.2\%$). The mantle and head/viscera were separated for one paralarvae (ML = 5 mm), and their results were comparable: mantle: $\delta^{15}\text{N} = 5.2\%$; head/viscera: $\delta^{15}\text{N} = 4.9\%$. There was no significant difference between the mean mantle muscle $\delta^{15}\text{N}$ values of male ($n = 95$, $\bar{x} = 13.3\%$, $\text{SD} = 1.0\%$) and female ($n = 18$, $\bar{x} = 12.9\%$, $\text{SD} = 1.2\%$) *O. bartramii* greater than 300 mm ML in years 1998–2001 (2 sample *t*-test, $P = 0.147$). There was also no significant difference between the mean mantle muscle $\delta^{15}\text{N}$ values of male ($n = 18$, $\bar{x} = 10.2\%$, $\text{SD} = 0.26\%$) and female ($n = 12$, $\bar{x} = 10.3\%$, $\text{SD} = 0.45\%$) *O. bartramii* from the single 1996 station (2 sample *t*-test, $P = 0.461$).

The mean $\delta^{15}\text{N}$ for non-paralarval *O. bartramii* (omitting the 1996 samples because they were collected from a far removed site, and their small size and narrow size range) was significantly different between years 1998 and 2001 (Table 2) (ANOVA, $n = 133$, $df = 3$, $P = 0.012$). The mean size (mm) of *O. bartramii* was also significantly different between years 1998 and 2001 (Table 2) (ANOVA, $n = 133$, $df = 3$, $P < 0.001$).

The $\delta^{15}\text{N}$ values for all *O. bartramii* samples combined showed a sigmoidal relationship (4-parameter logistic curve, adjusted $r^2 = 0.81$) with mantle length (Fig. 2).

S. oualaniensis

The mean $\delta^{15}\text{N}$ of mantle muscle of all non-paralarval *S. oualaniensis* was 8.1‰ ($n = 156$, $\text{SD} = 1.1\%$). The mean $\delta^{15}\text{N}$ value for paralarvae (whole animal samples) was 6.1‰ ($n = 14$, $\text{SD} = 1.4\%$). There was also no significant difference between the mantle muscle $\delta^{15}\text{N}$ values

Table 2 The mean and standard deviation of mantle length values (mm) of non-paralarval *O. bartramii* individuals used for SIA during years 1996, 1998–2001

Year	Number of individuals	Mean ML, mm (SD)	Mean $\delta^{15}\text{N}\%$ (SD)
1996	30	224 (31)	10.3 (0.3)
1998	49	299 (62)	13.3 (2.1)
1999	51	359 (116)	12.2 (1.7)
2000	10	348 (72)	13.6 (0.6)
2001	23	398 (72)	13.1 (1.2)

ML mantle length

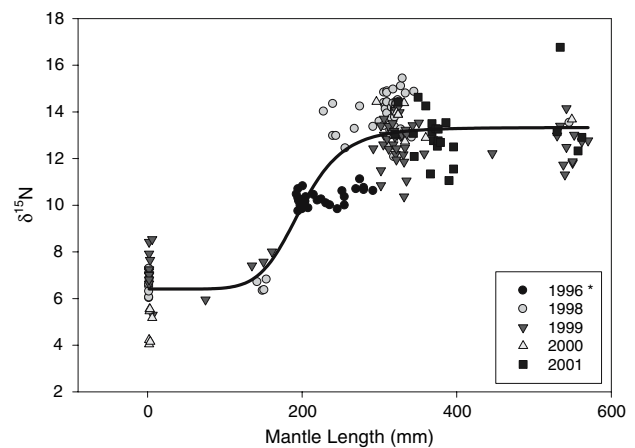


Fig. 2 The $\delta^{15}\text{N}$ values for mantle muscle taken from *O. bartramii* during all 4 years of sampling for SIA. The curve is a four parameter logistic curve, computer fit with Sigmaplot 9. *All squids were caught on the HOKUSEI MARU except the northern 1996 samples. $f = y_0 + a \times \exp(-\exp(-(x-x_0)/b))$; $a = 6.8482$, $b = 38.0979$, $x_0 = 183.2625$, $y_0 = 6.4261$

