Mar Biol (2007) 150:647–658
DOI 10.1007/s00227-006-0360-y

RESEARCH ARTICLE

A rapid ontogenetic shift in the diet of juvenile yellowfin tuna from Hawaii

Brittany S. Graham · Dean Grubbs · Kim Holland · Brian N. Popp

Received: 19 September 2005 / Accepted: 3 April 2006 / Published online: 27 June 2006

Abstract Within the tropical and subtropical oceans, tuna forage opportunistically on a wide variety of prey. However, little is known about the trophic ecology of the smallest size classes which play an important role in stock assessments and fisheries management. The foraging behavior of yellowfin tuna, *Thunnus albacares* (23.5–154.0 cm FL), collected from nearshore Fish Aggregating Devices (FADs) around Oahu was studied using stable isotope and stomach contents analyses. Emphasis was placed on small juveniles. Yellowfin tuna changed their diets significantly between 45 and 50 cm forklength (ca. 1.5 kg). Smallest size classes fed on planktonic organisms inhabiting the shallow mixed layer, primarily larval stomatopod and decapod crustaceans, whereas larger tuna fed on teleosts and adult *Oplophorus gracilirostris*, a vertically migrating mesopelagic species of shrimp. When interpreting the variation in prey δ^{15}N values, we considered both their relative trophic position and δ^{15}N values of the nitrogen at the base of the food web. Based on the distinct diet shift of the yellowfin tuna, demonstrated by both isotope and stomach content analyses, we propose a critical mass threshold was reached at about 45 cm FL that enabled sufficient endothermic capability to allow tuna to access prey dwelling in deeper, colder water. These ontogenetic changes in foraging range and commensurate shift in diet of small tunas would affect their vulnerability to fishing pressure.

Communicated by G.F. Humphrey, Sydney

B. S. Graham (✉)
Department of Oceanography, University of Hawaii, Honolulu, HI 96822, USA
e-mail: grahamb@hawaii.edu

D. Grubbs · K. Holland
Hawaii Institute of Marine Biology, University of Hawaii, Kaneohe, HI 96744, USA

B. N. Popp
Department of Geology and Geophysics, University of Hawaii, Honolulu, HI 96822, USA

Introduction

Yellowfin tuna diets have been described from many locations throughout the world's tropical and subtropical oceans, especially in the Pacific Ocean. With few exceptions, previous stomach content studies concluded that yellowfin tuna are opportunistic predators that feed on a tremendously diverse forage base, although the majority of the diet often comprises only a few families of epipelagic teleosts and crustaceans (e.g., Reintjes and King 1953; Alverson 1963). However, little is known about the feeding behavior or diet of the smallest size classes of tropical tunas. Only one published study included an analysis of yellowfin tunas less than 50 cm FL. Maldeniya (1996) noted that yellowfin tuna less than 40 cm FL fed on planktonic crustacean while those greater than 50 cm FL were piscivores. Brock (1985) also sampled small yellowfin tuna (ranging from 25 to 150 cm in fork length) but in the analysis of stomach contents, he pooled all size classes and only differentiated between tuna caught near a Fish Aggregating Device (FAD) or away from a FAD. Understanding the diet of the smallest size classes is especially pertinent because these small tunas dominate the structure-associated communities that

aggregate around natural and manmade floating objects, including FADs (Menard et al. 2000).

Several studies have successfully used stable isotope methods to examine fish trophic interactions (e.g., Fry 1988; Hobson and Welch 1992), temporal and spatial variations in food web dynamics (e.g., Deegan and Garritt 1997; O'Reilly et al. 2002), migration (e.g., Doucett et al. 1999; Fry et al. 2003), diet and habitat specialization (e.g., Harrigan et al. 1989), and ontogenetic shifts (e.g., Renones et al. 2002; Post 2003). These studies are based on the observation that the carbon, sulfur, and nitrogen isotopic compositions of an organism's tissue reflect its food or nutrient source. During assimilation, isotopic fractionation (preferential incorporation) of ^{15}N and ^{13}C relative to prey items occurs at each successive trophic level (e.g., Minagawa and Wada 1984). Trophic-related fractionation results in an average 3.4 and 0.7‰ increase in $\delta^{15}N$ and $\delta^{13}C$ values of the consumer at each subsequent trophic level, respectively (Deniro and Epstein 1978, 1981). Unlike stomach content analyses, stable isotope values of an organism's tissues provide information on the time-integrated assimilated diet. Metabolic activity affects tissue turnover rates which, in turn, affect stable isotope values in different tissues (Fry and Arnold 1982; Tieszen et al. 1983). More metabolically active tissues will reflect changes in diet more rapidly than tissues with slower turnover rates (e.g., Hobson and Clarke 1992; Hesslein et al. 1993). Accordingly, values from different tissues within the same organism can provide an archive of feeding history that can differ in temporal resolution.

Tuna are the only teleosts to have evolved physiological mechanisms for 'whole body' thermoregulation wherein the skeletal musculature is kept warmer than the surrounding water. This ability is a function of the development of pertinent vascular structures, an internal heat source, and the thermal inertia associated with increasing mass (Holland et al. 1992; Dickson 1994; Dickson et al. 2000). Physiological and behavioral thermoregulation allows tuna to gain independence from thermal constraints and increase their foraging range to include both the upper mixed layer and the cooler waters below the thermocline. However, little information is available on when endothermic capabilities are achieved, and if the onset of thermoregulation actually coincides with an increase in foraging range within the pelagic ecosystem and a commensurate change in prey type or prey diversity.

In this study, we investigated the feeding habits and trophic dynamics of yellowfin tuna associated with nearshore FADs by coupling stable isotope and traditional stomach content analyses of the same individuals. Over a two-year study, we examined the stomach contents and measured white muscle and liver tissue isotopic compositions to provide a robust temporal indication of tuna foraging behavior. Furthermore, to examine possible ontogenetic shifts in tuna foraging behavior and biology, we examined a range of size classes of yellowfin tuna with special emphasis placed on small size classes. We found that between 45 and 50 cm forklength (ca. 1.5 kg), yellowfin tuna rapidly shift their diets. Because tunas are capable of physiological thermoregulation, we propose that the ontogenetic onset of the thermoregulatory capability is driving the observed shift in diet.

Methods

We collected tuna from the nearshore FADs located around Oahu, Hawaii (Fig. 1). All fish were caught at FADs that were less than 30 km from the island of Oahu. Depth of moorings for FADs used in this study range between 523 and 2,084 m with an average mooring depth of 1,349 m. Tuna were collected with conventional rod and reel fishing gear using a variety of baits and artificial lures. The majority of tuna sampled were caught between 0600 and 1200 h. In waters around Hawaii, yellowfin tuna spawn from May until October with major spawning activity occurring from June until August (Itano 2000). Tunas were collected during all months of the year and the largest and smallest size classes were sampled at the same time during several occasions. We measured fork lengths for all tuna and converted the lengths to mass (g) using length-weight relationships previously developed for yellowfin tuna collected from around Hawaii

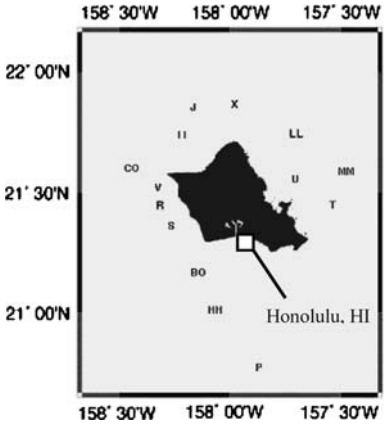

Fig. 1 Study region. The location of the nearshore fixed FADs around the island of Oahu where yellowfin tuna were sampled from October 2002 to May 2004

(Uchiyama and Kazama 2003). Collection of samples occurred between October 2002 and July 2004.

