THE TROPHIC ECOLOGY OF TWO OMMASTREPHID SQUID SPECIES, 
OMMASTREPHES BARTRAMII AND STHENOTEUTHIS OUALANIENSIS, IN THE 
NORTH PACIFIC SUB-TROPICAL GYRE

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Abstract

This paper examines the trophic ecology of the squids *Ommastrephes bartramii* and *Sthenoteuthis oualaniensis*, using stomach contents and stable isotopic techniques. Simple energetics models were constructed using some of the data collected.

Samples for stomach contents were collected from 1996-2001 and 323 *O. bartramii* and 302 *S. oualaniensis* were captured. Fish and cephalopod remains dominated the stomach contents. Myctophids were found most abundantly in both squids, *Symbolophorus evermanni* was recovered at the highest percentage (7.5%) in *O. bartramii*, while *M. lychnobium* or *spinosum*, *Lobianchia gemelleri*, and *Myctophum selenoides* were all recovered at similar proportions (~5%). Of the Myctophidae found in *S. oualaniensis* stomachs, *S. evermanni* was the most abundant (37%), followed by *C. warmingii* and *H. proximum/rheinhardti* (both ~15%), and *M. lychnobium* (3%). Beaks from Onychoteuthidae occurred most frequently (14%) in *O. bartramii*, while Histiotethidae, Enoploteuthidae, and unidentified beaks all occurred at similar frequencies (10-12%). In *S. oualaniensis*, Enoploteuthidae occurred most frequently (17%) followed by Onychoteuthidae (10%). The diet of *O. bartramii* was more general while *S. oualaniensis* diet was more specialized on certain prey groups.

From 1998-2001 samples were taken from captured squids for stable isotope analyses, 143 *O. bartramii* and 160 *S. oualaniensis*. SIA was conducted on the mantle muscle of *O. bartramii* that were divided into five categories based on mantle length, (1-7 mm) was 6.4%, (75-100 mm) was 6.9%, (200-300 mm) was 11.1%, (300-400) was 13.3%, (400-570 mm) was 12.8%. The δ¹⁵N values for all *O. bartramii* mantle muscle samples showed a logistic increase with mantle length. The mean δ¹⁵N value for *S. oualaniensis* sub-adult and adult mantle muscle (128 to 324 mm) was 8.2 ‰. The mean δ¹⁵N value for paralarvae was 6.2‰. The δ¹⁵N values for all *S. oualaniensis* mantle muscle samples showed an exponential increase with mantle length.

Eye lenses, and blood samples were also taken from each squid species and showed similar patterns of δ¹⁵N increase with mantle length respectively, blood was unavailable in the smaller size ranges of *O. bartramii*.
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Chapter I: Stomach contents analysis (SCA)

Introduction

This paper examines the trophic ecology of *Ommastrephes bartramii* and *Sthenoteuthis oualaniensis*, both species are members of the order Teuthoidea, suborder Oegosida, family Ommastrephidae. Most oceanic squids in this family are large fast moving species that are key members in the pelagic ecosystem (Roper, 1984). Ommastrephid squids play an important dual role in the pelagic realm as both predator on wide ranging meso- and epipelagic species, and as prey for large top end predators such as swordfish, sharks, tuna, cetaceans and many others (King and Ikehara, 1956; Okutani et al., 1976; Seki, 1993).

*O. bartramii* is a cosmopolitan squid within the temperate and subtropical regions of the Pacific, Atlantic, and Indian Oceans (Clarke, 1966; Roper et al., 1984). In the North Pacific, *O. bartramii* generally occurs in a region between 25 and 50° N latitude. These squid undergo a yearly roundtrip migration from their feeding grounds near the Subarctic Boundary (36-46° N) during the summer/fall and head south to subtropical spawning grounds (20-35° N) during the fall/winter (Murata and Nakamura, 1998). Spawning is known to occur in at least two general geographical areas, southeast of Honshu, Japan, and in waters near the Hawaiian Archipelago (Saito and Kubodera, 1993; Young and Hirota, 1990; Bower, 1996). *O. bartramii* appears to be a vertical migrator, occupying near surface waters during the night, and descending to depths during the day. Two studies done on the daytime depth distribution of *O. bartramii* have indicated two depth ranges in the North Pacific, 150-300m in the in the open ocean (Murata and Nakamura, 1998) and 400-700m near the Ogaswara Islands off Japan (Nakamura, 1993), the difference presumed to be due to water clarity at the different latitudes. *O. bartramii* is a prey item for a host of pelagic marine mammals, and sperm whales (*Physeter macrocephalus*) in particular seem to feed heavily on this squid (Kajimura and Loughlin, 1988; Okutani et al., 1976; Okutani and Satake, 1978). In addition, *O. bartramii* is a mainstay in the diet of the blue shark (*Prionace glauca*) and commercially important species such as swordfish (*Xiphias gladius*), blue marlin (*Makaira mazara*), striped
marlin \((Tetrapurus audax)\), yellowfin tuna \((Thunnus albacares)\), bigeye tuna \((T. obesus)\), and bluefin tuna \((T. thynnus)\) among others (Nakamura, 1985; Reintjes and King, 1953; King and Ikehara, 1956; Pinkas et al., 1971; Matsumoto, 1984). In addition to being forage for commercially valuable species, \(O. bartramii\) is itself a commercially exploited species. In the North Pacific, \(O. bartramii\) was the target of an international drift-net fishing fleet of approximately 500 vessels. Annual landings between 1985-1990 were between 248-378,000 tons (Murata and Nakamura, 1998). Catch rates have been reduced following the moratorium on drift-net fishing in 1992, and \(O. bartramii\) remains an under-utilized resource.

\(Sthenoteuthis oualaniensis\) ranges from the tropical to sub-tropical waters of the Indian and Pacific oceans, and occasionally extends into temperate waters (Roper, 1984). There are purported to be up to five separate forms of \(S. oualaniensis\) (Nesis, 1993), the "typical" form that occupies the Hawaiian waters is the form being discussed here. \(S. oualaniensis\) is a permanent inhabitant of Hawaiian waters, and apparently spawns throughout the year, with an increase in spawning during the summer months (Harman and Young, 1985). Adult and sub-adult \(S. oualaniensis\) are thought to vertically migrate, inhabiting near-surface waters during the night and descending to depth during the day. Daytime observations of \(S. oualaniensis\) have occurred in submersibles at depths of 400 and 1080 m in the Indian Ocean (Moiseev, 1991). Distribution data from around the Hawaiian islands suggest that these squids occur deeper than 650 m during the day (Young and Hirota, 1998). Smaller \(S. oualaniensis\) are important prey items for a range of daytime feeding seabirds (Ashmole and Ashmole, 1967; Seki and Harrison, 1989) indicating that the younger squids are still abundant in near-surface waters during the day. \(S. oualaniensis\) is also a major component in the diets of commercial fish species such as yellowfin tuna \((T. albacares)\), bigeye tuna \((T. obesus)\), bluefin tuna \((T. maccopyii)\), and skipjack tuna \((K. pelamis)\) (Reintjes and King, 1943; Kornilova, 1981; Brock, 1985; Young et al., 1997). \(S. oualaniensis\) has also been the target of fisheries in Japan and China (Okutani and Tung, 1978) as well as being targeted for food and bait in the Hawaiian tuna handline fishery (Yuen, 1979).

There are limited data on the specific feeding habits of both \(O. bartramii\), and \(S. oualaniensis\) in the Central North Pacific. Seki (1993) did some general stomach contents
analyses on *O. bartramii*, but did not categorize the prey items past family in most cases. Shchetinnikov (1992) performed a detailed analysis on *S. oualaniensis* in the Eastern Pacific and found heavy predation by *S. oualaniensis* on young *Dosidicus gigas* which is a member of the same subfamily. Because *O. bartramii* and *S. oualaniensis* are also members of the same subfamily, heavy predation of one on the other was expected at the inception of this study. In addition, few studies have looked at the feeding habits of both of these species during the same study in the same area (Dunning and Brandt, 1985), and none have done so in the Central North Pacific.

These two species overlap in habitat during the winter when *O. bartramii* migrate south to spawn. Much of the area of overlap appears to be a marginal habitat for *S. oualaniensis* during the winter, as females there do not seem to mature properly (Young and Hirota, 1998). Interactions of closely related squid species, such as *O. bartramii* and *S. oualaniensis*, may have profound impacts on shaping the life histories of these animals. As potential competitors, or even predator and prey, the impacts that these species have on each other may be reflected in differing overall life history strategies. Direct effects such as competition for resources or predator prey interactions would only occur in areas of temporal and spatial overlap such as in near Hawaiian waters during the winter. Young and Hirota (1998) found that, in Hawaiian waters, the mean size of *S. oualaniensis* commonly caught at jigging stations was about 40% of the mantle length (ML) of the smallest *O. bartramii* females. This size difference places much of the *S. oualaniensis* population well within the size range of potential squid prey for *O. bartramii*.

Studying *O. bartramii* and *S. oualaniensis* where their populations overlap may reveal interactions such as competition for food resources or predator prey interactions that affect of determine habitat and life history patterns for these squids. Studying the feeding ecology of these squids will add to our understanding their effect on the ecosystem since they are viewed as major predators of mesopelagic fauna. Investigations of the feeding ecology of *O. bartramii* and *S. oualaniensis* can elucidate their impact on energy transfer in the oceanic ecosystem.
Materials and methods

Bias and difficulties in SCA

In order to use stomach contents to analyze diet of squids, the squid must first be captured. Due to the ban on drift nets, the sampling methods available for capturing squids for this study were few, and pole and line capture was the most effective method available although the method introduces some bias. This method used light attractants, that alter normal environmental conditions and can cause aberrant feeding behavior. In addition, using a sampling method that relies on attraction generally captures only hungry or partially sated animals. The sampling milieu of pole and line jigging for squids is highly restricted to the near-ship environment and is limited for practical purposes to a few tens of meters in depth.

The frequency of occurrence, in which the number of stomachs containing specific prey is recorded as a percent of all stomachs, is a simple and direct measurement of the feeding spectrum of predators (Frost, 1946; Hunt and Carbine, 1951). Identification of prey was determined for this study using some of the hard indigestible parts of prey that resist physical destruction by the squid predators. This method does not provide relative contribution of prey to the whole diet because if a species occurs once or multiple times in a stomach, it is still only recorded as being present in the stomach.

The percent abundance of prey refers to the percentage of each prey type out of all prey items counted (Crisp, et al., 1978; Ikusemiju and Olaniyan, 1977). Determining abundance relies on the ability of the investigator to record prey items as discrete units, which can be difficult in squid that finely chop their prey with their beaks. As in the frequency of occurrence method, otoliths and beaks of prey that are resistant to physical destruction were used. Percent abundance tends to overestimate the energetic contribution of large numbers of small items of prey. Another problem with this technique is that episodic feeding by a few individuals has the same percentage impact as low level feeding by many individuals, but these instances are very different ecologically. Some of these problems can be ameliorated when this technique is coupled with data on frequency of occurrence of prey items.

Otoliths and beaks were used as the basis for much of the analyses done in this study. Otolith morphology has been shown to be highly species specific in many cases
(Scott, 1903; Fitch and Brownell, 1968; Schmidt, 1968; Morrow, 1979; Clarke, 1986; and others), although some studies have shown that intra-specific variation can be substantial enough to cause uncertainties in identification in some species (Norden, 1961; Casteel, 1974). Cephalopod beaks also have been used effectively for species identification (Clarke, 1966). Using otoliths and beaks found in the stomachs and not fresh material, such as tissue, to identify prey items helps to reduce bias from aberrant feeding around the ship. Presumably only the freshest material in the stomachs has been subject to the effects of the ship and attraction lights. Free otoliths and beaks must have been retained in the stomach for some time in order to be released from their surrounding tissue. Erosion of otoliths in a fairly acidic gastric environment can cause difficulties in identification. Because cephalopod digestive tracts are nearly neutral (Bidder, 1966; Wallace et al., 1981) calcareous structures such as otoliths retain the details of morphology. Another possible bias concerns otolith and beak accumulation in the stomachs of these squids, representing multiple meals (see Pitcher, 1980; Hunter 1983). In cephalopods, indigestible material apparently does not remain in the stomach for long, although the exact residence time is unknown (Bidder, 1966; Wallace et al., 1981; Andrews and Tansey, 1983). Of course, for an otolith or beak to be present in the stomach, the head or buccal mass of the prey must be consumed. Squids do not always consume the heads of the fishes that they eat (Bradbury and Aldrich, 1969). Porteiro et al. (1990) found that Loligo forbesi consumed the heads of smaller fishes while rejecting the heads of larger fish, biasing stomach results based on otoliths towards smaller fishes. Also, squids may not have the stomach capacity to consume all of a large prey that is captured. Finally, using hard parts that resist digestion to determine prey types may introduce bias against prey species that lack hard parts and are rapidly digested (Pierce et al., 1994).

Dietary measurements-

In this study niche breadth is used to quantify the degree of specialization to which a squid utilizes available resources (see Krebs, 1989; Cortes et al., 1996; Heithaus, 2001). Resource states, based on prey taxa, were used in this study. Levins’ measure of
niche breadth is used to measure the uniformity in the distribution of prey items among all the prey categories (Levins, 1968) and is calculated as:

\[ B = \frac{1}{\sum p_j^2} \]

\(B\) = Levins' measure of niche breadth
\(p_j\) = Proportion of items in the diet that are of food category \(j\) (\(\sum p_j = 1.0\))

\(B\) can range from 1 (minimum niche breadth, specialized diet) to a maximum of \(n\) (maximum niche breadth, all categories used equally) where \(n\) is the total number of resource categories. For ease of comparisons \(B\) can be standardized to a scale from 0 (minimum niche breadth) to 1 (maximum niche breadth) according to Hurlbert (1978).

Levins' standardized niche breadth is calculated as:

\[ B_\lambda = \frac{B - 1}{n - 1} \]

\(B_\lambda\) = Levins' standardized niche breadth
\(B\) = Levins' measure of niche breadth
\(n\) = number of possible resource categories

This measure of niche breadth ignores the possibility (and probability) that resources vary in their abundance within the ecosystem and across geographical regions. Varying degrees of rarity were not considered in this project owing to the extreme difficulty in assessing relative abundance of micronekton species in the pelagic environment.

MacArthur and Levins' measure is one of the first, and simplest, methods to calculate resource overlap between species. MacArthur and Levins' measure is calculated as:

\[ M_{jk} = \frac{\sum p_{ij}p_{jk}}{\sum p_{ij}^2} \]

\(M_{jk}\) = MacArthur and Levins' niche overlap of species \(k\) on species \(j\)
\(p_{ij}, p_{jk}\) = Proportion that resource \(i\) is of the total resources used by species \(j\) and \(k\)
n = The total number of resource categories

In essence this equation takes the niche breadth of one species and divides it by a combined niche breadth calculated using the proportions of prey items from both species. By applying this equation to two species using the same resource category matrix the equation yields overlap of one species resource usage onto another species usage. This measure is not symmetrical between species. Most other measures of overlap yield a symmetrical value of overlap of two species, representing the actual space of overlap between two species. Using a value of asymmetrical resource overlap to estimate impact of one species on another may be more indicative of most actual species interactions. MacArthur and Levins' measure has been used effectively to determine resource overlap (e.g. Macpherson, 1981; Ellis et al., 1996; Heithaus, 2002) and is used here since initial assumptions were that there would by asymmetrical overlap between *O. bartramii* and *S. oualaniensis*. There are several measures of niche overlap that have been developed over the years, and there is debate over which measure is best (Hurlbert, 1978; Linton et al., 1981; Krebs, 1989). Many of the measures of resource overlap contain varying degrees of bias to differing numbers of resource categories, differences in sample sizes between species being compared, and resource evenness (Krebs, 1989).

*Cruises*

Most specimens were collected during six cruises that were conducted aboard the fishery training vessel HOKUSEI MARU during the years of 1996 through 2001. The length of each cruise was 15 days and each cruise started on February 5th and ended on February 20th. This study evolved as a subset of the original study on the general ecology of *Ommastrephes bartramii*. Cruise tracks differed per year but were generally located between 172° W and 158° W longitude and 18° N and 32° N latitude (Fig. 1). In most years the cruise tracks had to be altered to some extent during the cruise because of prevailing weather conditions. The general plan of each cruise was to proceed north to the beginning of the Subtropical Frontal Zone (usually located near 30° N) where individuals of *O. bartramii* are abundantly caught, and then move south towards the main islands of the Hawaiian archipelago where *O. bartramii* is caught in less abundance but
where *S. oualaniensis* is caught more abundantly. Additional specimens of *O. bartramii* were captured in August 2000 on a separate cruise aboard the HOKUSEI MARU at 41° N, 155° E. These specimens were stored aboard the HOKUSEI MARU at -20° C and were off-loaded with the regular specimens after the 2001 cruise.

Additional samples were collected during two cruises aboard the R/V Townsend Cromwell conducted in November and January of 1999 in connection with the National Marine Fisheries Service (NMFS) Honolulu laboratory. In the context of this study these were cruises of opportunity and samples were collected on a non-interference basis. These cruises involved a substantial portion of the total cruise times centered over Cross Seamount.

**Sample collection**

*Squids (Ommastrephes bartramii and Sthenoteuthis oualaniensis)*

Squids were caught using four different capture methods, pole and line jigging, automatic jigging, handlines and a submerged driftline method. Fishing operations began roughly around 2100 hrs and continued usually until 0400 hrs depending on weather and sea conditions. The use of each method of capture also was dependent on weather and sea conditions with the most reliable and frequently used method being pole and line jigging. Each fishing station was conducted as a drift station with the ship’s engine shut down in order to minimize disturbance. All station positions reflect the latitude and longitude at the point where the fishing operations began.

Pole and line jigging consisted of between three and eight people fishing per station using fishing poles rigged with 20 lb test line and a variety of sizes of squid jigs ranging from small to large (5-15 cm). Jigs consisted of a fusiform plastic or straight metal lure with two rows of hooks located opposite from the end of attachment to the line (Fig. 2). Large squid jigs had barbed hooks while smaller sized jigs had straight barbless hooks.

Three automated jigging machines were available onboard but their use was highly dependent on conditions. Automatic jigging machines consisted of a central
motorized unit with drums of spooled line with interconnected jigs located on either side (Fig. 3). Roughly 30-40 jigs were set on each line with the end of the line fixed to a 10 kg weight that allowed the line to spool freely. The lines were automatically reeled in and out to a depth of about 40-50 m on average. The drums were elliptical which imparted a slightly erratic motion to the jigs as they were reeled in or out. As the squids were hooked the upward pull of the machine kept the squids on the jig (they were not barbed) and as they were brought out of the water and over the rollers that guide the line, their weight caused them to drop off the jig into a receiving basket. This method of capture, although convenient because of its automation, lacked the finesse of hand jigging and would often result in the arms and tentacles of the squids being pulled free and the squid dropping back into the water.

Handlines consisted of about 50 m of line spooled around an H shaped wooden frame. A large squid jig was attached at the end of the line along with a 2 to 4 kg weight. The jig was fished between 30-40 m and tied off to the ship. The jig was allowed to soak for up to several hours with periodic checks to determine if a squid had been caught. A submerged drift-line was also used (Fig. 4). The drift-line consisted of roughly 300 m of black polypropylene line. At one end of the main line a large weight and a 50 m line with a buoy were attached. Buoy and weight were put overboard and the main line was allowed to sink/drift away from the ship. Long-line clips with three m of monofilament line ending in a barbed squid jig were attached to the main line at 10 m increments. After five clips with jigs were placed on the main line a long line clip with a light source was attached. In between every 10 jigs a longline clip with a weight was clipped to the main line. This pattern was repeated until 20 jigs had been rigged on the main line. Once all the jigs were attached to the main line another large weight was attached and the shipboard side of the drift-line was submerged. The drift-line was usually deployed once or twice a night, weather permitting, and was soaked for two to three hours each deployment. No recording devices were attached to the line.

Potential Prey of *O. bartramii* and *S. oualaniensis*
Potential prey of *O. bartramii* and *S. oualaniensis* were obtained on all six of the HOKUSEI MARU cruises. During years 1996-1999 mesopelagic fishes and squids were captured using a large midwater otter trawl. The trawl had an operational rectangular mouth opening of 200 m². A depth telemeter was fixed to the top edge of the mouth of the net, in the center. One or two tows at 800 m (an hour at depth for each tow) was made at every station during the daytime, weather permitting. Seas of over three m were considered too large to tow in due to the stress on the towing cable and winch. During years 2000-2001 the large trawl was replaced with a smaller frame trawl. This trawl consisted of a square steel frame roughly three meters on each side and trailing a net with approximately 10 mm stretch mesh. The depth telemeter used on the large trawl was also used on the frame trawl. The frame trawl was towed to a depth of 900 meters and required less turn-around time than the large trawl, consequently it was frequently used twice at each station.

Three tows were taken during the November cruise aboard the Townsend Cromwell using a ≈ five m² Isaac’s Kidd midwater trawl with a 202 μm mesh net. These tows were taken at night and ranged in depth from 160 to 300 m.

*Sample manipulation and preparation*

*Squids*

Mantle length (measured from the dorsal anterior free mantle margin to the posterior end of the fins), sex, and species were determined onboard for all jig caught squids during all cruises. Each stomach (with cecum attached) was removed from each squid and placed in a small plastic bag, labeled, and then frozen at -20°C for later analysis in the lab. In the lab stomachs were thawed, excess water was blotted, and then the stomachs were weighed. The caecum, and any amount of esophagus or intestine, was trimmed off and the remaining stomach and contents were weighed again. The contents were removed and any excessive water was blotted and the contents were weighed. Using these three measurements the weights of the material in the stomach, the stomach itself, and the cecum were obtained for all squids captured. After weighing, the contents were placed into a 333 um Nitex strainer and rinsed with running water. The contents were
then examined under a dissection microscope for identifiable parts. Squid food items are thoroughly chopped by the action of their beaks, making identification of prey difficult. Because the squids rely primarily on enzymatic digestion (Hochachka, 1994), calcium carbonate parts such as otoliths survive the feeding process in exceptional condition. Beaks of prey squids also survive well although often broken into pieces. To identify prey items within the stomachs, fish otoliths and cephalopod beaks were removed and stored separately from each other, in 100% ethanol or 50% isopropanol, respectively for later identification. To avoid the loss of small particles stuck in the folds of the stomach, the stomach lining was removed after the contents had been fully examined. The stomach lining was examined under the dissection microscope using transmitted light that shone through the lining and made any particles caught in the folds highly visible, many small otoliths were found in this manner. Sagittal otoliths recovered from the stomachs of the squids were compared with sagitta in an otolith library formed from fish caught during the cruises. Henceforth, any mention of otoliths will be referring to sagitta. Cephalopod beaks recovered from the stomachs of the squids were also identified by comparisons to a library of beaks.

Normalized stomach fullness was calculated using a regression derived by Tung (1978) from values of stomach weights for given mantle lengths of S. oualaniensis. This regression was assumed to approximate a value of fullness that the stomach at a certain size of squid can hold. Fullness of each stomach was normalized as a percentage of the regression value for 100% fullness. These values were regressed against mantle length for male and female O. bartramii and S. oualaniensis to estimate an average percentage of fullness.

Levins’ measure of niche breadth (Levins, 1968) was calculated separately for otoliths and beaks recovered in the stomachs of both squid species. Fish and squid represented the two distinct and major sources of food for these squids and were thus considered separately to examine potential differences. This value was then standardized using the method from Hurlbert (1978). MacArthur and Levins’ measure of niche overlap (1967) was also calculated separately for both species using the otolith and beak data. When calculating niche breadth only otoliths and beaks that could be identified were used, and categories were assigned to the lowest identifiable taxon.
The number of resource categories was determined as the total number of resources (fishes or squids) that were represented in all the stomachs of both squid species during the entire study. This was assumed to be the number of resource categories even though other resource categories may exist for both fishes and squids that were not represented in the stomachs sampled.

**Potential Prey of *O. bartramii* and *S. oualaniensis***

Potential prey collected in the trawls were placed into gallon-size ziplock bags, labeled and frozen at -20°C. Only specimens that were in a condition that would allow for identification (photophores mostly intact, body mostly intact, etc..) were kept. As a result the identification library was incomplete. Not all otoliths and beaks could be identified using the libraries constructed for this study.

Fishes caught in the trawls were taken back to the lab and identified to lowest possible taxon. Fishes in the family Myctophidae were generally classified to species level when possible, other fishes were usually only categorized to the family level. All fish caught in good condition during the years of 1996-1998 were used as the basis for the otolith library. The otoliths (sagitta, asteriscus, and lapillus) of all fish from these years were removed using a dissection microscope, scissors, and micro-forceps. The otoliths were cleaned of debris and placed in cardboard microslide containers and covered with a glass slide. Otoliths were collected from a total of 250 fish comprising 20 separate fish families including 17 species of myctophids.

**Results**

*General information*

Over the six years of this study, 323 *O. bartramii* were captured, 267 males (82.3%) and 56 females (17.3%). The male *O. bartramii* had a mean mantle length of 329 mm (SD = 25 mm), while the females had a mean mantle length of 535 mm (SD = 41 mm). There were 302 *S. oualaniensis* caught, 40 males (13.2%) and 262 females (86.7%). The male *S. oualaniensis* had a mean mantle length of 155 mm (SD = 15 mm),
while the females had a mean mantle length of 198 mm (SD = 40 mm). The total number of squids caught varied per year (Table 1.).

**Table 1.** The number of squids of both species caught during each year (the number of *S. oualaniensis* caught during 1999 includes specimens taken aboard the R/V Townsend Cromwell).

<table>
<thead>
<tr>
<th>Year</th>
<th><em>Ommastrephes bartramii</em></th>
<th><em>Sthenoteuthis oualaniensis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>65</td>
<td>5</td>
</tr>
<tr>
<td>1997</td>
<td>46</td>
<td>19</td>
</tr>
<tr>
<td>1998</td>
<td>53</td>
<td>73</td>
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<tr>
<td>1999</td>
<td>71</td>
<td>48</td>
</tr>
<tr>
<td>2000</td>
<td>9</td>
<td>31</td>
</tr>
<tr>
<td>2001</td>
<td>16</td>
<td>31</td>
</tr>
</tbody>
</table>

A stomach was considered empty if there was no weighable material (i.e., <0.001 g). Of the male *O. bartramii* stomachs examined, 19 (7.1%) were empty, while nine (16%) of the female stomachs were empty. Of the male *S. oualaniensis* stomachs examined 12 (30%) were empty, while 44 (17%) of the female stomachs were empty.

The stomach content weights for *O. bartramii* ranged from < 1 to 95.9 g in males (x = 9.7 g, SD = 13.7), and from < 1 to 435 g in females (x = 32.3 g, SD = 66.7). The stomach content weights for *S. oualaniensis* ranged from < 1 to 10.8 g in males (x = 2.18 g, SD = 2.95), and from < 1 to 31.0 g in females (x = 4.21, SD = 5.32). Stomach fullness did not increase appreciably with mantle length for either species (Fig. 5,6). *S. oualaniensis* showed an increase in maximum fullness between 125 and 200 mm ML (Fig. 6). Average percentages of fullness for *S. oualaniensis* males and females were 24 and 13%, and for *O. bartramii* males and females averages were 8 and 5% (Fig. 5,6).