of non-paralarval male ($n = 15$, $\bar{x} = 7.2\%$, $\text{SD} = 0.6\%$) and female ($n = 26$, $\bar{x} = 7.3\%$, $\text{SD} = 0.4\%$) *S. oualaniensis* in a similar size range < 161 mm ML ($P = 0.41$). The mean $\delta^{15}\text{N}$ for non-paralarval *S. oualaniensis* was significantly different between years and increased from 1998 to 2001 (Table 2) (ANOVA, $n = 156$, $df = 3$, $P < 0.001$). The mean size (mm) of *S. oualaniensis* was also significantly different between years 1998 and 2001 (Table 2) (ANOVA, $n = 156$, $df = 3$, $P < 0.001$) (Table 3).

The $\delta^{15}\text{N}$ values for all *S. oualaniensis* mantle muscle samples showed an exponential increase (single exponent, 3-parameter) with mantle length (Fig. 3, $r^2 = 0.56$).

Species comparisons

The $\delta^{15}\text{N}$ values for mantle muscle of all non-paralarval *S. oualaniensis* and *O. bartramii* were significantly different (two-sample *t*-test, $P < 0.001$) with *O. bartramii* being 4.3‰ greater on average than *S. oualaniensis*. The $\delta^{15}\text{N}$ values for mantle muscle of all paralarval

Table 3 The mean and standard deviation of mantle length values (mm) of non-paralarval *S. oualaniensis* individuals used for SIA during years 1998–2001

Year	Number of individuals	Mean ML (SD)	Mean $\delta^{15}\text{N}\%$ (SD)
1998	32	168 (17)	7.4 (0.8)
1999	46	188 (40)	8.6 (1.4)
2000	35	201 (32)	8.2 (0.9)
2001	43	234 (56)	8.6 (0.9)

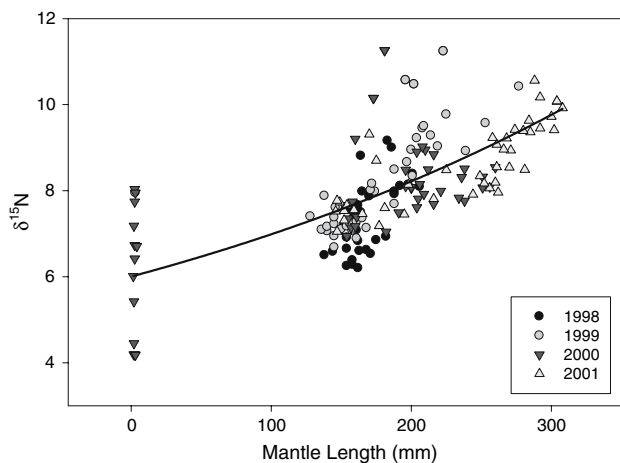


Fig. 3 The $\delta^{15}\text{N}$ values for mantle muscle taken from *S. oualaniensis* during all four years of sampling for SIA. The curve is a single three parameter exponential curve, computer fit to the data using Sigmaplot 9. $f = y_0 + a \times b^x$, $y_0 = 4.2081$, $a = 1.8726$, $b = 1.0037$

S. oualaniensis and *O. bartramii* were not significantly different (two-sample *t*-test, $P < 0.528$) with *O. bartramii* being only 0.3‰ greater on average than *S. oualaniensis*. The $\delta^{15}\text{N}$ values for all squid samples combined (regardless of species) showed a sigmoidal relationship (4-parameter logistic curve, adjusted $r^2 = 0.76$) with mantle length (Fig. 4).