Gut content analyses

Stomach contents were preserved in a solution of 10% phosphate-buffered formalin for at least 72 h and then stored in 50% ethyl alcohol until lab analysis. In the laboratory, we: (1) rinsed the stomachs and removed all contents, (2) identified each prey item to the lowest possible taxon, (3) recorded the number and digestion state of each taxon, and (4) measured the wet volume of each prey taxon using water displacement in graduated cylinders (Wolfert and Miller 1978; Hyslop 1980). As part of a larger tuna feeding study, the size classes were chosen arbitrarily in 25 cm intervals of <50, 50–74.9, 75–99.9, and 100+ cm. Due to the large sample size of very small yellowfin associated with the FADs, the smallest class was divided into two equal intervals (20–34.9 and 35.0–49.9 cm) (Table 1).

The proportion of empty stomachs and the mean stomach repletion in a sample can be used as indices of relative foraging success. These indices were calculated for each size class of tuna and were used to discern differences in foraging success based on predator size. Stomach repletion was estimated as the volume of prey in milliliters per kilogram of body weight. We defined empty stomachs as those with less than 0.1 ml of prey per kg of body weight.

We calculated percent abundance (%N) and percent volume (%V) of each prey taxon by dividing the total number or volume of a given taxon by the total number or volume of prey in all stomachs pooled. Pooled metrics such as %N and %V are the most commonly used dietary measures in feeding studies; however, they are significantly flawed. Since they are single measures from pooled samples, no confidence intervals can be calculated (Chipps and Garvey, in press). Also, prey items in individual stomachs are not independent; therefore, sacrificial pseudoreplication is committed when prey items are pooled across stomachs (Hurlbert 1984). Mean percent abundance (%MN) and mean percent volume (%MV) are less biased and allow the computation of confidence intervals; therefore, these were used as the primary measures to compare diets. To calculate mean percent volume (%MV), the percent volume (%V) of all prey taxa were calculated for each individual stomach by dividing the volume of each prey taxon by the total volume of prey in that stomach. We then calculated the mean of these values for all samples within each size class (including zeros) to yield single estimates of mean percent volume (%MV) and the associated standard error for each taxon. Mean percent abundance (%MN) was calculated similarly using the numbers rather than volumes of prey taxa.

Shifts in diet were estimated by the degree of dietary overlap between size classes using Moriseta's original index. When Moriseta's index was at least 0.60, we consider overlap between diets to be significant (Zaret and Rand 1971). No single dietary measure can adequately characterize the diet of a population (Larimore 1957). Therefore, we calculated overlap between size classes using all four dietary measures, %N, %V, %MN, and %MV for prey families.

Table 1 Interval and sample sizes for each size class used in the gut content analyses

Size class	Fork length interval (cm)	Stomach contents		
		N	% With prey	Repletion–ml/kg (SEM)
0	<35.0	198	92.93	8.07 (1.07)
1	35.0–49.9	160	75.63	3.53 (0.60)
2	50.0–74.9	34	67.65	3.50 (1.16)
3	75.0–99.9	9	66.67	6.53 (4.01)
4	100.0+	3	100.00	5.40 (3.80)

The proportion of empty stomachs mean and stomach repletion are also provided

SEM standard error of the mean

Stable isotope analyses

After collection, tuna were transported on ice to the shore-based laboratory and stored at –20°C until processed further. Fish collected from the FADs were sampled for an array of tissues, but data reported here are only for white muscle and liver tissues. White muscle tissue was always collected from the posterior, epaxial region of the tuna. The carbon and nitrogen isotopic compositions of recently ingested prey items found in the stomach contents were also analyzed.

Tissue and prey samples were lyophilized, lipid-extracted, and homogenized [Wig-L-Bug® ball and capsule amalgamator (Crescent Industries, Auburn, ME, USA) or mortar and pestle]. The variable amount of lipid in fish can confound interpretation of the carbon isotope composition because lipids are depleted in ^{13}C relative to the bulk tissue (e.g., Doucett et al. 1999). Therefore, lipids were removed by an automated high pressure and temperature extraction system (ASE 200®, Dionex, CA, USA) using hexane according to manufacturer's recommendations (Dionex Application Note 342, http://www.1.dionex.com/en-us/webdocs/application/industry/environmental/extraction/AN342.pdf).

Carbon and nitrogen isotopic compositions of tuna and prey samples were determined using an on-line

carbon–nitrogen analyzer coupled with an isotope ratio mass spectrometer (Finnigan ConFlo II/Delta-Plus, Bremen, Germany). Isotope values are reported in standard δ-notation relative to an international standard. Standards are V-PDB and atmospheric N_2 for carbon and nitrogen, respectively. A glycine standard was used to ensure accuracy of all isotope measurements. Furthermore, several samples were measured in duplicate or triplicate, and the analytical error associated with these measurements was typically ≤0.2‰.

We examined the relative contributions of different prey items to tuna diets by coupling $\delta^{15}N$ values and stomach content data using a simple mass balance approach:

$$\delta^{15}N_{YFT}=\left(\sum(\%MV_x\delta_x)\right)/T\%MV+TF, \quad (1)$$

where $\delta^{15}N_{YFT}$ is the $\delta^{15}N$ value of the tuna tissue, $\%MV_x$ the diet fraction of prey item X, and δ_x the isotope value of prey item X, T%MV the total fraction of the diet, and TF the trophic fractionation between the diet and tuna (3.4‰ after DeNiro and Epstein 1981).

Results

Gut content analyses

We analyzed the stomach contents of 404 FAD-associated yellowfin tuna. Tuna ranged from 23.5 to 154.0 cm in fork length; however, most (89%) were less than 50 cm. Overall, 80.6% of the tuna stomachs contained food and the mean repletion was 7.4 ml of prey per kg of body weight. There were no trends in stomach repletion or the proportion of tuna with prey as a function of tuna size class (Table 1).

Seventy-nine prey families were identified from the stomachs of yellowfin tuna in this study. The dominant prey taxa were combined into seven functional groups (Fig. 2). Two ontogenetic shifts in diet were apparent. First, as tuna size increased, there was a shift from a predominantly crustacean diet to a mixed diet of crustaceans and teleost fishes. Epipelagic and reef teleosts increased from 12.1%MV and 7.7%MN for size class 0 to 61.0%MV and 48.8%MN for class 4. Second, although crustaceans were important components in the diet of all size classes, the crustacean taxa consumed changed dramatically as tuna size increased. In terms of %MV, the pelagic larvae of decapod and stomatopod crustaceans composed 76 and 56% of the diet for classes 0 and 1, respectively, but represented only <17% of the diet of each of the three larger size classes. The opposite trend was seen for the mesopelagic shrimp, *Oplophorus gracilirostris* (Fig. 2a, b). This species contributed less than 1% to the diet of the smallest size class and was less than 6% of the diet of size class 1; yet, it contributed more than 25% to the diets of each of the three largest size classes.