The weight of the stomach organ increased with increasing size of both species of squids (Fig. 7). The weight of the stomach organ increased exponentially with mantle length with similar trends in both *O. bartramii* and *S. oualaniensis* (Fig. 7). Stomach
organ weight increased with mantle length for each squid according to the following regressions.

\[
\text{Stomach weight}_{O. bartramii} = -3.0008 + [1.6922 \times 1.0051^{(\text{mantle length})}]
\]
\[
\text{Stomach weight}_{S. oualaniensis} = -8.9057 + [6.8234 \times 1.0024^{(\text{mantle length})}]
\]

Fish and cephalopod remains dominated the stomach contents; rarely some decapod crustacean material was found but consisted of such small and unidentifiable portions that it was ignored. Other items such as thecate gastropods, amphipods, copepods, and unidentifiable pieces of plastic were occasionally found. These smaller items (excluding the plastics) were assumed to be transit items (i.e., from the stomach of prey) as they were probably too small for the squids to feed on (Shchetinnikov, 1992). In 15 of the \(O. bartramii\) stomachs mesh-like masses of gelatinous tissue were found which were possibly the remains of pyrosomes, but could not be identified conclusively. Similar mesh-like prey were not found in \(S. oualaniensis\). One \(O. bartramii\) stomach was filled with an egg mass from a pelagic octopus, probably \(Argonanta\) sp. A total of 2561 otoliths and 424 beaks were recovered during this study. For \(O. bartramii\), otoliths were found in 264 (81.5%) of the stomachs and 169 (52.2%) stomachs contained cephalopod beaks. For \(S. oualaniensis\), otoliths were found in 211 (69.6%) stomachs while beaks were found in 96 (31.7%) of the stomachs. The fish otoliths recovered from the stomachs of both squid species were categorized into 17 families. Of these 17 families only otoliths from the members of Myctophidae, Anoplogasteridae, and Omosudidae were identified to species level. Within the Myctophidae 16 species were identified. Four species of myctophids could only be identified to genus, they where \(Hygophum proximum\) or \(H. rheinhardti\), and \(Myctophum lychnobium\) or \(M. spinosum\). These four species were placed within two species groups. The beaks found in the stomachs of both species of squids represented 18 families of cephalopods from which 29 genera were identified, of these, 11 were identified to the species level. Otoliths or beaks occurring at < 1% frequencies in both species were ignored. Otoliths or beaks which made up < 1% of the total abundance in both species were ignored.
**Otolith data (O. bartramii)-**

**Frequency:**

Otoliths belonging to the family Myctophidae were the most frequently occurring otoliths in both squid species. Myctophid otoliths were found in 63% of *O. bartramii* stomachs while unidentified otoliths occurred next most frequently (37%), followed by otoliths from Gonostomatidae (16%) (Fig. 8). Otoliths of seven families of fishes occurred in *O. bartramii* stomachs at frequencies of greater than or close to 5% (Fig. 8). Otoliths from eight species groups within the family Myctophidae were found at high (> 5%) frequencies in *O. bartramii*, while six other myctophid species groups were found at > 1% frequencies (Fig. 9).

**Abundance:**

There were 1624 otoliths recovered from all the *O. bartramii* stomachs. The myctophid and unidentified categories comprised 63% of all otoliths in *O. bartramii* (Fig. 10). The three next most abundant family categories of otoliths found in *O. bartramii*, belonged to Gonostomatidae (11%), Melamphaeidae (7%), and Sternoptychidae (5%) (Fig. 10). The remaining 12% of the otoliths found in *O. bartramii* stomachs belonged to the families Photichthyidae, Evermannellidae, Chauliodontidae, Gempylidae, and Scopelarchidae. Of the otoliths from the Myctophidae, *Symbolophorus evermanni* was recovered at the highest percentage (7.5%) in *O. bartramii*, while *M. lychnobium* or *spinosum*, *Lobianchia gemellerii*, and *Myctophum selenoides* were all recovered at similar proportions (≈ 5%) (Fig. 11).

**Fish eye lenses (O. bartramii)-**
Fish eye lenses were collected from *O. bartramii* stomachs during the cruises in 1996-1999. There were a total of 925 fish eye lenses collected during those years, compared with 1511 otoliths and 255 beaks over the same period.

**Otolith data (S. oualaniensis)**

**Frequency:**

In *S. oualaniensis* 62% of stomachs contained myctophid otoliths, 19% contained unidentified otoliths, and 4% contained gonostomatid otoliths (Fig.8). Otoliths from three species groups within myctophidae (*S. evennanni, C. warmingii, and H. proximum/rheinhardtii*) were found at greater than or close to 20% occurrence, while seven other species within Myctophidae occurred at > 1% (Fig. 9). The only other otoliths that occurred at a frequency greater than 1% in *S. oualaniensis* stomachs were from Evermanellidae (1.65%).

**Abundance:**

There were 937 otoliths recovered from the *S. oualaniensis* stomachs. The myctophid and unidentified categories comprised 97% of the otoliths in *S. oualaniensis* (Fig.10). Gonostomatidae and Evermanellidae made up the remaining 3% of otoliths in *S. oualaniensis*. Of the otoliths from Myctophidae found in *S. oualaniensis* stomachs, *S. evennanni* was the most abundant (37%), followed by *C. warmingii* and *H. proximum/rheinhardtii* (both ≈ 15%), and *M. lycnobiunm* (5%) (Fig.11).

**Beak data (O. bartramii)**

**Frequency:**

At the family level, beaks from Onychoteuthidae occurred most frequently (14%) in *O. bartramii*, while Histiotutheidae, Enoploceuthidae, and unidentified beaks all occurred at similar frequencies (10-12%). Ommatrephidae and Pyroteuthidae occurred next most frequently (5%) while all other categories of beaks found in *O. bartramii* occurred at < 5% (Fig. 12). When the beaks were categorized to the lowest possible taxa, *Onychoteuthis* sp. occurred most frequently in *O. bartramii* (14%), while *Histiotheuthis*
sp., and unidentified beaks occurred next most frequently (10%). *Abraliopsis* sp. A and *Pyroteuthis addolux* occurred in *O. bartramii* with similar frequencies (5%), while all the other categories had frequencies below 3% (Fig. 13).

**Abundance:**

There were 288 cephalopod beaks recovered from the *O. bartramii* stomachs. At the family level, beaks of Onychoteuthidae were most abundant (24%). Beaks of Histiotethidae, Ommastrephidae, Enoploteuthidae, were all recovered at similar abundances (10-12%) in *O. bartramii* stomachs (Fig. 14). When beaks were categorized to the lowest possible taxa, *Onychoteuthis* sp. accounted for 24% of beaks in *O. bartramii* followed by *Histiotethis* sp.(12%). *Pyroteuthis addolux* and *Abraliopsis* sp. A both accounted for similar percentages (6%) of the beaks in *O. bartramii*. Nine more categories of beaks were recovered in *O. bartramii* at varying frequencies between 2-3% (Fig. 15).

**Beak data (S. oualaniensis)-**

**Frequency:**

At the family level in *S. oualaniensis*, Enoploteuthidae occurred most frequently (17%) followed by Onychoteuthidae (10%), while Ommastrephidae and unidentified beaks occurred in similar frequencies (≈ 5%) (Fig. 12). When beaks were categorized to the lowest possible taxa in *S. oualaniensis* stomachs, *Abraliopsis* sp A. occurred most frequently (12%) followed by *Onychoteuthis* sp. (10%), and *Enoploteuthis* sp. and *Abraliopsis* sp. (≈ 2%) (Fig. 13).

**Abundance:**

There were 136 cephalopod beaks recovered from the *S. oualaniensis* stomachs. At the family level beaks of Enoploteuthidae were most abundant (46%) in *S. oualaniensis* stomachs, followed by Onychoteuthidae (22%), unidentified beaks (12%) and Ommastrephidae (11%) (Fig. 14). When beaks recovered in *S. oualaniensis* stomachs were categorized to the lowest possible taxa *Abraliopsis* sp. A, and *Onychoteuthis* sp. accounted for the greatest percentages of beaks, 33 and 22% respectively (Fig. 13).
Abraliopsis sp. accounted for 7% of beaks in S. ovalaniensis while three other genera occurred at < 3% for each category (Fig. 15).

Ommastrephid prey remains (O. bartramii and S. ovalaniensis)-

O. bartramii:
The remains of three O. bartramii were identified in O. bartramii stomachs, all three consisted of freshly consumed material. No identifiable remains of S. ovalaniensis were found in O. bartramii stomachs. The remains of 16 unidentified ommastrephid squids were found in O. bartramii stomachs, 15 of these consisted of freshly consumed material such as arms and tentacles. The freshly consumed materials were considered to be artifacts because O. bartramii were frequently seen attacking others that had been hooked, and there were numerous pieces of caught animals (arms, tentacles) being lost overboard during fishing.

S. ovalaniensis:
The remains of two S. ovalaniensis were identified in S. ovalaniensis stomachs, one was fresh material while the other appeared to have been consumed at a significant time prior to capture. No identifiable remains of O. bartramii were found in S. ovalaniensis stomachs. The remains of 16 unidentified ommastrephid squids were found in S. ovalaniensis stomachs, 13 of these consisted of freshly consumed material such as arms and tentacles. The freshly consumed materials were considered to be artifacts because there were numerous pieces of caught animals (arms, tentacles) being lost overboard during fishing.

Gender comparisons (O. bartramii)-
Otoliths:

Otoliths occurred in 230 (86.1%) of the male *O. bartramii* stomachs, and 33 (58.9%) of the female stomachs. Otoliths occurred at higher frequencies in male than female *O. bartramii* stomachs for all families of fishes except Chauliodontidae, Apogonidae, Giganturidae, and Melanostomiatidae (Fig. 16). A similar trend was seen in myctophid otoliths that occurred in higher frequencies in male *O. bartramii* in all species groups except, *C. warmingii, D. perspiculatus,* and *B. longipes* (Fig. 17). Of the total otoliths recovered from *O. bartramii*, 1460 (90%) were from male stomachs while 164 (10%) were from females. Myctophids made up a greater relative abundance of the otoliths recovered from female *O. bartramii* (66%) than those recovered from males (48%) (Fig. 18). The abundance of unidentified otoliths was also higher in otoliths from female *O. bartramii* (18%) than in males (13%) (Fig. 18). The remaining 52% of the otoliths in the male *O. bartramii* stomachs showed similar values for the categories reported for both sexes combined (Fig. 10). The relative abundance of otoliths recovered from female *O. bartramii* showed greater proportions than those recovered from males for Chauliodontidae, *C. warmingii, L. tenuiformis, D. perspiculatus, M. nitidulum,* and *L. luminosa* (Fig. 19), while showing a general decrease in the proportions of otoliths recovered relative to males in all other categories (Fig. 18, 19).

Beaks:

For *O. bartramii*, beaks occurred in 143 (55.8%) males, and 26 (46.4%) females. Beaks of Onychoteuthidae occurred more frequently in male (17%) than in female (2%) *O. bartramii* stomachs (Fig. 20). In categories where both male and female *O. bartramii* showed high relative frequencies of beaks, the males usually showed greater frequencies of occurrence for beaks than females (Fig. 20). Female *O. bartramii* did show higher frequencies of occurrence in categories of beaks that did not occur frequently in males such as Omnistrephidae (family level comparison), *Nototodarus hawaiensis, Octopoteuthis nielsenii,* and *Mastigoteuthis* sp. (lower taxon-level comparisons) (Fig. 21). Of the total beaks recovered from *O. bartramii*, 245 (85.0%) were found in males while 43 (14.9%) were found in females. In categories where both male and female *O. bartramii* showed high relative abundances of beaks, the males usually showed greater
abundances than females (Fig. 22,23). Female *O. bartramii* did show greater abundances of beaks in categories of beaks that did not show high proportions in males (Fig. 22,23).

**Gender comparisons (S. oualaniensis)**

**Otoliths:**

For *S. oualaniensis*, otoliths occurred in 191 (72.9%) females, and 40 (71.4%) males captured. At the family level, otoliths of Myctophidae and unidentified otoliths were the only categories that occurred more frequently in *S. oualaniensis* females than in males (Fig. 24). Myctophid otoliths occurred more frequently in *S. oualaniensis* females than in males for all species groups except *M. lychnobium* or *spinosum*, *L. luminosa*, and *L. tenuiformis* (Fig. 25). Of the total otoliths recovered from *S. oualaniensis*, 850 (91%) were from females and 87 (9%) were from males. Myctophid and unidentified otoliths made up similar percentages of otoliths found in both female and male *S. oualaniensis* stomachs (97 and 95% respectively) with members of Evermanellidae, Gonostomatidae, and Nomeidae making up the remaining percentages (Fig. 26). Within Myctophidae both genders of squid contained similar percentages of *S. evermanni* and the species group *M. lychnobium* or *spinosum* (Fig. 27). Female *S. oualaniensis* contained larger percentages of *C. warmingii* and had more unidentified otoliths, while males contained more *H. proximum* or *H. rheinhardtii* (Fig. 27).

**Beaks:**

For *S. oualaniensis*, beaks occurred in 89 (34.0%) females, and 7 (17.5%) males. In *S. oualaniensis* stomachs, beaks were found in higher frequencies in females than in males for all categories except Ommastrephidae and unidentified beaks (Fig. 28). In female *S. oualaniensis* stomachs beaks of *Abraliopsis* sp. A were found at roughly twice the frequency found in males (Fig. 29). Of the total beaks recovered from *S. oualaniensis*, 124 (91.1%) were found in females while 12 (8.8%) were found in males. In the family level categories where both male and female *S. oualaniensis* showed high relative abundances of beaks, the males usually showed higher abundances for beaks than females, except for Enoploteuthidae prey (Fig. 30). Female *S. oualaniensis* showed
higher abundances of all lower taxon categories than males except for *P. addolux* (Fig. 31).

*Niche breadth and overlap:*

Levins’ standardized measure of niche breadth in *O. bartramii* was greater than niche breadth in *S. oualaniensis* using both otoliths and beaks as indices (Table 2). Niche breadth for *O. bartramii* males was greater than females using otoliths, but niche breadth was greater in female *O. bartramii* than in males using beaks (Table 2). Niche breadth for *S. oualaniensis* females and males was approximately equal using otoliths or beaks (Table 2). The niche breadth for the different size classes of *O. bartramii* males showed no consistent trend, although the largest size class showed the lowest niche breadth, while niche breadth increased with size class in *S. oualaniensis* (Table 2). The value of niche breadth for the large size class of *O. bartramii* was greatly affected by one stomach that contained 24 otoliths of *S. evermanni* (7% of all the otoliths in this category). Excluding this individual resulted in a niche breadth value of 0.33 for the large size class of *O. bartramii*. The niche breadth of the largest size class of *S. oualaniensis* was greater than the breadth calculated for the species as a whole (Table 2.).
Table 2. Comparisons of Levins' modified measure of niche breadth \((B)\) for different categories of squids using different prey indices (unidentified items excluded).

<table>
<thead>
<tr>
<th>Prey indices</th>
<th>Ommastrephes bartramii</th>
<th>Sthenoteuthis oualaniensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (otoliths)</td>
<td>0.56</td>
<td>0.086</td>
</tr>
<tr>
<td>Male (otoliths)</td>
<td>0.40</td>
<td>0.085</td>
</tr>
<tr>
<td>Female (otoliths)</td>
<td>0.23</td>
<td>0.083</td>
</tr>
<tr>
<td>Total (beaks)</td>
<td>0.22</td>
<td>0.097</td>
</tr>
<tr>
<td>Male (beaks)</td>
<td>0.19</td>
<td>0.096</td>
</tr>
<tr>
<td>Female (beaks)</td>
<td>0.39</td>
<td>0.096</td>
</tr>
<tr>
<td>Large size class (otoliths)</td>
<td>0.23 (396-336 mm)[males]</td>
<td>0.12 (300-201 mm)</td>
</tr>
<tr>
<td>Medium size class (otoliths)</td>
<td>0.41 (335-320 mm)[males]</td>
<td>0.08 (198-161 mm)</td>
</tr>
<tr>
<td>Small size class (otoliths)</td>
<td>0.34 (319-228 mm)[males]</td>
<td>0.06 (160-120 mm)</td>
</tr>
</tbody>
</table>

The overlap of *S. oualaniensis* on *O. bartramii* was greater than the overlap of *O. bartramii* on *S. oualaniensis* using both fish otoliths and squid beaks (Table 3). The overlap of *O. bartramii* males on females was greater using cephalopod beaks but less using fish otoliths. Overlap was virtually the same for both male and female *S. oualaniensis* using both otoliths and beaks (Table 3). Niche overlap of the small *O. bartramii* size class increased with size of *S. oualaniensis* (Table 3). Niche overlap of the different size classes of *S. oualaniensis* on the small *O. bartramii* increased with increasing size of *S. oualaniensis* (Table 3).
Table 3. Comparisons of MacArthur and Levins measure of niche overlap for both species of squids using different prey indices and different size categories of squids. *O. bartramii*, small = 319-228 mm ML; *S. oualaniensis*, small = 160-120 mm, medium = 198-161 mm, large = 300-201 mm. sm= small, md = medium, lg= large

<table>
<thead>
<tr>
<th>Overlap of one group on another</th>
<th>Fish Otoliths</th>
<th>Cephalopod beaks</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. bartramii</em> on <em>S. oualaniensis</em></td>
<td>0.22</td>
<td>0.46</td>
</tr>
<tr>
<td><em>S. oualaniensis</em> on <em>O. bartramii</em></td>
<td>1.16</td>
<td>0.93</td>
</tr>
<tr>
<td><em>O. bartramii</em> male on female</td>
<td>0.31</td>
<td>0.85</td>
</tr>
<tr>
<td><em>O. bartramii</em> female on male</td>
<td>0.52</td>
<td>0.45</td>
</tr>
<tr>
<td><em>S. oualaniensis</em> male on female</td>
<td>0.95</td>
<td>0.80</td>
</tr>
<tr>
<td><em>S. oualaniensis</em> female on male</td>
<td>0.97</td>
<td>0.80</td>
</tr>
<tr>
<td>sm <em>O. bartramii</em> on sm <em>S. oualaniensis</em></td>
<td>0.08</td>
<td>---</td>
</tr>
<tr>
<td>sm <em>O. bartramii</em> on md <em>S. oualaniensis</em></td>
<td>0.11</td>
<td>---</td>
</tr>
<tr>
<td>sm <em>O. bartramii</em> on lg <em>S. oualaniensis</em></td>
<td>0.15</td>
<td>---</td>
</tr>
<tr>
<td>sm <em>S. oualaniensis</em> on sm <em>O. bartramii</em></td>
<td>0.32</td>
<td>---</td>
</tr>
<tr>
<td>md <em>S. oualaniensis</em> on sm <em>O. bartramii</em></td>
<td>0.35</td>
<td>---</td>
</tr>
<tr>
<td>lg <em>S. oualaniensis</em> on sm <em>O. bartramii</em></td>
<td>0.36</td>
<td>---</td>
</tr>
</tbody>
</table>

Otoliths from myctophid species that were found in substantial numbers in both *O. bartramii* and *S. oualaniensis* stomachs were significantly larger (otolith length along the long axes of sagitta) in *O. bartramii* stomachs (Table. 4).
Table 4. List of the mean length (long axis of sagitta) and number of otoliths of different species of myctophids found in *O. bartramii* and *S. oualaniensis* stomachs along with the P-values obtained for a two sample t-test against the hypothesis that the mean of otoliths in *O. bartramii* > otoliths in *S. oualaniensis*.

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Mean length (mm) and numbers for <em>O. bartramii</em></th>
<th>Mean length (mm) and numbers for <em>S. oualaniensis</em></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. evermannii</em></td>
<td>4.42 (25)</td>
<td>4.04 (107)</td>
<td>0.001</td>
</tr>
<tr>
<td><em>C. warmingii</em></td>
<td>3.60 (36)</td>
<td>2.87 (58)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>H. proximum</em> or <em>rheinardti</em></td>
<td>1.61 (20)</td>
<td>1.47 (63)</td>
<td>0.006</td>
</tr>
<tr>
<td><em>M. lychnobium</em> or <em>spinosum</em></td>
<td>3.64 (42)</td>
<td>2.70 (29)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>M. selenoides</em></td>
<td>3.10 (23)</td>
<td>2.53 (9)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Otolith size comparisons between male and female *O. bartramii*, conducted for the two categories of myctophids (*L. tenuiformis* and *C. warmingii*) that had enough numbers for comparison, did not yield significant differences at the 95% confidence level. There were, however, differences in otolith sizes recovered from the stomachs of female and male *S. oualaniensis* for two of the four groups that contained enough otoliths for comparison (Table 5).

Table 5. List of the mean length (long axis of sagitta) and number of otoliths of different species of myctophids found in female and male *S. oualaniensis* stomachs along with the P-values obtained for a two sample t-test against the hypothesis that the mean size of otoliths in females > otoliths in males.

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Mean length (mm) and numbers for females</th>
<th>Mean length (mm) and numbers for males</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. warmingii</em></td>
<td>2.98 (52)</td>
<td>1.40 (6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>S. evermanni</em></td>
<td>4.11 (87)</td>
<td>3.69 (17)</td>
<td>0.03</td>
</tr>
<tr>
<td><em>H. proximum</em> or <em>rheinardti</em></td>
<td>1.49 (52)</td>
<td>1.32 (9)</td>
<td>0.056</td>
</tr>
<tr>
<td><em>M. lychnobium</em> or <em>spinosum</em></td>
<td>2.64 (22)</td>
<td>2.89 (6)</td>
<td>0.365</td>
</tr>
</tbody>
</table>
Discussion

General discussion

In this study Teleostei and Cephalopoda comprised the main dietary components of both *O. bartramii* and *S. oualaniensis* by abundances and frequencies. These items are typical of ommastrephid prey items in general, and prey of *O. bartramii* and *S. oualaniensis* in particular (Young, 1975; Arraya, 1983; Nixon, 1987; Seki, 1993; Schetinnikov, 1992). Crustacean material was virtually absent in the stomachs of both species, which has been observed for *O. bartramii* (Seki, 1993; Naito et al., 1977; Arraya, 1983; Dunning and Brandt, 1985) and for adult *S. oualaniensis* (Shchetinnikov, 1992; Tung, 1976). Shchetinnikov (1992) found that larger *S. oualaniensis* (> 155mm ML) switched from a diet of mostly crustaceans to that of fishes. Adult *S. oualaniensis*, however, have been observed feeding on crustaceans in certain specific areas on the windward sides of the main Hawaiian Islands and off Okinawa (Taguchi et al., 1985; Tung, 1976). Murata (1990) found crustaceans in the stomachs of *O. bartramii*, but this was because his analysis included smaller immature squids. Attempts were made to sample individual *S. oualaniensis* that might show crustacean feeding habits in adult, but this behavior was not observed.

Cannibalism has been reported frequently in schooling squids (Nixon, 1987) and has been reported commonly in *O. bartramii* and *S. oualaniensis* (Seki, 1993; Araya, 1983; Nigmatullin et al., 1983). Cannibalism was virtually absent in this study for either species. When material was found in the stomachs that suggested cannibalism, the material was almost always fresh and usually consisted of arms and tentacles. Arms and tentacles of the squids were frequently pulled off during attempted landings and thrown back into the water by the fisherman, or attached to the jigs and used as bait. Squids on deck waiting to be processed were also frequently seen chewing on themselves or a nearby squid, although attempts were made to minimize this occurrence. Squids, especially *O. bartramii* females, were often observed attacking other squids caught on jigs. Apparent cannibalism in this study was likely an artifact of sampling squids by jig fishing; Breiby and Jobling (1985) reported similar observations. Seki reported extensive cannibalism in gillnet caught *O. bartramii*, although this could have resulted from squid
feeding on “gilled” squid prior to their own entanglement. Dunning and Brandt (1985) found little or no evidence of cannibalism under similar fishing conditions.

The stomach organ weights scaled exponentially with body length in both species. As expected this indicates that as the squid grows its ability to consume larger meals increases. Attraction based methods generally catch animals that are hungry. The largest amount of material was found in one female that consumed 435 g of material (almost entirely fresh squid material from other *O. bartramii*), which is only about 68% of total calculated fullness using the equation from Okutani and Tung, 1978. The percentages of fullness were greater for males than females in each species. Assuming that the categories with the greater numbers of individuals represent more robust data sets, the *O. bartramii* males and *S. oualaniensis* females show that the squids are between 5-13% full during the times when they were sampled.

**Otolith data**

Otolith frequency data suggest that *O. bartramii* is a more generalized predator than *S. oualaniensis*. The data show that many groups of fish prey show up at moderate frequencies in *O. bartramii* stomachs while few fish prey groups show up at high frequencies in *S. oualaniensis*. The data on frequency of occurrence match the abundance data in both pattern and amount fairly well indicating that large bouts of feeding by certain individuals on select species probably did not bias the abundance data to a large degree.

Otoliths had a much greater frequency of occurrence and were present in greater numbers overall than cephalopod remains in both *O. bartramii* and *S. oualaniensis*. These squids are feeding predominantly on fishes. The fish prey of *S. oualaniensis* was almost exclusively made up of myctophids while *O. bartramii* fed on myctophids and several other groups of fishes. Gonostomatids occurred fairly frequently as did several other groups of mesopelagic fishes that were not fed upon by *S. oualaniensis*. Disparate feeding also occurred between *O. bartramii* and *S. oualaniensis* on prey items within the Myctophidae. *Symbolophorus evermanni* was a mainstay in the diet of *S. oualaniensis* (> 35% of all otoliths found), as were *C. warmingii* and the species group of *H. proximum* or *rheinhardtii*, while the diet of *O. bartramii* is spread over a wide variety of species with
none greatly dominating. No single species of fish in *O. bartramii* accounted for greater than about 10% of all the otoliths found. In *S. oualaniensis*, three categories of myctophids accounted for almost 70% of all the otoliths found, while in *O. bartramii* the three most abundant myctophid categories only accounted for 17% of the total. This prevalence of myctophid prey in *S. oualaniensis* is supported by other studies from Hawaiian waters and Taiwan (Young, 1975; Tung, 1976; Wormuth, 1976; Okutani and Tung, 1978) and from areas of the Indian Ocean and Red Sea (Zuev, 1971; Filipova, 1974; Nigmatullin *et al.*, 1983). The substantial amount of *S. evermanni* in the diet of *S. oualaniensis* is also confirmed by Shchetinnikov (1992) in the Eastern Pacific.

**Beak data**

Similar trends to those seen in the stomach content data based on otoliths were also seen when comparing the two squid predators using beaks. The beak data indicate that the feeding spectra of *S. oualaniensis* and *O. bartramii* differ in both the type of species fed upon, and the frequency and proportions of feeding. As was seen with otoliths, *O. bartramii* fed on more species of cephalopods usually at lower percentages than *S. oualaniensis*, which fed on fewer species but with greater percentages in the categories it did feed on. Nine of the categories that *O. bartramii* fed on were not even present in *S. oualaniensis* stomachs. Both squids fed with high percentages on *Onychoteuthis* spp. *O. bartramii* fed with a high percentage on *Histioteuthis* spp. which *S. oualaniensis* did not feed on at all while *S. oualaniensis* fed with higher percentages than *O. bartramii* on *Abraliopsis* sp A. and on *Abraliopsis* spp. which *O. bartramii* did not feed on. These results agree to some extent with previous studies that found members of Onychoteuthidae and Enoploteuthidae to occur in *O. bartramii* and *S. oualaniensis* stomachs (Murata, 1990; Shchetinnikov, 1992; Seki, 1993).
Gender differences

*O. bartramii*-

Results from the otolith data indicate that some differential feeding between adult males and the larger adult females of *O. bartramii* occurs. Females showed a higher abundance of myctophids (66%) than did males (48%). Males had a higher abundance for all other major family level categories except unidentified and Chauliodontidae. Within the Myctophidae the top three categories in males were not found in females. The three categories with the highest abundance percentages in females were found in lower percentages in males. The frequency of occurrence data also show a difference between males and females, prey categories in males occurred more frequently in all categories at the family level or within Myctophidae except for, Chauliodontidae, Apogonidae, Giganturidae, Melanostomiatidae, *D. perspiculatus*, *C. warmingii*, and *B. longipes*. The frequency of occurrence data have to be viewed with some skepticism because of the small sample size of females, 56 captured with only 33 stomachs containing otoliths. The abundance data may be more robust, with 164 total otoliths from females. The niche breadth for male *O. bartramii* was greater than for females but both were lower than that for the species as a whole. Dietary overlap between the sexes was also low indicating that the high values of niche breadth obtained with males and females combined is partly caused by differential feeding between the sexes, with the females feeding broadly enough to raise the overall niche breadth of the species. This result contrasts with a previous study done at a more general level farther north that did not find a difference in the feeding between sexes (Seki, 1993). Differential feeding by male and female *O. bartramii* was also found in the stomach content data using beaks. Males had higher abundance in a number of categories where both sexes fed, such as *Onychoteuthis* spp. Female *O. bartramii* had substantial abundances for categories where males did not feed at all such as *Octopoteuthis nielsen* and *Nototodarus hawaiensis*. The trend of greater niche breadths in male relative to female *O. bartramii* found using otoliths was opposite to that found using beaks. Using the beak data, niche breadth for male *O. bartramii* was almost half of that for females. Worded another way, the niche breadth for males (using beak data) was less than the value for the whole species while niche breadth in females was greater than the species value. The trend in dietary overlap for male and female *O.
*bartramii* using beaks was also different than that found using otoliths. Overlap of females on males was low while overlap of males on female *O. bartramii* was high (> 0.8). These results indicate that, for cephalopod prey, *O. bartramii* females have a more generalized pattern of feeding than do *O. bartramii* males, which seem to concentrate more heavily on fewer categories. Caution should be taken when viewing the results of male/female comparisons using the beak data because of the small numbers of beaks available from females (43) that were used for these comparisons.

