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TROPHIC DYNAMICS AND MOVEMENTS OF TUNA IN THE TROPICAL PACIFIC OCEAN INFERRED FROM STABLE ISOTOPE ANALYSES

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAI'I IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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We certify that we have read this dissertation and that, in our opinion, it is satisfactory in scope and quality as a dissertation for the degree of Doctor of Philosophy in Oceanography.

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DEDICATION

This dissertation is *dedicated to my parents* who have made everything possible. You have always supported me to wander, explore, and dream. I am forever grateful.

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ABSTRACT

Due to the mobility of marine vertebrates and their vast pelagic habitat, researchers have had to develop novel methods to determine the distribution, movements, and foraging behavior of top predators. The aim of this study was to apply bulk and compound-specific stable isotope analysis (CSIA) to examine the trophic dynamics and movements of tunas in the equatorial Pacific Ocean (eqPac). Yellowfin tuna collected around Hawaii displayed a rapid diet shift between 45-50 cm forklength. The trophic level (TL) was similar for the small (<45cm) and large (>50cm) size classes of yellowfin. Due to differences in the nitrogen source and biological transformations of this nitrogen, baseline $\delta^{15}N$ values are lower in the surface mixed layer (SML) than below the thermocline. Thus, the rapid ontogenetic shift in tuna did not a reflect a TL shift, but rather, a shift in vertical foraging habitat. I hypothesized that small juveniles are physiologically confined to the SML and larger tuna forage over a greater vertical range. Throughout the eqPac, the TL of larger size classes of yellowfin was consistent (4.2 ±0.4). Establishing the TL of tuna in the eqPac provides a reference point to examine historical and future changes in tuna trophic dynamics.

$\delta^{15}N$ and $\delta^{13}C$ values of animals can be used as natural tags to trace their movements through ecosystems. This method to studying animal movements requires isotopic variation at the base of the food web. Baseline $\delta^{15}N$ values show strong geographical patterns in the eqPac. If tuna migrated extensively within these isotope gradients, little isotopic variation would be observed in tuna because baseline $\delta^{15}N$ values

would be integrated. $\delta^{15}N$ values of yellowfin, bigeye, and skipjack tuna showed ca. 12‰ variation, but spatially coherent structure in the eqPac. CSIA results revealed that this $\delta^{15}N$ variation relates more to tuna movements than differences in their TL. Thus, the high isotopic variation observed in tuna reflects their restricted movements in the EqPac and differences between the species indicate different movement rates. In summary, this study established that isotope values of tunas are indicators of their foraging behavior, habitat utilization, and movements.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....V

ABSTRACT.....VIII

LIST OF TABLES.....XIII

LIST OF FIGURES.....XIV

CHAPTER ONE: **Introduction and Research Rationale**.....1

REFERENCES.....11

CHAPTER TWO: **A Rapid Ontogenetic Shift in the Diet of Juvenile Yellowfin Tuna From Hawaii.** (Co-authors: Dean Grubbs, Kim N. Holland, and Brian N. Popp). Published in: Marine Biology, 150, 647-658.

2.1. ABSTRACT.....23

2.2. INTRODUCTION.....24

2.3. MATERIAL AND METHODS.....26

2.4. RESULTS.....30

2.5. DISCUSSION.....35

2.6. ACKNOWLEDGEMENTS.....42

2.7. REFERENCES.....43

CHAPTER THREE: **Tissue Turnover Rates in Captive and Wild Populations of Yellowfin Tuna, *Thunnus albacares*: Implications for Estimating Movements of Tropical Tunas.** (Co-authors: Brian Fry, Brian N. Popp, Robert J. Olson, and Kim N. Holland).

3.1. ABSTRACT.....57

3.2. INTRODUCTION.....58

3.3. MATERIAL AND METHODS.....60

3.4. RESULTS.....66

3.5. DISCUSSION.....69

3.6. ACKNOWLEDGEMENTS.....77

3.7. REFERENCES.....78

CHAPTER FOUR: **Bulk $\delta^{15}N$ Cartography: a novel approach to estimating net movements of tropical tunas in the Equatorial Pacific Ocean.** (Co-authors: Brian Fry, Robert J. Olson, Valerie Allain, Felipe Galván-Magaña, Anders Nielsen, Brian N. Popp)

4.1. ABSTRACT.....92

4.2. INTRODUCTION.....93

4.3. MATERIAL AND METHODS.....98

4.4. RESULTS.....105

4.5. DISCUSSION.....109

4.6. CONCLUSIONS.....125

4.7. ACKNOWLEDGEMENTS.....126

4.8. REFERENCES.....127

CHAPTER FIVE: **Trophic dynamics of yellowfin tuna in the tropical Pacific Ocean inferred from compound-specific $\delta^{15}N$ analysis.** (Co-authors: Brian N. Popp, Brian Fry, Cecelia CS Hannides).

5.1. ABSTRACT.....151

5.2. INTRODUCTION.....152

5.3. MATERIAL AND METHODS.....158

5.4. RESULTS.....162

5.5. DISCUSSION.....165

5.6. ACKNOWLEDGEMENTS.....174

5.7. REFERENCES.....175

CHAPTER SIX: **Conclusions and Future Research**

6.1. SUMMARY.....189

6.2. SIGNIFICANCE & IMPLICATIONS.....191

6.3. FUTURE RESEARCH DIRECTIONS.....193

6.4. REFERENCES.....200

APPENDIX 1: Bulk $\delta^{15}N$ values, $\delta^{13}C$ values, and C/N ratios of tuna and prey items collected from Hawaiian waters (Chapter 2).....217

APPENDIX 2: Tissue-specific bulk $\delta^{15}N$ values, $\delta^{13}C$ values, and C/N ratios of yellowfin tuna analyzed from the captive diet switch (Chapter 3).....229

LIST OF TABLES

Table		Page
2.1	List of the size interval and sample sizes for each size class of tuna used in the gut content analyses, along with the proportion of empty stomachs and stomach repletion rates	50
2.2	Morisita's original index of dietary overlap between sizes classes of yellowfin tunas	50
2.3	List of the average $\delta^{15}N$ and $\delta^{13}C$ values (‰) prey items collected from yellowfin tuna stomachs	51
3.1	List of the calculated tissue turnover rates (N day^{-1}) and half-lives (in days) for captive and wild yellowfin tuna	84
3.2	Estimated trophic enrichment factors, or the difference between $\delta^{15}N_{organism}$ and $\delta^{15}N_{diet}$, from the captive and wild diet shift datasets	85
4.1	Summary and descriptive statistics for the $\delta^{15}N$ and $\delta^{15}C$ values of three tropical tuna species collected throughout the equatorial Pacific	140
4.2	Summary of the north-south net movement rates (km day^{-1}) for tropical tunas at 125°W and 145°W transects	141
5.1	List of the trophic level and $\delta^{15}N$ values of bulk tissues and amino acids of yellowfin tuna from the equatorial Pacific	182
5.2	List of the trophic level and $\delta^{15}N$ values of bulk tissue and individual amino acids of small and large size-classes of tuna and their prey from Hawaii	186
5.3	List of the trophic level and $\delta^{15}N$ values of bulk tissue, source amino acids (Gly, Phe), and trophic amino acids (Ala, Glu) of yellowfin tuna <45 cm fork length and >60cm FL from Hawaii	188

LIST OF FIGURES

Figure		Page
1.1	Schematic diagram of trophic level and $\delta^{15}N$ values (‰) in two simplified marine food webs with different sources of new nitrogen supporting the base of the food web	19
1.2	Plot of the east-west depth profile of water temperature (°C) across the equatorial Pacific Ocean, averaged from 2°S to 2°N	20
1.3	Location of the Equatorial Pacific study region that outlines the warm pool-cold tongue (WPCT) system	21
2.1	Location of study region and the nearshore fixed fish aggregating devices (FADs) around the island of Oahu	52
2.2	Plots of the $\delta^{15}N$ values of yellowfin tuna white muscle and liver tissue over a range of (a) fork lengths and (b) mass	53
2.3	Plots of the relative importance of major prey taxa, measured as (a) mean percent volume (%MV) and (b) mean present number (%MN) in the diets of five size classes of yellowfin tuna	54
2.4	Plots of the comparison between (a) %MV and %V and (b) %MN and %N of seven functional prey groups in the diets of small and large size classes of yellowfin tuna	55
3.1	Plots of the $\delta^{15}N$ values of captive yellowfin tuna vs. the time since the onset of the diet shift for (top panel) white muscle tissue and red muscle tissue and (bottom panel) liver tissue and gill filaments	86
3.2	Plots of (top panel) the $\delta^{15}N$ values of wild yellowfin tuna versus estimated tuna age in days and (bottom panel) exponential model fit to the natural diet shift observed in juvenile yellowfin	88

3.3 Plot of the comparison between the $\delta^{15}N$ values and models of captive and wild yellowfin tuna.....90

4.1 Locations of yellowfin, bigeye, and skipjack tuna and suspended particulate nitrogen samples collected and analyzed in the equatorial Pacific Ocean.....142

4.2 Plot of the $\delta^{15}N$ values of yellowfin, skipjack, and bigeye tuna vs. fork length from the Equatorial Pacific Ocean.....143

4.3 Plots of the geographical distribution of the $\delta^{15}N$ values of (top panel) yellowfin, (middle panel) skipjack, and (bottom panel) bigeye tuna in the equatorial Pacific.....144

4.4 Plots of the geographical distribution of the (top panels) $\delta^{13}C$ values and (bottom panels) C/N ratios for (left panels) yellowfin and (right panels) skipjack tuna in the equatorial Pacific Ocean.....145

4.5 Plots of the $\delta^{15}N$ values of yellowfin and skipjack tuna and particulate nitrogen vs. latitude for an equatorial transect at 125°W in the eastern equatorial Pacific.....146

4.6 Plots of the $\delta^{15}N$ values of bigeye tuna and particulate nitrogen vs. latitude for an equatorial transect at 125°W in the eastern equatorial Pacific.....147

4.7 Plots of the $\delta^{15}N$ values of yellowfin and skipjack tuna and particulate nitrogen vs. latitude for an equatorial transect at 140°W in the central equatorial Pacific.....148

4.8 Plots of the geographical difference between the $\delta^{15}N$ values of (top panel) yellowfin and bigeye (bottom panel) yellowfin and skipjack tuna in the equatorial Pacific.....149

4.9 Map of four distinct biogeochemical provinces within the equatorial Pacific Ocean inferred from stable isotope analysis.....150

5.1 Location of yellowfin tuna samples collected in the equatorial Pacific that were analyzed for bulk and compound-specific isotope analysis of individual amino acid analyses.....189

5.2 Plot of the frequency of the $\delta^{15}N$ values of amino acids isolated from yellowfin muscle samples collected from the equatorial Pacific.....190

5.3 Plot of average compound-specific $\delta^{15}N$ values of individual amino acids and CSIA-derived trophic level estimates of yellowfin tuna collected from the equatorial Pacific.....191

5.4 Plot of bulk tissue $\delta^{15}N$ values, compound-specific $\delta^{15}N$ values of individual amino acids, and CSIA-derived trophic level estimates of yellowfin and bigeye tuna collected from nearshore FADs around Hawaii.....193