Moriseta's original index (Table 2) indicated that significant overlap existed between the diets of size 0 and size 1 using three of the four metrics (%MV, %MN, and %N). No significant overlap existed between these (0 and 1) and the three larger size classes. The diet of size class 2 overlapped significantly with the diets of classes 3 and 4 using three of the four metrics. Overlap between classes 3 and 4 was significant using the two-pooled metrics, $\%V$ and $\%N$, but not using the two averaged metrics %MV or %MN. Overall, these results suggest that a significant dietary shift occurred around 50 cm FL.

In tunas less than 50 cm FL, larval stomatopod and decapod crustaceans, species associated with the shallow mixed layer dominate the diet with 68.5%MV, but only constituted 18.2%MV of the diet of tunas greater than 50 cm FL (Fig. 3a). Teleosts and *O. gracilirostris* dominate the diet of the larger tuna. *O. gracilirostris* is a vertically migrating species associated with the 700 m isobath around the Hawaiian Islands (Reid et al. 1991). During day, this species is found between 300 and 700 m depth but migrates to less than 200 m (<50 m when spawning) depth during the night (Ziemann 1975). The *O. gracilirostris* represented only 2.8%MV of the diet of tunas less than 50 cm FL but made up 26.4%MV of the diet of those larger than 50 cm FL (Fig. 3a). Fishes were only 17.1%MV of the diet of tunas less than 50 cm fork length but were 45.7%MV of the diet of larger tunas (Fig. 3a). The trends in terms of prey numbers (%MN) were very similar to those for volume (Fig. 3b).

Using the pooled data (%N and %V), the ontogenetic differences were exaggerated for the crustacean taxa but muted for the teleosts. Stomatopod and decapod crustacean larvae were 53.7% of the total prey volume and 81.8% of the total prey numbers for the smaller tuna compared to only 0.9% of the total volume and 10.6% of the total prey numbers of the larger tuna. In contrast, *O. gracilirostris* was only 13.4% of the total volume and 2.2% of the total prey numbers for the smaller tuna, but was 77.5% of the prey volume and 66.0% of the prey numbers for the larger tunas. While the numerical proportion of teleosts in the diets of larger tunas was nearly three times that of the smaller tunas (21.3–7.2%), the volumetric proportions were comparable (21.3 and 20.3%, respectively). These data illustrate the exaggerated influence one or a few

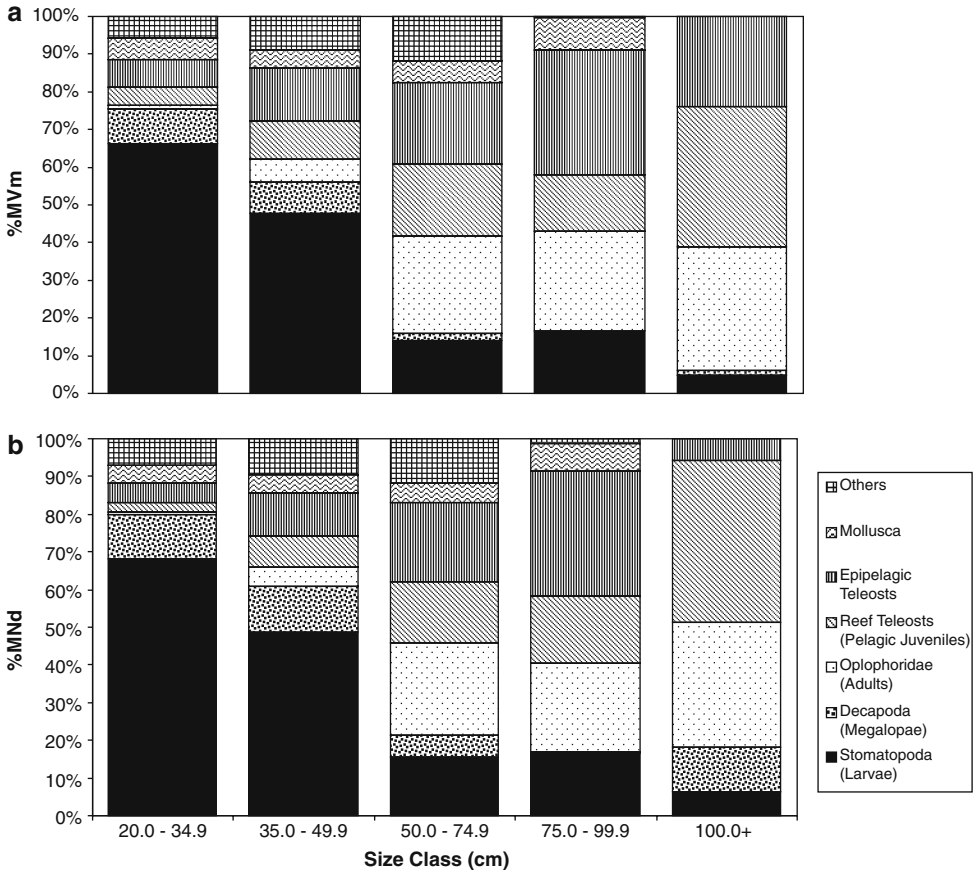

Fig. 2 Importance of major prey taxa, measured as (**a**) %MV and (**b**) %MN, in the diets of five size classes of yellowfin tunas

stomachs can have on dietary measures that use pooled data (%N and %V). While *O. gracilirostris* is important in the diet of larger yellowfin tunas, a few samples with very large numbers of this single prey taxon exaggerated the importance of this species in the diet in terms of %N. Likewise, a few small tunas with single, large teleosts in the stomach exaggerated the importance of teleosts in the diet in terms of %*V*.

Table 2 Moriseta's original index of dietary overlap between sizes classes of yellowfin tunas

Size	0	1	2	3	4
Class	(<35.0)	(35.0–49.9)	(50.0–74.9)	(75.0–99.9)	(100.0+)
Overlap of prey taxa in terms of %MN and %*N*					
0		0.80*	0.28	0.15	0.10
1	*0.36*		0.41	0.43	0.20
2	*0.03*	*0.53*		0.64*	0.55
3	*0.02*	*0.58*	*0.89**		0.46
4	*0.03*	*0.54*	*0.99**	*0.89**	
Overlap in prey taxa in terms of %MV and %*V*					
0		0.79*	0.29	0.19	0.18
1	*0.69**		0.40	0.45	0.25
2	*0.05*	*0.17*		0.59	0.65*
3	*0.03*	*0.19*	*0.96**		0.54
4	*0.05*	*0.18*	*0.94**	*0.95**	

Overlap estimates using pooled data (%*N* and %*V*) are italicized

*Indicates significant overlap ($P \geq 0.60$)

Stable isotope analyses

One hundred and six samples of white muscle and liver tissues collected from FAD-associated yellowfin tuna, ranging from 26 to 100 cm FL (ca. 0.3–18.3 kg), were analyzed for their carbon and nitrogen stable isotopic compositions. These tuna were a sub-set of the 404 individuals examined for gut content analyses. From an initial pilot study on 15 tuna tissue samples, lipid extraction did not statistically change δ^{15}N values ($P < 0.01$, Student's *t*-test). Based on a much larger dataset, lipid extraction also did not statistically affect the δ^{13}C value of WMT or LVR tissue in yellowfin tuna <45 cm FL ($P < 0.01$, Student's *t*-test). Therefore, after initially lipid-extracting tuna tissue samples, later samples were only lyophilized and homogenized before analysis.