*S. oualaniensis*- 

Results from the otolith data on diet of *S. oualaniensis* did not indicate a substantial difference in prey items between the sexes. The frequencies of occurrence and abundances of otoliths in the diet both showed similar trends between males and females, with small differences in select categories, *C. warmingii, M. lychnobium* or *spinosum, D. fragilis*, and *L. luminosa*. The niche breadth of each sex was small and virtually identical both to each other and to the value calculated for the species as a whole. Dietary overlap of males on females and females on males was high (> 0.9) in both cases indicating that the diets of both sexes are similar. The frequency of occurrence data may be suspect because, of the 40 male *S. oualaniensis* caught, only 19 contained otoliths. The abundance data may be more robust, with 87 otoliths in males.

There were little or no differences in feeding by female and male *S. oualaniensis* based on the beak data. The proportions of several of the cephalopod categories were higher in males than females except for *Abraliopsis* sp. A, and the categories where males did not feed at all. The frequencies of occurrence were similar between males and females except for *Abraliopsis* sp. A, *Onychoteuthis* sp., and the categories where occurrence was zero in males. The niche breadths for each gender were practically identical indicating that their range of food items is the same. The overlap of males on females was also nearly identical to the overlap of females on males. Both of these measures indicate that there are no gender differences in diet between female and male *S. oualaniensis*. The results should be viewed with some caution because the number of beaks used to calculate these relationships is small (12 beaks occurring in 7 male
stomachs out of 40 males total). However, this trend in the beak data is in agreement with that found from the more robust otolith data.

**Niche breadth and overlap**

The dietary measurements indicate that in Hawaiian waters *S. oualaniensis* is a more specialized predator than *O. bartramii*. Levins' measure relies only on the proportions of prey categories found in the stomachs and is not affected by the number of categories assigned by the researcher (although theoretically $B$ would approach the number of categories evaluated if all feeding was in equal proportions across all categories), and these values reveal a large margin between the niche breadths of each squid, 20.1 for *O. bartramii* and 3.85 for *S. oualaniensis*. The standardized values reveal an even larger margin between the two, with *O. bartramii* having a niche breadth of 0.56 and *S. oualaniensis* at 0.086. Niche breadth increased slightly with size class for *S. oualaniensis* indicating that as the animal grows larger its diet expands. Schetinnikov (1992) also reported dietary changes with size for *S. oualaniensis*. The niche breadth values for small and large *O. bartramii* were nearly identical when the individual with the large numbers of *S. evermanni* was removed. Although the difference between the smallest and largest breadths was similar for both species size classes (roughly 0.07 for each) the difference as a percent of the whole was much greater in *S. oualaniensis*.

These data use the same categories of prey to calculate the standardized niche breadth for feeding in each species; this process does not take into account the fact that there may be other potential prey categories that were not represented in the individuals sampled. Given the sample sizes caught for each squid, most of the prey categories are probably represented. The unidentified otoliths were not included in the calculations. Because the unidentified otoliths only represented around 13% of the total for both species, and encompassed at least 10 easily differentiable categories, the effect of each category on niche breadth values would be minor. Many families of fishes were present only in *O. bartramii* and since several species appear to be encompassed in these family categories, niche breadth in *O. bartramii* is likely broader, and the actual difference in niche breadth between these squids probably greater than reported here.
Measures of dietary overlap calculated from the proportions of all the otolith categories (excluding the unidentified otoliths) revealed unequal overlap between the two squid species. Values of overlap $> 0.7$ (calculated with MacArthur and Levins’ index) have been considered substantial in previous studies of other animals (Macpherson, 1981; Ellis et al., 1996; Heithaus, 2001). Some values of overlap from other studies ranged up to 1.8 but values above 1 were considered high (Heithaus, 2001). The overlap of \textit{O. bartramii} diet onto that of \textit{S. oualaniensis} was low (0.22), indicating that species fed upon by \textit{O. bartramii} were not fed upon to the same degree, or at all, by \textit{S. oualaniensis}. The overlap of \textit{S. oualaniensis} diet on that of \textit{O. bartramii} was high (1.16) indicating that species fed upon by \textit{S. oualaniensis} were present in the diet of \textit{O. bartramii} to a substantial degree. The results of these calculations indicate that diet composition of \textit{S. oualaniensis} is a subset of the diet of \textit{O. bartramii}. This result is confirmed by finding only one category of fish, Nomeidae, that was present in \textit{S. oualaniensis} and not in \textit{O. bartramii}. Nomeidae only occurred once in \textit{S. oualaniensis} with two otoliths being present. On the other hand, thirteen categories of fishes occurred exclusively in \textit{O. bartramii} but not in \textit{S. oualaniensis}, many of these categories occurred frequently and with high percentages, e.g. Sturnoptychidae, Melamphaeidae, etc.. Dietary overlap increased for both species as the larger size classes of \textit{S. oualaniensis} approached the smallest size class of \textit{O. bartramii}. The increases in overlap were small but consistent throughout the size classes.

The niche breadth of \textit{O. bartramii} was broader than that of \textit{S. oualaniensis} in the calculations based on cephalopod beaks. The difference in niche breadths was not as large as that found in the otoliths but breadth in \textit{O. bartramii} was still more than twice that of \textit{S. oualaniensis}. The calculations of niche overlap also showed the same trends with the beaks as they did with the otoliths. Overlap of \textit{O. bartramii} diet on \textit{S. oualaniensis} diet was low (0.46), while overlap of \textit{S. oualaniensis} on \textit{O. bartramii} was high (0.93). The trends in differences of niche breadth and dietary overlap between \textit{O. bartramii} and \textit{S. oualaniensis} found in the beak data were the same as those found in the otolith data but the magnitude of the differences was less extreme. The beak data indicate (as did the otolith data) that \textit{O. bartramii} feeds moderately on many categories in a broad
range of prey items while *S. oualaniensis* feeds more heavily on a few categories in a relatively narrow range of prey items.

Statistically significant differences occurred in the sizes of otoliths found in *O. bartramii* versus *S. oualaniensis*. In the five species groups of myctophids where there were substantial numbers of otoliths for comparison, larger mean lengths of otoliths were found in every group in *O. bartramii* stomachs. Shchetinnikov (1992) found three feeding stages in *S. oualaniensis* with regard to prey size: when the squids were small (40-100 mm ML) prey size remained constant, in medium sized squids (100-150 mm ML) the size of prey increased proportionally with squid predator, and in larger squids (150-365 mm ML) the prey size increased more rapidly than did predator size. A trend of prey size increase with predator size within *S. oualaniensis* and *O. bartramii* was not evident in otolith length or beak length. The length of myctophid prey (calculated from regressions of otolith length versus fish length) for *C. warmingii*, *H. proximum/rheinhardti*, *M. selenoides*, and *S. evermanni*, ranged from 12 to 27% of the average length of *S. oualaniensis*. These values agree with the range of prey sizes relative to the group II sizes of *S. oualaniensis* (ML = 100-150 mm) found by Shchetinnikov (1992) of 16 to 21% for myctophids. *S. oualaniensis* shows a typical size selection of prey in the presence of *O. bartramii* feeding on the same groups.

Given the results of differential size selection of shared prey between *O. bartramii* and *S. oualaniensis*, and the differential feeding by male and female *O. bartramii*, a difference in size of prey within categories shared by *O. bartramii* genders might be expected. There were only two categories that contained sufficient numbers of otoliths to compare males and females of *O. bartramii* and neither prey category demonstrated a significant size difference between the genders. *S. oualaniensis* males and females did not exhibit differences in niche breadth and their dietary overlap was nearly identical, however, significant differences in the otolith sizes of two prey categories, *C. warmingii* and *S. evermanni* did occur. Feeding by *S. oualaniensis* on *H. proximum* or *rheinhardti* was not significantly different between genders at the 95% confidence level, although with a p value of 0.056 the differences were close to significant. In both cases of significant differences of otolith size between gender, females preyed upon larger sizes of myctophids. Given the lack of strong trends between overall *S. oualaniensis* size and
otolith sizes of *C. warmingii* and *H. proximum/rheinhardtii* these results apparently depict a gender specific feeding preference.

**Conclusions**

Understanding competition between wide ranging open ocean species is difficult given present limitations of the data. In addition, the habitat virtually excludes experimental manipulation, such as removal or exclusion experiments. Zuev *et al.*, (1975; quoted in Shchetinnikov, 1992) states “the systematic and ecological similarity of [*S. oualaniensis*, *O. bartramii*, and other ommastrephids] has led to division of the open ocean... through interspecific competition.” If so, one might expect these species to show strong competitive interactions in areas of sympatry.

The proportions and frequencies of items in the diets of *O. bartramii* and *S. oualaniensis*, as well as the dietary measurements calculated from these values, indicate that, although *S. oualaniensis* feeds on a subset of the *O. bartramii* diet, their feeding patterns are basically different. The stomach contents data show that *O. bartramii* feeds on a broader selection of species than *S. oualaniensis* and feeds more evenly across these categories. Not only does *O. bartramii* feed more broadly than *S. oualaniensis* on fishes and squids at the family level, it also feeds more generally at the species level. When the prey items are classified to genus and species levels, substantial fine scale differences in diet emerge. Even when feeding on the same species of fishes, dietary differences are evident in the size of fishes eaten, with larger fish being eaten by *O. bartramii* relative to those eaten by *S. oualaniensis*. The diets of both squids give good agreement with the diets of other studies that have looked at these species separately, arguing against a possible niche shift when they are located together geographically.

Whether these dietary differences are an effect of resolved competition, or simply a result of the size difference between these two species, presumably unrelated to competition, cannot be determined. However, the niche breadth of *S. oualaniensis* increased with size, and the dietary overlap increased between the smaller size *O. bartramii* and the increasing sizes of *S. oualaniensis*, indicating that size may be a major factor in controlling the feeding habits of these animals relative to each other. These
results raise the question of whether or not juveniles of similar size for each species represent the stage where competition between them occurs. Because of the difficulties in capturing the juvenile forms, little is known about the juvenile stage of either species, but the peak spawning periods differ between these squids and migration causes \( O. \ bartramii \) to be absent from \( S. \ oualaniensis \) habitats for a long period when these species would directly overlap in size.

In the size ranges of squids sampled direct interactions between these species, such as predation, were not observed in the areas sampled. This finding is contrary to studies in other areas that have shown substantial feeding on closely related squids in this family with comparable size differential, and contrary to expectations.

Apparently competition between \( O. \ bartramii \) and \( S. \ oualaniensis \) for food resources is low. Direct predation by one species on another was also not substantial during this study. These results call into question the underlying reasons for the geographical separation of these species, and the separation of known peaks in breeding seasons. Given the lack of evidence for direct interaction and/or competition for food resources between \( O. \ bartramii \) and \( S. \ oualaniensis \), competitive exclusion of one species by another does not seem likely. The present feeding patterns displayed by each species could be the result of past competition. Perhaps intense competition between the juvenile forms of these two species accounts for the evolution of their partial geographical and temporal separation. Female \( O. \ bartramii \) are rarely caught or seen at the southern end of the migration, suggesting that they are occupying a deeper nighttime habitat in their southern range. If so, this deeper nighttime habitat would spatially segregate them from male \( O. \ bartramii \) and from \( S. \ oualaniensis \), and lessen the probability of direct predation of large female \( O. \ bartramii \) on \( S. \ oualaniensis \).
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Chapter II: Stable isotope analysis (SIA)

Introduction

A brief history of SIA

The term “isotope” was first introduced in 1914 by Frederick Soddy to explain certain ambiguities in atomic research such as the observation that the same elements from different geographical areas had different atomic weights, and that atomic weight did not always increase with atomic number. Isotopes were defined as distinctive atoms of a certain element that had different atomic weights. There are actually three types of isotopes, radioactive isotopes (isotopes which are unstable and decay), radiogenic stable isotopes (those that are stable and formed from radioactive decay), and non-radiogenic
stable isotopes (Faure, 1986). Radioactive isotopes have been used to measure long-term processes because of their long half-lives and predictable rates of decay. Unlike radiogenic stable isotopes, non-radiogenic stable isotopes (which will henceforth be referred to only as stable isotopes) were all formed during the creation of the Universe. Because these isotopes have all been present since the beginning of time and do not decay, they have been used extensively as tracers of past geological and paleological conditions (Isaacs et al., 1963; MacNeish, 1967; Weiner et al., 1976).

Observations that the ratios of the stable isotopes of some compounds varied in natural substances were made fairly early on (Hoering, 1955). The utility of nitrogen stable isotopes in biological and ecological processes became evident when it was determined that the stable isotope ratio of nitrogen ($^{15}$N/$^{14}$N) in an animal is affected by its diet (DeNiro and Epstein, 1981). This process was at first looked upon as a complication to using stable isotopes as tracers of paleological processes (Steele and Daniel, 1978). An enrichment of roughly 3.4‰ was determined for the $\delta^{15}$N at each trophic level that the food material passes through (Mingawa and Wada, 1984, Peterson and Fry, 1987). Mingawa and Wada (1984) proposed that this enrichment of nitrogen was caused by the preferential excretion of $^{14}$N in urea and ammonia, and the retention of $^{15}$N in the tissues.

These discoveries have led to the use of stable isotopes in a plethora of studies on food webs and trophic relationships (Hobson and Welch, 1992; Kling et al., 1992; Hobson et al., 1994), animal migrations (Fry, 1988; Kline et al., 1998; Marra et al., 1998), and nutrient biogeochemical cycling (Hoch et al., 1996; Karl et al., 1997). There has been a substantial effort devoted to investigating the variation of isotopic composition at the phytoplankton/bacterial level in the ocean (Hoch et al., 1992; Waser et al., 1998a, 1998b) Much of the higher trophic level work in the marine realm has been relegated to coastal and nearshore 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 (Rau, 1983; Sholto-Douglas et al., 1991; Rau et al., 1992; Kline et al., 1998; Takai et al., 2000). Stable isotopes of nitrogen represent a potentially powerful ecological tool to determine trophic and foodweb relationships in the open ocean, however interpretation of isotopic data can be difficult.
Limitations of using SIA

One of the most obvious drawbacks of using stable isotope analysis to study trophic relationships is that it only reflects the metabolic processes that resulted in the signal of the isotope you are using. Stable isotope analysis does not identify prey types or feeding patterns, which can be critical in understanding material flow in an ecosystem. Because stable isotope signatures are an integrated value of feeding many potential prey of a given predator cannot be ruled out. Using stable isotopes alone there is no way to determine which species preyed upon by a predator and which aren't if potential prey items have similar signatures.

To use stable isotopes effectively in evaluating trophic positions the “baseline” signal within the environment must be determined, that is, if every trophic level causes a fixed enrichment in an isotope, one must first determine the starting value of the isotope was in the environment. However, this starting value can be difficult to determine. The cycling of basal resources in an ecosystem (carbon, nitrogen, phosphorus) will affect the isotopic signature of the nutrient pools, in turn affecting the isotopic signature of the primary producers that make use of these nutrients (Michener and Schell, 1994). The different metabolic pathways used to fix nitrogen in plants have been shown to effect isotopic fractionation and therefore the baseline $\delta^{15}N$ value for the ecosystem. Fractionation of nitrogen during assimilation depends on the enzymatic processes involved in assimilating a particular nitrogen source molecule ($\text{NO}_3^-$, $\text{NO}_2^-$, $\text{NH}_3^+$, and $\text{N}_2$). Each of these species of nitrogen undergoes different active/diffusive pathways into the cell for assimilation, and then follows different metabolic pathways to become fixed as cellular nitrogen (Fogel and Cifuentes, 1993). These pathways, both physical and enzymatic, can fractionate nitrogen to different extents depending on the ambient concentrations of each nitrogen source molecule (Miyake and Wada, 1971; Wada and Hattori, 1978; Hoch et al., 1992; Waser et al., 1998; and others). A range of fractionation values for $\delta^{15}N$ has been reported under these conditions varying from -4 to 27% (summarized in Fogel and Cifuentes, 1993).
Other difficulties arise from processes that occur as material is passed up to higher trophic levels. One problem is that animals have different assimilation efficiencies for different types of compounds (Karasov, 1990) and that some compounds such as certain amino acids can be either synthesized or incorporated directly into the animals tissues. Synthesized material can undergo different fractionation from deposited material (Deniro and Epstein, 1977; Monson and Hayes, 1982). Different chemical compositions within tissues of an organism also can result in different fractionations (DeNiro and Epstein, 1981; Hesslein et al., 1993; Pfeiler et al., 1998). Different metabolic pathways of amino acid synthesis can show different amounts of fractionation of material (Macko et al., 1987). “Isotopic routing” is another phenomenon that can cause difficulties in stable isotopic studies of trophic interactions (Schwarz, 1991). The biochemical components of the diet do not constitute a uniform pool isotopically. Each component within the diet may undergo different routes to different tissue compartments within the predators tissues (Tieszen and Fagre, 1993) therefore, the isotopic value obtained from a certain tissue of the predator may not be directly related to the isotopic value of the bulk diet. A subset of isotopic routing is the phenomenon of protein sparing where an animal with a varying diet will use some portions of the diet for tissue synthesis and other portions for metabolism (Castellani and Rea, 1992). This would cause the isotopic value of the predator to be reflected to a greater extent in the high protein portions of the diet rather than the bulk diet itself.

The condition of the predator has also been shown to have an effect on the stable isotope signal. Hobson et al. (1993) showed that starving birds showed in increase in $\delta^{15}$N with loss of muscle mass. They hypothesized that the birds catabolized their own tissues for metabolism, becoming progressively enriched through deamination and excretion of lighter nitrogen. A related problem with the use of stable isotopes in ecology is that metabolic turnover rates can effect the stable isotope signal of animals which have changed their diets either through dietary shifts, migration, or seasonal changes (Ben-David et al., 1997; Minami and Ogi, 1997; Kline et al., 1998; Marra et al., 1998). Different tissues within an organism show different turnover times with which they incorporate a new isotopic signal from the diet (Hobson and Clarke, 1992; Hesslein et al., 1993). Some tissues such as those in the blood and liver have fast turnover times, e.g.
they incorporate a new and different signal in the diet quite rapidly (Tiezsen et al., 1983; Hobson and Clarke, 1992) while tissues such as muscle take longer to incorporate a dietary signal (Hobson and Clarke, 1992; Hesslein et al., 1993). Turnover times can also affect the isotopic signal being measured if the signal was incorporated during growth of the organism rather than during tissue maintenance (Frazer et al., 1997).

Preparation and storage of samples is another source of potential problems when using SIA. Tissues used in SIA need to be protected from bacterial degradation and the concomitant fractionation that this process would impart to these tissues. Traditional methods of preservation and storage of tissues can effect their isotopic composition. Formalin, mercuric chloride, and di-methyl sulfoxide (DMSO), and certain lysis buffers have all been shown to significantly alter the $^{15}$N composition of muscle and blood (Hobson et al., 1997; Bosley and Wainright, 1999). Freezing, drying, storage in 70% ethanol and acidification did not have any effect on isotopic composition of samples (Hobson et al., 1997; Bosley and Wainright, 1999).

Finally, the behavior of the organisms being studied can affect isotopic signatures. Animals with widely varying or specialized feeding habits, both within and between populations of a single species, can affect the outcome of trophic level results using SIA to look at species level comparisons (Vander-Zanden et al., 2000; Beaudoin et al., 1999). SIA used alone without observational or stomach content data would be unable to explain such individual variation.

**Benefits of SIA**

Even with all the difficulties involved in using SIA to look at food webs and trophic relationships SIA remains a powerful tool in ecology. This technique can assign an objective value to an animals' trophic position in an ecosystem. Assigning trophic relationships based on stomach contents and/or observations of feeding is difficult, time consuming, has been highly criticized do to the subjective nature of these techniques (Paine, 1988). SIA has shown to be an effective method of assigning trophic position in simple lake ecosystems (Estep and Vigg, 1985; Kling et al., 1992). Because SIA is an integrated signal over a certain time period (depending on the tissue sampled) it provides a view of feeding which is unlikely to be biased by collection methods. The uncertainties
involved with fractionation in different tissues and their associated turnover times have
been the subject of several studies and the ambiguities are likely to become better
understood in the near future (Hobson and Clark, 1992; Hobson and Clark, 1993;
Hesslein et al., 1993).

Recently, studies have taken advantage of the fact that isotopic signatures that are
laid down in a non-metabolic material can give insight into the trophic history of an
organism. A material within an organism that is non-metabolic will not have a turnover
time due to maintenance and will only possess an isotopic signature that is effectively
locked in place during formation and will remain unchanged within that portion of the
tissue. Researchers have begun to use this retrospective method to look at historical
signals of migration and seasonal changes in certain animals such as birds and whales
which can lock isotopic signals within their non-metabolic structures such as feathers and
baleen respectively (Hobson and Schell, 1998; Best and Schell, 1996; Mizutani et al.,
1992). There have even been attempts to look at long-term trophic changes within
ecosystems by using the isotopic signals of archived non-metabolic hard parts such as
fish scales (Wainright et al., 1993), which opens up the possibility of studying decadal
scale or longer ecological processes in history.

The amount and complexity of problems associated with SIA should not be
viewed as reasons to curb the usage of this technique. These problems should be cited
and acknowledged as information that a prudent researcher will take into account when
designing and conducting research involving the use of SIA.

**Materials and Methods**

**Notation**

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

\[ \delta X = \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \times 1000 \]

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

*Squids (Ommastrephes bartramii and Sthenoteuthis oualaniensis)*

Squids were collected as noted in the section on SCA.

*Potential prey items of O. bartramii and S. oualaniensis*

Potential prey items were collected as noted in the section on SCA.

Large fishes

Tuna (*Thunnus obesus, Thunnus albacares, Thunnus alalunga* and *Katsuwonus pelamis*) along with Mahi Mahi (*Coryphaenus hippurus*) were caught during the two cruises aboard the Townsend Cromwell in 1999. Tuna were caught using both a longline and rod and reel. Mahi were caught using a rod and reel while chumming the waters at station, and by trolling as the ship was underway.

Plankton

Three plankton tows were conducted during the day at each station, weather permitting. Tows were conducted in seas up to four or five meters before conditions were deemed inappropriate for towing operations. A one m² ring plankton net with a 333 μm mesh was suspended from a custom modified bridle system used to minimize the effect of equipment on the avoidance of maneuverable plankton such as squid paralarvae. Two tow points were welded to the outside of the one m² ring on opposite sides. Wire bridles ran from the two points on the ring to a spreader bar about one meter in length. From the spreader bar a pair of short bridles attached to the main towing cable. A pair of five meter lengths of rope ran from the tow points on the ring to a 20 kg weight. In this system, therefore, the side attachment points of the bridle and weight allowed the net to tow without the attachment mechanism directly in front of the net opening. A plastic cod end was attached to the tail end of the net. Each plankton tow was approximately 25 minutes 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 minutes until it was just below the surface at which point it towed for another 5 min, and then taken aboard.

**Particulate organic matter (POM)**

A positive pressure filtration system was designed to filter approximately 12 L of water for each sample. This quantity of water was needed to obtain enough nitrogen for stable isotope analysis. A 64 cu. in. nitrogen cylinder was used as the pressure source. The nitrogen cylinder was connected to three 15 L polycarbonate carboys. Holes were drilled in the carboy caps and a polycarbonate barbed fitting was sealed into each one with silicone sealant. Each carboy was connected in series to its neighbor with tygon tubing while the third carboy was connected to the nitrogen cylinder using a high-pressure two-stage regulator. A 25 mm inline filter was connected to the nozzle of each carboy and 25 mm Whatmann GF/F’s (< 0.7 μm pore size) were used to filter the water. The whole system was pressurized to 15 psi; sufficient pressure to filter the water in a reasonable amount of time (2-3 hrs) while maintaining the integrity of the filters. Surface water was collected by fixing a 5 L bucket to 20 m length of rope that was thrown from the bow of the ship into the water. The bucket was thrown away from the ship as far as possible to avoid possible contaminants from the ship such as oil or particles. Once retrieved the sample of water was visually inspected to make certain that there were no large particles, and the water was used to fill the carboys. When filtration was competed carboys, filter holders, and their respective tubing, were rinsed with a 1 M HCl solution and then rinsed again with distilled water to insure that growth did not take place within the system. The system was designed to collect a maximum of three samples of particulate organic matter per station. Sometimes certain areas of the system developed leaks that prevented the full compliment of samples from being taken at each station.

**Sample manipulation and preparation**

**Squids**

During the cruises from 1998-2001 samples were also taken from captured squids for stable isotope analyses. A total of 143 *O. bartramii* and 160 *S. oualaniensis* were
sampled. After a squid was caught, weighed, and had its stomach removed, a 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 cartilage or excessive photophore material (the dorsal photophore in *S. oualaniensis* was specifically avoided). In 1998 the muscle segments were rinsed lightly with distilled water and wrapped in foil that was then placed in a labeled bag. The samples were then frozen at −20°C for later manipulation and analysis in the lab. This procedure was time consuming and resulted in corrosion of the foil around the sample that complicated further manipulations. For the years 1999-2001 the muscle samples were placed in 20 ml glass scintillation vials that were capped, labeled and frozen as before. For analysis the muscle sample was thawed and removed from the vial. 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 been unexposed to any outside materials. The remaining sample was placed back into the vial and refrozen. The sectioned muscle sample was then cut into smaller pieces to increase surface area, placed into a clean 20 ml glass scintillation vial and dried at 60°C overnight. These smaller pieces were then ground into a fine powder using a Wig-L-Bug dental amalgamater (Crescent Dental MFG. Co, Model 5AR). The resulting powder was then weighed out to between 300 and 1000 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 considering the C:N ratios of the samples used.

In addition to muscle, various other tissues were also sampled from select squids in order to compare the isotopic fractionation and turnover times between tissues and to investigate possible ontogenetic signals in secreted hard structures. The eyes of many of the squids were removed and frozen for later analysis. In the lab select eyes were thawed and the lenses removed and any residual tissue or fluid was cleaned. The distal lens was removed and discarded leaving the larger proximal lens. Using micro-forceps, separate distinct layers or “shells” of a lens were separated from each other. Starting from the innermost shell (smallest) of a lens, the shells were sequentially separated, ending with
the outermost (largest). The shells were placed in foil lined plastic scintillation caps and the caps were labeled. After separation the thickness of each shell was measured using an ocular micrometer fixed in a dissection microscope. Each shell was then dried, ground, and weighed as stated above. During growth the eye lens must be constantly re-adjusted to maintain the proper gradient of refractive index. This presumably occurs by shrinkage from loss of water. Determination of an accurate correction factor for this effect was not possible. The shrinkage effect would be a slight overestimate of ML in the retrospective values.

Samples of blood were also taken from large squids. Blood samples were taken before all other measurements in order to minimize the amount of blood lost to bleeding from the incision into the mantle. Blood was collected from two areas in the squids, the efferent branchial vessels coming from the gills, and the cephalic vein before it divides into the vena cavae. Ten cc syringes fixed with 18 gauge needles were used to collect the blood. The syringes were rinsed with 100% methanol and then twice with distilled water before sampling a new animal. Squids of around 200mm ML or larger were usually required to obtain enough blood, however squids of smaller sizes (180-190mm ML) would occasionally yield a sufficient quantity. Blood sample sizes ranged from less than one ml to over 20 ml from the largest squids caught. Blood samples were dried and ground as above.