6.1 Plot of bulk tissue $\delta^{15}N$ values of small juvenile tuna (<45cm FL).....210

6.2 Plot of bulk and individual amino acid $\delta^{15}N$ values for juvenile tuna with anomalously high $\delta^{15}N$ values, typical juveniles, and larger yellowfin collected from nearshore FADs around Hawaii.....211

6.3 Plot of $\delta^{13}C$ versus $\delta^{15}N$ values yellowfin tuna collected from different regions within the central and western equatorial Pacific.....212

6.4 Plot of bulk $\delta^{15}N$ values of a range of higher-trophic level predators from French Polynesia and New Caledonia.....213

6.5 Total mercury concentrations (ug/g) wet weight (ww) of a range of top predators collected from the (a) eastern tropical Pacific and (b) Hawaii.....214

6.6 Total mercury concentrations (ug/g) wet weight (ww) of small and large size classes of yellowfin tuna and two major prey items collected from nearshore FADs around Hawaii.....216

CHAPTER 1

Introduction and Research Rationale

1.1 Application of Stable Isotope Analysis to Pelagic Marine Ecosystems

Tropical tunas play a key role in the pelagic environment as top predators (Cox et al. 2002; Olson and Watters 2003) and can access a large horizontal and vertical habitat range because of their unique physiological, morphological, and behavioral adaptations for high-performance swimming (Brill and Bushnell 1991; Dewar and Graham 1994) and their ability to elevate internal body temperatures (Carey 1973; Stevens and Carey 1983; Dewar et al. 1994). These specialized pelagic predators are also the primary species exploited by industrialized fishing in the tropical oceans (Bayliff et al. 2005; Sibert et al. 2007). Commercial fisheries have removed 50 million tons of tuna from the Pacific Ocean since the mid-20th century (Sibert et al. 2006), which has lead to a debate on the affect of industrialized fisheries in altering marine ecosystems (e.g., Pauly et al. 1998; Springer 2000; Sibert et al. 2006). Selective removal of large predators from food webs can impart changes to the food web structure through trophic cascades (Carpenter et al. 1985; Estes et al. 1998; Pace et al. 1999; Pauly et al. 2000; Springer et al. 2000; Myers et al. 2007). If the majority of top predator biomass is reduced in the pelagic marine ecosystem, then there could be a release in predation and within-trophic guild competition (e.g. Springer et al. 2000). In turn, these changes could lead to niche expansions and retractions in other functional groups within the pelagic food web, and,

ultimately, lead to nutrient and biomass redistribution (Estes et al. 1998; Springer et al. 2000). For example, Zeidberg and Robison (2007) have suggested that the recent range expansion and increase in the biomass of humboldt squid (*Dosdicus gigas*) in the eastern tropical Pacific relates to the reduction of predation by their primary predators, large tuna and billfish, which have been removed by industrialized fishing. Before we can begin to assess the impacts of industrialized fishing on the pelagic food web structure and function, we must first determine and quantify the trophic relationships of the top predators targeted by the fisheries.

Knowledge of the trophic ecology of pelagic fishes in the Pacific Ocean has historically been derived from diet studies (e.g., Alverson 1963; Reintjes & King 1953; Brock 1985). However, stomach contents provide only a snapshot of the most recent foraging event. Stable isotope analysis (SIA) of a pelagic organism's tissues can be used as an alternative and complimentary tool to provide information on the time-integrated, assimilated diet (e.g., Fry 1988; Harrigan et al. 1989; Fantle et al. 1999). These studies are based on the observation that the stable isotopic composition of an organism's tissue reflects that of its food or nutrient source. During assimilation, discrimination of ^{15}N and ^{13}C occurs at each successive trophic level (Minagawa and Wada 1984). Isotopic discrimination results in ~ 3.4‰ and 0.7‰ increase in $\delta^{15}N$ and $\delta^{13}C$ values of the consumer relative to prey at each subsequent trophic level, respectively (DeNiro and Epstein 1978, 1981; Post 2002). This isotopic fractionation is commonly referred to as the trophic enrichment factor or TEF. Because the TEF for ^{15}N is larger, $\delta^{15}N$ values have been used extensively to reveal trophic relationships in a wide range of ecosystems (e.g., Fry et al. 1988; Hobson et al. 1999; Hobson et al. 2005; Cheral and Hobson 2005).

The $\delta^{15}N$ value of a consumer, however, is a function of not only the trophic level of the consumer, but also the $\delta^{15}N$ value of the primary producers at the base of the food web, which can vary widely but systematically in marine ecosystems (Altabet and François 1994; Waser et al. 2000; Altabet 2001; Voss et al. 2001).

The nitrogen isotopic composition of the primary producers set the $\delta^{15}N$ value at the base of the food web (or the isotopic baseline) and are dependent upon the $\delta^{15}N$ values of their nutrient source (e.g. nitrate, ammonium, N_2), subsequent biological transformations (e.g., denitrification in oxygen minimum zones), isotopic fractionation associated with nitrogen assimilation, nutrient pool size, and degree to which primary producers drawdown the nitrogen pool (e.g., Altabet and François 1994; Altabet 2001; Sigman and Casciotti 2001; Deutsh et al. 2001). Both the nutrient source and the nutrient uptake by marine phytoplankton are affected by physical and chemical oceanographic processes. For example, in upwelling regions deep water nitrate, which has a $\delta^{15}N$ value between 5-7‰ (Cline and Kaplan 1975; Sigman et al. 1997) is delivered to the euphotic zone where it is available for biological uptake (Saino and Hattori 1987). Alternatively, in areas where the water column is highly stratified and nutrients are limiting in the euphotic zone, nitrogen fixation might play an important role as the primary source of N fuelling the food web (Karl et al. 1997; Carpenter et al. 1997; Deutsch et al. 2001). The $\delta^{15}N$ value of dissolved N_2 is near 0‰ (atmospheric N_2) and the isotopic fractionation that occurs with biological uptake is minimal (see review by Sigman and Casciotti 2001). Thus, primary production based mainly on N_2-fixation results in a $\delta^{15}N$ value around 0‰ (-3 to 3‰; Wada and Hattori 1976; Carpenter et al. 1997; Mahaffey et al. 2005). As the new production (*sensu* Eppley and Peterson 1979) in these contrasting systems is

incorporated into the food web, the consumers foraging in these environments will exhibit the $\delta^{15}N$ values of the N supporting the system (Fig. 1.1). Within this framework, the $\delta^{15}N$ value of top predators foraging in different regions could provide information on the dominant forms of nitrogen fuelling the food web. However, the $\delta^{15}N$ values at the base of the food web, as indicated by either particulate N or of primary producers, reflect many biological transformations (e.g., Granger et al. 2003; Sigman et al. 2005). Thus, to begin using predator $\delta^{15}N$ values to distinguish dominant processes involved in influencing the $\delta^{15}N$ values at the base of the food web, close consideration needs to be paid to the dominant biological, chemical, and physical characteristics in a given region and interpretations of the sources of fixed N fuelling local production needs to be presented cautiously.

1.2 Chemical Tags and Tuna Movements

In order to develop the most successful tuna fisheries management strategies and determine population dynamics, movement patterns for these highly-mobile pelagic predators must also be accurately determined. The inability to directly observe migratory predators and the massive extent of their pelagic habitat has hindered our understanding of their movements. Recently these challenges have spurred the development of new technologies and novel methods to remotely examine animal movements, especially by tracking fish carrying acoustic (Carey and Olson 1982; Holland et al. 1990; Brill et al. 1999; Dagorn et al. 2000) or sophisticated satellite tags (Holland and Sibert 1994; Block et al. 1998; Lutcavage et al. 1999). These electronic tagging studies are generally limited

to studying individual movements, but the launch of extensive electronic tagging programs (e.g. Block et al. 2005) and advancements in tag-to-tag communication (Holland *personal communication*) will circumvent the difficulties of interpreting movement patterns based on only a small number of individuals.

While foraging in specific environments, individuals and populations can acquire unique elemental or isotopic signals or "natural chemical tags" that then can be used to examine their short and long-distance movements and dispersal patterns (e.g., Wolf and Martínez del Rio 2000; Hobson et al. 1999; Rubsenstein and Hobson 2004; Hobson et al. 2005). A natural tag approach to study pelagic fish movements requires considerable systematic geographical variation in the tracer of interest at the base of the food web. Ecologists studying trans-continental avian migration have clearly demonstrated that individuals and populations acquire natural chemical tags (e.g., D/H isotopic ratios of feathers) while foraging in terrestrial habitats, which reflect the isotopic value of water and that can be used to track movements to new habitats (Wolf and Martínez del Rio 2000; Rubsenstein and Hobson 2004; Hobson et al. 2005). Accordingly, hydrogen isotope cartography in terrestrial ecosystems has been used to successfully determine the temporal connections between different migratory populations (Hobson et al. 2001; Bearhop et al. 2005), locate migration corridors and breeding regions (Hobson et al. 1999), and map species dispersals (Hobson et al. 2005; Bearhop et al. 2005). In coastal ecosystems, stable isotope analysis was applied to estimate shrimp residency and movements in coastal marshes and the results highlighted essential juvenile habitat and, in turn, what habitat restoration efforts should focus on to maintain coastal shrimp fisheries (Fry et al. 2003). Therefore, an isotope tag or isotope cartography approach can

potentially "source" organisms to particular regions or habitats and, in turn, aid in species conservation and management (Fry et al. 2003; Hobson et al. 2005; Bearhop et al. 2005).

The potential to use isotope cartography to study the movement patterns of highly-mobile predators in the pelagic ecosystems has not previously been attempted. As stated earlier, this approach requires considerable systematic geographical variation in $\delta^{15}N$ and/or $\delta^{13}C$ values at the base of the food web. The carbon isotopic composition of consumers often reflects the algal sources of production, which generally have high $\delta^{13}C$ values associated with rapid growth rates of large phytoplankton and low values associated with slowly growing small phytoplankton (Cifuentes et al. 1988; Popp et al. 1998). A much larger isotopic variation in baseline $\delta^{15}N$ values is expected because of variations in the isotope values of the nitrogen sources (e.g., N_2 vs. NO_3^-) and the isotopic fractionation associated with the biologically-mediated transformations of these nitrogen sources (e.g., NO_3^- assimilation, denitrification, nitrification). Surface particulate organic matter (POM), sinking POM, and deep-sea sediments are often considered proxies for the base of the food web, and their $\delta^{15}N$ values show coherent geographical patterns in many regions, for example the equatorial Pacific Ocean (Altabet 1988; Farrell et al. 1995; Altabet 2001; Voss et al. 2001). Altabet (2001) showed a 10‰ variation in the $\delta^{15}N$ values of POM from 12°S to 12°N, along an equatorial transect in the central Pacific. As pelagic predators forage within and move through pelagic ecosystems with gradients in $\delta^{15}N$ values of primary producers, the $\delta^{15}N$ values of phytoplankton should be propagated up the food web and be reflected in the predators' tissues. Thus, the $\delta^{15}N$ values of predators can provide insight into their historical foraging habits and movements. In order to successfully apply this chemical tag approach to pelagic predators, extensive

sample coverage of tropical tunas is required. Our research group, Drs. V. Allain, R. Olson, B. Popp, F. Galvan-Magana, and B. Fry, have been collaborating with other scientists and fishers to sample tunas, associated pelagic fishes, and their stomach contents in the equatorial Pacific Ocean using observers onboard purse-seine and long-line commercial fishing vessels.