Carbon isotopic values of white muscle tissue of tuna <45.0 cm (–16.1 ± 0.3‰) were not significantly different than tuna ≥45.0 cm (–16.5 ± 0.4‰) ($P < 0.001$, Student's *t*-test). White muscle and liver tissue δ^{13}C

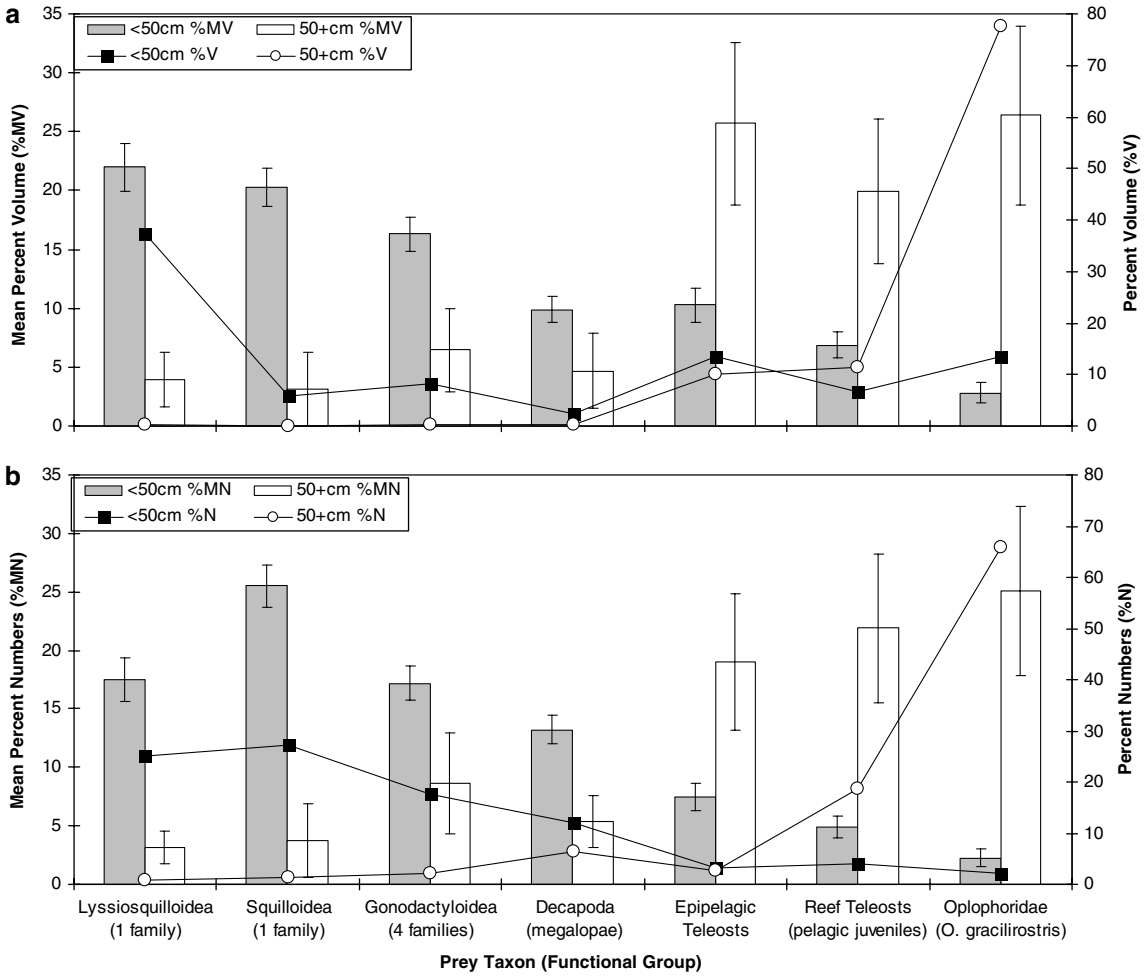

Fig. 3 Comparison of the relative importance of seven functional prey groups in the diets of small (< 50 cm FL) and large (≥50 cm FL) yellowfin tunas. **a** %MV and %*V*. **b** %MN and %N

values showed no trend with fork length or mass. However, a distinct positive shift in δ^{15}N occurs in the white muscle tissue of yellowfin tuna between a fork length of 45 and 50 cm (Fig. 4a). Nitrogen isotope values of white muscle tissue of tuna < 45.0 cm (6.7±0.6‰) are significantly less than tuna ≥45.0 cm (10.2±1.8‰) ($P < 0.001$, Student's *t*-test) (Table 3). Tuna larger than 45 cm FL exhibited a large range of δ^{15}N values relative to smaller size classes (Fig. 4a). Converting fork length to mass resulted in isotope ratios for white muscle that showed a logistic increase reaching an asymptote in the largest size classes (Fig. 4b). Carbon isotopic values of liver of tuna < 45.0 cm (–17.3 ± 0.5‰) were not significantly different than tuna ≥45.0 cm (–17.8 ± 0.8‰) ($P < 0.001$, Student's *t*-test). Liver δ^{13}C values showed no trend with fork length or mass. Liver δ^{15}N values of tuna < 45.0 cm (6.1±0.8‰) were significantly less than tuna ≥45.0 cm (8.4±1.5‰) ($P < 0.001$, Student's *t*-test) (Fig. 4a). A logistic increase was modeled for yellowfin δ^{15}N liver values and mass, which demonstrated a similar trend to white muscle tissue (Fig. 4b). There was not a statistical difference between the slopes (i.e., regression coefficients) of the liver and white muscle tissue datasets. The increase in average δ^{15}N values between small (< 45.0 cm FL) and larger tuna (≥45.0 cm FL) was around 3.5‰ for white muscle and 2.3‰ for liver tissue, respectively. However, a large increase (~5‰) in the δ^{15}N value of both the white muscle and liver tissues is observed immediately after 45 cm FL.