Potential prey items of *O. bartramii* and *S. oualaniensis*

Fishes caught by trawl during cruises in 1999-2001 were used for SIA and to determine otolith size versus fish weight relationships. Only species that could be identified were used for these analyses. Identified fish were measured for their standard lengths (measurement starts at foremost point on fish and ends at the fork of the tail). The fish were also weighed, their otoliths were removed and measured using an ocular micrometer and dissection microscope, and a muscle sample was excised. When taking the muscle sample, the skin and scales were removed and a fillet of meat was taken from the dorsal musculature near the dorsal fin, care was taken to avoid bone tissue. The muscle sample was then dried, ground and weighed as above.
Large fishes

Muscle samples were taken from all tuna (Thunnus obesus, T. albacares, T. alalunga, and Katsuwonus pelamis) and mahi (Coryphaenus hippurus) caught which were not being utilized for other research. Samples of white muscle were excised from just behind the pectoral fin of the tuna and mahi mahi. All samples were placed in labeled 20 ml glass scintillation vials and frozen at -20°C. In the laboratory samples of white muscle of both species were thawed, trimmed, dried, ground and weighed following the same protocol as previous muscle samples.

Plankton

Plankton samples were kept in 10 L buckets of seawater chilled with bags of ice to keep them from overheating during sorting. Samples were examined for paralarvae of squids and any paralarvae were identified. O. bartramii and S. oualaniensis paralarvae were placed in 20 ml glass scintillation vials, labeled, and frozen at -20°C for later analysis. The remaining plankton sample was filtered through a 333 um Nitex mesh and the resulting bolus of material was placed in a labeled plastic ziplock® bag and frozen at -20°C.

In the laboratory paralarval mantle lengths of O. bartramii and S. oualaniensis were measured using an ocular micrometer on a dissection microscope. Because of the small size of the paralarvae the specimens were not subjected to the grinding process and whole animals were dried and weighed for isotopic analysis as noted above. The mantle and head/viscera were separated from a single large O. bartramii paralarva.

The plankton samples were thawed and a “representative” sub-sample was taken from the mass of zooplankton. A small amount of the whole bolus of plankton was taken, dried, ground and weighed as above. Typically, no attempt was made to categorize the zooplankton or to estimate the contribution of one group or another to the sample as a whole. From one tow in 2000 four categories of organisms were separated, euphausids, hyperid amphipods, chaetognaths, and radiolaria. Multiple individuals of each group were combined and treated as above.
Particulate organic matter

The filters were dried in 20 ml scintillation vials as above. The filters were then placed in flattened foil boats and rolled and then placed into a small glass tube. The foil and filter were then tamped down on both ends by small glass rods that fit inside the tube, this resulted in a compact foil covered filter which was then analyzed.

Elemental analyses and mass spectrometry

All samples which were investigated for the isotopes $^{15}\text{N}$ and $^{13}\text{C}$ were analyzed using the same system located in 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 (Continuos Flow) interface. The ConFlo II allowed for fully automated dual element analysis (C and N) from each combustion including determination of C and N elemental concentrations.

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

Results

Filters
The $\delta^{15}N$ values for filtered POM samples collected between 1999 and 2001 ranged from -0.7 to 6.8\%. The mean $\delta^{15}N$ values for filtered material varied by up to 3.04\% between years (Table 2-1). There was significant difference (one-way ANOVA, $p < 0.001$) between the $\delta^{15}N$ of POM collected during 1999 and other years. A two sample t-test showed that the $\delta^{15}N$ of POM collected during the 1999 Hokusei Maru cruise was not significantly different from the $\delta^{15}N$ of POM collected during the cruises aboard the Townsend Cromwell ($p = 0.057$). There were no clear trends in $\delta^{15}N$ with latitude or longitude, although variation between years was evident (Figs. 1, 2).
Table 1. The mean and standard deviations of $\delta^{15}$N values for all POM collected on separate ships during different years. HM= Hokusei Maru, TC= Townsend Cromwell, 1999 total is the combined values for the HM and TC cruises. The Townsend Cromwell cruises took place in November and January; all other samples were taken during February.

<table>
<thead>
<tr>
<th>Year (Ship)</th>
<th>Mean $\delta^{15}$N (SD)</th>
<th>Min-max</th>
<th># of filters</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999 (TC)</td>
<td>2.0 (0.8)</td>
<td>0.5-4.0</td>
<td>14</td>
</tr>
<tr>
<td>1999 (HM)</td>
<td>1.0 (1.3)</td>
<td>-0.7-3.0</td>
<td>8</td>
</tr>
<tr>
<td>1999 (total)</td>
<td>1.7 (1.1)</td>
<td>-0.7-4.0</td>
<td>22</td>
</tr>
<tr>
<td>2000 (HM)</td>
<td>3.5 (1.5)</td>
<td>0.8-5.7</td>
<td>16</td>
</tr>
<tr>
<td>2001 (HM)</td>
<td>4.0 (1.9)</td>
<td>2.1-6.8</td>
<td>13</td>
</tr>
<tr>
<td>All years combined</td>
<td>2.8 (1.6)</td>
<td>-0.7-6.8</td>
<td>51</td>
</tr>
</tbody>
</table>

Plankton

The $\delta^{15}$N values for plankton samples collected during 1999 and 2000 ranged from 0.1 to 6.5%. The mean $\delta^{15}$N values for bulk plankton material varied by 0.7% between 1999 and 2000 (Table 2-2). The $\delta^{15}$N values for the bulk plankton did not show significant differences between 1999 and 2000 using two sample t-tests at $p = 0.294$. 
Table 2. The mean $\delta^{15}$N values for plankton collected during 1999 and 2000 aboard the Hokusei Maru. The plankton category for 2000 includes the various subgroups listed separately. The number of samples in the categories with specific taxa represent number of combined individuals.

<table>
<thead>
<tr>
<th>Year (category)</th>
<th>Mean $\delta^{15}$N (SD)</th>
<th>Min-max</th>
<th># of samples/individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999 (bulk)</td>
<td>3.9 (1.1)</td>
<td>2.3-6.4</td>
<td>10</td>
</tr>
<tr>
<td>2000 (bulk)</td>
<td>4.6 (1.8)</td>
<td>0.1-6.5</td>
<td>15</td>
</tr>
<tr>
<td>2000 (Euphausidaceae)</td>
<td>5.4 (-)</td>
<td>(-)</td>
<td>5</td>
</tr>
<tr>
<td>2000 (Hyperiidae)</td>
<td>5.1 (-)</td>
<td>(-)</td>
<td>5</td>
</tr>
<tr>
<td>2000 (Radiolaria)</td>
<td>0.1 (-)</td>
<td>(-)</td>
<td>10</td>
</tr>
<tr>
<td>2000 (Chaetognatha)</td>
<td>5.6 (-)</td>
<td>(-)</td>
<td>5</td>
</tr>
</tbody>
</table>

Potential prey items of (O. bartramii and S. oualaniensis)

SIA was conducted on 113 individuals comprising 7 families of fishes and 2 families of squids that are known or potential prey items for O. bartramii and/or S. oualaniensis. Thirteen species of myctophids were analyzed.
Table 3. The mean $\delta^{15}$N values for different categories of known and potential prey items of *O. bartramii* and/or *S. oualaniensis*. Standard deviations are listed in parentheses. All species of myctophid listed are included in the category Myctophidae. Heavily outlined cells represent myctophid species.

<table>
<thead>
<tr>
<th>Category</th>
<th>$\delta^{15}$N (SD)</th>
<th>Min-max</th>
<th># of individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myctophidae</td>
<td>8.4 (1.8)</td>
<td>5.1-13.1</td>
<td>81</td>
</tr>
<tr>
<td>Gonostomatidae</td>
<td>11.1 (1.7)</td>
<td>8.5-12.6</td>
<td>11</td>
</tr>
<tr>
<td>Melamphaeidae</td>
<td>12.6 (1.2)</td>
<td>11.2-14.1</td>
<td>6</td>
</tr>
<tr>
<td>Sternopycthidae</td>
<td>9.5 (1.0)</td>
<td>7.8-10.8</td>
<td>7</td>
</tr>
<tr>
<td>Malacosteidae</td>
<td>9.9 (0.7)</td>
<td>9.5-10.4</td>
<td>2</td>
</tr>
<tr>
<td>Astronesthidae</td>
<td>10.6 (-)</td>
<td>(-)</td>
<td>1</td>
</tr>
<tr>
<td>Omosudis omosudis</td>
<td>10.0 (-)</td>
<td>(-)</td>
<td>1</td>
</tr>
<tr>
<td>Abraliopsis sp.</td>
<td>8.3 (0.7)</td>
<td>7.8-8.8</td>
<td>2</td>
</tr>
<tr>
<td>Histioteuthis hoylei</td>
<td>11.0 (-)</td>
<td>(-)</td>
<td>1</td>
</tr>
<tr>
<td>Abralia trigonura</td>
<td>10.1 (-)</td>
<td>(-)</td>
<td>1</td>
</tr>
<tr>
<td>Bolinichthys longipes</td>
<td>7.3 (0.7)</td>
<td>6.6-8.4</td>
<td>5</td>
</tr>
<tr>
<td>Ceratoscopelus warmingii</td>
<td>8.9 (2.2)</td>
<td>6.5-13.1</td>
<td>27</td>
</tr>
<tr>
<td>Diaphus trachops</td>
<td>8.6 (0.1)</td>
<td>8.5-8.7</td>
<td>2</td>
</tr>
<tr>
<td>Electrona risso</td>
<td>10.3 (-)</td>
<td>(-)</td>
<td>1</td>
</tr>
<tr>
<td>Hystrophum proximum</td>
<td>7.5 (0.8)</td>
<td>6.3-9.4</td>
<td>13</td>
</tr>
<tr>
<td>Hystrophum rheinhardtii</td>
<td>7.5 (0.7)</td>
<td>6.9-8.4</td>
<td>4</td>
</tr>
<tr>
<td>Lampadinae luminosa</td>
<td>9.6 (0.5)</td>
<td>9.3-10.0</td>
<td>2</td>
</tr>
<tr>
<td>Lobianchia gemelleri</td>
<td>8.3 (1.1)</td>
<td>6.4-10.2</td>
<td>13</td>
</tr>
<tr>
<td>Lampamyctus nobilis</td>
<td>7.0 (-)</td>
<td>(-)</td>
<td>1</td>
</tr>
<tr>
<td>Lampamyctus tenuiformis</td>
<td>10.6 (1.4)</td>
<td>9.0-12.1</td>
<td>5</td>
</tr>
<tr>
<td>Myctophum lychnobium</td>
<td>6.7 (1.3)</td>
<td>5.2-8.2</td>
<td>4</td>
</tr>
<tr>
<td>Myctophum selenoides</td>
<td>10.8 (-)</td>
<td>(-)</td>
<td>1</td>
</tr>
<tr>
<td>Symbolophorus evermanni</td>
<td>7.1 (0.6)</td>
<td>6.4-7.5</td>
<td>3</td>
</tr>
</tbody>
</table>

Individuals of Sternopycthidae, Gonostomatidae, *L. tenuiformis*, *M. lychnobium*, and Melamphaeidae (minus one outlier) all showed linear increases in $\delta^{15}$N with length (fork length) (Fig. 3). The remaining groups of myctophids did not show clear increases in $\delta^{15}$N with length (Fig. 4).
Large fishes

SIA was conducted on 53 individuals from two families of fishes comprising five species of large epipelagic fishes (Table 2-4).

Table 4. The mean $\delta^{15}$N values for different categories of epipelagic fishes, standard deviations are listed in parentheses. The tuna category is comprised of the four species listed in the heavily outlined cells.

<table>
<thead>
<tr>
<th>Fish Category</th>
<th>$\delta^{15}$N (SD)</th>
<th>Min-max</th>
<th># of individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Coryphaena hippurus</em></td>
<td>9.2 (1.2)</td>
<td>7.9-12.2</td>
<td>23</td>
</tr>
<tr>
<td>Tuna (combined)</td>
<td>10.6 (1.2)</td>
<td>8.2-13.3</td>
<td>30</td>
</tr>
<tr>
<td><em>Thunnus obesus</em></td>
<td>10.6 (1.0)</td>
<td>8.7-13.3</td>
<td>21</td>
</tr>
<tr>
<td><em>Thunnus albacares</em></td>
<td>10.0 (1.6)</td>
<td>8.2-12.7</td>
<td>7</td>
</tr>
<tr>
<td><em>Thunnus australis</em></td>
<td>11.8 (-)</td>
<td>(-)</td>
<td>1</td>
</tr>
<tr>
<td><em>Katsuwonus pelamis</em></td>
<td>12.4 (-)</td>
<td>(-)</td>
<td>1</td>
</tr>
</tbody>
</table>

The values of $\delta^{15}$N were significantly different (two sample t-test, $p < 0.001$) between the combined tuna values and mahi mahi (*Coryphaena hippurus*). There were no significant differences in between species comparisons of the mean values of $\delta^{15}$N for *Thunnus obesus* and *Thunnus albacares* (two sample t-test, $p = 0.391$). There was no relationship of $\delta^{15}$N with fork length for all tuna combined or for the mahi mahi (Fig. 5).

Squids (*O. bartramii and S. oualaniensis*)

*O. bartramii*

SIA was conducted on the mantle muscle of 174 separate individuals of *O. bartramii* that were divided into five categories based on mantle length (Table 2-5). The 1-7 mm category is composed of 27 paralarvae where the whole animals were analyzed.
The mantle and head/viscera were separated for one paralarvae (ML = 5mm) and their results were comparable, mantle: $\delta^{15}N = 5.23\%$; head/viscera: $\delta^{15}N = 4.92\%$.

Table 5. The mean $\delta^{15}N$ values of mantle muscle for different categories of O. bartramii, standard deviations are listed in parentheses. Whole animals were analyzed in the category of 0-7 mm ML.

<table>
<thead>
<tr>
<th>Mantle length (mm)</th>
<th>Mean $\delta^{15}N$ (SD)</th>
<th>Min-max</th>
<th># of individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-7</td>
<td>6.4 (1.2)</td>
<td>4.0-8.5</td>
<td>27</td>
</tr>
<tr>
<td>75-200</td>
<td>6.9 (0.7)</td>
<td>6.0-8.0</td>
<td>8</td>
</tr>
<tr>
<td>200-300</td>
<td>11.1 (1.5)</td>
<td>9.7-14.4</td>
<td>40</td>
</tr>
<tr>
<td>300-400</td>
<td>13.3 (1.0)</td>
<td>10.4-15.4</td>
<td>107</td>
</tr>
<tr>
<td>400-570</td>
<td>12.8 (1.4)</td>
<td>10.0-16.8</td>
<td>19</td>
</tr>
</tbody>
</table>

The 30 squid taken from northern latitudes exhibited a narrow range of $\delta^{15}N$ values (std dev = 0.3) over a mantle length range of 193 to 292 mm. The $\delta^{15}N$ values of mantle muscle tissue showed a sigmoidal relationship (4-parameter logistic curve, $r^2 = 0.8$) with mantle length for all O. bartramii samples (Fig. 7). No statistically significant differences were found between $\delta^{15}N$ values and latitude or longitude of collection stations within individual years for mantle muscle tissue taken from male O. bartramii with mantle lengths of > 300 mm (one-way ANOVA, 1998- $p = 0.206$, 1999- $p = 0.057$, 2001- $p = 0.82$). Between-year samples, however, did show some differences. The $\delta^{15}N$ values were significantly higher for mantle muscle taken from males > 300 mm ML captured in 1998 and 2000 (n = 44, $\mu = 14.0\%$, SD = 0.75) than for males > 300 mm ML captured in 1999 and 2001 (n = 62, $\mu = 12.8\%$, SD = 0.85) (2-sample t-test, $p < 0.001$). There were no significant differences in male mantle muscle $\delta^{15}N$ values taken from males > 300 mm ML between years 1998 and 2000 or 1999 and 2001 (2-sample t-test, $p = 0.980$ and $p = 0.315$ respectively). There was no significant difference between the mantle muscle $\delta^{15}N$ values of male and female O. bartramii captured in 1999 (2-sample t-test, $p = 0.178$) or 2001 (2-sample t-test, $p = 0.46$). Only one female sample was obtained during 1998 and another in 2000.

SIA was conducted on 181 segments of eye lenses from 14 different O. bartramii individuals (six males, five females and three juveniles). The $\delta^{15}N$ values of eye lens
samples ranged from a minimum of 3.6% to a maximum of 16.8% for all individuals. The \( \delta^{15}N \) values of eye lens samples showed a sigmoidal relationship (4-parameter logistic curve) with estimated mantle length (based on eye lens radius) for each male and female sample, and for all samples combined (Table 2-5, Fig. 8). The \( r^2 \) for the logistic curve fitted to the \( \delta^{15}N \) of the eye lens segments versus estimated mantle length from all individuals combined was 0.8. However, the mean \( r^2 \) for each specific eye lens segment curves was 0.9 indicating considerable difference between individuals.

Table 6. The \( r^2 \) values of the sigmoidal relationship for \( \delta^{15}N \) of eye lens segments and estimated mantle length (based on eye lens radius), and number of samples for different \( O. bartramii \) individuals.

<table>
<thead>
<tr>
<th>Sex, date caught, ML</th>
<th>( r^2 )-value</th>
<th>Min-max</th>
<th># of samples (eye segments)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, 2-8/9-00, 296 mm</td>
<td>0.99</td>
<td>4.8-10.9</td>
<td>12</td>
</tr>
<tr>
<td>Male, 2-8/9-00, 318 mm</td>
<td>0.99</td>
<td>4.6-14.5</td>
<td>11</td>
</tr>
<tr>
<td>Male, 2-7/8-00, 333 mm</td>
<td>0.99</td>
<td>4.2-12.7</td>
<td>8</td>
</tr>
<tr>
<td>Male, 2-8/9-00, 354 mm</td>
<td>0.98</td>
<td>6.5-11.7</td>
<td>9</td>
</tr>
<tr>
<td>Male, 2-9/10-00, 360 mm</td>
<td>0.88</td>
<td>6.1-12.6</td>
<td>14</td>
</tr>
<tr>
<td>Male, 2-11/12-01, 370 mm</td>
<td>0.98</td>
<td>5.1-13.7</td>
<td>14</td>
</tr>
<tr>
<td>Female, 2-16/17-99, 446 mm</td>
<td>0.97</td>
<td>3.7-16.7</td>
<td>20</td>
</tr>
<tr>
<td>Female, 2-11/12-01, 534 mm</td>
<td>0.94</td>
<td>4.2-15.8</td>
<td>21</td>
</tr>
<tr>
<td>Female, 2-9/10-00, 540 mm</td>
<td>0.97</td>
<td>4.2-14.5</td>
<td>19</td>
</tr>
<tr>
<td>Female, 2-11/12-01, 557 mm</td>
<td>0.81</td>
<td>7.4-10.3</td>
<td>20</td>
</tr>
<tr>
<td>Female, 2-11/12-01, 570 mm</td>
<td>0.98</td>
<td>3.9-16.8</td>
<td>17</td>
</tr>
</tbody>
</table>

The \( \delta^{15}N \) values of eye lens segments for the three juvenile \( O. bartramii \) did not show a sigmoidal relationship with estimated mantle length, but a nearly straight and horizontal line. This shape is similar to the corresponding portion of the \( \delta^{15}N \) versus
mantle length curve for mantle muscle and therefore supports the initial shape of the sigmoidal curve (Fig. 9).

SIA was conducted on 61 samples of *O. bartramii* blood each from a separate individual (Fig. 10). The $\delta^{15}$N values ranged from 9.7 to 14.7‰ with a mean value of 12.2‰, SD = 0.9. A separate logistic curve could not be fit to the blood samples because the smaller animals caught did not have enough blood for analysis. There was a significant difference between the mean $\delta^{15}$N values of blood taken from individuals in 1998 and 2000 ($n = 10, \mu = 13.4\%$, SD = 0.6) and 1999 and 2001 ($n = 51, \mu = 12.0\%$, SD = 0.8) (2-way t-test, $p < 0.001$).

*S. oualaniensis*

SIA was conducted on the 167 separate *S. oualaniensis* individuals (153 subadults/adults and 14 paralarvae). The mean $\delta^{15}$N value for sub-adult and adult mantle muscle (ML range, 128 to 324 mm) was 8.2‰, SD = 1.12 with a range of 6.2-11.2‰. The mean $\delta^{15}$N value for paralarvae (whole body sample) was 6.2‰, SD = 1.44 with a range of 4.2 to 8.0‰. The $\delta^{15}$N values for all *S. oualaniensis* mantle muscle samples showed an exponential increase (single exponent, 3-parameter) with mantle length (Fig. 11, $r^2 = 0.56$). Samples taken in 1999-2001 analyzed separately showed a stronger exponential relationship between $\delta^{15}$N and mantle length, and samples in 1998 showed a weaker relationship (Table 2-6.).

**Table 7.** The $r^2$ values, and number of samples of (mantle muscle) from all sizes (whole body) taken for each year. The $r^2$ values are for single exponent 3-parameter exponential relationships between mantle length and $\delta^{15}$N.

<table>
<thead>
<tr>
<th>Year</th>
<th>$r^2$</th>
<th>Min-max</th>
<th># of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>0.38</td>
<td>6.1-9.2</td>
<td>32</td>
</tr>
<tr>
<td>1999</td>
<td>0.75</td>
<td>4.2-11.2</td>
<td>50</td>
</tr>
<tr>
<td>2000</td>
<td>0.82</td>
<td>4.2-9.0</td>
<td>38</td>
</tr>
<tr>
<td>2001</td>
<td>0.79</td>
<td>5.4-10.6</td>
<td>47</td>
</tr>
</tbody>
</table>
A subgroup of samples from different years showed high $\delta^{15}N$ values for the given mantle lengths. Station 2-12/13-00 (21.17°N, 152.19°W) had two (out of six caught at that station) female samples as well as three paralarvae (all from that station) that were isotopically heavy for their size (Fig. 11). Samples taken from a nearby station the following year 2-10/11-01 (21.20°N, 152.30°W) did not show any substantially higher $\delta^{15}N$ values from average. Squids from two northwestern stations, 2-9/10-99 (28.59°N, 164.59°W) and 2-14/15-99 (28.28°N, 163.12°W) also showed an isotopically heavy mean $\delta^{15}N$ for their size (Fig. 12). There were statistically significant increases in adult mantle length and $\delta^{15}N$ of muscle for S. oualaniensis caught during successive years (one-way ANOVA, $p < 0.001$ for ML and $\delta^{15}N$ against year) (Table 2-7).

**Table 8. The mean and standard deviation of mantle length values (mm) of S. oualaniensis individuals used for SIA during years 1998-2001.** The mean $\delta^{15}N$ values for 1999 were calculated without the two isotopically heavy stations, n=32.

<table>
<thead>
<tr>
<th>Year</th>
<th># of individuals</th>
<th>Mean ML (SD)</th>
<th>Mean $\delta^{15}N$%o (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>32</td>
<td>168 (17)</td>
<td>7.1 (1.3)</td>
</tr>
<tr>
<td>1999</td>
<td>46</td>
<td>187 (41)</td>
<td>7.7 (0.9)*</td>
</tr>
<tr>
<td>2000</td>
<td>44</td>
<td>204 (32)</td>
<td>8.0 (1.2)</td>
</tr>
<tr>
<td>2001</td>
<td>43</td>
<td>234 (56)</td>
<td>8.5 (1.2)</td>
</tr>
</tbody>
</table>

Because of the relationship of $\delta^{15}N$ with mantle length, residuals of the $\delta^{15}N$ data against mantle length (calculated using the exponential relationship noted above) were used to explore geographical and temporal relationships of the $\delta^{15}N$ values for S. oualaniensis mantle muscle. There were no statistical differences in the residuals between years when the two northwestern stations in 1999 were excluded (one-way ANOVA, $p = 0.242$) (Table 2-8). There were also no statistical differences in residuals of all years combined against binned latitude (one-way ANOVA, $p = 0.247$) or binned longitude (one-way ANOVA, $p = 0.142$) (Table 2-8).
Table 9. The mean and standard deviation of residual values of δ15N (mantle muscle) for S. oualaniensis mantle samples for year, latitude, and longitude. *The mean residual values for 1999 were calculated without the two isotopically heavy stations.

<table>
<thead>
<tr>
<th>Year/Latitude/Longitude</th>
<th>number of samples</th>
<th>mean of residuals (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>32</td>
<td>-0.351 (0.717)</td>
</tr>
<tr>
<td>1999*</td>
<td>32</td>
<td>-0.004 (0.624)</td>
</tr>
<tr>
<td>2000</td>
<td>44</td>
<td>-0.108 (0.869)</td>
</tr>
<tr>
<td>2001</td>
<td>43</td>
<td>-0.189 (0.547)</td>
</tr>
<tr>
<td>18-20°N</td>
<td>17</td>
<td>0.061 (0.636)</td>
</tr>
<tr>
<td>20-22°N</td>
<td>80</td>
<td>-0.283 (0.691)</td>
</tr>
<tr>
<td>22-24°N</td>
<td>16</td>
<td>-0.077 (0.629)</td>
</tr>
<tr>
<td>25-28°N</td>
<td>28</td>
<td>-0.033 (0.851)</td>
</tr>
<tr>
<td>28-30°N</td>
<td>10</td>
<td>-0.047 (0.545)</td>
</tr>
<tr>
<td>152-156°W</td>
<td>79</td>
<td>-0.129 (0.672)</td>
</tr>
<tr>
<td>156-158°W</td>
<td>20</td>
<td>-0.326 (0.714)</td>
</tr>
<tr>
<td>158-161°W</td>
<td>31</td>
<td>-0.309 (0.831)</td>
</tr>
<tr>
<td>161-165°W</td>
<td>21</td>
<td>0.099 (0.580)</td>
</tr>
</tbody>
</table>

There was a statistical difference (two-sample t-test, p<0.001) between the mean residual values for δ15N (mantle muscle) of all samples of S. oualaniensis (n = 151) and samples taken from the two anomalous stations in 1999 (n = 18). There was also a statistical difference between actual mean δ15N values (mantle muscle) of the two isotopically heavy stations in 1999 (n = 18) and all the other 1999 stations combined (n = 32) (two-sample t-test, p < 0.001).

SIA was conducted on 158 eye lens segments from 14 S. oualaniensis (13 females, 1 male). The data from individuals was separated into three classes (typical, atypical, anomalous) based on their relationships of δ15N to estimated mantle length (estimated from eye lens radius). The typical class of individuals (n = 11) showed an exponential relationship (three parameter exponential, r² = 0.77) between δ15N and estimated mantle length (Fig. 12). The atypical class (n = 4) also showed an exponential relationship between δ15N of eye lens segments and estimated mantle length, but the curvature was much greater than the typical class (three parameter exponential, r² = 0.90). The anomalous class consisted of a single female individual whose δ15N values of its eye lens segments were much greater than any individual in the other classes (Table. 2-10) and did not show an exponential relationship between δ15N values of its eye lens.
segments and estimated mantle length (Fig. 12). The exponential curves for eye lens segments in the typical and atypical classes were different in curvature and elevation from the combined mantle muscle $\delta^{15}N$ versus mantle length curve.

Table 10. The number of samples, minimum and maximum $\delta^{15}N$ values of eye lens segments for *S. oualaniensis* individuals from the three different classes and the $r^2$ values for their exponential relationship with estimated mantle length (based on eye lens radius).