1.3 Study Region: Equatorial Pacific Ocean

Industrial fisheries have removed 50 million tons of tuna from the Pacific Ocean since the 1950s (Sibert et al. 2006). The majority of this exploited biomass is tropical tunas removed from the equatorial Pacific Ocean (i.e., 20°N to 20°S). This equatorial region is a temporally and spatially dynamic system that is dominated by two large biogeochemical provinces, an oligotrophic region in the west and a high-nutrient, low chlorophyll (HNLC) region in the east. These two regions are produced by physical processes in the ocean basin (e.g., LeBorgne et al. 2002). Trade winds generally blow to the west across the equatorial Pacific Ocean, piling up warm surface water in the west. This added volume of warm water depresses the thermocline in the western equatorial Pacific (Wyrtki 1975, McPhaden 1999) (Fig. 1.2). In the eastern and central equatorial Pacific, the trade winds drive a divergence of surface water at the equator, which is then advected poleward by Ekman transport. These forces set up a strong, persistent upwelling zone that extends from the coast of South America to the eastern boundary of the warm pool (warm pool-cold tongue system (WPCT)) (Fig. 1.3). The cold tongue region of the equatorial Pacific is characterized as a high nutrient-low chlorophyll

(HNLC) region because micronutrients (e.g., iron) or effective microzooplankton grazing restricts the maximal unitization of macronutrients by marine phytoplankton in the euphotic zone (Minas et al. 1986; Coale et al. 1996; Landry et al. 1997). In contrast to the cold tongue, the warm pool is comparable to the oligotrophic subtropical gyres (e.g., Station ALOHA; Karl et al. 1997) in that the biological production is lower than in the cold tongue and is controlled by macronutrient input into the euphotic zone (Le Borgne et al. 2002). The spatial extent of both the warm pool and cold tongue can vary temporally, especially in relation to El Niño/Southern Oscillation (ENSO) events (Wyrtki 1975, McPhaden 1999). By correlating relative abundance of skipjack tuna to the cold tongue-warm pool boundary, Lehodey et al. (1997) demonstrated large-scale movements of skipjack tuna were linked to zonal shifts in the WPCT boundary. ENSO events drive the eastward edge of the warm pool to shift east into the central Pacific (Wyrtki 1975). Thus, tuna movements in the warm pool could be linked to zonal shifts in the cold tongue-warm pool interface and the relaxation of equatorial upwelling that occurs with ENSO events. Using a combination of the geographic variation in the $\delta^{15}N$ values of tuna or isotope cartography with compound-specific analyses, I will characterize the large-scale trophic dynamics and the general movement patterns of tropical tunas in the different production regimes, contrasting the low-productivity, warm pool to the higher productivity observed in the cold tongue (LeBorgne et al. 2002).

1.4 Research Objectives

Within this dynamic tropical Pacific Ocean system, my primary research objectives are to (1) use stable isotopic compositions to determine the trophic ecology of tropical tuna collected from Hawaii and the western, central, and eastern equatorial Pacific; (2) estimate tissue ^{15}N turnover rates for tropical tunas and apply these rates to estimate tuna net movements; (3) establish oceanic isotope cartography and with these isotope maps, characterize regional residency and movement patterns in tropical tunas; and (4) apply compound-specific stable isotope analysis to investigate tuna trophic dynamics and movements patterns. The results from this study should provide basin-wide information on tropical tuna trophic dynamics, the nutrient sources fueling tuna production, and net (zonal) movement rates of tuna populations, which can then be incorporated into ecosystem and stock assessment models to further support sustainable tuna fisheries in the equatorial Pacific Ocean. Ultimately, the chemical tag approach should "source" or link pelagic predators to geographical regions in which they forage and by doing so, help define suitable spatial stratification for stock assessment models. Moreover, by locating the regions where tropical tunas exhibit the greatest site fidelity, this method could provide data to support regional and local conservation policies.

This dissertation is divided into six chapters, of which this introduction is the first chapter. Chapter 2 concerns the trophic ecology of yellowfin tuna (*Thunnus albacares*) collected from nearshore fish-aggregating devices (FADs) around Oahu, Hawaii. Based on a distinct diet shift observed in these tuna between 45-50 cm fork length, demonstrated by both isotope and stomach content analyses, I proposed that a critical mass threshold was reached, which enabled sufficient endothermic capability to allow tuna to access prey dwelling below the thermocline. Chapter 2 has been published in

Marine Biology (Graham, B.S, D. Grubbs, K. Holland, and B.N. Popp. 2007. Rapid ontogenetic dietary shift in juvenile yellowfin tuna. Marine Biology 150:647-658). In Chapter 3, I discuss an experiment I performed at the Hawaiian Institute of Marine biology where I maintained captive juvenile yellowfin on a diet enriched by 9.3‰ relative to their natural diet and used these data to determine nitrogen turnover rates (N day^{-1}) in various tissues. I compared the tissue turnover rates estimated for the captive tuna to those estimated for the wild population of yellowfin that was discussed in Chapter 2. Also, in this chapter I applied the white muscle turnover rate to an observed latitudinal gradient in the bulk $\delta^{15}N$ values of adult yellowfin collected in the eastern tropical Pacific (Popp et al. 2007) to constrain N-S net movement rates of the yellowfin population. Chapter 3 will be submitted to the *Journal of Experimental Biology* under the authorship of B.S. Graham, B. Fry, B.N. Popp, R.J. Olson, and K.N. Holland.

In Chapter 4, I present geographical $\delta^{15}N$ maps for 3 species of tropical tunas, yellowfin, skipjack, and bigeye tuna that incorporate over 2000 individual tuna samples. In this chapter, I use this "isotope cartography" approach to discuss tuna residency, inter-species differences in $\delta^{15}N$ values, and the nutrient dynamics and mechanisms driving the observed geographical $\delta^{15}N$ patterns. In addition, with the tissue turnover rates calculated from Chapter 3, I examine the N-S, directional movements of the 3 tuna species along two transects in the central and eastern equatorial Pacific. The development of isotope cartography in the pelagic ecosystem and examining movements based on isotope datasets are both unique and significant components of my dissertation. Chapter 4 is in preparation for submission to scientific journal under the authorship of B.S. Graham, R.J. Olson, V. Allain, B.N. Popp, F. Galvan, and B. Fry.

In chapter 5, I combined the extensive bulk stable isotope dataset discussed in Chapter 2 and 4 with a compound-specific isotope (CSIA) dataset of yellowfin tuna to evaluate the mechanisms producing variations in their bulk $\delta^{15}N$ values in the tropical Pacific Ocean. Specifically, I demonstrated that variations in yellowfin bulk $\delta^{15}N$ values reflect changes in the isotopic baseline and that the trophic level of yellowfin tuna does not vary significantly across the equatorial Pacific. On a regional scale, I demonstrated that the trophic level does not differ between small and large size classes of yellowfin from Hawaii. Instead, the rapid ontogenetic shift in bulk $\delta^{15}N$ values described in Chapter 2 reflects a change in vertical foraging habitat, with small juveniles being physiologically confined to the surface mixed layer and large size classes foraging over a greater vertical range. Furthermore, resuls of bulk and compound-specific analyses also provided initial evidence of at least two general foraging strategies among the larger size classes of tuna collected around Hawaii. Therefore, in Chapter 5, I show that CSIA is effective method to examine trophic dynamics over varying spatial scales and present isotopic data that supports physiological and behavioral vertical niche partitioning by yellowfin tuna. Finally, in Chapter 6, I provide a research summary, significance, and outline several directions for future research.

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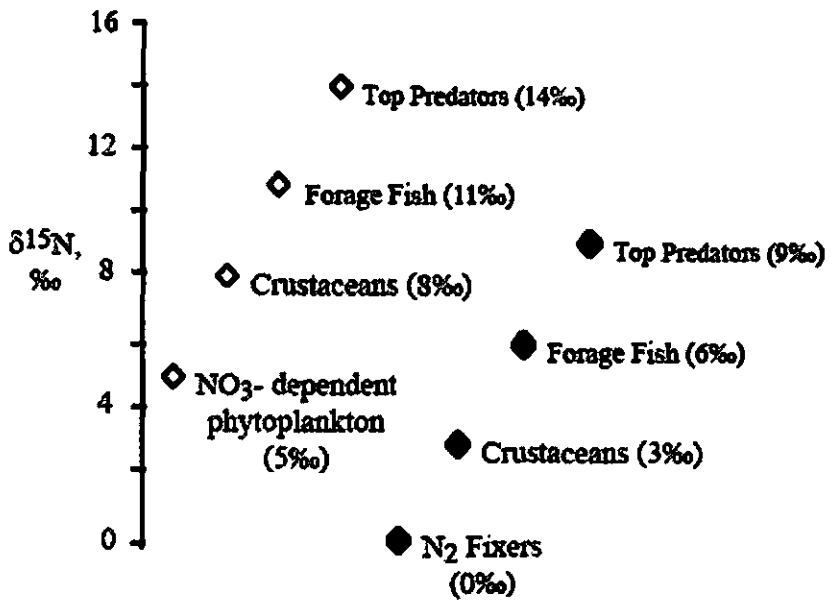

Figure. 1.1 Generalized $\delta^{15}N$ values for two simplified marine food web. In these four-trophic level systems, a general trophic enrichment factor (TEF) of 3‰ was assumed. The difference between the food webs is the source of new nitrogen supporting the base of the food web, or marine phytoplankton. If the entire pool of upwelled nitrate is consumed, the marine phytoplankton $\delta^{15}N$ value ≈ 5‰. In another ecosystem, where the nutrients are low and the surface mixed layer is highly stratified, a competitive advantage can exist for N_2-fixers and their $\delta^{15}N$ value is assumed to be ≈ 0‰. These baseline $\delta^{15}N$ values then set the stage for the $\delta^{15}N$ values at the subsequent trophic levels.