A range in δ^{15}N values was observed among the different prey species (Table 3). Stomatopod and decapod larvae, which were a major component of tuna in size groups 1 and 2, exhibited δ^{15}N values that averaged 3.4‰ less than δ^{15}N values of the tissues of the smallest tuna size classes. Using the simple diet-mixing model (Eq. 1, with the stomach content analysis providing estimates of 69% of the diet–$\%MV_x$), the observed yellowfin tuna δ^{15}N value closely matched the predicted value for small size classes (Table 3). For larger tuna, the

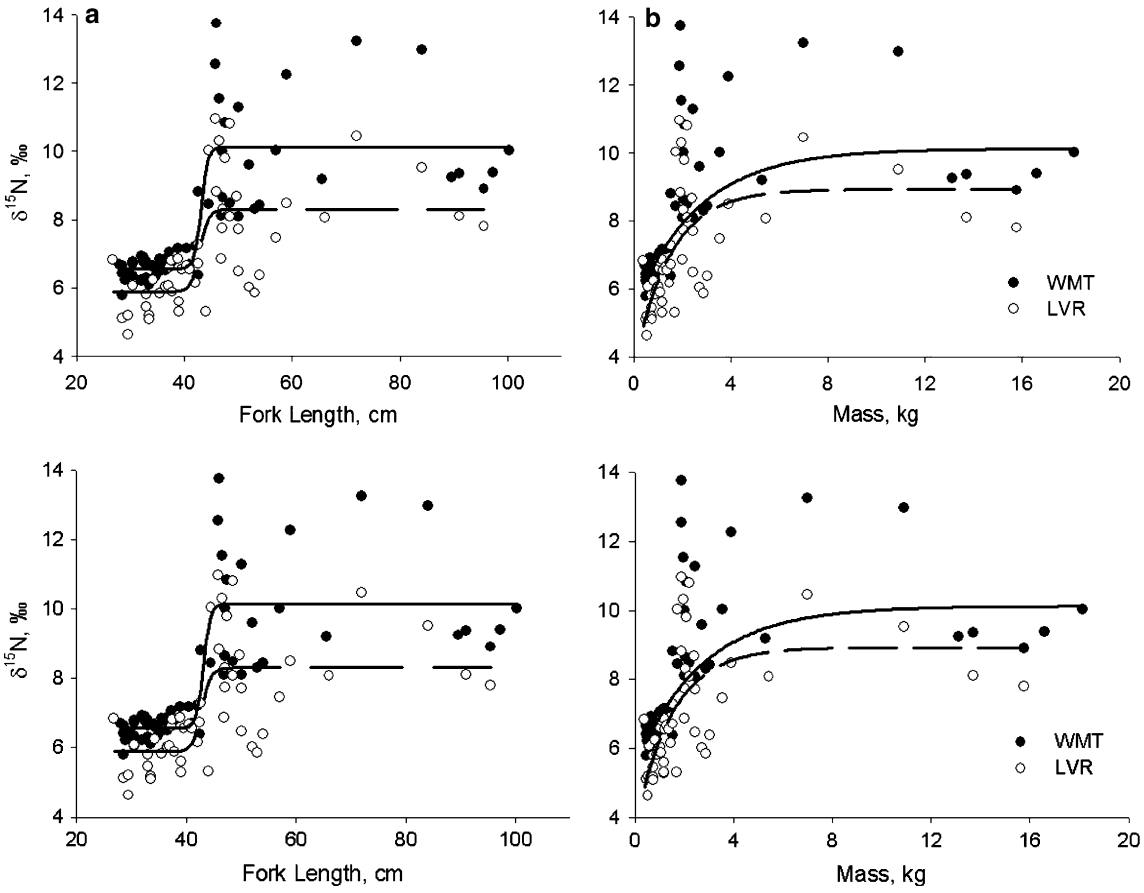

Fig. 4 **a** δ^{15}N values of yellowfin tuna white muscle (*WMT*) and liver (*LVR*) tissues over a range of fork lengths. A 4-parameter sigmoid model ($f = y_0+a/(1+\exp(-(x-x0)/b))$) was fitted to the yellowfin WMT (*solid line*; $f = 6.6+a/(1+\exp(-(x-43.1)/0.64))$; $R^2 = 0.71$) and LVR (*dashed line*; $f = 5.9+a/(1+\exp(-(x-42.9)/0.82))$; $R^2 = 0.49$). **b** Fork lengths were converted to mass using the length-weight relationships of Uchiyama and Kazama (2003). Illustrated is a 3-parameter exponential growth model ($f = y_0+a/(1-e^{-bx})$) fitted to the yellowfin data for WMT (*solid line*; $f = 4.0+a/(1-e^{-0.70x})$; $R^2 = 0.60$) and LVR (*dashed line*; $f = 3.3+a/(1-e^{-0.87x})$; $R^2 = 0.41$)

isotope-mixing model does not predict the observed average δ^{15}N value. It is clear from the model that a diet component with higher δ^{15}N values is missing. However, given the variation in prey δ^{15}N values, a combination of diet inputs into the mixing model can produce a wide range of predicted yellowfin δ^{15}N values. Prey δ^{13}C values were influenced by lipid-extraction and are reported as either lipid-extracted or non-lipid-extracted values (Table 3). Carbon isotope values of prey did vary (range: –20.0 to –16.7), but did not help to discriminate tuna dietary inputs because there were little δ^{13}C differences between small size classes (e.g., WMT $\delta^{13}C_{<45.0\text{ cm FL}} = -16.1 \pm 0.3$) and large size classes (e.g., WMT $\delta^{13}C_{\geq 45.0\text{ cm FL}} = -16.5 \pm 0.4$).

Discussion

Small juvenile yellowfin tuna, associated with Oahu FADs, consumed different diets than the larger size classes found at the same FADs. Nitrogen isotope data demonstrated that the diet shift occurs around 45–50 cm FL. Based on plots of mean cohort length for 4 years classes, tuna with a 45–50 cm FL were estimated to be 10–12 months old (Grubbs, unpublished data). At this size, yellowfin growth rate is such that a period of ~2 months is required to grow 5 cm (Lehodey and Leroy 1999). This rapid ontogenetic diet shift was corroborated by stomach content analysis performed on the same individuals. Moreover, the variability in δ^{15}N values in tuna greater than 45 cm FL indicate that foraging niche width increases dramatically at this size (Bearhop et al. 2004). Yellowfin tuna diets changed from one dominated by crustacean larvae to a more varied diet composed of adult oplophorid shrimp and teleosts fishes. These different prey types have a range of nitrogen isotope values, which then drive the shift in δ^{15}N values of yellowfin tuna.

Prey isotope composition is a reflection of two main influences: the position of the prey in the food web and

Table 3 Average $\delta^{15}N$ and $\delta^{13}C$ (and standard deviations) of prey types collected from yellowfin tuna stomachs during this study

Prey item	$\%MV_{<35\ cm}$ [b]	$\%MV_{\geq 50\ cm}$ [b]	$\delta^{15}N$, ‰	$\delta^{13}C$, ‰
Oplophoridae (6)	1	26	6.6 (1.0)	–18.4(0.6), –16.9(0.1)[LE]
Lyssiosquillidae (1)[a]	28	4	5.4	–20.0
Other stomatopoda (8)[a]	39	10	4.0(0.5)	–18.9(0.5), –18.1(0.3)[LE]
Squillidae (1)[a]			5.2	–20.0
Decapod Megalopae (1)[a]	10	5	3.6	–19.8
Cephalopoda (Ommastrephidae; 1)	6	5	6.6	–18.7
Reef teleosts (Mullidae; 3)	3	13	5.2(0.1)	–18.9(0.4), –17.6(0.2)[LE]
Dactylopteridae (1)[a]	2	5	4.7	–18.9
Epipelagic teleosts (Exocoetidae; 2)	7	23	3.5–3.7	–19.0, –18.5, –18.4, –18.1[LE]
Tetraodontiformes (Ostraciidae; 2)	4	4		
small individual (1)			3.2	–18.2
large individual (2)			5.5–5.6	–18.8, –18.3[LE]
Observed tuna white muscle $\delta^{15}N$ averages:				
$\delta^{15}N_{<35\ cm}$ = 6.5 ± 0.3‰				
$\delta^{15}N_{\geq 50\ cm}$ = 10.2 ± 1.6‰				
Predicted tuna white muscle $\delta^{15}N$ averages:				
$\delta^{15}N_{<35\ cm}$[c] = (((0.28×5.4) + (0.39×4.0) + (0.10×3.6) + (0.07×3.6))/0.84) + 3.4 = 7.8‰				
$\delta^{15}N_{\geq 50\ cm}$[d] = ((0.26×6.6) + (0.10×4.0) + (0.13×5.2) + (0.23×3.6))/0.72) + 3.4 = 8.4‰				