<table>
<thead>
<tr>
<th>Sex, date caught, ML</th>
<th>class</th>
<th>Min-max $\delta^{15}N$ (#)</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, 2-12/13-00, 158 mm</td>
<td>typical</td>
<td>3.7-5.3 (9)</td>
<td>0.92</td>
</tr>
<tr>
<td>Female, 2-14/15-99, 200 mm</td>
<td>typical</td>
<td>4.2-9.0 (11)</td>
<td>0.93</td>
</tr>
<tr>
<td>Female, 2-12/13-00, 209 mm</td>
<td>typical</td>
<td>4.3-6.7 (13)</td>
<td>0.94</td>
</tr>
<tr>
<td>Female, 2-7/8-00, 210 mm</td>
<td>typical</td>
<td>3.6-5.8 (10)</td>
<td>0.78</td>
</tr>
<tr>
<td>Female, 2-14/15-99, 223 mm</td>
<td>typical</td>
<td>5.4-9.8 (12)</td>
<td>0.96</td>
</tr>
<tr>
<td>Female, 2-13/14-00, 251 mm</td>
<td>typical</td>
<td>4.2-6.2 (12)</td>
<td>0.88</td>
</tr>
<tr>
<td>Female, 2-12/13-01, 255 mm</td>
<td>typical</td>
<td>2.8-9.4 (12)</td>
<td>0.97</td>
</tr>
<tr>
<td>Female, 2-12/13-01, 302 mm</td>
<td>typical</td>
<td>3.8-8.8 (11)</td>
<td>0.96</td>
</tr>
<tr>
<td>Female, 2-12/13-01, 304 mm</td>
<td>typical</td>
<td>3.3-9.6 (10)</td>
<td>0.98</td>
</tr>
<tr>
<td>Female, 2-12/13-01, 308 mm</td>
<td>typical</td>
<td>3.1-9.8 (12)</td>
<td>0.96</td>
</tr>
<tr>
<td>Female, 2-14/15-99, 324 mm</td>
<td>typical</td>
<td>4.2-10.0 (10)</td>
<td>0.96</td>
</tr>
<tr>
<td>Female, 2-12/13-00, 160 mm</td>
<td>atypical</td>
<td>4.2-8.9 (11)</td>
<td>0.95</td>
</tr>
<tr>
<td>Female, 2-12/13-00, 173 mm</td>
<td>atypical</td>
<td>4.6-8.5 (10)</td>
<td>0.94</td>
</tr>
<tr>
<td>Female, 2-12/13-01, 181 mm</td>
<td>anomalous</td>
<td>11.3-12.6 (10)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

SIA was conducted on 21 blood samples, each from a separate *S. oualaniensis* individual, only two of these individuals were under 200 mm ML. The $\delta^{15}N$ values from the blood samples had a mean of 8.0‰, SD= 1.2 and ranged from 6.4 to 10.1‰ (11.3‰ for the anomalous individual). The blood samples from 2000 and 2001 were significantly different (two-sample t-test, p=0.025) with blood from 2001 being heavier (2000: n = 14, $\mu\delta^{15}N = 7.8‰$, SD = 1.1; 2001: n = 6, $\mu\delta^{15}N = 8.7‰$, SD = 0.736). There was an exponential increase in blood $\delta^{15}N$ with mantle length ($r^2 = 0.70$, Fig. 13) that differed only slightly from the mantle muscle $\delta^{15}N$ versus ML curve.
Species comparisons

The mean $\delta^{15}N$ values for all animals sampled increased with certain taxa (Fig. 14). The $\delta^{15}N$ values for mantle muscle of *S. oualaniensis* and *O. bartramii* were significantly different (two-sample t-test, $p < 0.001$) with *O. bartramii* being 4.5% greater on average than *S. oualaniensis*. Unless otherwise stated all comparisons are based on adult and sub-adults squid. The $\delta^{15}N$ value (mean of means) from the muscle of the top five most abundant fish prey items found in *O. bartramii* stomachs was 9.7%, which is 3.55% less than the mean value for all muscle taken from *O. bartramii*. The $\delta^{15}N$ value (mean of means) of the top five most abundant fish prey items found in *S. oualaniensis* was 7.5% which is 0.6% less than the mean muscle value for all *S. oualaniensis*.

The $\delta^{15}N$ values for blood of *S. oualaniensis* and *O. bartramii* were significantly different (two-sample t-test, $p < 0.001$) with *O. bartramii* being 4.2% greater on average than *S. oualaniensis*. *O. bartramii* and *S. oualaniensis* each had significantly higher $\delta^{15}N$ values for the mantle than the blood. In *O. bartramii* the mantle muscle was 0.6% heavier than the blood (paired t-test, $n = 21$, $p < 0.001$) and in *S. oualaniensis* the mantle was 0.59% heavier than the blood (paired t-test, $n = 20$, $p < 0.001$) (Figs. 15, 16).

The $\delta^{15}N$ values, within single individuals, for outer eye lens segments of *O. bartramii* were significantly different from mantle muscle values ($n = 13$, $p < 0.001$). For *O. bartramii* there a mean 1.0% difference between mantle and outer eye lens segments. The $\delta^{15}N$ values, within single individuals, for the outer eye lens segments in *S. oualaniensis* were significantly different from the mantle muscle values (paired T-tests, $n = 14$, $p = 0.004$). For *S. oualaniensis* there was a mean difference of 1.1% between mantle and outer eye segments.
Discussion

Filters

The $\delta^{15}N$ values collected for the POM are within the range of other published values for PON in the North Pacific (Wada et al., 1975; Wada and Hattori, 1976; Wada and Hattori, 1991; Karl et al., 1997). The mean of all years (2.8‰) is similar to annual means (3.1‰) for $\delta^{15}N$ of sinking particles reported by Karl et al. (1997) in the same waters. The $\delta^{15}N$ values of POM collected in 1999 were significantly lighter from other years indicating a possible increased contribution of N$_2$ fixation during that year. The total range of values was 7.6‰, but because of the wide range of processes that can contribute to $\delta^{15}N$ signatures of POM (see Michener and Schell, 1994) this range does not necessarily translate into differences in trophic levels. The $\delta^{15}N$ values reported here are lighter than those reported for bulk phytoplankton in the North Pacific Ocean reported by Miyake and Wada (1967) which had a mean of 5.6‰. The samples of filtered material were designed to represent an approximate baseline signal for the trophic calculations in this ecosystem and were assigned a trophic level of one.

Plankton

The mean $\delta^{15}N$ value of bulk plankton was 1.1‰ heavier than the mean value for POM; much less than the 3.4‰ indicative of a trophic level enrichment. Because of the complexity of the planktonic assemblage, bulk plankton values may not be a wholly applicable value. This variability is evident in the values obtained for separate groups of plankton. Values for colonial radiolaria were among the lightest found in this study. The combined value for euphausids, chaetognaths, and hyperiids (5.4‰) agrees with other published results (Kaehler et al., 2000) and was 2.6‰ greater than the estimated trophic level one value, however these organisms cannot all be considered herbivores.

Potential prey
In general, the myctophid categories were lighter than the other mesopelagic categories. The myctophid fishes had a mean $\delta^{15}N$ value that was 4.5‰ heavier than the mean plankton value, and 3.0‰ heavier than the combined $\delta^{15}N$ values for euphausids, chaetognaths, and hyperiids. The range within the myctophids was 8.0‰, and much of this variation was due to C. warmingii, L. tenuiformis and L. gemellerii indicating substantial variation between related species and between individuals within a species. Three species of myctophids exhibited increases in $\delta^{15}N$ with length that could account for a certain amount of variation in some species such as L. tenuiformis. Very little published information exists on $\delta^{15}N$ values of myctophids, Kline (1997) lists “Northern lanternfish” as having values of approximately 13.5‰ with a variation of about 0.5‰ and an assigned trophic level of close to 4. Gould et al. (1997) found “lanternfish” in the transition zone of the North Pacific to have $\delta^{15}N$ values of 10.1 to 11.2‰, but did not assign a trophic level. In this study the mean value for myctophids indicate a trophic position of 2.6. Clarke (1972) estimated that the Myctophidae occupy the third trophic level based on his biomass estimations.

Other mesopelagic fishes (gonostomatids, melamphaeids, and sternoptychids) exhibited stronger relationships of $\delta^{15}N$ with length for muscle tissue. The $\delta^{15}N$ values of the gonostomatids and melamphaeids were 2.7 and 4.2‰ greater than the average myctophid value and indicated trophic levels of 3.4 and 3.8 respectively. Gonostoma spp. have been shown to feed on euphausids in the Gulf of Mexico (Lancraft et al., 1988) however the $\delta^{15}N$ values of the gonostomatids (a mixture of Gonostoma species) measured in this study were much higher than one would anticipate if they had substantial feeding on euphausids.

Large fishes

The tuna and mahi mahi showed no increase of $\delta^{15}N$ with size and the data were highly scattered. The difference in diving profiles of bigeye and yellowfin, and the greater abundance of mesopelagic prey species in the stomachs of deeper diving bigeye tunas (Tsuchiya, 1998), indicates fundamental foraging differences between these species. These foraging differences did not manifest as different stable isotopic signatures obtained for these two species.
**Squids (O. bartramii and S. oualaniensis)**

*O. bartramii*

The overall δ₁⁵N values for *O. bartramii* are in general agreement with other published results. Takai *et al.* (2000) report δ₁⁵N 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) report δ₁⁵N values of between 10.0-15.1‰ for 44 *O. bartramii* (170-446 mm) 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 δ₁⁵N (mantle muscle and eye segments) with size and the sigmoidal shape of the curve.

Body size is 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 δ₁⁵N with increasing size across taxa have been documented in several marine ecosystems (Sholto-Douglas *et al.*, 1991; France *et al.*, 1998; Fry and Quinones, 1994; Kline, 1997). Sholto-Douglas *et al.* (1991), however, found that δ₁⁵N values for anchovy (*Engraulis capensis*) and round herring (*Etrumeus whiteheadi*) decreased with increasing fish length. Two hypotheses were put forward for this reverse trend, a physiological basis (i.e., changes in growth or assimilation efficiencies), or a trophic basis, (i.e., reflecting a shift from zoophagy to phytophagy). The range of δ₁⁵N in the lengths of fishes they tested was only 1.2‰, much less than was observed in this study. Kline *et al.* (1998) observed species-specific increases in δ₁⁵N with increasing fish length (∼ 60-480 mm) for four species of salmonids in waters off Northern Alaska. The δ₁⁵N versus length data for all four species were fitted with second-order polynomial regressions that detailed rapid increase in δ₁⁵N with length followed by a flattening of the curve and a plateau at the greatest fish lengths. The greatest range of δ₁⁵N 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 δ₁⁵N 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 (≈ 7‰, Trophic level (TL) ≈ 2) to adult (≈ 13‰, TL ≈ 4).

The δ¹⁵N mantle data for *O. bartramii* shows an initial plateau followed by a rapid increase in trophic position followed by a trophic plateau beginning around 250-300 mm mantle length. The latter plateau corresponds to a trophic position of 4.1. Size appears to be a major factor in determining TL in this species. However, the logistic relationship seen in *O. bartramii* mantle data (rather than an exponential relationship seen in *S. oualaniensis*) indicates that the causes are more complex. The shapes of the curves will be discussed in more detail under the Species Comparisons section.

Mean mantle length of *O. bartramii* males increased in every year sampled for δ¹⁵N but the difference was not always significant. There was no significant difference between mean mantle lengths in 1998-2000 (one-way ANOVA, p = 0.055; mean ML: 1998 = 318 mm, 1999 = 321 mm, 2000 = 329 mm). There was a significant difference in mean mantle length of *O. bartramii* males caught in 2001 (mean ML = 366 mm) versus other years (one-way ANOVA, p < 0.001). Overall *O. bartramii* did not show a change in δ¹⁵N with size above 300 mm ML using mantle muscle, however overall differences in mean mantle length could indicate differential yearly growth regimes. The differences in mean δ¹⁵N of mantle muscle samples for *O. bartramii* did not, however, coincide with the mean mantle length differences between years as δ¹⁵N for 2001 was more similar to 1999 while 1998 was more similar to 2000.

Aside from mantle length, the main source of variation in δ¹⁵N values of mantle muscle was variation between years, in spite of the fact that within some years samples were taken over a wide geographical range. Uncertainty exists as to the nature of the observed yearly differences in *O. bartramii*. The 1999 POM was significantly lighter than 2000 and 2001, and the mantle values for 1999 *O. bartramii* males were lighter than those sampled in 2000 but not different from mantles sampled in 2001. Thus, heavier mantle values in 2000 correspond with heavier POM in 2000 but the trend is opposite in 2001. The results, therefore, are equivocal as to whether or not yearly changes in mantle values represent a yearly change in the baseline trophic signal of the environment. Changes in food chain length may occur with differences in species assemblages above the baseline signal that could account for yearly variation.
The retrospective $\delta^{15}$N values of the eye lenses reveal an almost identical logistic relationship to estimated mantle length as seen in the $\delta^{15}$N values of muscle versus mantle length. The fit of the logistic curve, however, was better for each individual than for all individuals combined, indicating that variation between individuals has a greater effect on this relationship than variation within individuals. Individual curves are considered in the next chapter. The interpretation of the sigmoidal curve is discussed under Species Comparisons.

While offset somewhat from mantle values the blood samples followed the mantle patterns during these years. Blood has been found to have a fast turnover rate in birds when incorporating isotopic signals ($\delta^{13}$C) (Hobson and Clarke, 1992, 1993). Within the blood Hobson and Clarke (1992, 1993) found that the half-life the blood plasma of crows was 2.9 days. Whole cellular fraction had a tenfold longer turnover time than plasma (29 days). Muscle tissue in avians was found to have a much longer half-life than blood plasma, and Hesslein et al. (1993) found that it took over 100 days for a new $\delta^{15}$N signal to be wholly incorporated after a switch in diet in broad whitefish. While cephalopod blood is very different (it does not contain cells for oxygen transport) the blood signal could be incorporated in a relatively short time frame relative to the muscle. If so, the consistent relationship in $\delta^{15}$N between blood and muscle indicates that either there is little difference in isotopic signal in waters that O. bartramii passes through on its southerly migration, or that at the time of capture the O. bartramii individuals had been residing in local waters long enough for the muscle tissue to incorporate a local signal.

**S. oualaniensis**

The $\delta^{15}$N values reported here are generally in agreement with published values. Takai et al. (2000) reported mean $\delta^{15}$N of 10.0%o (5 individuals; mean ML = 217mm) for S. oualaniensis caught off Japan (26.30°N, 144.00°E). This value is higher than the mean value obtained for adults and sub-adults for this study, but well within the range of variation for adult and sub-adult Hawaiian squids. Squid from the same family (Todarodes pacificus) with a similar size range (207 mm, SD = 7) taken from the Japan Sea also had $\delta^{15}$N values (10.5%o) comparable to S. oualaniensis of that size. Takai et al.
(2000) found large regional differences in $\delta^{15}$N values for *S. oualaniensis* throughout both the Pacific and other oceans. The greatest $\delta^{15}$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 %o), a fact they attributed to denitrification of upwelled nitrate in the area (Cline and Kaplan, 1975). The exponential increase in $\delta^{15}$N with ML is different from *O. bartramii* and will be discussed under Species Comparisons.

There were no obvious differences in $\delta^{15}$N with geography in this study, with the exception of the two stations in 1999 that showed enrichment of $\delta^{15}$N relative to all other stations. Two squids from a single station in 2000 also showed high $\delta^{15}$N values but nothing unusual was found in the stomachs of these squids. A distinction between geographically induced differences and intrinsic individual variation is difficult to determine without a better understanding of individual feeding history. Individual variation due to feeding specialization has been shown to cause $\delta^{15}$N to range by $>6$%o in northern pike within the same lake (Beaudoin *et al.*, 1999). The specialization found by Beaudoin *et al.* was seen not only in the isotopic values but also in feeding determined from stomach contents.

The increase of $\delta^{15}$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 $\delta^{15}$N with mantle length was stronger in individual years than for all the data combined, except in 1998, which had a weak relationship. The weak exponential relationship between $\delta^{15}$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.

The $\delta^{15}$N values taken for the blood samples of *S. oualaniensis* support the general trend of exponential increase in $\delta^{15}$N with increased size. The blood samples were statistically different from the mantle samples but there was a uniform difference over the mantle sizes tested. This would indicate that isotopic signals that are presumably incorporated over a short time period in the blood (weeks?) are similar to those presumably incorporated over a longer time period in the muscle (months?).
The $\delta^{15}N$ values taken from retrospective analyses of the eye lens segments of the "typical" group show an exponential increase with mantle size (estimated from eye lens radius) similar to that seen for mantle muscle, although falling well below the curve for mantle muscle at smaller sizes. The reasons for this discrepancy at smaller sizes are unknown. All of the individual retrospective relationships between $\delta^{15}N$ and estimated mantle length were stronger than for the combination of all individuals. The differences between the two steeply increasing "atypical" individuals and the more gradually increasing "typical" individuals which came from the same station could be a result of individual feeding specialization, beyond where the curves join near 75 mm ML, by the heavier group of animals. Many of the values from estimated sizes of ca. 110-150 mm ML fell in the same $\delta^{15}N$ range as the "typical" group. Another explanation for the "atypical" values seen in these individuals is different growth rates between the two groups. If fractionation is less during periods of rapid growth (Fantle et al., 1999) the animals with slower growth rates would have higher $\delta^{15}N$ values. The "anomalous" individual is problematic and will be considered in more detail in the next chapter. For other squid the $\delta^{15}N$ values observed for the eye lenses represent an increase in trophic position with size. More detailed individual analyses of these groups is conducted in Chapter III.

*Species comparisons*

The $\delta^{15}N$ values seem to describe trophic positions fairly well in this study. The increase in $\delta^{15}N$ from POM up to large animals such as squids and tunas agrees in general with existing data on the trophic position of these organisms. The fact that *O. bartramii* occupy a higher trophic level than tunas, and that the largest sized *S. oualaniensis* approach the trophic level of the tunas sampled may be counterintuitive when considering the size differences. However, size alone, is insufficient to determine trophic levels especially when the animals in question belong to unrelated taxa.

In general, adult *O. bartramii* is more then a trophic level above *S. oualaniensis* (4.6‰, 1.3 TLs), which is compatible with *S. oualaniensis* as prey for *O. bartramii* (a situation which was presumed at the beginning of this study). From the stomach analyses,
however, *S. oualaniensis* is rarely preyed upon by *O. bartramii*. This situation re-affirms the need to couple SIA with known feeding data. The higher trophic position of *O. bartramii*, relative to *S. oualaniensis*, can be partially explained by prey items found during the SCA. The five most abundant prey species found in *O. bartramii* had a mean $\delta^{15}N$ value that was almost exactly a trophic level below the mean value of adult *O. bartramii*. This is somewhat surprising given the result that *O. bartramii* was a generalist feeder and did not specialize heavily on any one prey item. The five most abundant prey items in *S. oualaniensis*, however, were only about one fifth of a trophic level below the mean value of *S. oualaniensis*, in spite of the fact that *S. oualaniensis* is a more specialized predator; only five prey species make up 75% of the otoliths recovered from *S. oualaniensis* stomachs. The prey fishes sampled for SIA, however, were too large to adequately represent *S. oualaniensis* and *O. bartramii* prey sizes. The otoliths for the five most abundant species collected from the stomachs of adult/sub-adult *S. oualaniensis* were on average only 58% of the length of otoliths from the same species used for SIA, compared to otoliths from adult/sub-adult *O. bartramii* which were 74% of the size sampled for SIA. Using the genus group of *M. lychnobium* and *M. spinosum* this effect would cause a 1.0% difference in *S. oualaniensis* and a 0.8% difference in *O. bartramii*. The fishes sampled for SIA, therefore, were most likely heavier (isotopically) than actual prey fed upon by both squids, which still places *S. oualaniensis* only about half a trophic level above, and *O. bartramii* about one and a half trophic levels, above their primary prey (using 3.4‰ per trophic level). Fishes sampled for SIA were closer in size to those fed upon by *O. bartramii* and therefore more accurately reflect the dietary $\delta^{15}N$ signal of *O. bartramii* than of *S. oualaniensis*. There is also the possibility that a substantial component of the diet of *S. oualaniensis* was missing from the stomach content study.

The patterns of $\delta^{15}N$ change with size for the mantle samples of *O. bartramii* and *S. oualaniensis* were different, a sigmoidal curve versus an exponential curve. Among the most likely factors that would contribute to these differences are: differences in feeding, growth rate, and migration. *S. oualaniensis* and *O. bartramii* clearly show differential feeding patterns according to SCA but data are few for *O. bartramii* in the size range where increases in $\delta^{15}N$ versus ML are most rapid.
The migrational pattern of *O. bartramii* may lead to the animal being subjected to isotopically different ecosystems. When *O. bartramii* migrates from near Hawaiian waters, north to the feeding grounds it may enter into an ecosystem that has a totally different baseline signal for $\delta^{15}$N. Nitrogen fixation by organisms such as *Trichodesmium* sp. can introduce low (close to zero) $\delta^{15}$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 $\delta^{15}$N values (Altabet, 1989; Liu and Kaplan, 1989) and in the transition zone (the northern end of *O. bartramii* migration) may play a more important role in supplying the nitrogen demands of the phytoplankton resulting in a higher baseline trophic signal. Wada and Hattori (1976) found a range of PON in the North Pacific from -1.7 to 9.7‰ while Miyake and Wada (1967) found a range of 3.4 to 7.2‰ for PON. Two studies have shown possible migratory patterns of $\delta^{15}$N in whale baleen (Best and Schell, 1996; Hobson and Schell, 1998) on the order of 2-4‰. Hypothetically the migration of these squids from a lighter ecosystem to a heavier one (see Wada and Hattori, 1991) and then back again should result in a more bell shaped curve of the eye lens segments. Migration south shows no obvious effect on $\delta^{15}$N in spite of seemingly mandatory changes in diet with region. The greatest range of $\delta^{15}$N for the eye lenses of *O. bartramii* was 13‰, which is a large change to explain using migration alone. 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), also did not show substantially different values, 12.1 and 11.7‰ respectively. However, if growth rates are taken into consideration as well, the situation changes. If *O. bartramii* individuals are putting on the majority of their mass over a relatively short time period at the feeding grounds, then the northern signal will be incorporated and the muscle signal will remain relatively high even though the animals are moving south again because the squid are presumably not growing as rapidly (much of the growth would be in reproductive tissue) and hence incorporating little of the lighter southern signal into muscle. The signal of the tissues, therefore, will be a combination of rapid growth of new tissue then maintenance turnover of old tissue. If turnover times of the tissues in question are too long then the southern signal will not be observed from either growth or turnover. The time periods that these animals remain in each area would have a large effect on the
shape of the eye lens segments $\delta^{15}\text{N}$ curve with length. In conjunction with these hypotheses, feeding on different prey with heavier signals during rapid growth of the squid predator could play a role in shaping the sigmoidal curve, regardless of the baseline ecosystem signal.

Little is known about the size at which $O.\ bartramii$ begins its migration northward. At 175 mm ML $S.\ oualaniensis$ begins to exhibit a variety of increasing $\delta^{15}\text{N}$ trajectories based on retrospective eye lens data (see next chapter) presumably due to growth or diet differences. The steep trajectory of $O.\ bartramii$ appears to start around the same size and could have the same cause since, by this size, they should be well into their northern migration and encountering different feeding regimes. Around this size growth (in length) should also be at a maximum, and much faster than in $S.\ oualaniensis$, which could contribute as well.

The northern group of $O.\ bartramii$ showed no increase in $\delta^{15}\text{N}$ with ML suggesting that, at this locality, prey type was independent of size and that squid had sufficient time to accommodate prey values with their tissues (this will be examined more closely in Chapter III). Presumably $\delta^{15}\text{N}$ increase with size resumed as migration continued.

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 $S.\ oualaniensis$ doesn't get quite large enough to reach a trophic plateau.

The $\delta^{15}\text{N}$ values for blood samples showed similar patterns for $S.\ oualaniensis$ and $O.\ bartramii$ to those found using muscle and eye lens segments. The mean difference between $O.\ bartramii$ and $S.\ oualaniensis$ using blood was 4.2‰ (1.2 TLs) compared to 4.6‰ (1.3 TLs) obtained for muscle of adults/sub-adults. The difference between muscle and blood within $O.\ bartramii$ and within $S.\ oualaniensis$ averaged 0.6‰ and 0.6‰ respectively and was consistent between most individuals. This indicates that a distinct difference in fractionation occurs between these tissues. Correcting for this tissue bias, no differences were apparent that might reflect different turnover times between the supposedly slow muscle and fast blood tissues. The implications are that $O.\ bartramii$
individuals sampled may have been present in the area on long enough time scales to wholly incorporate this signal in both tissues, or that there is no signal difference between waters near Hawaii and the northern range of *O. bartramii*’s migration.

**Isotopes at indicators of trophic level**

This study brings into question the efficacy of using the value of 3.4\% as the standard increase in $\delta^{15}$N per trophic level. The bulk plankton values obtained from the net tows were fairly close in value to the mean $\delta^{15}$N values obtained for the POM. Even the range of values for the bulk plankton and the POM were similar in most cases. Certain organisms such as chaetognaths had $\delta^{15}$N values which, using the 3.4\% per TL, would identify them incorrectly as herbivores. Other problems with using this standard increase occurred with the *O. bartramii* paralarvae showing very similar values to juveniles, and the 0.5 TL calculated for *S. oualaniensis* and the 1.5 TL for *O. bartramii* over their prey. These problems point to greater inherent isotopic variability between trophic positions at lower trophic levels. The greater isotopic variability between trophic positions at lower trophic levels probably reflects the complexity of the planktonic ecosystem. The effects of nutrient recycling, new production, and mixotrophy, and increased assimilation efficiencies should be most notable at lower trophic levels as well. Presumably, the varied diet of larger animals and the long integration times of their tissues, produce long-term averages that progressively damp out many of the initial factors that affect $\delta^{15}$N at the higher trophic levels. This, however, is confounded by individual trophic variability and taxa/tissue specific biases at higher trophic levels. Turnover times probably change with height in the trophic pyramid, for example, the large variability of the myctophids (~ 8\%) suggests that there may be factors other than diet that are involved in determining the isotopic signal and would therefore compromise the canonical 3.4\% value.

**Conclusions**

Several limitations with using stable isotope analyses as a method to elucidate the trophic ecology of organisms were encountered during this study. If $\delta^{15}$N is reflective of trophic level, and the trophic level of an animal is dictated by its size, then the $\delta^{15}$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. This study has also shown that using different tissues will not necessarily yield similar results. While some tissues such as the mantle, blood, and eye lenses all supported the general trends of change in $\delta^{15}\text{N}$ with size for both $S. oualaniensis$ and $O. bartramii$, there were substantial differences in the increase determined for $S. oualaniensis$ mantle and eye lens tissue.

These discrepancies between and within some tissues require that either specific tissues be carefully chosen when addressing certain questions, or that whole organisms be used throughout a study.

The variability in using SIA 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 clearly indicates different trophic ecology for these squids. However, the wide difference in trophic level determined by $\delta^{15}\text{N}$ was unexpected. Both squids fed heavily on Myctophidae, although $O. bartramii$ also clearly feeds on certain piscivorous fishes. The SIA results indicate that these animals are substantially more than a trophic level apart based on the mantle, blood, and terminal eye segments, although individual variability was great. Obviously, placing undue confidence in one or a few measurements is risky. One must also be cautious, therefore, and rely on average values until the causes of variability are better understood.

Future studies of SIA should include rigorous controlled experimental studies to determine the exact sources of $\delta^{15}\text{N}$ variability. Unfortunately this approach is not always possible. This study, for example, examined animals that do not yield easily, if at all, to experimental manipulation. Nevertheless, SIA remains one of the few techniques to assess trophic interactions within ecosystems that are difficult at best to study. In such cases, constraining the results of SIA by using different tissues and supporting techniques is crucial for obtaining useful results.

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. To better determine how these general values (and their variability) reflect the feeding ecology of an organism it is also necessary to look at specific individual variation to see how it compares to the larger general trends. This is the subject of the next chapter.
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$\delta^{15}$N

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$\text{Mantle length (mm)}$

$\delta^{15}$N (muscle)

$\text{unusually heavy individuals}$

$r^2 = 0.56$
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Chapter III: Individual isotopic analyses

Introduction

The differential fractionation of $\delta^{15}N$ and $\delta^{13}C$ by different tissues has been recognized since the inception of stable isotopic techniques in ecology (DeNiro and Epstein, 1981). More recently, researchers have begun to experimentally quantify tissue dependent differences both in scope and relative timeframe of signal incorporation (turnover time) (Hesslein et al., 1993). Mizutani et al. (1991,1992) assessed the enrichment of non-metabolic structures (feathers) in a variety of captive birds to determine the efficacy of using these structures as indicators of past feeding during the time periods of feather formation. They found that the feathers did not show an age dependent change, and that values had slight species dependent enrichments. Hobson
(1993) attempted to use liver, muscle and bone collagen to determine short, intermediate, and long-term trophic levels of seven species of seabirds. Each tissue was fairly effective in separating the birds by trophic level corresponding to known feeding habits. The isotopic signals from baleen of migratory whales have also been used in attempts to determine past feeding history (Best and Schell, 1996; Hobson and Schell, 1998). Both studies noted substantial oscillations in $\delta^{13}C$ and $\delta^{15}N$ with baleen length (age), one in southern right whales (*Eubalaena australis*) and the other in Arctic bowhead whales (*Balaena mysticetus*). Neither Best and Schell (1996) nor Hobson and Schell (1998) could isolate a single cause of the isotopic isolations and Hobson and Schell (1998) theorized that the shifts were due to either an annual movement between areas of different baseline $\delta^{15}N$ signal, an annual shift in one third of a trophic level, or that the animals underwent seasonal fasting which resulted in enrichment via protein catabolism.