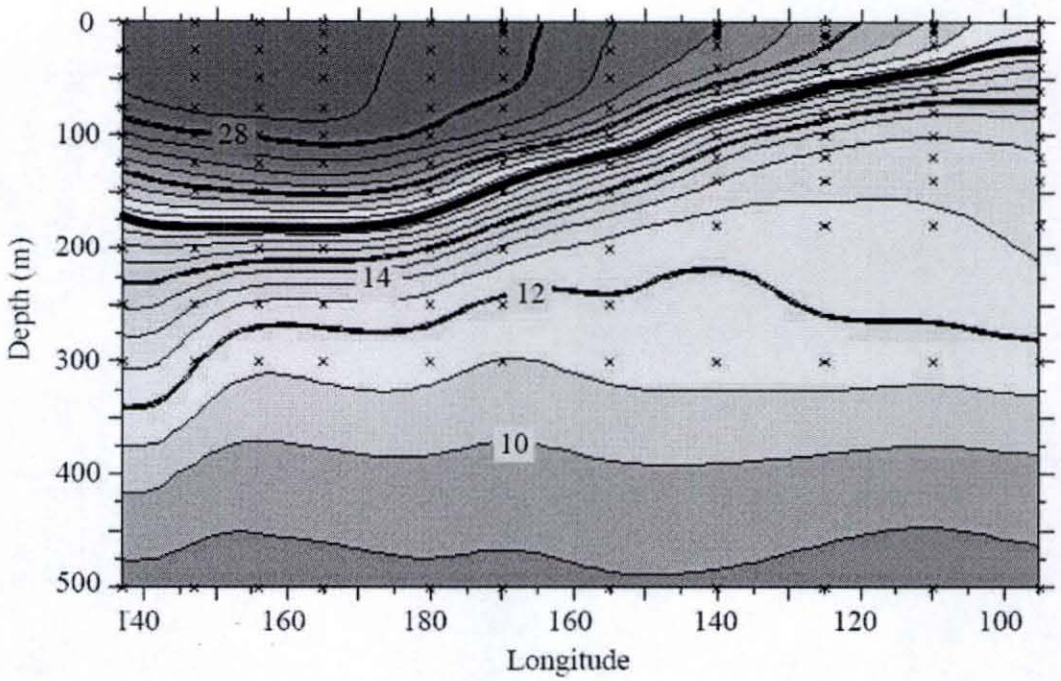

Figure 1.2 An east-west depth profile of water temperature (°C) across the equatorial Pacific Ocean (averaged from 2°S to 2°N). Adapted from the TAO/TRITON project (PMEL/NOAA; http://www.pmel.noaa.gov/tao/jsdisplay/).

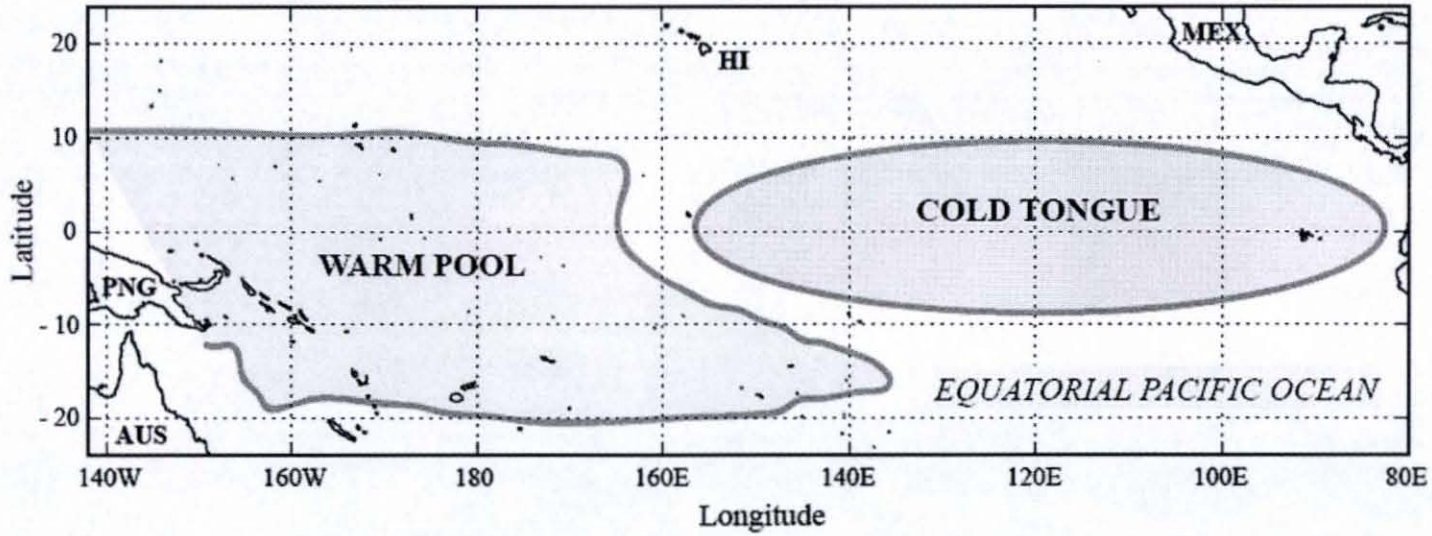

Figure 1.3. Equatorial Pacific Ocean study region. The warm pool-cold tongue (WPCT) equatorial Pacific system is a persistent feature of the equatorial Pacific, but the extent of the warm pool and cold tongue varies seasonally and interannually due to climatic events, especially with ENSO events. The cold tongue region is characterized by persistent upwelling, high nutrients, low chlorophyll, and bio-limiting iron concentrations (i.e., HNLC region). The warm pool is characterized by deep thermocline depths, low nutrients, and the largest commercial catch of tropical tunas. In order to examine tropical tuna trophic dynamics and movements, muscle samples of tropical tunas, their prey, and stomach contents of tuna were collected by onboard fishing observers throughout this

region. This extensive sampling effort was coordinated by scientists at the Secretariat of the Pacific Community (SPC), Inter-American Tropical Tuna Commission (IATTC), and the Mexican Interdisciplinary Center of Marine Sciences (CICIMAR).

CHAPTER 2

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

Graham, BS, D Grubbs*, KN Holland, and BN Popp (2007) Mar Biol 150:647-658

2.1 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 45-50 cm forklength (c.a. 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 $\delta^{15}N$ values we considered both their relative trophic position and $\delta^{15}N$ 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

* Dr. Dean Grubbs collected, analyzed, and synthesized the stomach content data presented in this chapter.

dwelling in deeper, colder water. These ontogenetic changes in foraging range, and commensurate shift in diet of small tunas can affect their vulnerability to fishing pressure.

2.2 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 very diverse forage base, although the majority of the diet often comprises only a few families of epipelagic teleosts and crustaceans (e.g., Alverson 1963; Reintjes & King 1953). 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 cm 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 composition of an organism's tissue reflects 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 45-50 cm forklength (c.a. 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.

2.3 Material and Methods

We collected tuna from the nearshore FADs located around Oahu, Hawaii (Fig. 2.1). 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 ranged between 523-2084 m, with an average mooring depth of 1,349m. 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 hours and 1200 hours. 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 (Uchiyama and Kazama 2003). Collection of samples occurred between October of 2002 and July 2004.

2.3.1 Gut Content Analyses

Stomach contents were preserved in a solution of 10% phosphate-buffered formalin for at least 72 hours 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 25cm intervals of <50cm, 50-74.9cm, 75-99.9cm, 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 (20-34.9cm and 35.0-49.9cm) (Table 2.1).

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 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 Morisita's original index. When Morisita'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.

2.3.2 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 tissue. 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) 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, USA) using hexane according to manufacturer's recommendations (Dionex Application Note 342, http://www1.dionex.com/enus/webdocs/application/industry/-environmental/extraction/AN342.pdf).

Carbon and nitrogen isotope 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). 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:

$$\delta^{15}N_{YFT} = \sum(f_x\delta_x) + TEF \quad (1)$$

where $\delta^{15}N_{YFT}$ is the $\delta^{15}N$ value of the tuna tissue, f_x is the diet fraction of prey item X and δ_x is the isotope value of prey item X, and TEF is trophic enrichment factor between the diet and tuna (3.4 ‰ after DeNiro and Epstein 1981).

2.4 Results

2.4.1 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 2.1).

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. 2.3). 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. 2.3a and 2.3b). 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.

Morisita's original index (Table 2.2) indicated 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 were 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 of 18.2%MV of the diet of tunas greater than 50 cm FL (Figure 2.4a). Teleosts and *O. gracilirostris* dominate the diet of the larger tuna. *Oplophorus gracilirostris* is a vertically migrating species associated with the 700 meter isobath around the Hawaiian Islands (Reid et al. 1991). During night, this species is found between 300 and 700 meters deep but migrates to less than 200 meters (<50 m when spawning) deep during the day (Zeimann 1975). *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. 2.4a). 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. 2.4a). The trends in terms of prey numbers (%MN) were very similar to those for volume (Figure 2.4b).

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% to 7.2%), the volumetric proportions were comparable (21.3% and 20.3% respectively). These data illustrate the exaggerated influence one or a few 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.

2.4.2 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 changed $\delta^{15}N$ values ($p < 0.01$, Student's t-test). Based on a much larger dataset, lipid extraction also did not statistically affect the $\delta^{13}C$ value of WMT or LVR tissue in yellowfin tuna < 45cm 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 isotope 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 $\delta^{13}C$ values showed no trend with fork length or mass. However, a distinct positive shift in $\delta^{15}N$ occurs in the white muscle tissue of

yellowfin tuna between a forklength of 45 and 50 cm (Fig. 2.2a). The mean nitrogen isotope value of white muscle tissue of tuna <45.0 cm (6.7 ± 0.6 ‰) was significantly less than for tuna ≥45.0 cm (10.2 ± 1.8 ‰) ($p < 0.001$, Student's t-test) (Table 2.3). Tuna larger than 45 cm FL exhibited a large range of $\delta^{15}N$ values relative to smaller size classes (Fig. 2.2a). Converting fork length to mass resulted in isotope values for white muscle that showed a logistic increase reaching an asymptote in the largest size classes (Fig. 2.2b). Carbon isotope 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 $\delta^{13}C$ values showed no trend with fork length or mass. Liver $\delta^{15}N$ 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. 2.2a). A logistic increase was modeled for yellowfin $\delta^{15}N$ liver values and mass which demonstrated a similar trend to white muscle tissue (Fig. 2.2b). There was not a statistical difference between the slopes (i.e. regression coefficients) of the liver and white muscle tissue datasets ($p < 0.01$, Student's t-test). The increase in average $\delta^{15}N$ 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 (approximately 5 ‰) in the $\delta^{15}N$ value of both the white muscle and liver tissue is observed immediately after 45 cm FL.

A range in $\delta^{15}N$ values was observed among the different prey species (Table 2.3). Stomatopod and decapod larvae, which were a major component of tuna in size groups 1 and 2, exhibited $\delta^{15}N$ values that averaged 3.4 ‰ less than $\delta^{15}N$ values of the tissues of the smallest tuna size classes. Using the simple diet mixing model (eqn. 1, with the stomach content analysis providing estimates of 69 % of the diet), the observed

yellowfin tuna $\delta^{15}N$ value closely matched the predicted value for small size classes (Table 2.3). For larger tuna, the isotope mixing model does not predict the observed average $\delta^{15}N$ value. It is clear from the model that a diet component with higher $\delta^{15}N$ values is missing. However, given the variation prey $\delta^{15}N$ values, a combination of diet inputs into the mixing model can produce a wide range of predicted yellowfin $\delta^{15}N$ values. Prey $\delta^{13}C$ values were influenced by lipid-extraction and are reported as either lipid-extracted or non-lipid-extracted values (Table 2.3). 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 $\delta^{13}C$ differences (<0.4‰, see above) between small size classes and large size classes.

2.5 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 four year 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 approximately 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 tuna $\delta^{15}N$ 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 $\delta^{15}N$ values of yellowfin tuna.