Number of samples analyzed are indicated after prey names

[a]Samples include many individuals in a single analysis

[b]Mixing model (Eq. 1) input, %MV, is the diet fraction

[c]Predicted tuna $\delta^{15}N$ value based on 84% of observed diet components

[d]Predicted tuna $\delta^{15}N$ value based on 72% of observed diet components

[LE]Lipid-extracted $\delta^{13}C$ values

the isotope values of the nitrogenous nutrient source at the base of the food web. In isotope ecological theory, a trophic level is represented by a shift of ~3.4‰ in $\delta^{15}N$ values between prey and predator (DeNiro and Epstein 1981; Minagawa and Wada 1984). Thus, the average observed difference of 3.5‰ in nitrogen isotope values between the smallest tuna size classes and larger tuna could represent differences in the trophic status of the prey consumed. However, spatial and temporal changes in the nutrient source at the base of a food web can also affect the overall nitrogen isotope values of consumers (e.g., O'Reilly et al. 2002). Thus, foraging location of an organism can affect its $\delta^{15}N$ value. In the open ocean ecosystem, the flux of limiting nutrients to the surface mixed layer regulates primary production (Epply and Peterson 1979). If the system is nitrogen-limited, the $\delta^{15}N$ value of the nitrogen source can dominate the nitrogen isotopic composition of the downward flux of particulate organic matter, an end product of primary production in the surface waters (Altabet 1988). In the oligotrophic open ocean north of Oahu, primary production in surface waters is nitrogen-limited and appears to be controlled by two significant sources of new nitrogen: (1) physically controlled upward flux of nitrate from deep water and (2) biological fixation of nitrogen (N_2) gas in near-surface waters (Karl et al. 1997). In the same study area, Dore et al. (2002) investigated the relative seasonal importance for these two modes of primary production using continuous time series measurements of particulate nitrogen (PN) flux and their $\delta^{15}N$ values spanning a ten-year period. Results showed that the nitrogen isotopic composition of PN in the surface mixed layer is primarily driven by N_2 fixation, leaving the surface PN pool with low $\delta^{15}N$ values, whereas PN collected from waters below the mixed layer (ca. 150 m) had higher $\delta^{15}N$ values (Dore et al. 2002). Therefore, in stratified waters north of Oahu, deep water (>150 m) PN $\delta^{15}N$ values are higher (>~3‰) than the surface mixed layer, which are near atmospheric N_2 (–1–1‰). Microbial processing and zooplankton scavenging of the labile fraction of PN results in an increase in $\delta^{15}N$ values of sinking particles because, analogous to trophic enrichment, organisms will excrete light nitrogen leaving the substrate enriched in ^{15}N (DeNiro and Epstein 1981; Checkley and Miller 1989). The reduced nitrogen released by these feeding activities can be incorporated into the food web through the microbial loop (e.g., Lee and Wakeham 1992 and references therein). In combination, these processes would result in a positive $\delta^{15}N$ depth gradient in the nitrogen isotopic composition of particulate organic matter (Saino and Hattori 1980). Accordingly, mesopelagic prey that feed at deeper depths should have higher $\delta^{15}N$ values than organisms that feed at a similar trophic level in surface waters (e.g., Rau et al.

1989). If the δ^{15}N values of the N sources are conserved, then prey δ^{15}N values may not be just a function of trophic position, but also of the location of forage habitat in the water column.

Even though it is still unclear if trophic position or nutrient dynamics are driving the observed differences in prey δ^{15}N values, tuna N isotope values shifted with an increase in mass. Three hypotheses could explain the observed ontogenetic diet shift: (1) seasonal shifts in prey types, (2) ontogenetic shifts in predator gape size and agility, or (3) an increase in foraging depth facilitated by the onset of endothermic capability. During the growth of a young-of-year tuna, seasonal fluctuations in prey could result in a diet shift. However, it is unlikely that the dietary shift was due to seasonal changes in prey availability because all major taxa of larval stomatopod and decapod crustaceans were common prey of small yellowfin tuna during every month of the study, but rare in the stomachs of large yellowfin tuna.

If the same prey were available throughout the year, then tuna either actively selected different prey types or different size-classes of tuna had different foraging habitats. The first possibility could be explained by differences in gape size, gillraker size, and swimming agility, whereas the second could be explained by physiological and behavioral differences that enable the larger predators to exploit forage areas not accessed by the smaller size classes. A fish's gape and gillrakers typically increase with fork length. Therefore, ontogenetic shifts in diet could reflect an increase in the maximum and a limit to the minimum prey sizes that can be consumed at a given size (e.g., Renones et al. 2002). However, the most common prey (numerically and volumetrically) in this study were small relative to the gape regardless of the size of the predator. Moreover, tuna in the smallest size class regularly contained single prey items that were relatively large. For example, yellowfin less than 30 cm FL consumed carangid fishes that were 19 cm long. These observations suggest the observed ontogenetic changes in diet were not determined by an increase in gape size. Moreover, the size of the dominant stomatopod family in the diet of the smaller tunas (Lyssiosquillidae) is comparable to the size of the *O. gracilirostris* and larger than many of the larval reef teleosts that dominated the prey field for the larger tunas. This suggests that the ontogenetic changes in diet were not a function of gape size and gillraker size. Furthermore, the swimming ability of even the smallest tunas in this study are likely far superior to that of most of the major prey taxa consumed.

Our third hypothesis is based on the observation that ontogenetic diet shifts are often the result of shifts in habitat use. This diet shift is especially important for predators in sized-structured populations with size-dependent variation in mortality (Werner and Gilliam 1984; Hampton 2000). An increase in endothermic capability associated with an increase in mass would allow yellowfin tuna to expand their foraging range and thereby encounter additional and different food resources. If foraging range increases, then one would predict that the trophic niche width would also increase. In terms of the diversity of prey, stomach content analysis indicated that prey diversity is actually higher for the smaller tunas, though rarefaction suggests this may be a reflection of differences in sample sizes between the largest and smallest size classes in this study (Grubbs, unpublished data). Stable isotope analysis does not provide information on the prey diversity consumed, but variability in consumer δ^{15}N values is a powerful technique to assess foraging niche width (Bearhop et al. 2004). If prey abundance does not vary widely seasonally and there is range in prey δ^{15}N values, then the wider range of δ^{15}N values observed in tuna ≥45 cm FL is due to the ingestion of prey from an increasing range of trophic levels or foraging depths. Therefore, even though prey species diversity does not appear to be greater in larger size classes of yellowfin tuna, the prey that are consumed by larger tunas occupy a larger trophic breadth in the foodweb.