Retrospective isotopic analyses should reveal much about individual feeding history, and the extent to which individuals within the same species may differ from each other. Individual variation in recent feeding history has been shown by isotopic studies. For example, Beaudoin *et al.* (1999) looked at the individual feeding specialization of the northern pike (*Esox lucius*) in five lakes in northern Alberta Canada. Both the stomach contents analysis (SCA) and the SIA revealed considerable within population variation, with some individuals differing by up to two TLs within a lake population. Beaudoin *et al.* (1999) found that specialization on fish versus benthic invertebrates between individual pike in certain lakes was not only a long-term trait (not ephemeral random feeding events) but depended on the size of the pike and the presence of other fish assemblages between lake systems; indicating size dependent trophic shifts in response to a competitive void. Vander Zanden *et al.* (2000) however found that size of lake trout (*Salvelinus namaycush*) and individual specialization did not account for the majority of isotopic variation in the species but that the majority of variation occurred between populations of different lakes.

Clearly SIA based investigation of trophic interactions can sample tissues that hold historical records. These historical records can be used to determine historical feeding and to investigate the isotopic variation that occurs within and between individuals of a population. In chapter II some overall trends emerged for both species of
squids based on composites of many individuals. The general trends were most likely controlled by the animals' trophic position however substantial variation within the trends was observed. The focus of this chapter is to make a more detailed retrospective analysis of eye lens and gladius material to address the individual variation within the more general composite trends observed.

**Materials and Methods**

See chapter II for details on the materials and methods.

**Results**

*Ommastrephes bartramii* $\delta^{15}N$ (adults)

**2-14/15-99 #14 F (570):**

The $\delta^{15}N$ of the lens segments of this squid ranged from 3.9 to 16.8‰; the mantle value was 12.8‰ and the blood was 12.2‰. The lens values increased from 159mm ML up to 372mm ML and then stayed relatively stable until after 458 mm ML where the last value decreased to 13.7‰ (Fig. 1).

**2-16/17-99 # 5 F (446):**

The $\delta^{15}N$ of the lens segments of this squid ranged from 3.7 to 16.6‰; the mantle value was 12.2‰ and the blood was 11.6‰. The lens values decreased once after the first point to their lowest value at 128mm ML then increased fairly consistently (with one peak of 14.3‰ at 245mm ML) to 16.6‰ at 304mm ML and then stayed relatively stable until after 363mm ML where the last value decreased to 13.3‰ (Fig. 2).

**2-7/8-00 #1 M (333):**

The $\delta^{15}N$ of the lens segments of this squid ranged from 4.2 to 12.7‰; the mantle value was 12.1‰. The lens values increased immediately from the first point at 125mm
ML (4.2%) to the 270mm ML (12.7%) and then decreased to the last point at 333mm ML (12.1%) (Fig. 3).

2-8/9-00 #1 M (318):

The δ¹⁵N of the lens segments ranged from 4.6 to 14.5%; the mantle value was 13.0%. The lens values decreased after the initial point at 107mm ML (4.9%) and then increased steadily to 225mm ML (12.9%), after that the lens values continued to increase at a slower rate culminating at 318mm ML (14.5%) (Fig. 4).

2-8/9-00 #3 M (296):

The δ¹⁵N of the lens segments ranged from 4.8 to 10.9%; the mantle value was 14.4%. The lens values stayed relatively constant from 96mm ML (4.8%) until 150mm ML (5.4%), then increased to 221mm ML (10.9%) then decreased to 296mm ML (10.0%) (Fig. 5).

2-8/9-00 #4 M (354):

The δ¹⁵N of the lens segments ranged from 6.5 to 11.7%; the mantle value was 14.4% the blood value was 13.1%. The lens values stayed relatively constant until 141 mm ML (6.5%) and increased to 209mm ML (10.0%) where the values continued to increase at a slower rate up to 354mm ML (11.7%) (Fig. 6).

2-9/10-00 #4 (540):

The δ¹⁵N of the lens values ranged from 4.2 to 14.5%; the mantle value was 13.7% and the blood was 12.5%. The lens values showed a slight initial increase to 238 mm ML (5.3%) followed by a rapid increase and plateau at 296mm ML (11.4%) to 343mm ML with another increase and plateau at 430mm ML to 517mm ML (14.5%) followed by a final decrease at 540mm ML (12.5%) (Fig. 7).

2-9/10-00 #5 (360):
The $\delta^{15}N$ of the lens values ranged from 6.2 to 12.5\%, the mantle value was 12.9\% and the blood was 12.9\%. The lens values showed an initial decrease to 134mm ML (6.2\%) and then increased steadily, with a peak at 206mm ML (9.0\%), up to 256mm ML (12.5\%) followed by a general decrease to 360mm ML (11.1\%) (Fig. 8).

2-11/12-01 #2 (370):

The $\delta^{15}N$ of the lens values ranged from 5.1 to 13.7\%; the mantle value was 12.8\% and the blood was 12.6\%. The lens showed an initial plateau to 157mm ML (5.3\%), a general increase to 273mm ML (13.7\%) followed by a final decrease to 370mm ML (12.2\%) (Fig. 9).

2-11/12-01 #5 (534):

The $\delta^{15}N$ values of the lens ranged from 4.2 to 15.8\%; the mantle value was 16.8\%. The lens showed an overall increase from 140mm ML (4.2\%) to 534mm ML (15.8\%) with a dip in the trend at 357mm ML (11.2\%) (Fig. 10).

2-11/12-01 #1 (557):

The $\delta^{15}N$ of the lens values ranged from 7.4 to 10.2\%; the mantle value was 14.4\% and the blood was 10.8\%. The lens values showed an initial decrease to 172 (7.4\%) then generally increased to 274mm ML (10.2\%) followed by a general plateau (with fluctuations) to 557 mm ML (9.8\%) (Fig. 11).

*Ommastrephes bartramii* $\delta^{15}N$ (northern samples)

#21 (220):

The $\delta^{15}N$ of the lens values ranged from 4.76 to 7.30\%, and the mantle value was 10.20\%. The lens showed an initial decrease from 130mm ML (5.82\%) to 173mm ML (4.76\%) followed by an increase to 220mm ML (7.30\%) (Fig. 12).

#26 (201):
The δ¹⁵N of the lens values ranged from 5.03 to 7.11‰, and the mantle value was 10.22‰. The lens showed an increase from 117 mm ML (5.03‰) to 201mm ML (7.11‰) (Fig. 13).

#28 (255):

The δ¹⁵N of the lens values ranged from 5.51 to 7.47‰, and the mantle value was 10.69‰. The lens showed an initial plateau from 144mm ML (5.51‰) to 193mm ML (5.86‰) followed by an increase to 255mm ML (7.47‰) (Fig. 14).

Ommastrephes bartramii δ¹⁵N juveniles

2-16/17-98 #25 (142):

The δ¹⁵N of the lens values ranged from 5.14 to 5.44‰, and the mantle value was 6.33‰. The lens showed a slight increase from 94mm ML (5.14‰) to 149mm ML (5.44‰) (Fig. 15).

2-16/17-98 #26 (151):

The δ¹⁵N of the lens values ranged from 4.88 to 5.27‰, and the mantle value was 6.36‰. The lens showed fairly constant values from 96mm ML (5.27‰) to 151mm ML (5.05‰) (Fig. 16).

2-11/12-99 #5 (161):

The δ¹⁵N of the lens values ranged from 4.84 to 7.07‰, and the mantle value was 8.01‰. The lens showed fairly constant values from 94mm ML (5.14‰) to 142mm ML (5.11‰) followed by a final increase to 161mm ML (7.07‰) (Fig. 17).

2-11/12-99 #6 (150):

The δ¹⁵N of the lens values ranged from 5.23 to 6.20‰, and the mantle value was 7.57‰. The lens showed the highest value from the initial sample at 82mm ML (6.2‰) and showed an overall oscillatory decline to 150mm ML (5.33‰) (Fig. 18).
Sthenoteuthis oualaniensis δ¹⁵N (adults)

2-14/15-99 #1 (324):

The δ¹⁵N values of the lens ranged from 4.24 to 10.00‰, and the mantle value was 11.14‰. The lens showed a slight increase from 129mm ML (4.24‰) to 172mm ML (4.7‰) followed by a rapid increase to 286mm ML (10.00‰) and a small decrease to 324mm ML (9.83‰) (Fig. 19).

2-14/15-99 #3 (200):

The δ¹⁵N values of the lens ranged from 4.20 to 9.04‰, and the mantle value was 10.46‰. The lens showed a decrease from 71mm ML (4.95‰) to a plateau between 95mm ML (4.20‰) and 109mm ML (4.22‰) followed by a rapid increase to 200mm ML (9.04‰) (Fig. 20).

2-14/15-99 #10 (223):

The δ¹⁵N values of the lens ranged from 5.36 to 9.75‰, and the mantle value was 11.24‰. The lens values remained relatively constant from 77mm ML (5.55‰) to 129mm ML (5.71‰), with one peak at 112mm ML (5.90), followed by an increase to 223mm ML (9.75‰) (Fig. 21).

2-7/8-00 #1 (210):

The δ¹⁵N values of the lens ranged from 3.56 to 5.85‰, and the mantle value was 8.95‰. The lens values decreased initially from 105mm ML (3.87‰) to 118mm ML (3.56‰) and then showed an overall increase to 210mm ML (5.85‰), with one peak at 166mm ML (5.22‰) (Fig. 22).

2-12/13-00 #1 (209):
The $\delta^{15}$N values of the lens ranged from 4.31 to 6.66‰, and the mantle value was 7.92‰. The lens values remained fairly constant from 90mm ML (4.44‰) to 115mm ML (4.44‰) and then showed an overall increase to 209mm ML (6.66‰) (Fig. 23).

2-12/13-00 #2 (160):

The $\delta^{15}$N values of the lens ranged from 4.18 to 8.86‰, and the mantle value was 9.20‰. The lens values initially decreased from 78mm ML (4.62‰) to 84mm ML (4.18‰) then increased slightly to 111mm ML (4.87‰) followed by a rapid increase to 160mm ML (8.96‰) (Fig. 24).

2-12/13-00 #3 (181):

The $\delta^{15}$N values of the lens ranged from 11.35 to 12.59‰, the mantle value was 11.26‰, and blood was 9.44‰. The lens values initially decreased from 94mm ML (11.99‰) to 115mm ML (11.35‰) then increased slightly to 168mm ML (12.59‰) and remained constant to 181mm ML (12.53‰) (Fig. 25).

2-12/13-00 #4 (173):

The $\delta^{15}$N values of the lens ranged from 4.56 to 8.50‰, the mantle value was 10.15‰. The lens values initially decreased from 91mm ML (5.12‰) to 110mm ML (4.56‰) then increased to 173mm ML (8.50‰) (Fig. 26).

2-12/13-00 #6 (158):

The $\delta^{15}$N values of the lens ranged from 3.72 to 5.32‰, the mantle value was 7.74‰. The lens values increased from 85mm ML (3.72‰) to 158mm ML (5.32‰) with a dip at 123mm ML (4.33‰) (Fig. 27).

2-13/14-00 #3 (251):

The $\delta^{15}$N values of the lens ranged from 4.20 to 6.21‰, the mantle value was 8.07‰ and blood was 7.68‰. The lens values increased from 111mm ML (4.20‰) to 251mm ML (6.20‰) with a peak at 166mm ML (4.80‰) and 221mm ML (6.21‰) (Fig. 28).
The δ¹⁵N values of the lens ranged from 2.79 to 9.40‰, the mantle value was 8.48‰ and blood was 7.71‰. The lens values increased from 108mm ML (2.79‰) to 225mm ML (9.40‰) (Fig. 29).

The δ¹⁵N values of the lens ranged from 3.35 to 9.65‰, the mantle value was 10.08‰ and blood was 9.20‰. The lens values showed a slight general increase from 98mm ML (3.35‰) to 159mm ML (3.82‰), followed by a rapid increase to 304mm ML (9.65‰) (Fig. 30).

The δ¹⁵N values of the lens ranged from 3.84 to 8.80‰, the mantle value was 9.41‰. The lens values remained relatively constant from 101mm ML (4.00‰) to 161mm ML (3.99‰), followed by a rapid increase to 302mm ML (8.80‰) (Fig. 31).

The δ¹⁵N values of the lens ranged from 3.08 to 9.78‰, the mantle value was 9.92‰ and blood was 9.23‰. The lens values decreased initially from 95mm ML (3.35‰) to 112mm ML (3.08‰), followed by an overall increase to 308mm ML (9.78‰) (Fig. 32).

Discussion

Ommastrephes bartramii δ¹⁵N (adults)

The retrospective δ¹⁵N values of the eleven adult O. bartramii eyes sampled, ten showed a more or less sigmoidal relationship (with some variation) of eye lens segment δ¹⁵N with estimated mantle length (Figs. 1-10). The earliest values attainable were in the eye region corresponding to 100mm ML and were in the vicinity of 4.0-6.0‰. The eye
lens values showed a rapid increase, mostly between ML of 175 and 300. While individual variation occurred in size at which ascent began, along with the steepness and smoothness of the ascent, the overall patterns were the same. This perhaps is not surprising since the curve placed through the combined eye lens data was very similar to the data taken for mantle muscle of different sized individuals as seen in the previous chapter.

Although most squids showed the rapid increase in eye lens segment $\delta^{15}N$ at around 175mm ML some squids deviated from this trend. Squids 2-8/9-00 #3, 2-9/10-00 #5 showed rapid increases in eye lens segment $\delta^{15}N$ earlier than the other squids, at or before 150mm ML (Figs. 5, 8). Squid 2-8/9-00 #3 had a typical sigmoidal pattern but it showed a plateau at an earlier size than typical *O. bartramii*, roughly 225mm ML and at a lower $\delta^{15}N$ value (Fig. 5). Squid 2-9/10-00 #5 did not show a typical smooth sigmoidal pattern but had a number of deviations within the overall curve (Fig. 8). Squid 2-11/12-01 #5 also did not show a typical smooth sigmoidal pattern but increased rapidly and then showed a dip around 350mm ML before rising to follow its previous trajectory (Fig. 10). Squid 2-9/10-00 #4 began its rapid increase in eye lens $\delta^{15}N$ later than other squids, close to 250mm ML, then showed an initial plateau followed by an increase and another plateau (Fig. 7). Squid 2-11/12-01 #1 had the most deviant eye lens segment $\delta^{15}N$ pattern (Fig. 11). This eye only showed a slight increase from 175mm ML to roughly 300mm ML and then remained relatively constant (with slight variations) at low $\delta^{15}N$ values to the final segment (Fig. 11). Although the initial value ($\approx 8\%$ at roughly 150mm ML) seems high this is presumably due to the lack of data corresponding to smaller sizes.

Six squids each show a decrease of $\delta^{15}N$ in their final (i.e.- outer) eye lens segment (Figs. 1, 2, 3, 7, 8, 9). The decrease is usually small ($\approx 1\%$) except in two of the females (Figs. 1, 2) where the decrease to the outer segment was closer to 3%. The larger decreases however, followed unusually high values. All of the decreases occurred while the squids were at or south of the subtropical front on their return migration. In three of these cases (Figs. 1, 2, 9) the eye lens segments get progressively lighter and have endpoints close to the muscle and blood $\delta^{15}N$ values which may indicate that the eye, muscle and blood have fully accommodated to values in the new ecosystem. These three squids (Figs. 1, 2, 9) all showed decreases in eye lens segment $\delta^{15}N$ over the final 100 to
150mm ML and, assuming a 2mm growth per day, would indicate that these squids have been in the area for 50 to 75 days. Two squids did not decrease toward their final eye lens segments, although a plateau in the data had essentially been reached (Figs. 4, 6). These squid may be recent arrivals to the area where they were caught although only in one (Fig. 6) did the end points differ strongly from the muscle.

The low and nearly flat curve of squid 2-11/12-01 #1 (Fig. 11) recalls a similar but very high eye-lens curve of *S. oualaniensis* (see below) and may indicate that, occasionally, some unaccounted for source of bias interferes with accurate measurement of δ¹⁵N values. The eleven curves show strong individual variation in the size and rate at which squid climb the trophic pyramid.

**Ommastrephes bartramii δ¹⁵N (Northern samples, sub-adults)**

The final eye lens segments of all three northern squids have similar δ¹⁵N values (Figs. 12-14) in agreement with the constancy of mantle values seen in Chapter 2 for all squids taken at this locality. The variable immediate history (outer eye segment values) of these squid suggests that the constancy of the mantle δ¹⁵N values was due to the locality (and its prey population), and that the squid were in transit through this area. Therefore, regardless of size, these squids incorporated the signal of the area they passed through which was fairly uniform over a wide range of sizes. The mantle values are high relative to final lens values and may indicate that lens values take longer to incorporate the signal.

**Ommastrephes bartramii δ¹⁵N (juveniles)**

Three of the four juvenile *O. bartramii* eye lens segments did not show increases in δ¹⁵N with mantle length, and all four had muscle δ¹⁵N values that were greater than the outer eye-lens segment of each squid (Fig. 15-18). The lack of increase of δ¹⁵N is consistent with the initial plateaus seen in the initial portions of the eye lens curves of many of the adult *O. bartramii*. The higher δ¹⁵N values of mantle muscle relative to outer eye-lens segments in these small squids is consistent with the possibility that the outer lens segments lag behind the mantle during periods of rapid change.
Sthenoteuthis oualaniensis $\delta^{15}N$

*S. oualaniensis* does not undergo any known seasonal migration, and no geographical patterns in $\delta^{15}N$ were seen in the POM material during this study. The retrospective analysis of $\delta^{15}N$ in most *S. oualaniensis* sampled showed an exponential increase in eye-lens segment $\delta^{15}N$ with calculated ML (Figs. 20, 21, 23, 24, 26-32). Some individuals showed a steep and sudden increase in $\delta^{15}N$ with size (Figs. 20, 24, 26, 29). Other individuals exhibited a more gradual increase in $\delta^{15}N$ with size (Figs. 21, 30, 31, 32). The most gradual increase in eye lens segment $\delta^{15}N$ with size was shown by three caught in 2000 (Figs. 23, 27, 28). Three individuals showed marked deviations from this exponential increase in eye lens segment $\delta^{15}N$ with size, 2-14/15-99 #1, 2-7/8-00 #1, and 2-12/13-00 #3 (Figs. 19, 22, 25). Squid 2-7/8-00 #1 (Fig. 22) shows an overall exponential increase, however there is a large peak in $\delta^{15}N$ for the eye lens at 166mm ML that is well above the general trend for this individual. Squid 2-14/15-99 #1 (Fig. 19) also showed an overall exponential increase, however the presence of an initial plateau, and a final plateau formed by the outer eye segment makes this retrospective eye lens curve resemble those obtained for many of the *O. bartramii* adults. Because squid 2-14/15-99 #1 (Fig. 19) was the largest *S. oualaniensis* caught (324mm ML) and overlaps the size range of many *O. bartramii* adults, somewhere above 300mm ML these squids may reach a size dependent $\delta^{15}N$ plateau as seen in the *O. bartramii* $\delta^{15}N$ versus ML data, but not in the *S. oualaniensis* data. Another four *S. oualaniensis* (2-14/15-99 #3, 2-12/13-00 #1, 2-12/13-00 #4, and 2-13/14-00 #3) had patterns of eye lens segment $\delta^{15}N$ increase with calculated size that also showed sigmoidal attributes (Figs. 20, 23, 26, 28) but these individual curves still fit the exponential model better. A damped sigmoidal pattern could be typical for this species but it did not show up on the $\delta^{15}N$ versus ML data because the small sample size of squids > 300mm ML, and variability in the timing (size of ML) and extent of increase, obscured the pattern.

The most bizarre pattern seen is from squid 2-12/13-00 #3 (Fig. 25) with all the retrospective values uniformly high. The muscle $\delta^{15}N$ value was comparable to the eye and both were as high as any mantle or eye value obtained at any size. The blood value for squid 2-12/13-00 #3 also corresponds to values of muscle and blood found for other *S. oualaniensis* of this size. Although, 5 of the 9 squid (including paralarvae) from this same
station had unusually high values, no satisfactory explanation has been found for the trends observed in squid 2-12/13-00 #3 (Fig. 25). It could be possible that this animal was under extreme nutrient stress throughout its lifespan, causing elevated $\delta^{15}$N values due to catabolization of its own tissues, or that it had some biochemical deficiency that caused increased fractionation when forming its tissues.

**Conclusions**

The overall increase in $\delta^{15}$N with size seen, both within a single individual and between individuals, for *O. bartramii* and *S. oualaniensis* is most likely controlled by ontogenetic trophic level increases. Although a geographical isotopic signal from a different ecosystem would have an effect on *O. bartramii* values, the increase in eye lens segment $\delta^{15}$N with size for *S. oualaniensis*, a geographical non-migrator, emphasizes the importance of size alone. The hypothesis that increased $\delta^{15}$N corresponds to increased size is also supported by the eye lens segment $\delta^{15}$N comparisons between juvenile, northern sub-adult, and adult *O. bartramii*. The decrease of $\delta^{15}$N in the outer-most eye lens segments of many *O. bartramii* may indicate a geographical $\delta^{15}$N signal corresponding to the animals return to Hawaiian waters. Support for a geographical signal in *O. bartramii* is obtained from the largest *S. oualaniensis* captured. This large *S. oualaniensis* showed a plateau in the $\delta^{15}$N eye lens at ca. 2% less than the median value for a similar sized *O. bartramii*. This difference may indicate a slight convergence of *O. bartramii* values towards those of *S. oualaniensis* as *O. bartramii* enters Hawaiian waters.

Presumably the eye lens retrospective data mostly reflect a combination of an individual squid's growth rate, feeding regime, and feeding behavior. Since trophic level and $\delta^{15}$N values are related to the size of the squid, then the individual growth rate of the squid could have an effect on the shape of the curve from the retrospective eye-lens data. Food quality (which will affect growth rate of the predator) can also have an effect on the eye-lens data. Fantle *et al.* (1996) found that blue crab, *Callinectes sapidus*, showed rapid growth with little $\delta^{15}$N fractionation (0.1%) when fed a high protein diet, and slow growth with substantial fractionation (up to 3.1%) when fed low protein diets. Fantle *et*
al. (1996) postulated that the protein poor diets did not supply enough energy and the crabs catabolized their own tissues to meet metabolic requirements. Adams and Sterner (2000) also found an effect of dietary nitrogen content on δ15N enrichment in daphnids. They found that δ15N enrichment in Daphnia magna was linearly related to the C:N ratio of the food, and that enrichment ranged from 0 to 6%. Finally, individual feeding specialization can also have a marked effect on δ15N variation between individuals within a population, (Beaudoin et al., 1999). Age data, which can be determined by growth rings in the squid statoliths, could be beneficial in understanding retrospective δ15N analyses. Anomalously old or young squids (very different times of hatching from the norm) would have different growth rates that could explain peculiar δ15N retrospective signals.

There are several reasons for confidence that the major features of the δ15N data provide useful and robust data: A general size relationship of increasing δ15N with increasing size was observed across several taxa. Consistent relationships between δ15N and ML were seen within both O. bartramii and S. oualaniensis. The δ15N data from the mantles and the eye-lens segments showed similar logistic patterns versus ML in O. bartramii. The δ15N data from the mantles, eye-lens segments, and blood showed similar exponential patterns versus ML in S. oualaniensis. The δ15N data from the eye-lens segments of individual adult O. bartramii fit strongly to logistic curves. The δ15N data from the eye-lens segments of individual adult and sub-adult S. oualaniensis fit strongly to exponential curves.

With a few exceptions, the δ15N patterns seem to reflect the feeding history of the squids. The exceptions, however, suggest that caution must be used in the interpretation of all of the data. The interactions between trophic level, feeding behavior, nutrient stress, tissue fractionation, and geographical variation on the stable isotopic signatures of an animal are still poorly understood. Separating and quantifying the effects of these factors requires controlled experimental manipulations. Such experiments should enable increased usage of stable isotopes for understanding a wide array of ecological questions.
References


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Figure 14. $\delta^{15}\text{N}$ versus length of eye lens segments of a female northern *O. bartramii*. $\delta^{15}\text{N}$ of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 15. $\delta^{15}N$ versus length of eye lens segments of a juvenile *O. bartramii*. $\delta^{15}N$ of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 16. $\delta^{15}$N versus length of eye lens segments of a juvenile *O. bartramii*. $\delta^{15}$N of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 17. $\delta^{15}N$ versus length of eye lens segments of a juvenile *O. bartramii*. $\delta^{15}N$ of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 18. δ¹⁵N versus length of eye lens segments of a juvenile *O. bartramii*. δ¹⁵N of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 19. $\delta^{15}N$ versus length of eye lens segments of a female *S. oualaniensis*. $\delta^{15}N$ of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 20. $\delta^{15}N$ versus length of eye lens segments of a female *S. oualaniensis*. $\delta^{15}N$ of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 21. δ¹⁵N versus length of eye lens segments of a female *S. oualaniensis*. δ¹⁵N of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 22. $\delta^{15}\text{N}$ versus length of eye lens segments of a female *S. oualaniensis*. $\delta^{15}\text{N}$ of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 23. $\delta^{15}N$ versus length of eye lens segments of a female *S. oualaniensis*. $\delta^{15}N$ of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 24. $\delta^{15}N$ versus length of eye lens segments of a female *S. ovalaniensis*. $\delta^{15}N$ of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 25. $\delta^{15}$N versus length of eye lens and gladius segments of a female *S. oualaniensis*. $\delta^{15}$N of mantle muscle and blood plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 26. $\delta^{15}N$ versus length of eye lens and gladius segments of a female *S. oualaniensis*. $\delta^{15}N$ of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 27. $\delta^{15}$N versus length of eye lens segments of a male S. oualaniensis. $\delta^{15}$N of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 28. $\delta^{15}N$ versus length of eye lens segments of a female *S. oualaniensis*. $\delta^{15}N$ of mantle muscle and blood plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 29. $\delta^{15}N$ versus length of eye lens segments of a female *S. ovalaniensis*. $\delta^{15}N$ of mantle muscle and blood plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 30. $\delta^{15}N$ versus length of eye lens segments of a female *S. oualaniensis*. $\delta^{15}N$ of mantle muscle and blood plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 31. $\delta^{15}$N versus length of eye lens segments of a female *S. oualaniensis*. $\delta^{15}$N of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 32. $\delta^{15}N$ versus length of eye lens segments of a female *S. oualaniensis*. $\delta^{15}N$ of mantle muscle and blood plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Chapter IV: Energetics and Trophodynamics

Introduction

Ommastrephid squids play key roles in the open ocean ecosystem and are of commercial value as a food resource (Roper et al., 1984). Traditional finfish stocks have been steadily declining (Caddy and Rodhouse, 1998), while cephalopod landings have been increasing to a maximum of 3.3 mmt in 1997 (FAO, 2000). Researchers have speculated that in some areas depleted fish stocks are undergoing a widespread species replacement by squids (Pauly et al., 1998; Balguerias et al., 2000). Although squids are less efficient than fishes in some ways (e.g. locomotion is less efficient than the traditional undulatory motion of fishes) (O’Dor and Webber, 1986; O’Dor and Webber, 1991) squids can undergo rapid somatic growth (Forsythe and van Heukelem, 1987; O’Dor and Hoar, 2000). The ramifications on oceanic ecosystems of widespread replacement of fish by short-lived rapidly growing squids, with energetically costly locomotion, is unclear. One approach to understanding these effects is through the construction of ecosystem models based on energy flow.

Unfortunately knowledge of many crucial factors necessary for models of oceanic squids, such as biomass, production, and life history parameters, are lacking (Piatkowski
et al., 2001). In addition, because of the difficulty in capturing and rearing large ommastrephid squids, little data on energetics and growth of these organisms are available. Demont and O’Dor (1984) studied the effects of activity, temperature and mass on the metabolism of the ommastrephid Illex illecebrosus in the laboratory. Webber and O’Dor (1985) studied the metabolism of the same animal in a controlled recirculating water tunnel. Clarke et al. (1994) applied the consumption rates calculated for I. illecebrosus (DeMont and O’Dor, 1984) to a related ommastrephid Illex argentinus and constructed a preliminary energy budget based on growth and the biochemical composition of certain tissues.