Prey isotope composition is a reflection of two main influences: the position of the prey in the food web and 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 approximately 3.4 ‰ in $\delta^{15}N$ values between prey and predator (DeNiro and Epstein 1981; Minagawa and Wada 1984). 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 (Eppley 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 controlled by N_2 fixation, leaving the surface PN pool with low $\delta^{15}N$ values, whereas PN collected from waters below the mixed layer (c.a. 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 to 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 as observed by Rau et al. (1989). If the $\delta^{15}N$ values of the N sources are conserved, then prey $\delta^{15}N$ values may not be just a function of trophic position, but also 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 $\delta^{15}N$ 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 can give an indication of the trophic breadth of the prey. Variability in consumer $\delta^{15}N$ 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 $\delta^{15}N$ values, then the wider range of $\delta^{15}N$ values observed in tuna $\geq$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 $\delta^{15}N$ 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 $\delta^{15}N$ values between 9 to 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 $\delta^{15}N$ values observed in larger size classes of tuna is likely due to the ingestion of prey of an increasing trophic diversity or different foraging strategies. 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 forklength 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 ~10cm FL or 163g (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 to 2.5 kg 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 often or 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 tunas 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 $\delta^{15}N$ 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 with changing habitat. The apparently tightly constrained forage depth of the small-size classes of tuna could have important implications for the management of tropical tuna fisheries.

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Table 2.1. Interval and sample sizes for each size class used in the gut content analyses. The proportion of empty stomachs mean and stomach repletion are also provided. SEM = standard error of the mean.

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)

Table 2.2. Morisita's original index of dietary overlap between sizes classes of yellowfin tunas. a) Overlap of prey taxa in terms of %MN and *%N*. b) Overlap in prey taxa in terms of %MV and *%V*. Overlap estimates using pooled data (%N and %V) are italicized. * indicates significant overlap (≥0.60)

a)

Size Class	0 (<35.0)	1 (35.0–49.9)	2 (50.0-74.9)	3 (75.0–99.9)	4 (100.0+)
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**	

b)

Size Class	0 (<35.0)	1 (35.0–49.9)	2 (50.0-74.9)	3 (75.0–99.9)	4 (100.0+)
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**	

Table 2.3. Average $\delta^{15}N$ and $\delta^{13}C$ (and standard deviations) of prey types collected from yellowfin tuna stomachs during this study. Numbers of samples analyzed are indicated after prey names.

Prey Item	%MV $_{<35cm}$ [b]	%MV $_{\geq 50cm}$ [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:

$\delta^{15}N_{<35\ cm} = 6.5 \pm 0.3$ ‰

$\delta^{15}N_{\geq 50\ cm} = 10.2 \pm 1.6$ ‰

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 ‰

[a] Samples include many individuals in a single analysis.

[b] Mixing model (Eqn 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] $\delta^{13}C$ values are lipid-extracted values.

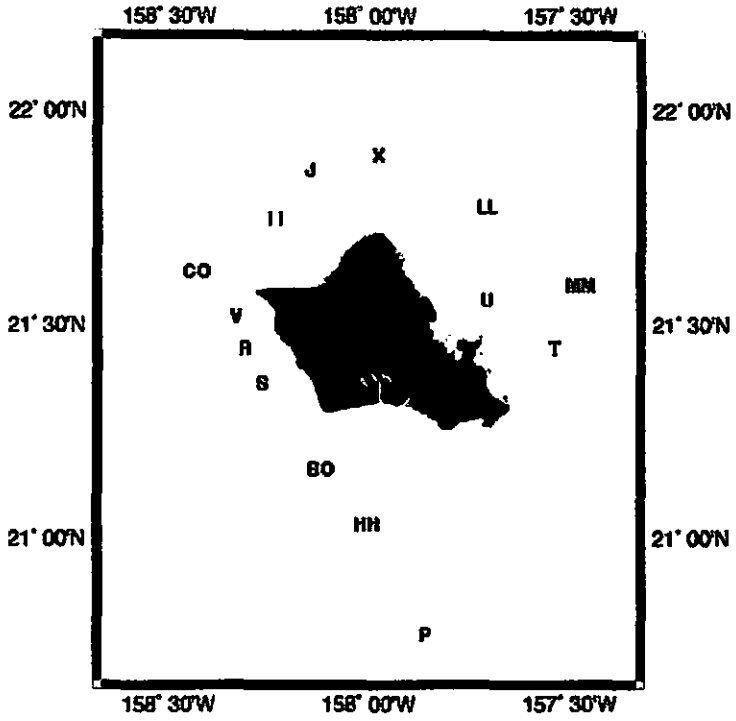


Figure 2.1. Study region. The location of the nearshore fixed FADs around the island of Oahu where yellowfin tuna were sampled from October 2002 to May 2004.

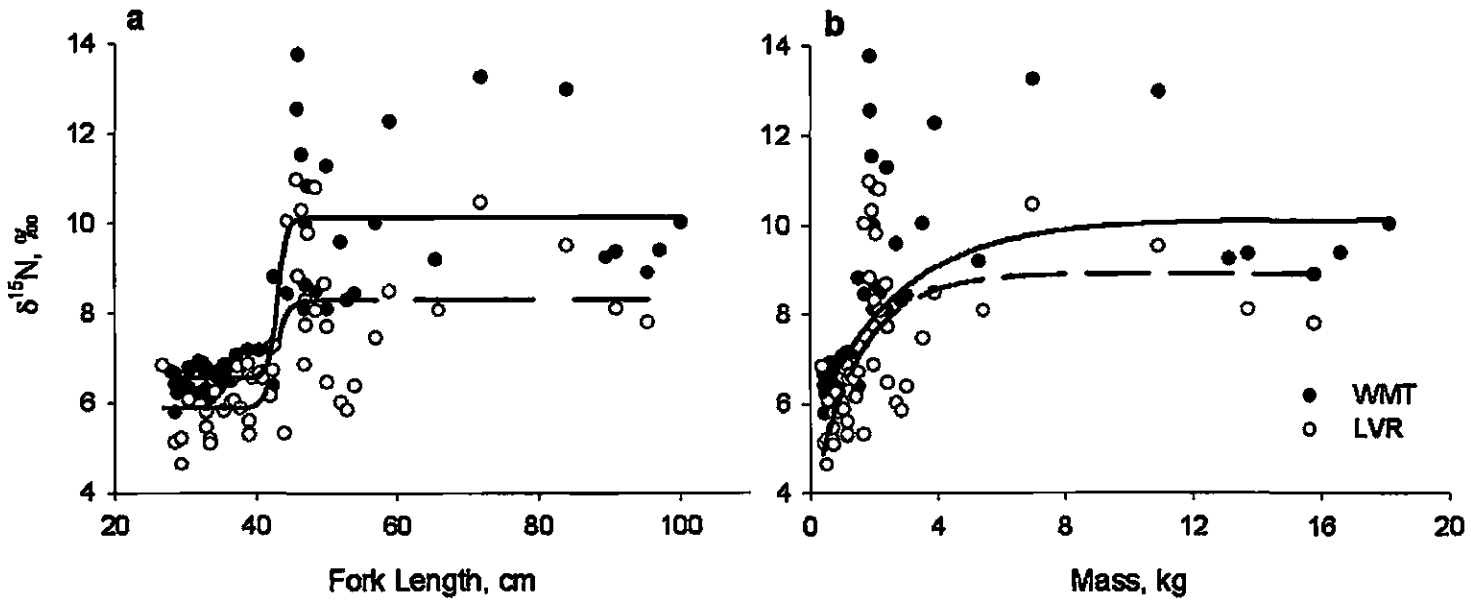


Figure 2.2. a) $\delta^{15}N$ values of yellowfin tuna white muscle (WMT) and liver (LVR) tissue over a range of fork lengths. A 4-parameter sigmoid model ($f=y_0+a/(1+\exp^{(-(x-x0)/b)})$) was fitted to the yellowfin WMT (solid line; $f = 6.6+a/(1+\exp^{(-(x-43.1)/0.64)})$; $R^2 = 0.71$) and LVR (dashed 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; $f=3.3+a/(1-e^{-0.87x})$; $R^2 = 0.41$).