The simple isotope mixing model suggests that there could either be a missing high δ^{15}N diet component in the stomach contents of tuna ≥50.0 cm FL or an important prey component was not analyzed for stable isotope analysis (e.g., additional epipelagic fish). The majority of tunas in this study were collected during the morning hours. It is possible that additional prey taxa were consumed in the afternoon and early evening hours but were fully digested by the time the tuna were captured. Even though these prey were not numerically or volumetrically important to the yellowfin tuna analyzed for stomach content analysis in this study, some mesopelagic prey collected from around Hawaii had δ^{15}N values between 9 and 11‰ (Parry 2003). Nitrogen isotope variability in tuna ≥45.0 cm FL could also be explained by the immigration of these tuna from other foraging regions that may have different isotope baselines, but there is no evidence that tuna around 50 cm FL migrate from other regions. Therefore, the wider range of δ^{15}N values observed in size classes of tuna immediately following 50 cm FL are likely due to the ingestion of prey of an increasing trophic diversity. This increase in foraging niche width and distinct diet shift observed in yellowfin tuna diets leads us to propose the hypothesis that an increase in

foraging range (depth) is facilitated by the onset of endothermic capabilities.

Tunas have evolved the ability to physiologically thermoregulate their body temperature, and are thereby able to pursue prey from the surface mixed layer into deeper, colder water, and to return to the surface mixed layer (Holland et al. 1992; Holland and Sibert 1994; Block et al. 1997; Dagorn et al. 2000). Dickson et al. (2000) demonstrated that the capacity for heat production and retention in red muscle increased with fork length in juvenile black skipjack tuna (*Euthynnus lineatus*) although the actual production and retention of heat has not yet been measured. This ontogenetic shift toward endothermy was a result of an increase in the size of red muscle components or heat exchangers, but there also appears to be a minimum size of functional endothermy at around ~10 cm FL or 163 g (Dickson 1994; Dickson et al. 2000). Even if nascent endothermic capabilities are present in very small size classes, the high surface area-to-volume ratio in small individuals might overwhelm the ability of the counter-current heat exchangers to buffer the effects of changes in ambient temperature associated with vertical diving excursions (Dickson et al. 2000). Thus, the potential for endothermy might be present in very small size classes but is not completely realized until a threshold mass is reached. When the mass threshold is reached, the tunas are then capable of making deep excursions, penetrating the thermocline, and gaining access to deeper food resources.

In recent reviews of ultrasonic and archival tagging data, depth distributions of yellowfin tuna appear to be independent of body size (Brill et al. 1999; Brill and Lutcavage 2001). However, in all these studies, the smallest tagged fish were at least 5 kg in mass, which is larger than most of the tuna included in the present study. The shift in diet from our study appears to begin at a mass of 1.5 kg. Current experiments being conducted around Oahu have placed acoustic tags simultaneously on tuna of 1–2.5 and >5 kg. Preliminary data from these electronic tags indicate that, even within this quite small range of sizes, the smallest individuals do not dive as deep as larger ones (Holland, unpublished data). These preliminary results support our hypothesis that different sizes of small yellowfin tuna separate their vertical foraging ranges, which result in the changes in diet shown by both isotope and stomach content analyses.

The specific mass required to shift from potential to realized endothermy might not be uniform among all tropical tuna species. Also, functional thermoregulation may not be as important in regions where environmental conditions such as thermocline depth and water temperature are different than those in Hawaii (Karl and Lucas 1996). Preliminary nitrogen isotope data of juvenile yellowfin tuna as small as 35 cm FL collected from waters around New Caledonia do not indicate a significant difference from the δ^{15}N values of larger size classes collected in the same region (Graham, unpublished data).

High mortality rates observed in small size classes of tunas associated with FADs in both eastern tropical Atlantic and western tropical Pacific (Menard et al. 2000; Hampton 2000) could be a result of these tunas being physiologically constrained to forage in surface waters. In areas where there is a distinct shift in diet with forage range, as in waters around Oahu, the vulnerability of these tuna to fishing effort or gear types could change over time. The apparently tightly constrained forage depth of the small-size classes of tuna could have important implications for the management of tropical tuna fisheries.

Acknowledgments We thank J. Tanimoto, T. Rust, and D. Cuny for assistance with preparatory isotope analysis work, and G. Ivey for assistance with stomach content analysis. We thank D. Itano for being invaluable in field collection and B. Fry for thoughtful comments throughout our research. Two anonymous reviewers helped improve the manuscript. This research was funded by Cooperative Agreement NA17RJ1230 between the Joint Institute for Marine and Atmospheric Research (JIMAR) and the National Oceanic and Atmospheric Administration (NOAA). The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA of any of its subdivisions. B. S. Graham was supported by a Pelagic Fisheries Research Program graduate assistantship. This is SOEST contribution number 6788.

References

Alverson F (1963) The food of yellowfin and skipjack tunas in the eastern tropical Pacific Ocean. Inter Am Trop Tuna Comm Bull 7:293–396

Altabet M (1988) Variations in nitrogen isotopic composition between sinking and suspended particles: implications for nitrogen cycling and particle transformation in the open ocean. Deep Sea Res 35:535–554

Bearhop S, Adams CE, Waldron S, Fuller RA, Macleod H (2004) Determining trophic niche width: a novel approach using stable isotope analysis. J Anim Ecol 73:1007–1012

Block BA, Keen JE, Castillo B, Dewar H, Freund EV, Marcinek DJ, Brill RW, Farwell C (1997) Environmental preferences of yellowfin tuna (*Thunnus albacares*) at the northern extent of its range. Mar Biol 130:119–132

Brill RW, Block BA, Boggs CH, Bigelow KA, Freund EV, Marcinek DJ (1999) Horizontal movements and depth distribution of large adult yellowfin tuna (*Thunnus albacares*) near the Hawaiian Islands, recorded using ultrasonic telemetry: implications for the physiological ecology of pelagic fishes. Mar Biol 133:395–408

Brill RW, Lutcavage ME (2001) Understanding environmental influences on movements and depth distributions of tunas