The trophic level concept is central to many ecosystem models ranging from the early days of ecosystem modeling (Lindeman, 1942) to more recent applications of global fisheries models (Pauly and Christensen; 1995). Some researchers argue against the trophic level concept (Peters, 1980) in favor of models based on growth (Peters, 1983). Cousins’ (1985), however, argues that a model of marine ecosystems based on a trophic continuum circumvents the failings of discrete trophic levels and static food chains. Regardless of the nomenclature all ecosystem energy models rely on a trophic concept (e.g. the transfer of energy from its basal resource to some endpoint) and forms of species linkages (predation, parasitism, etc.). While ecological models use assigned trophic levels for their calculations (Pauly and Christensen, 1995; MacFarlane et al., 2002; Leonov and Kanatov, 2002) few, if any, seem to use isotopically-defined trophic levels. While many studies use stable isotopes to determine trophic levels and food web structure (Sholto-Douglas et al., 1991; Gould et al., 1997; Kaehler et al., 2000; and many others) few seem to model the concomitant energy fluxes between such linkages. This chapter takes an initial step in combining stable isotopic data with energy budgets to create simple energetics models for the ommastrephid squids Ommastrephes bartramii and Sthenoteuthis oualaniensis to aid in understanding the ecology of these squids.
Materials and Methods

The ecological impact of these squids was estimated using a combination of published energetics data, and growth models for other squids along with measured parameters for *O. bartramii* and *S. oualaniensis* obtained in this study.

A general energy budget was constructed for both sexes of *O. bartramii* and *S. oualaniensis*.

\[
I_e = E_g + E_x + G + R + SDA + M_b + M_a
\]

- \( I_e \) = ingested energy
- \( E_g \) = egestion
- \( E_x \) = excretion
- \( G \) = growth
- \( R \) = energy into reproduction
- \( SDA \) = specific dynamic action
- \( M_b \) = basal metabolism
- \( M_a \) = active metabolism

Because the energy budget must be applied over the lifetime of the squid, a growth equation is needed. The growth of these squids was estimated using a growth equation derived for *O. bartramii* (Murata, 1990). The growth curve for *O. bartramii* was calculated by Murata (1990) from samples of catch data (150-470 mm ML). There are obviously problems with using an estimated growth rate, however the data of Murata seems as reliable as the other available data (Yatsu *et al.*, 1997; Yatsu and Mori, 2000). This growth equation was also applied to *S. oualaniensis* in lieu of data for this species. The growth equation of Murata (1990), ML = 55.31/1+e(1.084-0.0151t) (t = age in days), gives a logistic growth curve. Murata’s equation was modified to yield a maximum squid mantle length equal to the maximum mantle lengths of males and females of *O. bartramii* and *S. oualaniensis* caught during this study. Using maximum mantle lengths to determine the growth curve more closely estimates the squids growth over their entire lifespan. The numerator was replaced by a constant (determined for each category) and another constant was subtracted from the entire equation to yield a mantle length of zero at time zero. These modifications resulted in an equation for each category of squid that started at zero and increased logistically to a maximum that corresponded to the maximum measured ML for each category:
Mantle lengths were calculated over 365 days and converted into grams of biomass (BM) using regressions of measured mantle length versus weight from cruises 1997 and 1999 for _O. bartramii_ (BM<sub>_O. bartramii_</sub> = 9x10<sup>-5</sup>*ML<sup>2.8178</sup>, _r<sup>2</sup> = 0.97) and 1999 only for _S. oualaniensis_ (BM<sub>_S. oualaniensis_</sub> = 5x10<sup>-4</sup>*ML<sup>2.549</sup>, _r<sup>2</sup> = 0.81) (Figs. 1, 2).

Determining the total energy budget for these squids required four steps. Egestion (E<sub>g</sub>) and excretion (E<sub>e</sub>) were assumed to be small (Clarke et al., 1994) and were ignored. This energy budget was then modified to take into account the energy lost at lower trophic levels. A total energy budget was calculated for each sex and species of squid.

**Step 1:**

The energy required for somatic growth (G) and reproductive mass (R) was calculated in this step. Clarke _et al._ (1994) calculated the mean energy content of major tissues in both sexes (wet weight of testes, spermatophoric complex, ovary, nidamental gland, digestive gland, mantle, head, and viscera) of a congeneric species, _Illex argentinus_. The mean energy content of tissue for male (mean biomass 469 g) _I. argentinus_ (was 1.31 kcal/g and was 1.57 kcal/g for females (mean biomass 800 g). These values were determined for _I. argentinus_ near maturity but are applied here over the entire lifespan. Mean energy content was applied to the daily biomass increase for both _O. bartramii_ and _S. oualaniensis_. Somatic growth and reproductive mass were assumed to account for the total daily biomass increase. Each daily biomass increase was then multiplied by the average energy content of male or female tissue.

\[
G + R = (BM_{d+1} - BM_d) \times \text{Energy content (male or female)}
\]

**Step 2:**

\[
ML_t = \left(\frac{a}{1 + e^{(1.084 - 0.0151t)}}\right) - b
\]

_S. oualaniensis_ male: 
- a = 28  b = 7  ML<sub>final</sub> = 210 mm

_S. oualaniensis_ female: 
- a = 44  b = 10  ML<sub>final</sub> = 324 mm

_O. bartramii_ male: 
- a = 53  b = 13  ML<sub>final</sub> = 394 mm

_O. bartramii_ female: 
- a = 88  b = 22  ML<sub>final</sub> = 650 mm
Specific dynamic action (SDA) was calculated in this step. SDA was assumed to be 20% of energy incorporated into mass (G+R) based on previous findings for cephalopods and invertebrates (O’Dor and Wells, 1987; Parry, 1983).

\[ SDA = (G + R) \times 0.20 \]

Step 3a:

The total metabolic requirements (M_{total}) for each squid were calculated at daily increments using the equation derived from *Illex illecebrosus* by Webber and O’Dor (1985). Webber and O’Dor measured O₂ consumption of squid of known size (200 to 550 g) in a swim channel with controlled flow speed and temperature (15 ± 0.2°C). They developed an equation of whole animal O₂ consumption based on biomass (W) and speed (S): \( \text{mLO}_2/\text{hr} = (1.58 \times W^{0.725}) \times (1.016^{0.47 \cdot \text{ML}}) \). For this model swimming speed was approximated as 0.5 body lengths (ML) per second, which is an average speed calculated from three telemetered adult female *O. bartramii* (Nakamura, 1993). This assumes that the squids swim at a constant relative speed throughout their lives.

\[ M_{\text{total}} = (1.58 \times W^{0.725}) \times (1.016^{0.47 \cdot \text{ML}}) \]

Step 3b.

Basal metabolism (Mb) was calculated by assuming \( S = 0 \) for each daily size.

\[ M_b = (1.58 \times W^{0.725}) \]

Step 3c.

Active metabolism (Ma) was then calculated as \( M_{\text{total}} \) minus \( M_b \).

\[ M_a = M_{\text{total}} - M_b \]

Step 3d.
Because Webber and O'Dor kept a constant temperature of roughly 15° C the $M_b$ was modified to take into account the effect of temperature fluctuations encountered by *O. bartramii* and *S. oualaniensis*. Two temperature regimes were used in this model, tropical and temperate. The tropical regime consisted of a surface temperature of 25° C and 5° C at depth while the temperate regime consisted of surface temperature of 15° C and 5° C at depth. Both squid species were subjected to tropical surface temperatures until they reached 100mm ML at which point they began their diurnal vertical migrations and spent 12hrs at 25° C and 12hrs at 5° C. The start of vertical migration behavior was assigned to squids of 100mm ML based on the sizes of juvenile squids found in seabirds feeding near the surface (Ashmole and Ashmole, 1967; Seki and Hanison, 1989) which indicate that squids larger than 100mm ML are absent from surface waters. *S. oualaniensis* remained in the tropical regime throughout its life. *O. bartramii* moved out of the tropical regime and into the temperate regime by day 92, and then returned to the tropical regime by day 275, these time periods were chosen to roughly approximate the migratory pattern reported by Murata and Nakamura (1998) with the northerly migration occurring in May/June and the southerly migration occurring in November/December.

The temperature effect was calculated using the temperature modes and time periods described above coupled with an estimated $Q_{10}$ value of 2.6. This value was chosen as representative of that measured for a variety of animals (Schmidt-Nielsen, 1984; Hochachka and Somero, 2002), including cephalopods (Wells *et al.*, 1983; Seibel *et al.*, 1997), within their normal temperature range. Specifically, Seibel *et al.* (1997) found that a $Q_{10}$ value of 2.62 was applicable to some vertically migrating cephalopods. While some studies have found $Q_{10}$ values as high as 7, such values are generally considered an artifact of measurement outside the species normal range (see discussion in Seibel *et al.*, 1997). The temperature effect ($T_e$) was then calculated as 2.6 times $M_b$ for temperatures of 25° C and 0.38 for temperatures of 5° C.

$$M_b^* = (1.58 * W^{0.725}) * T_e$$

Step 3e.
The whole animal oxygen consumption, modified for temperature, \((M_{\text{total}*})\) for each half day increment was obtained by adding in the daily active metabolism, and the modified basal metabolism.

\[
M_{\text{total}*} = M_a + M_b
\]

Step 3f.

The temperature modified whole animal metabolism calculated in step 6 was converted to an energy requirement in kcal/day by adding up the two 12 hr periods in each day and multiplying by a standard conversion factor of 0.00463 kcal/mlO₂ (Elliot and Davidson, 1975).

\[
(M_{\text{total}*})^* (0.00463 \text{ kcal/mlO}_2)
\]

Step 4.

To obtain a daily energy requirement (kcal/day) based on somatic growth, reproductive growth, specific dynamic action, and metabolism (active and basal), the results of steps 1, 2, and 3 were combined.

\[
\text{Daily energy requirement} = \text{step 1} + \text{step 2} + \text{step 3f}
\]

The next steps combine the isotopic data (see chapter 2) with the energy budget. The overall daily energy requirement calculated in step 8 was modified to take into account the energy needed at lower trophic levels to supply the squids energy requirement. This calculation is shown in the following three steps.

Step A.

The regressions of squid mantle length versus \(\delta^{15}\text{N}\) determined from this study (See chapter 2) were used to assign a daily \(\delta^{15}\text{N}\) value to each squid.

A1. The \(\delta^{15}\text{N}\) versus ML regression for \(S.\ oualaniensis\) was calculated as:

\[
\delta^{15}\text{N}_{S. \ oualaniensis} = 4.2081 + (1.8276*1.0037^{\text{ML}})
\]
A2. The $\delta^{15}N$ versus ML regression for *O. bartramii* was calculated using a Gompertz 4-parameter sigmoidal curve:

$$\delta^{15}N_{O. bartramii} = 6.4261 + 6.8482(e^{-\frac{ML-183.2625}{38.0979}})$$

Step B.

Each daily $\delta^{15}N$ value calculated in step A was converted into a fractional trophic level (TL) for each different category of squid as follows: the $\delta^{15}N$ value of 2.8‰ was chosen as the baseline trophic level because of the values for POM found in chapter 2. A $\delta^{15}N$ value of 3.4‰ was used to define one trophic level step (see chapter 2). The trophic level was then defined as:

$$TL = ((\delta^{15}N - 2.8\%_0/3.4\%_0)+1)$$

Step C.

Daily energy requirement (step 4) was modified by a trophic level factor representing the energy needed through lower trophic levels. The trophic level factor was calculated using the trophic level of the squid at that day (step B) and an ecological efficiency of 10% for each TL. The trophic level factor was calculated as an exponential of 0.1 per trophic steps below that of the squid at each day (eg- TL of 4 equals 3 steps, or energy transfers).

$$\text{Energy requirement}_{(TL)} = \text{step4} \cdot (0.1^{(TL-1)})$$

A daily consumption rate was also calculated for each squid. The daily consumption rate was calculated as a percent of body mass using the daily energy requirement (step 4) and an average energy yield of prey species per gram. An average energy yield (kcal/g) for seven species of myctophid prey of *O. bartramii* and *S. oualaniensis* (*Bolinichthys longipes, Ceratoscopelus warmingii, Hygophum rheinhardtii, Lampanyctus tenuiformis, Lobianchia gemelleri, Myctophum selenoides*, and
*Taaningichthys minimus* was calculated as 1.0 kcal/g from Childress (1990). This energy yield was then used to calculate the amount of average myctophid material in a percentage of squid predator body weight to meet an adult squid's energy needs during each day of each model.

**Results**

*Male S. oualaniensis*

The modeled male *S. oualaniensis* grew to 210 mm ML and 0.42 kg in 365 days. Daily caloric energy requirement increased to day 95 when vertical migration began and there was a break in the graph showing energy requirement per day (Fig. 3). After day 95 the energy requirement values increased asymptotically to a maximum of 24 kcal/day (Fig. 3). The total lifetime energy requirement for a male *S. oualaniensis* was calculated at $5.7 \times 10^3$ kcal.

Modifying the energy requirement for trophic level produced a similar, but accentuated, curve to the unmodified energy requirement curve (Fig. 4). The energy requirement modified for trophic level of male *S. oualaniensis* increased to day 97 where there was a break in the curve and then the values increased asymptotically to 906 kcal/day. The total metabolic impact of male *S. oualaniensis* converted for trophic level over 365 days was $1.7 \times 10^5$ kcal.

The percent of body weight required to meet the energy demands of a male *S. oualaniensis* started at 280% on day one and decreased exponentially to 5.6% on day 365 (Fig. 5).

*Female S. oualaniensis*

The modeled female *S. oualaniensis* grew to 324 mm ML and 1.3 kg in 365 days. Daily caloric energy requirement increased to day 66 when vertical migration began and there was a break in the graph showing the energy requirement per day (Fig. 6). After day 66 the energy requirement values increased asymptotically to a maximum of 56 kcal/day
The total lifetime energy requirement for a female *S. oualaniensis* was calculated at $1.4 \times 10^4$ kcal.

Modifying the energy requirement for trophic level produced a similar, but accentuated, curve to the unmodified energy requirement curve (Fig. 7). The energy requirement modified for trophic level of female *S. oualaniensis* increased to day 66 where there was a break in the curve and then the values increased asymptotically to $8.7 \times 10^3$ kcal/day. The total metabolic impact of male *S. oualaniensis* converted for trophic level over 365 days was $1.3 \times 10^6$ kcal.

The percent of body weight required to meet the energy demands of a male *S. oualaniensis* started at 39.8% on day one and decreased exponentially to 4.5% on day 365 (Fig. 8).

**Male *O. bartramii***

The modeled male *O. bartramii* grew to 394 mm ML and 1.8 kg in 365 days. Daily caloric energy requirement increased to 10 kcal/day on day 55 and decreased to 7 kcal/day when vertical migration began (Fig. 9). After day 55 the energy requirement increased again to 22 kcal/day on day 92 and decreased to 16 kcal/day when the northern migration crosses into temperate waters (Fig. 9). After day 92 the energy requirement increased to 44 kcal/day on day 275 and then increased to 74 kcal/day when the southern migration crosses into tropical waters. After day 275 daily energy requirement increased slightly to a maximum of 77 kcal/day. The total lifetime energy requirement for a male *O. bartramii* was calculated at $1.4 \times 10^4$ kcal.

Modifying the energy requirement for trophic level produced a similar, but accentuated, curve to the unmodified energy requirement curve (Fig. 10). The TL modified energy-requirement of male *O. bartramii* increased to 112 kcal/day on day 55 and decreased to 84 kcal/day when vertical migration began (Fig. 10). After day 55 the TL modified energy requirement increased to 932 kcal/day on day 92 and decreased to 747 kcal/day then the northern migration passed into temperate waters (Fig. 10). After day 92 the TL modified energy requirement increased to $5.5 \times 10^4$ kcal/day on day 275 and increased to $8.5 \times 10^4$ when the southern migration passed into tropical waters. After day
275 the TL modified energy-requirement increased slightly to 8.9x10^4 kcal/day. The total metabolic impact of male *O. bartramii* converted for trophic level over 365 days was 1.7x10^5 kcal.

The percent of body weight required to meet the energy demands of a male *O. bartramii* started at 380% on day one and decreased exponentially to 4.1% on day 365 (Fig. 11).

**Female *O. bartramii***

The modeled female *O. bartramii* grew to 650 mm ML and 7.6 kg in 365 days. Daily caloric energy requirement increased to 12 kcal/day on day 35 and decreased to 10 kcal/day when vertical migration began (Fig. 12). After day 35 the energy requirement increased again to 79 kcal/day on day 92 and decreased to 63 kcal/day when the northern migration began (Fig. 12). After day 92 the energy requirement increased to 163 kcal/day on day 275 and then increased to 247 kcal/day when the southern migration began. After day 275 daily energy requirement increased slightly to a maximum of 250 kcal/day. The total lifetime energy requirement for a female *O. bartramii* was calculated at 5.0x10^4 kcal.

Modifying the energy requirement for trophic level produced a similar, but accentuated, curve to the unmodified energy requirement curve (Fig. 13). The TL modified energy requirement of female *O. bartramii* increased to 135 kcal/day on day 35 and decreased to 111 kcal/day when vertical migration began (Fig. 13). After day 35 the TL modified energy requirement increased to 6.9x10^4 kcal/day on day 92 and decreased to 5.6x10^4 kcal/day when the northern migration passed into temp waters (Fig. 13). After day 92 the TL modified energy requirement increased to 1.9x10^5 kcal/day on day 275 and increased to 2.8x10^5 when the southern migration passed into tropical waters. After day 275 the TL modified energy requirement increased slightly to 2.9x10^5 kcal/day. The total metabolic impact of female *O. bartramii* converted for trophic level over 365 days was 5.7x10^7 kcal.
The percent of body weight required to meet the energy demands of a female *O. bartramii* started at 189% on day one and decreased exponentially to 3.3% on day 365 (Fig. 14).

**Discussion**

Based on the food requirement (in % body weight per day) the squids would need, the models overestimate their energy requirement for roughly the first 100 days for each squid. During this period the modeled consumption requirements are mostly likely greater than the physiological capabilities of the squids. However, after the initial period of about 100 days the consumption estimates drop off dramatically and stabilize at more reasonable values. Tung et al. (1973) and Tung (1976) classified stomachs of *S. oualaniensis* (100-270 mm ML) caught off of Taiwan as “medium” fullness with contents weighing ≤3% of body weight, and “full” stomachs with contents weighing >3% of body weight. Therefore, according to these findings, one “full” stomach could come close to fulfilling the modeled energy requirements for *S. oualaniensis* males or females in the latter stages of the models. Shevtsov (1972), off the southeast of Japan, found 70% of *O. bartramii* (240-300 mm ML) stomachs with contents >1% of body weight, but did not quantify the value further, and did not comment on stomach “fullness”. According to Shevtsov’s results most *O. bartramii* captured would have enough in their stomachs to satisfy roughly a third to a quarter of their energy requirements in the latter stages of the model. Given that feeding studies rely on baited capture methods (jigging etc.) and will most likely sample hungry squids, once would expect stomach content weights to be low. These results, therefore, are consistent with modeled results. The poor fit of the model to the early life history of these squids is not unexpected given the growth model used was derived from sub-adults and adults (Murata, 1990).

The models indicate the effect that temperature could have on the energy demands of these squids. This effect is exemplified in a comparison between female *S. oualaniensis* and male *O. bartramii*. The size of the modeled male *O. bartramii* at 365 days was over 25% heavier than the modeled female *S. oualaniensis* at 365 days, yet their overall lifetime energy requirements were almost identical. This may be one contributing factor in the evolution of the migratory behavior of *O. bartramii*, and its larger body size,
in comparison to the smaller non-migratory *S. oualaniensis*. Although this model attempted to account for some aspects of behavior, such as diurnal vertical migration and geographical migration, beyond temperature it does not account for differential costs in vertical migration or horizontal migration versus normal swimming. These, and other more fine scale behaviors such as climb and glide swimming could affect energy demands.

The models also indicate the effect that trophic levels could have on the amount of energy needed from the base of the foodweb. Comparing male *O. bartramii* and female *S. oualaniensis*, and taking into account the energy needed through lower trophic levels, the male *O. bartramii* required roughly 11 times the energy. In general, accounting for energy needed from lower trophic levels resulted in an almost 30 fold increase in energy required for male *S. oualaniensis* and a 100 fold increase in energy for female *S. oualaniensis*. Accounting for trophic-level needs resulted in an increase in roughly 1000 times over lifetime energy requirements for *O. bartramii* males and females. Without concrete values of population size, estimates of the ecological impact for these animals are difficult to make. Historically, the North Pacific fishery for *O. bartramii* was harvesting roughly 300,000 mt/yr (Murata and Nakamura, 1998). With a 1:1 sex ratio of 650 mm ML females and 394 mm ML males, the overall energy required for this amount of *O. bartramii* (accounting for TL losses) in the North Pacific would be $1.3 \times 10^{15}$ kcal/yr. North Pacific production estimates range from 27 to $50 \times 10^{15}$ gC/yr (Behrenfeld and Falkowski, 1997) and assuming 10 kcal/gC (for a full explanation see Steele, 1975) the total energy in the North Pacific would be between 270 and $500 \times 10^{15}$ kcal/yr. Therefore using these energy models, *O. bartramii* could account for between 0.3 and 0.5% of the energy produced in the North Pacific. The population impact of these squids could actually be higher considering that many squids did not reach harvestable sizes due to natural mortality.

**Conclusions**

Combining measured metabolic functions and growth data, along with actual weight measurements into an energetics model provides a first estimate of the lifetime...
energy requirements of these squids. The daily energy requirements of the latter stages of the models agree to an extent with published feeding values of these squids.

Vertical migration, and in the case of *O. bartramii* horizontal migration, coincides with the time period when the squids energetic requirements begin to increase rapidly. The energy savings that *O. bartramii* obtains from crossing into colder northern waters is substantial and much of this energy is likely diverted into somatic and reproductive tissue growth. Given the lack of evidence for direct competition between these similar squids in Hawaiian waters, this migratory behavior could be the result of the evolution of large body size and faster growth in *O. bartramii* to take advantage of a more energy rich but less energy demanding environment, and not predominantly as a means of avoiding competition with *S. oualaniensis*.

Using the isotopically derived trophic level calculations within the framework of the energetics models gives an estimate of the total amount of energy these squids require from ecosystem. Given the debate on the ecological efficiencies within ecosystems, and the many assumptions used in the calculations, assessing the validity of the final energetic impact when modified for trophic level is difficult. Solid estimates of population sizes for these squids, along with fewer assumptions, would be required to make more accurate estimates of their ocean or worldwide impacts.

The future of incorporating isotopically derived trophic information into ecological/energetic models is virtually unexplored. The benefits of a method to unequivocally assign trophic positions, that are not constrained by discrete levels, are enormous. Stable isotopes could provide modelers with solid indications of the amount of energy transfers between a certain species and its basal resource, which would be invaluable in accurate modeling of foodweb energetics. Validation of this technique is necessary in a field setting however, and should be initially attempted in small, constrained ecosystems such as lakes or embayments. The ability to corroborate isotope data and actual measured energy transfers within a simple ecosystem is crucial to the widespread use of isotopically derived trophic information in energetic modeling.
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\[ y = 9 \times 10^{-5}x^{2.8178} \]

\[ R^2 = 0.9718 \]
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Chapter V: Conclusions

Summary

SCA and SIA were used effectively to study the trophic ecology of *O. bartramii* and *S. oualaniensis*, two ommastrephid squids, in Hawaiian waters. SCA and SIA can be used as complimentary techniques, each method alleviating certain weaknesses of the other. In this study each technique gave an incomplete depiction of the trophic status of these squids. Taken together these results set the framework for better understanding of each trophic niche these animals occupy in the pelagic ecosystem in Hawaiian waters. In addition the SIA was used retrospectively to infer historical trophic information. Using aspects from both the SCA and SIA portions of the study, a basic energy model was constructed for both sexes of squids. This energy model provided a reasonable first estimate of the energetic requirements of these squids and attempted to model the amount of energy needed from lower trophic levels to meet these demands.

The SCA indicated a fundamental difference in the feeding habits of the *O. bartramii* and *S. oualaniensis* caught during this study. While both squids fed heavily on myctophid fishes and onychoteuthid squids there were substantial differences in feeding on prey even at the family level. SCA results indicated generalized feeding in *O. bartramii*, and relatively more specialized feeding in *S. oualaniensis*. This feeding trend continued when the SCA results were analyzed at the species level for fish prey, and at the lowest possible taxa for cephalopod prey.

Within the family Myctophidae, *S. oualaniensis* fed heavily on *S. evermanni*, *C. warmingii*, and *H. proximum/rheinhardti*, while *O. bartramii* fed less frequently, and with less abundance, on a number of myctophid species. Although the results for squid prey were less pronounced than with fish prey, *O. bartramii* still fed less frequently, and with less abundance, on a wider variety of squid prey than did *S. oualaniensis*, with the exception of similar feeding on *Onychoteuthis* sp.
Using standard measures of niche breadth (Levins’ measure) and resource overlap (MacArthur and Levins) indicated the extent to which feeding on fish and squid prey differed between *O. bartramii* and *S. oualaniensis*. There were slight indications that resource overlap of *S. oualaniensis* on *O. bartramii* increased with increasing size category of *S. oualaniensis*. Within four categories of shared myctophid prey, otoliths found in *O. bartramii* were significantly larger than those found in *S. oualaniensis*. A similar situation was found between female and male *S. oualaniensis*, with larger otoliths recovered from female *S. oualaniensis*.

Given their overlapping habitat, confamilial status, and size differences one might expect these squid species to show prey/predator interactions, or at least strong competitive interactions in areas of sympatry. However, virtually no predation and little competition for food resources occurred between *O. bartramii* and *S. oualaniensis* in this study. The proportions and frequencies of items in the diets of *O. bartramii* and *S. oualaniensis*, as well as the dietary measurements calculated from these values, indicate that, although *S. oualaniensis* feeds on a subset of the *O. bartramii* diet, competition is low.

Even considering the differences in feeding from the SCA results, assigning relative trophic positions to these squids within the ecosystem would still be highly subjective, since even the trophic status of the prey are poorly known. For this reason, SIA were performed in an attempt to determine quantitative trophic relationships for *O. bartramii* and *S. oualaniensis*, both within the ecosystem and in relation to each other.

In general the $\delta^{15}$N data were, as expected, consistent with the broad categories of trophic levels generally assigned to various organisms. The POM was the lightest of any category, while zooplankton were slightly higher. Average $\delta^{15}$N values of myctophids and other mesopelagic fishes and squids were closer to average values of *S. oualaniensis* than would be expected based on SCA. *Ommastrephes bartramii* had the heaviest $\delta^{15}$N values of any organisms sampled, and their values were roughly a trophic step (3.4%) above those of their prey. Sampling methods of squid prey could have resulted in a heavier $\delta^{15}$N bias in the samples and may not accurately represent prey $\delta^{15}$N values for these squids. The $\delta^{15}$N values indicated that, on average, *O. bartramii* should be more
than 1 TL above *S. oualaniensis*. This is consistent with the different diets of these squid found in the SCA portion of this study.

Body size (ML) was the greatest factor in determining the $\delta^{15}N$ value for *O. bartramii* and *S. oualaniensis*. The general trend of $\delta^{15}N$ values for squid muscle (mantle) showed different patterns for *O. bartramii* and *S. oualaniensis* over the size ranges sampled. The $\delta^{15}N$ of *O. bartramii* muscle increased logistically with size and reached a plateau above 300 mm ML while the $\delta^{15}N$ of *S. oualaniensis* muscle increased exponentially up to the largest size sampled. These different patterns are probably due to the *O. bartramii* reaching a critical size where its trophic level stabilizes while *S. oualaniensis* does not reach this threshold size. A possible geographical component to the different patterns cannot be ruled out.