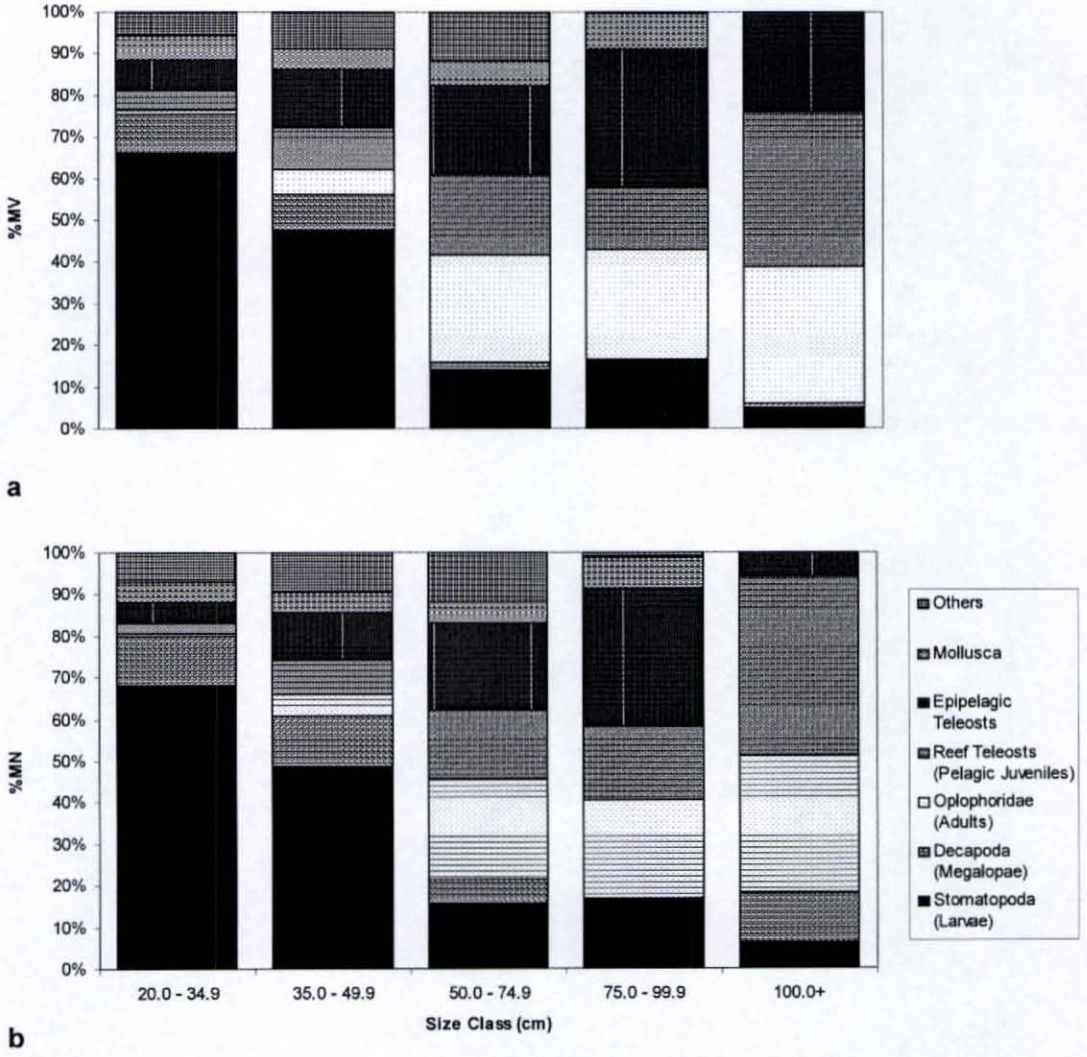
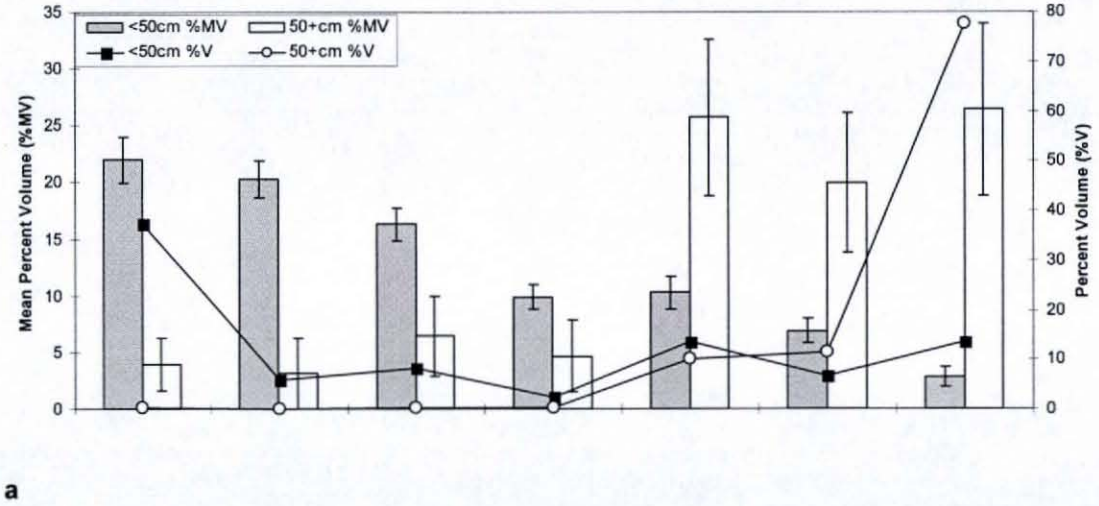
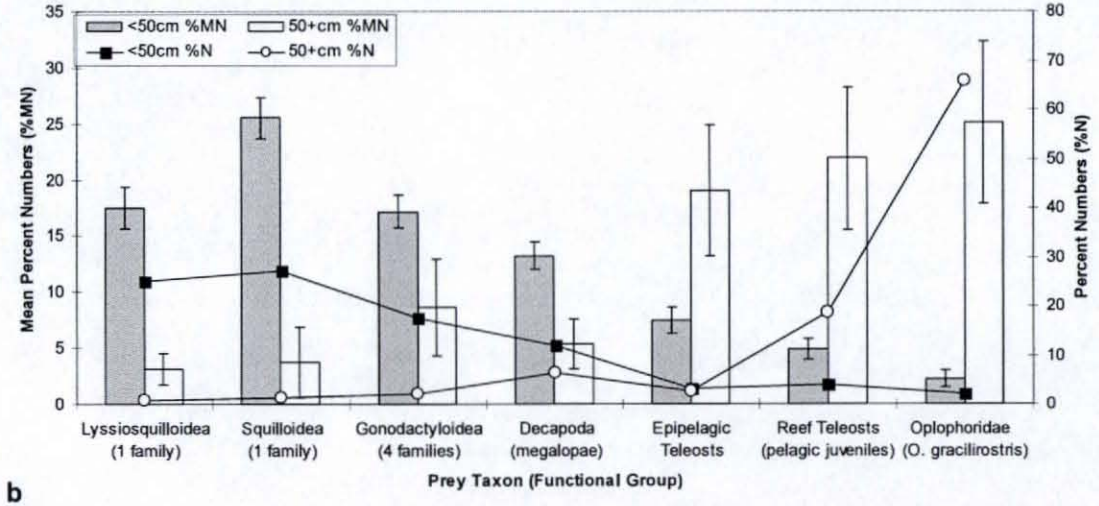


Figure 2.3. Importance of major prey taxa, measured as (a) %MV and (b) %MN, in the diets of five size classes of yellowfin tuna.


<50cm %MV
50+cm %MV
<50cm %V
50+cm %V
Mean Percent Volume (%MV)
Percent Volume (%V)
0
5
10
15
20
25
30
35
0
10
20
30
40
50
60
70
80

a


<50cm %MN
50+cm %MN
<50cm %N
50+cm %N
Mean Percent Numbers (%MN)
Percent Numbers (%N)
0
5
10
15
20
25
30
35
0
10
20
30
40
50
60
70
80
Lyssiosquilloidea (1 family)
Squilloidea (1 family)
Gonodactyloidea (4 families)
Decapoda (megalopae)
Epipelagic Teleosts
Reef Teleosts (pelagic juveniles)
Oplophoridae (O. gracilirostris)
Prey Taxon (Functional Group)

b

Figure 2.4. Comparison of the relative importance of seven functional prey groups in the diets of small (<50 cm FL) and large (≥50 cm FL) yellowfin tuna. a) %MV and %V. b) %MN and %N.

CHAPTER 3

Tissue turnover rates in captive and wild yellowfin tuna, *Thunnus albacares*: implications for estimating movements of pelagic predators

3.1 Abstract

To maximize the utility of stable isotope analysis to investigate foraging behavior and movement patterns of tropical tunas, we determined tissue-specific turnover rates by conducting a diet shift experiment on captive juvenile yellowfin tuna (*Thunnus albacares*). At the onset of the experiment, captive tuna were fed a diet enriched in ^{15}N relative to their previous, natural diet. The $\delta^{15}N$ values of animal tissues reflects that of the isotopic value of the animal's assimilated diet integrated over a time proportional to the tissue-specific turnover rate. Thus, tissue-turnover rates provide a temporal framework on which to base interpretations of stable isotopic data. We analyzed 4 tissues from a captive population. Liver represented the fastest (0.056 ^{15}N day^{-1}) and white muscle the slowest (0.011 ^{15}N day^{-1}) turnover rate, with tissue nitrogen half lives estimated as 12 and 63 days, respectively. These rates are the first recorded for a pelagic top predator fish and, in particular, an endothermic fish. A comparison of tissue turnover rates measured in captive and wild yellowfin tuna indicate that the rates are similar for liver, but those for muscle in the captive tuna were several-fold slower. The difference in muscle turnover rate may be due to more rapid growth in wild tuna that increases estimates of N turnover. Muscle turnover rates were used to interpret an observed latitudinal gradient in yellowfin tuna $\delta^{15}N$ values and to infer relatively

small (approximately 10km day^{-1} or less) net N-S movements of yellowfin tuna in the eastern tropical Pacific.

3.2 Introduction

Isotope values of pelagic predator tissues record information about their foraging habits and movement patterns. Stable isotopic values of an organism's tissues reflect its food and nutrient sources (DeNiro and Epstein 1978, 1981), and therefore, can reflect the animal's previous foraging behavior and movement history (Fry 1981; Schell et al. 1989; Hesslein et al. 1993). Ecologists studying trans-continental avian migration have clearly demonstrated that the isotope values or chemical tags of individuals and populations can be used to delineate diet, dispersion, and migration patterns, because the birds acquire distinct isotope signals while foraging in different terrestrial, freshwater, and coastal habitats (Chamberlain et al. 1994; Kelly and Finch 1998; Hobson et al. 1999). Natural isotopic gradients within the open ocean exist and have been applied to examine cetacean movent patterns in high latitude seas (e.g., Best and Schell 1996; Schell 2000: Lee et al. 2005). In the tropical Pacific Ocean $\delta^{15}N$ values of suspended particulate organic matter and deep-sea sediments indicate large and systematic isotopic variations (Farrell et al. 1995; Altabet 2001; Voss et al. 2001). Consequently, the potential exists for applying $\delta^{15}N$ values to examine movement patterns in tropical tunas. However, before stable isotope values of top marine predators can be effectively used for this purpose, the rate at which a predator's tissue acquires a local isotopic value must be established.

Following a shift to a diet with a different stable isotopic composition, an animal's isotope value will reach a relative steady state with the isotopic composition of the new food source(s) (Fry and Arnold 1982; Tieszen et al. 1983) plus some trophic enrichment factor (TEF). The TEF is the difference between the isotopic value of the animal and its diet. Metabolically active tissues will reflect the isotopic values of a new diet more rapidly than will tissues with slower turnover rates. The growth rate of an organism can also affect tissue turnover rate (e.g. Fry and Arnold 1982; Hesslien et al. 1991; Trueman et al. 2005), and some slow-growing cold-water fishes may reach complete isotope turnover only after periods of a year or more (Hesslein et al. 1993). Endothermic tropical tunas have a high average growth rate and standard metabolic rates and therefore, are expected to exhibit rapid turnover of their body tissues (Carey 1973; Stevens and Carey 1981; Dewar and Graham 1994; Dickson 2000). Accordingly, the use of isotope values as indicators of migration patterns in these mobile and metabolically active predators requires an understanding of their tissue turnover rates.

In this paper, we report the turnover rates in several tissues of captive juvenile yellowfin tuna (*Thunnus albacares*). These tissue turnover rates were determined by observing the rate at which those tissues approached the $\delta^{15}N$ value of a distinct experimental diet and are the first recorded for a top pelagic predator and a fish that exhibits behavioral and physiological thermoregulation (Carey 1973; Dewar et al. 1994). We compared the experimental turnover rates to those estimated from a natural diet shift observed in wild yellowfin tuna collected from the same region as the captive fish (Graham et al. 2007). Tissue-specific turnover rates for both the captive and wild diet shift datasets were estimated using exponential models (Teiszen et al. 1983; Hesslein et

al. 1993; MacAvoy et al. 2001). We discuss the application of tissue turnover rates to infer movement rates of these migratory predators in the pelagic marine ecosystem.

3.3 Material and Methods

3.3.1 Experimental Tank Description

A tuna tank at the Hawaiian Institute of Marine Biology (HIMB) was used for the diet shift experiment. The circular tank was 8.53m in diameter, filled to 2.13m depth, resulting in a seawater volume of $122m^3$ (122,000 L). The tank had a flow-through water circulation system. Seawater was pumped from an intake pipe just offshore of the HIMB facility, and into the tank through a PVC pipe elevated above the surface of the water. There was a positive correlation between filter back-flushing (cleaning) and the turnover rate of tank water. Therefore, the filter was back-flushed daily during the experiment to maintain the highest water turnover rate for this tank system. The average flow rate was 2.44 L s^{-1}, and ranged between 1.24 and 3.57 L s^{-1}. This is equivalent to a tank water turnover rate of 9.5 to 27.3 hours, respectively. The tank was manually cleaned approximately once per week during the duration of the experiment to reduce algal growth and bio-fouling. Tuna have low tolerance for physical abrasion (Wexler et al. 2003), so physical contact with the juvenile tuna was kept to a minimum to maximize survival rate during the 8 month experimental study.