and billfishes can significantly improve population assessments. Am Fish Soc Symp 25:179–198
Brock R (1985) Preliminary study of the feeding habits of pelagic fish around Hawaiian fish aggregation devices or can fish aggregation devices enhance local fisheries productivity? Bull Mar Sci 37:40–49
Checkley DM, Miller CA (1989) Nitrogen isotope fractionation by oceanic zooplankton. Deep Sea Res 36:1449–1456
Chipps SR, Garvey JE (in press) Quantitative assessment of food habits and feeding patterns. In: Guy C, Brown M (eds) Analysis and interpretation of freshwater fisheries data. American Fisheries Society, Bethesda
Dagorn L, Bach P, Josse E (2000) Movement patterns of large bigeye tuna (*Thunnus obesus*) in the open ocean, determined using ultrasonic telemetry. Mar Biol 136:361–371
Deegan L, Garritt R (1997) Evidence for spatial variability in estuarine food webs. Mar Ecol Prog Ser 147:31–47
DeNiro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42:495–506
DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim Acta 45:341–351
Dickson K (1994) Tuna as small as 207 mm fork length can elevate muscle temperatures significantly above ambient water temperature. J Exp Biol 190:79–93
Dickson K, Johnson N, Donley J, Hoskinson J, Hansen M, Tessier J (2000) Ontogenetic changes in characteristics required for endothermy in juvenile black skipjack tuna (*Euthynnus lineatus*). J Exp Biol 203:3077–3087
Dionex Application Note 342 (2000) Determination of PCBs in large-volume fish tissue samples using accelerated solvent extraction (ASE®). Dionex Corporation, CA
Dore JE, Brum JR, Tupas LM, Karl DM (2002) Seasonal and interannual variability in sources of nitrogen supporting export in the oligotrophic subtropical North Pacific Ocean. Limnol Oceanogr 47:1595–1607
Doucett R, Hooper W, Power G (1999) Identification of anadromous and nonanadromous adult brook trout and their progeny in the Tabusintac River, New Brunswick, by means of multiple-stable-isotope analysis. Trans Am Fish 128:278–288
Epply RW, Peterson BJ (1979) Particulate organic matter flux and planktonic new production in the deep ocean. Nature 282:677–680
Fry B, Arnold C (1982) Rapid $^{13}C/^{12}C$ turnover during growth of brown shrimp (*Penaeus aztecus*). Oecologia 54:200–204
Fry B (1988) Food web structure on Georges Bank from stable C, N, and S isotopic compositions. Limnol Oceanogr 33:1182–1190
Fry B, Baltzi DM, Benfield MC, Fleeger JW, Grace A, Haas HL, Quinones-Rivera ZJ (2003) Stable isotope indicators of movement and residency for brown shrimp (*Farfantepenaeus aztecus*) in coastal Louisiana marshscapes. Estuaries 26:82–97
Harrigan P, Zieman J, Macko S (1989) The base of nutritional support for the gray snapper (*Lutjanus griseus*): An evaluation based on a combined stomach content and stable isotope analysis. Bull Mar Sci 44:65–77
Hampton J (2000) Natural mortality rates in tropical tunas: size really does matter. Can J Fish Aquat Sci 57:100
Hesslein R, Hallard K, Ramlal P (1993) Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$. Can J Fish Aquat Sci 50:2071–2076
Hobson K, Clark R (1992) Assessing avian diets using stable isotopes I: turnover of ^{13}C in tissues. Condor 94:181–188
Hobson K, Welch H (1992) Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. Mar Ecol Prog Ser 84:9–18
Holland K, Brill R, Chang R, Sibert J, Fournier D (1992) Physiological and behavioral thermoregulation in bigeye tuna (*Thunnus obesus*). Nature 358:410–411
Holland K, Sibert J (1994) Physiological thermoregulation in bigeye tuna, *Thunnus obesus*. Environ Biol Fish 40:319–327
Hurlbert SH (1984) Pseudoreplication and the design of ecological field experiments. Ecol Monog 54:187–211
Hyslop EJ (1980) Stomach contents analysis – a review of methods and their application. J Fish Biol 17:411–429
Itano DG (2000) The reproductive biology of yellowfin tuna (*Thunnus albacares*) in Hawaiian waters and the western tropic Pacific Ocean: project summary. SOEST 00-01 JIMAR Contribution 00-328. Pelagic Fisheries Research Program, JIMAR, University of Hawaii, 69pp
Karl D, Letelier R, Tupas L, Dore J, Christian J, Hebel D (1997) The role of nitrogen fixation in biogeochemical cycling in the subtropical Pacific Ocean. Nature 388:533–538
Karl DM, Lucas R (1996) The Hawaiian Ocean Time-series (HOT) Program: Background, rationale and field implementation. Deep Sea Res II 43:129–156
Larimore WR (1957) Ecological life history of the warmouth (Centrarchidae). III. Nat Hist Surv Bull 27:83
Lee C, Wakeham SG (1992) Organic matter in the water column: future research challenges. Mar Chem 39:95–118
Lehody P, Leroy B (1999) Age and growth of yellowfin tuna (*Thunnus albacares*) from the western and central Pacific Ocean as indicated by daily growth increments and tagging data. In: Proceedings 12th Standing committee on tuna and billfish working, pp1–21
Maldeniya R (1996) Food consumption of yellowfin tuna, *Thunnus albacares*, in Sri Lankan waters. Environ Biol Fish 9:40–46
Menard F, Fonteneau A, Gaertner D, Nordstrom V, Stequert B, Marchal E (2000) Exploitation of small tunas by a purse-seine fishery with fish aggregation devices and their feeding ecology in an eastern tropical Atlantic ecosystem. ICES J Mar Sci 57:525–530
Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochim Cosmichim Acta 48:1135–1140
O'Reilly C, Hecky R, Cohen A, Plisnier P (2002) Interpreting stable isotopes in food webs: recognizing the role of time averaging at different trophic levels. Limnol Oceanogr 47:306–309
Parry M (2003) The trophic ecology of two Ommastrephid squid species, *Ommastrephes bartramii* and *Sthenoteuthis oualaniensis* in the North Pacific sub-tropical gyre. PhD thesis, University of Hawaii, Honolulu
Post D (2003) Individual variation in the timing of ontogenetic niche shifts in largemouth bass. Ecology 84:1298–1310
Rau GH, Heyraud M, Cherry RD (1989) $^{15}N/^{14}N$ and $^{13}C/^{12}C$ in mesopelagic shrimp from the Northeast Atlantic Ocean – evidence for differences in diet. Deep Sea Res Part A 36:1103–1110
Reid SB, Hirota J, Young RE, Hallacher LE (1991) Mesopelagic-boundary community in Hawaii: micronekton at the interface between neritic and oceanic ecosystems. Mar Biol 109:427–440
Reintjes J, King J (1953) Food of yellowfin tuna in the central Pacific. Fish Bull 54:90–110

Renones O, Polunin N, Goni R (2002) Size related dietary shifts of *epinephelus marginatus* in a western Mediterranean littoral ecosystem: an isotope and stomach content analysis. J Fish Biol 61:122–137

Saino T, Hattori A (1980) The ^{15}N natural abundance in oceanic suspended particulate matter. Nature 283:752–754

Tieszen L, Boutton T, Tesdahl K, Slade N (1983) Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. Oecologia 57:32–37

Uchiyama J, Kazama T (2003) Updated weight-on-length relationships for pelagic fishes caught in the central north Pacific ocean and bottomfishes from the Northwestern Hawaiian Islands. Pacific Islands Fisheries Science Center Administrative Report. H-03-01

Werner EE, Gilliam JF (1984) The ontogenetic niche and species interactions in size structured populations. Ann Rev Ecol System 15:393–425

Wolfert DR, Miller TJ (1978) Age, growth, and food of northern pike in eastern Lake Ontario. Trans Am Fish Soc 107:696–702

Zaret TM, Rand AS (1971) Competition in tropical stream fishes: support of the competitive exclusion principal. Ecology 52:336–342

Ziemann D (1975) Patterns of vertical distribution, vertical migration, and reproduction in the mesopelagic shrimp of the family Oplophoridae. PhD thesis, University of Hawaii, Honolulu