Data from individual retrospective eye lens tissue combined from all eyes sampled showed patterns of $\delta^{15}N$ increase against calculated ML that were similar to the general trends of the mantle data. The $\delta^{15}N$ values obtained from *O. bartramii* eye tissue increased in a logistic pattern and those obtained from *S. oualaniensis* eye tissue increased exponentially, however the curve of the latter was below that described for mantle muscle. The reasons for the discrepancy between *S. oualaniensis* tissues are not clear, however the data were not corrected for the changes in refractive index that occur in the eye lens during growth. These changes may be responsible for some of the discrepancy observed. The results of the eye lens tissue $\delta^{15}N$ for *S. oualaniensis* allowed for categorization of individuals into “typical”, “atypical”, and “anomalous” groups. The $\delta^{15}N$ values of the typical and atypical eye lenses all increased with calculated ML, although the atypical group increased more rapidly. The atypical case was interpreted as feeding specialization that began at a small size and remained throughout the animals life until around the time of capture, unfortunately the SCA data could not confirm this. The anomalous group consisted of the eye lens from a single squid and the $\delta^{15}N$ were abnormally high and did not exhibit a discernible pattern with calculated ML. No explanation was found for this pattern.

Blood samples taken from *O. bartramii* and *S. oualaniensis* were also similar to the results obtained from muscle and eye lens tissue. Blood samples were only available for *O. bartramii* at the larger end of the size spectrum sampled, so total validation of the
blood $\delta^{15}$N pattern was not possible, however the blood samples of the larger individuals were in general agreement with the pattern seen in the muscle and eye tissue. Blood was sampled from a wider relative size range of _S. oualaniensis_, and the $\delta^{15}$N values increased exponentially with ML.

The $\delta^{15}$N results for the mantle muscle, eye lens tissue, and blood, showed large individual variation within the overall patterns established for each squid. Retrospective analyses of eye-lens tissue for individual squid allowed some analysis of these causes of variation. In almost all squid sampled of both species, the mantle muscle was heavier than the blood, indicating that there may be an intrinsic fractionation between these two tissues. In general the mantle muscle was heavier than the outer eye segment also indicating that there is an intrinsic fractionation between these two tissues, although the exceptions were greater than in the comparison of muscle to blood.

The individual analyses for the eye lenses of each squid revealed similar patterns of $\delta^{15}$N increase with calculated size seen when data from each species was combined and analyzed as a group, except that the timing and slope of major changes showed great variation. The period of most rapid increase in $\delta^{15}$N for _O. bartramii_ eye lenses occurred between roughly 175 and 300 mm ML. Although most _O. bartramii_ eye lenses sampled conformed to the logistic $\delta^{15}$N increase with size, a few exceptions did occur, and variability within the logistic curve was common. Several of the eye lens curves showed a decrease in $\delta^{15}$N at the largest ML increment which may indicate a slight drop in TL as the squids move back into tropical waters during their southern migration. The eye lenses of juvenile _O. bartramii_ less than 175 mm ML did not show an increase in $\delta^{15}$N with calculated mantle length. This supported the mantle data, which suggested an unexpected TL plateau over this size range. Three sub-adult _O. bartramii_ (225 to 255 mm ML) captured at 46° N explained a peculiar intermediate plateau in $\delta^{15}$N muscle values versus ML for 30 squid taken at this latitude. The three sub-adult eyes sampled showed different histories that converged to the same final value indicating that the TL of these squid at this location was being dictated by local environmental factors, presumably prey type and availability.

Except for the peculiar anomalous squid, the $\delta^{15}$N values from individual eye lenses of _S. oualaniensis_ increased exponentially for almost all squids. Even so,
individual patterns showed high variability within the life of one individual as well as between individuals, indicating that movement up the trophic pyramid is not a steady process. The pattern of $\delta^{15}$N increase with ML from the eye tissue of the largest $S$. oualaniensis sampled (324 mm ML) had logistic qualities similar to the patterns seen in $O$. bartramii eye lenses, however the pattern still fit well to an exponential curve. This pattern suggests that the largest $S$. oualaniensis may show a trophic plateau as was seen in $O$. bartramii beginning at about the same size. Unfortunately insufficient numbers of large $S$. oualaniensis were caught to allow for confirmation of this trend.

With a few exceptions, the $\delta^{15}$N patterns seem to reflect the feeding history of the squids. There are several reasons for confidence that the major features of the $\delta^{15}$N data provide useful and robust data: A general size relationship of increasing $\delta^{15}$N with increasing size was observed across individual species of several non-cephalopod taxa. The $\delta^{15}$N data from the mantles and the eye-lens segments both showed logistic patterns versus ML in $O$. bartramii. The $\delta^{15}$N data from the mantles, eye-lens segments, and blood all showed exponential patterns versus ML in $S$. oualaniensis. Retrospective analysis of individual squids showed large variations in trophic position over fairly short time periods. Trophic position appears to be highly dynamic and is most likely a combination of many factors including, individual feeding behavior, prey availability, and fluctuations in food chain length. Retrospective analyses offer great potential for future studies in sorting out the causes of individual variation in TL.

Obtaining consistent $\delta^{15}$N values, generally reflective of trophic level, for $O$. bartramii and $S$. oualaniensis allowed the SIA results to be incorporated into energetic models for these squids. Combining modifications of existing metabolic and growth equations for $O$. bartramii into an energy budget produced daily energetic demands for $O$. bartramii and $S$. oualaniensis that conform to their physiological capabilities and published feeding results in the later stages of the model. The model results indicated the probable impact of migration behavior (diurnal and geographical) on the energetic demands of these squids. The model also demonstrated the possibly large effects that increasing trophic position with age can have on the overall energy required from the ecosystem to sustain these squids. The model also indicated that the energetic costs of a large geographical migration in $O$. bartramii, relative to no such migration in $S$. 

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oualaniensis, is largely offset by the colder northern waters. In addition, richer feeding
grounds in the north may make migration an energetically favorable option.

This study indicates the importance of using complimentary techniques such as
SCA and SIA when investigating difficult biological concepts such as trophic ecology.
Using SIA in a complex trophic environment like the North Pacific Subtropical gyre
remains challenging given the variables that can affect $\delta^{15}$N, however the results of this
study show that reproducible and ecologically relevant data can be obtained. The
interactions between trophic level, feeding behavior, nutrient stress, tissue fractionation,
and geographical variation on the stable isotopic signatures of an animal are still poorly
understood. Separating and quantifying the effects of these factors requires controlled
experimental manipulations. Such experiments should enable increased usage of stable
isotopes for understanding a wide array of ecological questions.

The average 3.4% increase in $\delta^{15}$N per trophic level does not appear to apply to
all organisms examined in this study. The S. oualaniensis sampled appear to be on
average lighter than their stomach contents would suggest. This discrepancy can be only
partially explained by sampling bias of prey items. Other discrepancies arise when using
the accepted increase, namely the high values obtained for paralarvae, some zooplankton,
and for certain myctophids. Perhaps there is a discontinuity between smaller pelagic food
webs and larger ones. This discontinuity, and the different energetic pathways that
connect the two systems, may also lead to problems with using the accepted trophic level
isotopic increase. Fractionation factors for lower trophic level organisms are almost
certainly different from higher trophic level organisms; assimilation efficiencies certainly
are. Also fractionation factors may change between different phyla of higher organisms,
confounding the tidy picture of a set numerical isotopic increase with trophic level.
Clearly more work needs to be done before stable isotopic analyses reach their full
potential as tools for elucidating ecosystem structure.
Appendix: $\delta^{13}$C Values

**Ommastrephes bartramii $\delta^{13}$C (adults)**

2-14/15-99 #14 F (570):

The $\delta^{13}$C of the lens segments of this squid ranged from -20.61 to -19.47%; the mantle value was -21.00% and the blood was -20.47%. The lens values increased initially from 159mm ML (-19.65%) and then decreased to 239mm ML (20.47%) where the values remained fairly constant to 314mm ML (-20.44%) and then increased to 372mm ML (-19.50%) followed by a rapid decrease to 417mm ML (-20.26%) and a more gradual decrease to 570mm ML (-20.50%) (Fig. 36).

2-16/17-99 #3 F (446):

The $\delta^{13}$C of the lens segments of this squid ranged from -20.55 to -19.27%; the mantle value was -21.48% and blood was -20.89%. The lens values generally decreased (with one peak at 152mm ML (-19.95%)) from 118mm ML (-20.1%) to 201mm ML (-20.37%) then increased to 304mm ML (-19.27%) (with one peak at 245mm ML, -19.55%) and finally decreased to 446mm ML (-20.55%) (Fig. 37).

2-7/8-00 #1 M (333):

The $\delta^{13}$C of the lens segments of this squid ranged from -20.95 to -19.44%; the mantle value was -20.92%. The lens values increased from the first point at 125mm ML (-19.73%) to 151mm ML (-19.44%) and then decreased to the last point (with one peak at 224mm ML, -19.76%) at 333mm ML (-20.95%) (Fig. 38).

2-8/9-00 #1 M (318):

The $\delta^{13}$C of the lens segments ranged from -20.41 to -18.90%; the mantle value was -20.46%. The $\delta^{13}$C of the gladius segments ranged from -20.29 to -19.70%. The lens values generally increased from 107mm ML (-20.14%) to 154mm ML (-18.90) then decreased to 318mm ML (-20.41%) (Fig. 39).
The gladius values increased to 40rnm ML (-19.95%) and then remained relatively constant to 200rnm ML (-20.46%) and finally increased to 318rnm ML (-19.70%) (Fig. 39).

2-8/9-00 #3 M (296):

The δ¹³C of the lens segments ranged from -20.39 to -19.34%; the mantle value was -20.63%.
The δ¹³C of the gladius segments ranged from -20.54 to -19.62%. The lens values showed an overall oscillatory decrease from 96rnm ML (-19.34%) to 209rnm ML (-20.39%) with two peaks around 150rnm ML (-19.37%) and 221 (-19.53%) (Fig. 40). The gladius values remained relatively constant to 70rnm ML (-19.97%) then decreased to 140rnm ML (-20.54%) and finally increased to 296rnm ML (-19.62%) (Fig. 40).

2-8/9-00 #4 M (354):

The δ¹³C of the lens segments ranged from -19.41 to -20.68%; the mantle value was -20.45% and the blood value -20.29%. The δ¹³C of the gladius segments ranged from -20.34 to -19.32%. The lens values increased slightly from 119 rnm ML (-20.43%) to 209rnm ML (-20.25%), and then increased up to 231rnm ML (-19.41) followed by a decrease to 354rnm ML (-20.68%) (Fig. 41). The gladius values increased from 244 rnm ML (-20.34%) to a plateau between 329rnm ML (-19.75) and 339rnm ML (-19.71) followed by a final increase to 354rnm ML (-19.32%) (Fig. 41).

2-8/9-00 #5 M (314):

The δ¹³C of the gladius values ranged from -20.19 to -19.49%; the mantle value was -20.46% and blood was -19.98%. The gladius values initially increased to 40rnm ML (-19.79%) and remained relatively constant to 314rnm ML (-19.49%) with one dip at 140rnm ML (-19.69%) (Fig. 42).

2-9/10-00 #1 (322):

The δ¹³C of the gladius values ranged from -20.39 to -18.89%; the mantle value was -20.41% and the blood was -20.21%. The gladius values showed an overall oscillatory increase from 207rnm ML (-20.26%) to 322rnm ML (-18.91%) (Fig. 43).

2-9/10-00 #2 (321):

The δ¹³C of the gladius values ranged from -20.24 to -18.65%; the mantle value was -20.54% and blood was -20.27%. The gladius values showed an overall oscillatory increase from 206rnm ML (-19.83%) to 321rnm ML (-18.65%) (Fig. 44).

2-9/10-00 #4 (540):
The $\delta^{13}$C of the lens values ranged from -20.97 to -19.28\%; the mantle value was -20.69\% and the blood was -20.43\%. The lens values showed an overall slight oscillating decrease from 140mm ML (-19.43\%) to 407mm ML (-19.67\%) followed by a rapid decrease to 540mm (-20.97\%) (Fig. 45).

2-9/10-00 #5 (360):

The $\delta^{13}$C of the lens values ranged from -21.58 to -20.16\%; the mantle value was -21.07\% and blood was -20.85\%. The lens values showed an overall decrease (with substantial oscillations) to 270mm ML (-20.53\%) followed by a rapid decrease to 360mm ML (-21.58\%) (Fig. 46).

2-11/12-01 #1 (557):

The $\delta^{13}$C of the lens values ranged from -21.79 to -20.03\%; the mantle value was -21.68\% and blood was -21.42\%. The lens values showed an initial increase from 150mm ML (-21.01\%) to 247mm ML (-20.03\%) then decreased to 354mm ML (-21.52\%) followed by a slight increase and plateau from 417 mm ML (-21.28\%) to 503mm ML (-21.33\%) with a final decrease to 557mm ML (-21.79\%) (Fig. 47).

2-11/12-01 #2 (370):

The $\delta^{13}$C of the lens values ranged from -20.91 to -20.22\%; the mantle value was -20.93\% and the blood was -20.57\%. The gladius values ranged from -21.50\% to -18.69\%. The lens showed an initial plateau (with oscillations) from 97mm ML (-20.36\%) to 217mm ML (-20.22\%) followed by a final decrease to 370mm ML (-20.91\%) (Fig. 48). The values showed an initial plateau from 255mm ML (-20.36\%) to 295mm ML (-20.43\%), a decrease to 310mm ML (-21.50\%) and a final increase up to 370mm ML (-18.69\%) (Fig. 48).

2-11/12-01 #5 (534):

The $\delta^{13}$C of the lens values ranged from -21.27 to -19.91\%; the mantle value was -20.60\%. The lens showed an overall decrease from 140mm ML (-20.14\%) to 340mm ML (-21.27\%), with two peaks at 163mm ML (-19.91\%) and 243mm ML (-20.02\%), followed by a general increase to 534mm ML (-20.42\%) (Fig. 49).

Ommastrephes bartramii $\delta^{13}$C (northern samples)

#21 (220):
The δ\(^{13}\)C of the lens values ranged from -21.05 to -19.86\%, and the mantle value was -20.08\%. The lens showed an overall increase from 130 mm ML (-21.05\%) to 220 mm ML (-19.86\%) with peaks at 153 mm ML (-20.55\%) and 173 mm ML (-20.38\%) (Fig. 50).

#25 (255):

The δ\(^{13}\)C of the lens values ranged from -20.60 to -20.13\%, and the mantle value was -20.43\%. The lens showed an initial increase from 144 mm ML (-20.36\%) followed by a decrease to a plateau between 168 mm ML (-20.58\%) to 224 mm ML (-20.56\%) followed by a peak at 243 mm ML (-20.28\%) and a final decrease at 255 mm ML (-20.43\%) (Fig. 51).

#26 (201):

The δ\(^{13}\)C values of the lens ranged from -20.85 to -20.32\%, and the mantle value was -20.38\%. The lens showed a decrease from 117 mm ML (-20.45\%) to 153 mm ML (-20.85\%), and then increased to 174 mm ML (-20.32\%) followed by a plateau to 201 mm ML (-20.38\%) (Fig. 52).

**Ommastrephes bartramii δ\(^{13}\)C juveniles**

2-16/17-98 #25 (142):

The δ\(^{13}\)C of the lens values ranged from -20.01 to -19.83\%, and the mantle value was -19.55\%. The lens showed a decrease from 94 mm ML (-19.83\%) to a plateau from 127 mm ML (-20.01\%) to 142 mm ML (-19.99) (Fig. 53).

2-16/17-98 #26 (151):

The δ\(^{13}\)C of the lens values ranged from -20.23 to -19.67\%, and the mantle value was -20.03\%. The lens decreased from 96 mm ML (-19.67\%) to 126 mm ML (-20.23\%) and then increased to 151 mm ML (-20.03\%) (Fig. 54).

2-11/12-99 #5 (161):

The δ\(^{13}\)C of the lens values ranged from 118.88 to -18.69\%, and the mantle value was -18.72\%. The lens showed fairly constant values from 94 mm ML (-18.88\%) to 161 mm ML (-18.82\%) (Fig. 55).

2-11/12-99 #6 (150):

The δ\(^{13}\)C of the lens values ranged from -19.25 to -18.78\%, and the mantle value was -19.82\%. The lens showed an initial increase from 82 mm ML (-18.97\%) to 93 mm ML (-18.78\%), a decrease to 111 mm ML (-19.25\%) and an oscillatory increase to 150 mm ML (-18.98\%) (Fig. 56).
**Ommastrephes bartramii δ^{13}C**

Preliminary analysis of the δ^{13}C values for the eye lens segments might indicate a geographical change in isotopic value of an ecosystem. Although the factors governing the signals of δ^{13}C and δ^{15}N in the environment, and within an organism, are wholly separate the carbon signal could potentially indicate movement into a different ecosystem. The δ^{13}C signals from the eye lenses and the muscle of the four juvenile *O. bartramii* caught in Hawaiian waters did not show any distinctive patterns and exhibited small fluctuations overall (the largest change in juvenile eye lens segments was 0.56‰) (Figs. 53-56). The eye lens segments from the three northern *O. bartramii* individuals also showed no distinctive general patterns with length, but did seem to start at lower δ^{13}C values than the juveniles. The muscle values for the northern squids were close to the lower end of δ^{13}C values seen for the four juvenile *O. bartramii* (≈20‰) (Figs. 50-52). The δ^{13}C signals of the eye lens segments from adult *O. bartramii* also did not share a common general trend, and most fluctuations of the δ^{13}C were small, on the order of 1.50‰ (36-49). In general the δ^{13}C values of the muscle were lighter than the muscle for juveniles, while the eye lens and gladius values fluctuated and overlapped the range for juveniles. The δ^{13}C values for the of 2-9/10-00 #2 and 2-8/9-00 #4 both steadily increased over the size ranges sampled, and could indicate a steady movement into another isotopic regime (Figs. 44, 41). For 2-8/9-00 #1, #3, and #5 showed very little change but all got heavier at the final segment (Figs. 39, 40, 42).

**Sthenoteuthis oualaniensis δ^{13}C (adults)**

2-14/15-99 #1 (324):

The δ^{13}C values of the lens ranged from -19.31 to -18.83‰, and the mantle value was -19.70‰. The lens showed a decrease from 129mm ML (-19.00‰) to 138mm ML (-19.31‰), an increase to 196mm ML (-18.83‰) followed by a decrease to 324mm ML (-19.70‰) (Fig. 57).

2-14/15-99 #3 (200):

The δ^{13}C values of the lens ranged from -19.32 to -18.83‰, and the mantle value was -19.19‰. The lens showed an increase from 71mm ML (-19.32‰) to 146mm ML (-18.83‰) and then decreased to 200mm ML (-19.30‰) (Fig. 58).

2-14/15-99 #10 (223):

The δ^{13}C values of the lens ranged from -20.30 to -18.81‰, and the mantle value was -19.17‰. The lens values increased from 77mm ML (-19.25‰) to 140mm ML (-18.81‰), with one dip at 112mm ML (-19.27), followed by an decrease to 223mm ML (-20.30‰) (Fig. 59).
The $\delta^{13}C$ values of the lens ranged from -19.00 to -18.07%, and the mantle value was -18.66%. The lens values showed an overall increase from 105mm ML (-19.00%) to 190mm ML (-18.07%) and then decreased to 210mm ML (-18.38%) (Fig. 60).

The $\delta^{13}C$ values of the lens ranged from -18.87 to -18.25%, and the mantle value was -18.65%. The lens values remained fairly constant, with some variation, from 90mm ML (-18.75%) to 209mm ML (-18.65%) with a peak at 198mm ML (-18.25%) (Fig. 61).

The $\delta^{13}C$ values of the lens ranged from -19.09 to -18.35%, and the mantle value was -18.35%. The lens values increased from 78mm ML (-19.09%) to 84mm ML (-18.54%), decreased to 94mm ML (-19.03%), increased slightly to 130mm ML (-18.35%), decreased again to 137mm ML (-18.60%) and finally increased to 160mm ML (-18.35%) (Fig. 62).

The $\delta^{13}C$ values of the lens ranged from -19.60 to -18.85%, the mantle value was -18.61%, and blood was -18.35%. The lens values decreased overall from 94mm ML (-19.00%) to 123mm ML (-19.60%) then increased to 160mm ML (-18.86%) then remained constant to 181mm ML (-18.85%) (Fig. 63). The values of the gladius showed an overall decrease from 106mm ML (-17.32%) to 161mm ML (-18.23%), then increased to a plateau between 166mm ML (-17.90%) and 176mm ML (-17.84%) and finally decreased to 181mm ML (-18.20%) (Fig. 63).

The $\delta^{13}C$ values of the lens ranged from -18.98 to -18.42%, the mantle value was -18.28%. The values of the gladius ranged from -17.66 to -16.93%. The lens values remained relatively constant from 91mm ML (-18.85%) to 122mm ML (-18.98%), increased to 138mm ML (-18.42%) then decreased overall to 173mm ML (-18.74%) (Fig. 64). The values of the gladius remained relatively constant from 95mm ML (-17.5%) to 145mm ML (-17.64%), increased to 165mm ML (-16.93%) then finally decreased to (-17.27%) (Fig. 64).

The $\delta^{13}C$ values of the lens ranged from -19.11 to -18.20%, the mantle value was -18.55%. The lens values increased from 85mm ML (-19.11%) to 104mm ML (-18.20%), decreased to 123mm ML (-19.05%) and finally increased to 158mm ML (-18.55%) (Fig. 65).
The δ¹³C values of the lens ranged from -19.03 to -18.23‰, the mantle value was -18.34‰ and blood was -18.21‰. The lens values increased from 111mm ML (-19.03‰) to 200mm ML (-18.38‰), decreased to 221mm ML (-18.71‰), increased to 234mm ML (-18.23‰) and finally decreased to 251mm ML (-18.60‰) (Fig. 66).

The δ¹³C values of the lens ranged from -19.81 to -18.26‰, the mantle value was -17.63‰ and blood was -17.63‰. The lens values remained relatively constant from 126mm ML (-18.48‰) to 210mm ML (-18.37‰) then decreased to 225mm ML (-19.81‰) (Fig. 67).

The δ¹³C values of the lens ranged from -19.68 to -17.90‰, the mantle value was -19.22‰ and blood was -18.71‰. The lens values increased from 98mm ML (-18.74‰) to 111mm ML (-17.90‰), followed by an overall decrease to 304mm ML (-19.68‰) (Fig. 68).

The δ¹³C values of the lens ranged from -19.48 to -17.78‰, the mantle value was -18.58‰. The lens values increased slightly from 101mm ML (-18.25‰) to 161mm ML (-17.99‰), followed by an overall decrease to 302mm ML (-19.48‰), with two peaks at 211mm ML (-17.78‰) and 243mm ML (-17.95‰) (Fig. 69).

The δ¹³C values of the lens ranged from -19.75 to -18.11‰, the mantle value was -18.88‰ and blood was -18.70‰. The lens values increased initially from 95mm ML (-18.84‰) to 112mm ML (-18.11‰) and remained relatively constant to 223mm ML (-18.24‰) followed by a decrease to 308mm ML (-19.75‰) (Fig. 70).

**Sthenoteuthis oualaniensis δ¹³C**

The δ¹³C values for the eye lens segments of *S. oualaniensis* stayed fairly constant at smaller sizes for many of the individuals, and decreased near the outer segments (Figs. 57-59, 61-64, 66-70). The overall variation in δ¹³C was low; all *S. oualaniensis* showed variations in the eye lens segments of < 2‰. Squid 2-12/13-00 #3, the squid with the high δ¹⁴N values for muscle and eye lens segments, showed a differing δ¹³C
pattern for eye lenses and for gladius values (Fig. 63). The gladius $\delta^{13}$C values showed an overall decrease while the eye lenses showed an initial decrease followed by an increase to a plateau. The gladius and eye lens segments for squid 2-12/13-00 #4 both showed similar $\delta^{13}$C patterns (Fig. 64). This difference between squids 2-12/13-00 #3 and #4 could indicate that there were physiological differences in #3 that may have caused the high $\delta^{15}$N values (Figs. 63, 64).
Figure 36. δ¹³C versus length of eye lens segments of a female *O. bartramii*. δ¹³C of mantle muscle and blood plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 37. $\delta^{13}C$ versus length of eye lens segments of a female *O. bartramii*. $\delta^{13}C$ of mantle muscle and blood plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 38. $\delta^{13}C$ versus length of eye lens segments of a male O. bartramii. $\delta^{13}C$ of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 39. $\delta^{13}C$ versus length of eye lens segments of a male *O. bartramii*. $\delta^{13}C$ of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 40. $\delta^{13}$C versus length of eye lens and gladius segments of a male *O. bartramii*. $\delta^{13}$C of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 41. $\delta^{13}$C versus length of eye lens and gladius segments of a male *O. bartramii*. $\delta^{13}$C of mantle muscle and blood plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 42. $\delta^{13}C$ versus length of gladius segments of a male *O. bartramii*. $\delta^{13}C$ of mantle muscle and blood plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 43. $\delta^{13}$C versus length of gladius segments of a male $O.\ bartramii$. $\delta^{13}$C of mantle muscle and blood plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 44. $\delta^{13}$C versus length of gladius segments of a male *O. bartramii*. $\delta^{13}$C of mantle muscle and blood plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 45. $\delta^{13}C$ versus length of eye lens segments of a female *O. bartramii*. $\delta^{13}C$ of mantle muscle and blood plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 46. $\delta^{13}C$ versus length of eye lens segments of a male *O. bartramii*. $\delta^{13}C$ of mantle muscle and blood plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 47. $\delta^{13}C$ versus length of eye lens segments of a female *O. bartramii*. $\delta^{13}C$ of mantle muscle and blood plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 48. $\delta^{13}C$ versus length of eye lens and gladius segments of a male *O. bartramii*. $\delta^{13}C$ of mantle muscle and blood plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 49. $\delta^{13}C$ versus length of eye lens segments of a female *O. bartramii*. $\delta^{13}C$ of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 50. $\delta^{13}$C versus length of eye lens segments of a male northern *O. bartramii*. $\delta^{13}$C of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 51. $\delta^{13}$C versus length of eye lens segments of a female northern O. bartramii. $\delta^{13}$C of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 52. $\delta^{13}C$ versus length of eye lens segments of a female northern *O. bartramii*. $\delta^{13}C$ of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 53. δ¹³C versus length of eye lens segments of a juvenile *O. bartramii*. δ¹³C of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 54. $\delta^{13}C$ versus length of eye lens segments of a juvenile *O. bartramii*. $\delta^{13}C$ of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 55. $\delta^{13}$C versus length of eye lens segments of a juvenile *O. bartramii*. $\delta^{13}$C of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 56. $\delta^{13}$C versus length of eye lens segments of a juvenile *O. bartramii*. $\delta^{13}$C of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 57. $\delta^{13}$C versus length of eye lens segments of a female *S. oualaniensis*. $\delta^{13}$C of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 58. $\delta^{13}$C versus length of eye lens segments of a female *S. oualaniensis*. $\delta^{13}$C of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 59. $\delta^{13}$C versus length of eye lens segments of a female *S. oualaniensis*. $\delta^{13}$C of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 60. $\delta^{13}C$ versus length of eye lens segments of a female *S. oualaniensis*. $\delta^{13}C$ of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 61. $\delta^{13}$C versus length of eye lens segments of a female S. oualaniensis. $\delta^{13}$C of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 62. $\delta^{13}$C versus length of eye lens segments of a female S. oualaniensis. $\delta^{13}$C of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 63. $\delta^{13}C$ versus length of eye lens and gladius segments of a female *S. oualaniensis*. $\delta^{13}C$ of mantle muscle and blood plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 64. $\delta^{13}C$ versus length of eye lens and gladius segments of a female *S. oualaniensis*. $\delta^{13}C$ of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 65. $\delta^{13}C$ versus length of eye lens segments of a male S. oualaniensis. $\delta^{13}C$ of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 66. $\delta^{13}$C versus length of eye lens segments of a female *S. oualaniensis*. $\delta^{13}$C of mantle muscle and blood plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 67. $\delta^{13}$C versus length of eye lens segments of a female *S. oualaniensis*. $\delta^{13}$C of mantle muscle and blood plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 68. δ^{13}C versus length of eye lens segments of a female *S. oualaniensis*. δ^{13}C of mantle muscle and blood plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 69. \(^{\delta^{13}}\)C versus length of eye lens segments of a female *S. oualaniensis*. \(^{\delta^{13}}\)C of mantle muscle plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.
Figure 70. $\delta^{13}$C versus length of eye lens segments of a female *S. oualaniensis*. $\delta^{13}$C of mantle muscle and blood plotted against mantle length at time of capture. Mantle length for eye lens segments estimated from regressions of eye lens radius and squid size. Date caught, squid number, and mantle length noted in bottom right corner.