3.3.2 Capture and Handling of Experimental Fish

Juvenile yellowfin tuna were collected between September 2003 and April 2004 at nearshore fish aggregating devices (FADs) using handlines and rods and reels with

barbless hooks. Yellowfin with no apparent abrasions or serious hook damage were measured (fork length estimated to the nearest 5cm), marked with a spaghetti tag and placed in a fiberglass transfer tank for transportation back to the HIMB facility. Transfer time between the capture and placement in the HIMB tank ranged between one and two hours. To reduce physical contact and possible abrasion of the fish, we did not make precise measurements of the length and weight of individual tuna at the time of capture or start of the experiment. Multiple groups of yellowfin tuna were introduced to the tank throughout the duration of the study. In previous studies of captive tuna, fish density was kept below 0.5 kg m^{-3} of seawater (Wexler et al., 2003), equivalent to approximately 60 juvenile yellowfin tuna in the HIMB tank system. However, because the water turnover rates in the HIMB tank were lower than previous tuna holding tanks, tuna stocking densities were kept on average below 0.15 kg m^{-3} (range, 0.05 to 0.2 kg m^{-3}).

The captive tuna were fed a diet of 70% squid and 30% smelt by weight. The fish fed aggressively and consumed the food as soon as it hit the water's surface. To reduce selective feeding on either type of food, a mixture of similar-sized pieces of the 70:30 squid:smelt diet was distributed to the tuna simultaneously and no prey selectivity was observed during feeding events. This mixture provided a diet adequate for tuna growth (i.e., protein and lipids) and a $\delta^{15}N$ value (13.4 ± 0.7‰, C:N = 4.5 ± 0.3) distinctly different than their natural diet (4.4‰, C:N = 6.3 ± 0.9, see also Graham et al. 2007). However, the $\delta^{13}C$ value of this diet was not distinctly different from the natural diet. In previous work on captive yellowfin, newly-captured fish were provided approximately 8% of body weight day^{-1} in a tank at approximately 27°C (Wexler et al. 2003). In this study, captive tuna were fed daily at random times during daylight hours and the number

of grams of food added to the tank per day was recorded. Due to the aggressive feeding by captive tuna, we were not able to track individual consumptions rates. Overall, the captive tuna were fed an average 7.4% of their body weight day^{-1} or approximately 70 grams of prepared food per fish day^{-1}, which is higher consumption rate than those estimated for wild yellowfin tuna in the eastern tropical Pacific (6.7% of their body mass day^{-1}; Olson et al. 1986).

3.3.3 Natural $\delta^{15}N$ Diet Shift

We also modeled the tissue turnover rates of wild yellowfin tuna that had undergone a natural diet switch. Graham et al. (2007) showed that juvenile yellowfin collected from nearshore FADs around Oahu, Hawaii exhibited a distinct ontogenetic change in diet that resulted in an ~4‰ increase in the $\delta^{15}N$ values of liver and white muscle tissues in < 2 months. The yellowfin in that study were collected between October 2002 and July 2004. All fish were measured (fork length, cm), the liver and white muscle tissue were sampled for stable carbon and nitrogen isotope analyses, and the stomachs were collected for gut content analysis. To model tissue turnover rates, age (days) was estimated from the measured lengths using a modified Von Bertalanffy growth model for yellowfin tuna collected in the western and central Pacific Ocean (Lehodey and Leroy 1999). Further details of the methods used are provided by Graham et al. (2007).

3.3.4 Stable Isotope Sampling of Captive Tuna

The captive yellowfin tuna were sacrificed at intervals after the diet switch to best try to characterize the rate of change in isotopic composition, and samples were taken of white muscle tissue (WMT), red muscle tissue (RMT), liver (LVR), and gill filaments (GLF). Some fish that had died in the tank (but had been feeding and were in otherwise good physical condition) were also sampled for WMT, RMT, and LVR. Samples of the experimental diet and recently-ingested food from stomach content of captive fish were homogenized and analyzed periodically to track the uniformity in the $\delta^{15}N$ values of the experimental diet.

All tissue and food samples were lyophilized and homogenized with a mortar and pestle. Lipids were not removed from the tissues or food samples. Nitrogen isotope values were determined using an on-line CN analyzer coupled with an isotope ratio mass spectrometer (Finnigan ConFlo II/Delta-Plus). Isotope values are reported in standard δ-notation relative to atmospheric N_2. Repeated analyses of laboratory reference materials with C/N values similar to fish protein indicated that the precision and accuracy of the isotopic measurements was $\leq$0.2‰. In addition, the precision in the $\delta^{15}N$ values of several tissue samples measured in triplicate was $\leq$0.2‰.

3.3.5 Tissue Turnover Model

There are at least two general approaches to determine tissue turnover rates based on stable isotope data. The most widely used method is an exponential fit to the isotopic data as a function of time (Teiszen et al. 1983; Hesslein et al. 1993; MacAvoy et al. 2001; Trueman et al. 2005). This traditional approach can be tailored to model only turnover (e.g. Teiszen et al. 1983) or turnover plus growth (e.g. Hesslein et al. 1993; MacAvoy et

al. 2001; Maruyama et al. 2001). Some studies have incorporated the effects of growth in their isotopic turnover models (Fry and Arnold 1982; Hesslein et al. 1993; MacAvoy et al. 2001; Suzuki et al. 2005; Cerling et al. 2007), but the experimental tuna were not weighed and measured accurately at the onset of the diet shift experiment, we only considered turnover in our analysis (i.e. no specific growth rate constant was included). To ensure comparability between datasets, we also applied a turnover-only model to the natural diet shift data.

Recently, Cerling et al. (2007) showed that an exponential fit to isotopic time-series has a few limitations, such as overemphasizing the initial isotopic value of an experiment. They introduced the reaction progress variable (RPV) model, which normalizes the data to a linear function. The key advantage of this new approach is that it allows multiple pools that turnover at different rates within a single tissue to be easily visualized and calculated (Ayliffe et al. 2004). As with the exponential model, the RPV model can also be adapted to incorporate growth plus turnover (Cerling et al. 2007).

With the relatively low frequency at which we sampled tank specimens (compared to the high sampling frequency in recent studies of other species, e.g. Ayliffe et al. 2004; West et al. 2004), the RVP model did not provide additional information beyond that obtained from the exponential fit. Therefore we applied an exponential model to the captive tuna datasets for each tissue type. The model predicts tissue $\delta^{15}N$ values (Y, ‰) at the time (X, days) the fish were sampled after the diet shift, as:

$$^{*}Y = (Y_0 - Y_n)e^{-kX} + Y_n , \qquad (1)$$

* The model derivation is included in Appendix B

where $b > 0$, Y_0 is the tissue isotope value at the start of the diet switch, Y_n is the expected tissue isotope value at the asymptote, *i.e.* for fish at steady state with their new diet, and b (‰ per day) is the tissue turnover rate attributed to metabolism. Tissue half-life (HL, days) is:

$$HL = \frac{\ln(0.5)}{-b} \quad . \tag{2}$$

Equation 1 was fitted by least squares to the experimental data sets for WMT, RMT, LVR, and GLF. Approximate 95% confidence intervals were computed by 1000 bootstraps for each data set. For each bootstrap, data were resampled at random, with replacement, and equation 1 was refitted. Bootstrap samples were taken from 3 or 4 subsets of data for each data set and the subset was based on the statistical design. For example, if the data were divided into 3 subsets, the isotope data was separated into three groups along the time axis (i.e., x-axis), and the first subgroup then contained one-third of the data starting from x = 0 to x = 1/3 of the full time course of the dataset. The statistical design required samples to be distributed throughout the time course of the experiment, and sample dependence on days was preserved by random resampling from within the data subsets. The samples of both types, sacrificed individuals and tank mortalities, were modeled together, but the data for the two different groups were also examined separately to determine if there were differences in the turnover rates.

We fitted another exponential model to the data sets for WMT and LVR in wild-caught yellowfin to obtain an independent estimate the tissue turnover rate (b) and Y_0. The model fit was different from the one applied to the captive tuna, because we did not want to assume a single asymptotic value (Y_n) was reached after the wild diet shift for all individuals sampled (i.e., there could have been individual specialization after the diet shift and emigration of individuals from distant regions). Accordingly, we fitted to only the data

corresponding to the youngest fish, shown by the black symbols in Figure 2, to avoid possible variability by older fish that could have recently immigrated to Oahu from regions with different isotope values (open symbols, Fig. 3.2) or exhibited diet specialization. The model predicts tissue $\delta^{15}N$ values (Y, ‰) at the age (X, days, estimated from the fork length) of the fish sampled, as:

$$Y = Y_0 + ae^{bX} , \qquad (3)$$

where Y_0 and b have the same meaning as for equation 1, $b > 0$, and $Y_0 + a$ is the y-intercept. Equation 3 was fitted by least squares to the wild data sets of WMT and LVR. Approximate 95% confidence intervals (95% CI) were computed in the same manner as described above for the experimental diet switch.

3.4 Results

3.4.1 Experimental Diet Shift

During the 8-month experimental study, $\delta^{15}N$ values of 38 captive yellowfin were determined. The longest individual residence time in the tank was 129 days, with the mean residence time of 34 days for the captive population. The estimated fork length (± SD) of the tuna introduced to the tank was 37cm ± 2cm (~950 to 1000g based on the weight-length-age relationship of Lehodey and Leroy (1999)). Also, a length of 37cm would equal an age of approximately 125 days (Lehodey and Leroy 1999). Of all individuals, 16 were sacrificed and 22 were 'samples of opportunity' (i.e. sampled shortly after mortality in the tank).

The initial isotopic value and diets of the yellowfin tuna held in captivity were determined from sacrificed specimens of similar size (≤35cm fork length) tuna that were captured in the same location as the fish that were examined in wild (Graham et al. 2007). These wild juvenile tuna had a WMT $\delta^{15}N$ value of 6.5 ± 0.3‰ (mean ± SD). A simple isotope mass balance model based on $\delta^{15}N$ values of the prey, weighted by the mass proportions in the stomach contents, provided a mean $\delta^{15}N$ value of 4.4‰ for approximately 84% of the diet in the wild (Graham et al. 2007). The estimated trophic enrichment factor (TEF), *i.e.* the isotopic difference between the mean $\delta^{15}N$ value of the diet and that of the wild tuna population was approximately 2.1‰.

The 70:30 squid:smelt experimental diet was isotopically consistent throughout the duration of the experiment, both in the samples of homogenized experimental diet (13.2 ± 0.9‰, n = 6) and in samples collected in the stomach contents of the sacrificed individuals (13.6 ± 0.3‰, n = 6). The $\delta^{15}N$ values of squid and smelt were similar, 13.2 ± 0.9‰ and 13.5± 0.5‰, respectively. Accordingly, a large isotopic difference (ca. 9.3‰) existed between the $\delta^{15}N$ value of the average experimental diet (grand average of homogenized diet and stomach samples is 13.4 ± 0.7‰, n = 12) and natural diet of the juvenile yellowfin tuna, thus enabling good resolution of diet shift dynamics.