Discussion

O. bartramii

The overall $\delta^{15}\text{N}$ values for *O. bartramii* are in general agreement with other published results for this species.

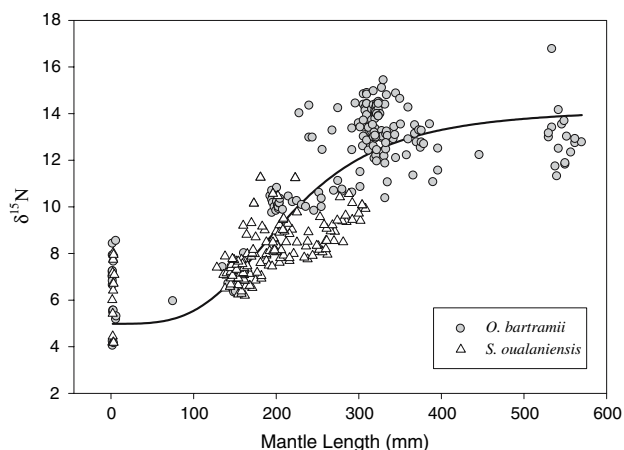


Fig. 4 The $\delta^{15}\text{N}$ values for mantle muscle taken from *S. oualaniensis* and *O. bartramii* during all years of sampling for SIA combined. The curve is a four parameter logistic curve, computer fit with Sigmaplot 9. $f = y_0 + a \times \exp(-\exp(-(x-x_0)/b))$; $a = 9.2234$, $b = -3.5732$, $x_0 = 215.1033$, $y_0 = 4.9834$

Takai et al. (2000) reported $\delta^{15}\text{N}$ values ranging from 11.8 to 12.1‰ for five female *O. bartramii* with mean mantle lengths of 360 mm (SD = 19 mm) captured off Japan. Gould et al. (1997) reported $\delta^{15}\text{N}$ values between 10.0 and 15.1‰ for 44 *O. bartramii* (170–446 mm ML) taken from albatross stomachs in the North Pacific transition zone (35–46°N, 170°E–148°W). The most distinctive feature of *O. bartramii*, besides the absolute values, was the increase in $\delta^{15}\text{N}$ with size and the sigmoidal shape of the curve.

Kline et al. (1998) observed species-specific increases in $\delta^{15}\text{N}$ with increasing fish length (≈ 60 –480 mm) for four species of salmonids in waters off Northern Alaska. The $\delta^{15}\text{N}$ versus length data for all four species were fitted with second-order polynomial regressions that detailed rapid increase in $\delta^{15}\text{N}$ with length followed by a flattening of the curve and a plateau at the greatest fish lengths. The greatest range of $\delta^{15}\text{N}$ values in one species was roughly 10‰, comparable to what was found over the whole size range of *O. bartramii* in this study. In a separate study Kline (1997) observed a sigmoidal relationship between $\delta^{15}\text{N}$ and length for combined nekton classes (fishes and squids) in Prince William Sound that ranged from 2 to 4 trophic levels (≈ 8 –16‰). This range coincides closely with the overall range of *O. bartramii* as it makes its transition from paralarvae (≈ 6 ‰) to adult (≈ 13 ‰). It appears that the sigmoidal increase observed for *O. bartramii* in this study could show ontogenetic trophic increases from paralarval stages all the way through adulthood which has been shown in previous studies (Cherel and Hobson 2005; Ruiz-Cooley et al. 2004).

The $\delta^{15}\text{N}$ mantle data for *O. bartramii* shows an initial plateau followed by a rapid increase in trophic position around 100 mm ML followed by a trophic plateau beginning around 250–300 mm mantle length. This rapid increase in $\delta^{15}\text{N}$ around 100 mm ML matches well with documented shifts in squid feeding around that size from epi-pelagic plankton to nektonic shrimps and fishes (Shchetinnikov 1992). Size appears to be a major factor in determining trophic position in this species. It is conceivable that *O. bartramii* reaches a trophic plateau at which increases in trophic position with size are not possible because of physical constraints of the squid itself (handling constraints) or availability of appropriate prey of higher trophic levels.

Aside from mantle length, the main sources of variation in $\delta^{15}\text{N}$ values of mantle muscle were variation between years and geographic area. Uncertainty exists as to the nature of the observed yearly differences in *O. bartramii* but one would expect a certain amount of variation between years. In spite of the 1996 samples being caught from a much different geographical region of the ocean, the $\delta^{15}\text{N}$ values fit well in the regression between paralarval samples, the few smaller sized *O. bartramii* caught in the

common sampling areas, and the full-sized adults. In this case at least it appears that the size of the animals was a greater factor in determining trophic position or $\delta^{15}\text{N}$ signal than geographic variation.

S. oualaniensis

The $\delta^{15}\text{N}$ values reported here are generally in agreement with published values for *S. oualaniensis*. Takai et al. (2000) reported mean $\delta^{15}\text{N}$ of 10.0‰ (5 individuals; mean ML = 217 mm) for *S. oualaniensis* caught off Japan (26.30°N, 144.00°E). This value is higher than the mean value obtained for non-paralarval samples for this study, but well within the overall range of variation for squids sampled. Squid from the same family (*Todarodes pacificus*) with a similar size range (207 mm, SD = 7) taken from the Japan Sea also had $\delta^{15}\text{N}$ values (10.5‰) comparable to *S. oualaniensis* of that size. Takai et al. (2000) found large regional differences in $\delta^{15}\text{N}$ values for *S. oualaniensis* throughout both the Pacific and other oceans. The greatest $\delta^{15}\text{N}$ values Takai et al. found for *S. oualaniensis* occurred off of Peru (14.00°S, 85.00°W) and were exceptionally high (16.3‰), a fact they attributed to denitrification of upwelled nitrate in the area (Cline and Kaplan 1975).

The increase of $\delta^{15}\text{N}$ with size for mantle muscle samples in *S. oualaniensis* did not plateau but increased exponentially over the entire size range of squid caught during this study. This indicates that *S. oualaniensis* does not reach a trophic plateau over the size ranges sampled, as occurred in *O. bartramii*. The exponential relationship of $\delta^{15}\text{N}$ with mantle length was stronger in individual years than for all the data combined, except in 1998, which showed a weak relationship. The weak exponential relationship between $\delta^{15}\text{N}$ and mantle length for 1998 is probably due to the lack of samples on each end of the size spectrum. In 1998 there were no paralarval samples taken, and few *S. oualaniensis* adults above 200 mm ML were captured.

Species comparisons

The $\delta^{15}\text{N}$ values seem to accurately describe relative trophic positions in this study. Using an established value of about 3.4‰ enrichment per trophic level (Mingawa and Wada 1986; Petersen and Fry 1987) adult *O. bartramii* in general are more than a trophic level above *S. oualaniensis* (4.3‰, 1.3 TLs), which would be expected if *S. oualaniensis* is a major prey for *O. bartramii*, a situation which was anticipated at the beginning of this study. Stomach analyses, however, have shown that *S. oualaniensis* is

rarely preyed upon by *O. bartramii* (Parry 2006). This situation re-affirms the need to couple isotopic analyses with known feeding data.

The patterns of $\delta^{15}\text{N}$ change with size for *O. bartramii* and *S. oualaniensis* were different, a sigmoidal curve versus an exponential curve. The most likely factor that would contribute to this difference is differences in feeding at the higher adult size range attained by *O. bartramii*. *S. oualaniensis* and *O. bartramii* clearly show differential feeding patterns according to stomach contents analyses (Parry 2006), but data are few for *O. bartramii* in the size range where increases in $\delta^{15}\text{N}$ versus ML are most rapid. It appears that, in similar size ranges, the two squids likely exhibit similar trophic ecology but as *O. bartramii* attains larger sizes than *S. oualaniensis* (at least in Hawaiian waters) it is able to exploit higher trophic levels which are unavailable to *S. oualaniensis*.

An alternate hypothesis for the difference in the different curves for $\delta^{15}\text{N}$ versus ML seen in each species is migration. *O. bartramii* migrates from near Hawaiian waters, north to feeding grounds in the Subarctic Frontal Zone. The northern habitat may be an ecosystem with a different baseline signal for $\delta^{15}\text{N}$. Nitrogen fixation by organisms such as *Trichodesmium* sp. can introduce low (close to zero) $\delta^{15}\text{N}$ nitrogen into the ecosystem (Mingawa and Wada 1986) and may be important in oligotrophic waters around Hawaii. Upwelled nitrate, on the other hand, has more enriched $\delta^{15}\text{N}$ values (Liu and Kaplan 1989) and in the northern habitat may play a more important role in supplying the nitrogen demands of the phytoplankton resulting in a higher baseline trophic signal. Values for *O. bartramii* caught off Japan (Takai et al. 2000) (39°30'N, 155°E) and in the North Pacific transition zone (Gould et al. 1997) (35–46°N, 170°E–148°W), however, did not show substantially different values, 12.1 and 11.7‰, respectively. Nevertheless, *O. bartramii* individuals probably gain the majority of their mass at the feeding grounds and should incorporate some of the northern signal. When the squid move south as they reach maturity, somatic growth may slow in favor of reproductive growth and hence incorporate little of the possibly lighter southern signal into muscle. The signal of the tissues, therefore, could result from a combination of rapid growth of new tissue in the north and then maintenance of old tissue in more southern waters. This hypothesis could explain the plateau seen in the $\delta^{15}\text{N}$ curve. The northern group of *O. bartramii* was substantially smaller than those caught closer to the study area and was more similar in size to *S. oualaniensis*. Similarly the $\delta^{15}\text{N}$ values of the northern group of *O. bartramii* were closer in value to those of large *S. oualaniensis* lending support to the feeding hypothesis for the $\delta^{15}\text{N}$ patterns with size rather than migration. One would expect that, if *O. bartramii* were indeed subjected to

a radically different, heavy isotopic signal from farther north, that these smaller *O. bartramii* would show a heavier signal for their size.

Conclusions

If $\delta^{15}\text{N}$ is reflective of trophic level, and the trophic level of an animal is dictated by its size, then the $\delta^{15}\text{N}$ signal will, to some extent, be dependent on a number of variables affecting the animal's size such as growth rate, food composition, and condition of the animal.

The variability in using stable isotopes requires that its use be coupled with other techniques that will provide a context or background within which the results can be analyzed. Stomach contents analysis or behavioral feeding studies would provide the best framework to base SIA results on. The differences in feeding by *S. oualaniensis* and *O. bartramii* revealed in the stomach contents (Parry 2006) clearly indicates different trophic ecology for these squids. Both squids fed heavily on Myctophidae, although *O. bartramii* also feeds on certain piscivorous fishes. The SIA results indicate that these animals are substantially more than a trophic level apart based on the mean mantle tissue values although individual variability was great.

The SIA results of this study indicate that the $\delta^{15}\text{N}$ values can show consistent general trends that are indicative of trophic interactions, in spite of the many factors that affect the results. Not surprisingly, substantial variability within these general patterns exists. A fundamental question of stable isotope analyses still exists; how much of the variability represents true trophic variability and how much represents other sources of bias? If this variability represents true trophic variability, then it suggests that trophic structure is much more complex than previously thought.

In general *O. bartramii* shows a final plateau in trophic level that begins at sizes that are near *S. oualaniensis*' maximum size in Hawaiian waters. *S. oualaniensis* does not show a plateau. If absolute size is the primary factor determining the presence of a plateau in these squids, then *S. oualaniensis* does not get quite large enough to reach a trophic plateau.

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