After a 5 day lag period when captive tuna adapted to feeding on dead prey or experimental diet, all of the $\delta^{15}N$ values of tissues in juvenile yellowfin increased, regardless of the residence time in the tank (Fig. 3.1). When considered separately, the $\delta^{15}N$ values of tank mortalities fell just below the $\delta^{15}N$ values of the sacrificed tuna for the same tank residence time suggesting slower turnover rates in the former group. However, many tank mortalities represented specimens with long residence times in the

tank during which times they were actively feeding. Therefore, we include isotopic data from these individuals in the calculations of tissue turnover rates. Tissue turnover rates derived from the two groups (sacrificed animals and fish that died) should be considered a conservative lower estimate of turnover rates in juvenile yellowfin tuna. The WMT exhibited the slowest tissue turnover rate (half-life = 63 days), followed consecutively by RMT (39 days), GLF (19 days), and LVR (12 days) (Fig. 3.1, Table 3.1). Based on the 95% confidence intervals, (i) WMT and RMT and (ii) GLF and LVR values do overlap, whereas (iii) WMT turnover is lower than LVR and GLF and (iv) RMT tissue turnover rate is lower than GLF (Table 3.1).

Tissue-specific TEFs observed in the field were not reached for WMT and RMT tissues in the experimental diet shift (Table 3.2). Moreover, the duration of the diet shift experiment was insufficient to allow the WMT and RMT data to reach an asymptote (Y_n) (Fig. 3.1). Although GLF and LVR tissues appeared to reach an asymptote after the diet shift (Fig. 3.1), the experimental TEF values did not approach those estimated from the wild population of juvenile yellowfin tuna (Table 3.2). Overall, TEFs near 0‰ were measured for the captive animals and values near 2‰ estimated for wild yellowfin (Table 3.2, Graham et al. 2007).

3.4.2 Natural Diet Shift

Wild yellowfin tuna $\delta^{15}N$ values indicate an abrupt change at approximately 140-160 days (Fig. 3.2). In this wild population of juvenile tuna, liver and white muscle tissue $\delta^{15}N$ values changed to reflect that of a different diet. During this natural diet shift, WMT (0.035 ^{15}N day^{-1}) exhibited a faster turnover rate relative to (a) the turnover rate of

LVR (0.026 ^{15}N day^{-1}) in the wild individuals and (b) the turnover rate of WMT (0.011 ^{15}N day^{-1}) in captive fish. However, the 95% CIs for the turnover rates of WMT and LVR for the wild juvenile tuna overlap (Table 3.1). WMT and LVR turnover rates calculated for the wild population allow comparison with the experimental tissue turnover rates (Fig. 3.3). The 95% CIs did overlap for the LVR turnover estimates in the captive and wild populations, but the WMT turnover rate from the natural diet shift was significantly different from the experimental WMT turnover estimate (Table 3.1, Fig. 3.3).

3.5 Discussion

Determining specific tissue turnover rates for tropical tunas was especially important given their unique physiological, morphological, and behavioral adaptations for high-performance swimming in the pelagic ecosystem (Brill and Bushnell 1991; Dewar et al. 1994). The ability of tunas to elevate internal body temperatures enhances many of their physiological and biochemical processes (Carey 1973; Stevens and Carey 1983). In many regards, the metabolic rates and physiology of tunas are more similar to endothermic animals than other ectothermic fishes – including very active groups such as salmonids (e.g. Carey 1973; Stevens and Carey 1983; Brill and Bushnell 1991; Dewar and Graham 1994). In a review of isotope turnover rates estimated for a range of ectothermic aquatic organisms, an inverse relationship was apparent between body size and N isotopic turnover rates (McIntyre and Flecker 2006). This finding is not

unexpected considering the mass dependence of animal's metabolic rate (e.g. Gillooly et al. 2001).

Yellowfin tuna tissue turnover rates are more rapid than what might be predicted by body mass alone and appear to approach rates closer to endothermic organisms (Tieszen et al. 1983). Although the yellowfin tuna examined in the present study were juveniles, their body mass is much larger than other fish species that have been subjected to an experimental isotope diet shift (see review in McIntyre and Flecker 2006). When comparing the juvenile tuna nitrogen turnover rates to values reported for other fish (Hesslein et al. 1993; MacAvoy et al. 2001; Maruyama et al. 2001; Miller et al. 2001; Bosely et al. 2002; Suzuki et al. 2005; McIntyre and Flecker 2006), tuna turnover rates are faster than would be predicted by mass alone. In addition to the effects of body size on metabolism, tissue turnover rates are positively correlated with environmental temperature (e.g. Bosely et al. 2002; McIntyre and Flecker 2006). Because tunas maintain an internal body temperature higher than the ambient environment and because the difference between body temperature and ambient temperature is variable and appears to be under the control of the individual fish (Carey 1973; Holland et al. 1992), the effect of ambient water temperature on tissue turnover rates may be less pronounced in tuna than in ectothermic fish. Thus, the higher tissue turnover rates observed in tuna may be another manifestation of endothermy in tunas and their unique physiology and adaptation for high-performance swimming in the tropical pelagic environment.

3.5.1 Experimental Diet Shift

In the experimental setting, turnover rates were distinct among some of the tissues examined. These data confirm that by sampling a suite of tissues from a top marine predator, information can be derived on food habits on varying temporal scales. If natural isotope gradients exist, then the $\delta^{15}N$ values of these tissues should also provide insight on the movements of highly-mobile marine predators. In the natural environment, organisms rarely reach a steady state with their diet because food resources can often vary spatially and temporally (e.g., Fry et al. 2003; O'Reilly et al. 2002). It can be generally assumed that a steady state isotopic composition corrected for the TEF value would occur after three half-lives. By that time, the majority of an animal's mass (88%) would acquire the new isotopic signal of the local prey or the local isotope 'tag'. Following this general assumption, if, as in this study, isotope values differ by 6‰ between two diets, 88% turnover will result in a fairly close approximation of the new diet with less than 0.7‰ difference remaining between diets. Applying these calculations to the juvenile tuna of this study, nitrogen in the tissue with the slowest turnover rate (white muscle tissue) would represent an accumulated foraging history of at least 6 to 7 months whereas the temporal resolution for liver would be 1 to 2 months. Isotopic analysis of gill filaments that have high protein synthesis and turnover rates to facilitate ionic exchange, waste removal, and respiration (Lyndon and Houlihan 1998) might be another viable option for examining short term shifts in diet. To investigate long-term trends in the diet of top pelagic predators, biologically conserved material (e.g. otoliths, spines, vertebrae) would be the most appropriate tissue to analyze (e.g. Estrada et al. 2006). Any observed isotopic disparity between these different tissues greater than seen

in an animal kept on a constant diet (i.e. between-tissue isotopic differences), would then indicate a recent diet shift or movements from a region with a different isotopic baseline.

The low TEF values recorded for wild and captive tuna, and the disparity between their TEF values could relate to nitrogen excretion and assimilation processes. Previous studies have demonstrated that TEF values are lower for organisms (a) with a NH_4^+ excretion pathway (i.e., ammonotelic organisms) (Vander Zanden and Rasmussen 2001; Vanderklift and Possard 2003), (b) with a diet higher in nitrogen content (e.g. carnivores) (Adams and Sterner 2000; Vander Zanden and Rasmussen 2001; Vanderklift and Possard 2003), (c) with fast growth rates (Trueman et al. 2005), and (d) in marine environments (Vanderklift and Possard 2003). Thus, low TEF values would be expected for tuna because they are ammonotelic marine predators with fast growth rates. The disparity in captive and wild tuna TEF values could relate to a number of factors, especially since an experimental setting often can not simulate natural environmental conditions. One measured difference between the wild and captive groups was in the nitrogen content of the diets, with a lower average C/N value in the captive diet than the wild diet. This higher nitrogen content of the captive diet could potentially explain the lower TEFs observed in this group of tuna. However, it is difficult to speculate on the mechanisms influencing the TEFs, especially N use efficiency (Vanderklift and Possard 2003; Trueman et al. 2005), because nitrogen excretion, N assimilation, and growth rates were not measured in this study. The disparity in TEFs observed in captive and wild individuals could have resulted from a number of factors, but it is quite clear that the duration of the experiment should have been extended to assess if the experimental TEFs would approach the natural values. However, it was difficult to maintain tuna in the tank

for these longer residence times, mainly because of incidental wall strikes and potentially vitamin deficiencies. These problems could be overcome with modest modifications of the HIMB facility such as painting stripes on the tank walls to provide better visual cues to the captive fish and adding readily-available vitamin supplements to the captive diet. Overall, many factors can contribute to variability observed in TEF values (Vanderklift and Possard 2003; Trueman et al. 2005), but this study supports a TEF value for yellowfin tuna WMT much less than the standard 3.4‰ reported in the literature (see Post 2002).

3.5.2 Natural Diet Shift

The ontogenic shift in wild juvenile yellowfin tuna $\delta^{15}N$ values demonstrated that tissues respond quickly to a dietary switch. Liver tissues in the field and experimental setting showed similar turnover rates, suggesting that the basal metabolic activity of the captive animals was not significantly different from those in the natural environment. However, a significant difference was observed between experimental and field estimates of white muscle turnover rates, with a much faster WMT turnover rate observed in the individuals in the wild relative to the captive group (Fig. 3.3). The most likely explanation for the faster turnover in WMT of the wild animals seems to be growth dilution (Fry and Arnold 1982; Hesslein et al. 1993; MacAvoy et al. 2001), with rapid growth dynamics present in wild animals but limited in the captive individuals. Another possibility is that the locomotor activity of the captive fish was lower than that of the wild fish and that this reduced the rate of WMT turnover.

3.5.3 Implications for Tracking Tuna Movements

Although there are very few published $\delta^{15}N$ data for top predators in the equatorial Pacific, coherent spatial patterns have been observed at the base of the food web (e.g. Farrell et al. 1995; Altabet 2001). In general, high $\delta^{15}N$ values are broadly associated with areas with a predominance of water column denitrification (e.g. oxygen minimum zone in the eastern tropical Pacific) (Farrell et al. 1995; Voss et al. 2001) or a high degree of nitrate utilization by phytoplankton in the upper euphotic zone (Altabet 2001), and low $\delta^{15}N$ values associated with enhanced nitrogen fixation (e.g. western Pacific warm pool and gyres) (Dore et al. 2001; Deutsch et al. 2007). If predator movements are relatively restricted within these different environmental regions, these baseline isotope values should propagate up through the food web and be expressed in the $\delta^{15}N$ values of the top pelagic predators. Our preliminary isotope data on top predators show spatial coherence and $\delta^{15}N$ differences as large as 12‰ in the equatorial Pacific Ocean (Graham et al. 2006) and as large as 8‰ in the eastern equatorial Pacific Ocean (Popp et al. 2007). Popp et al. (2007) used amino acid-specific nitrogen isotopic analyses of adult yellowfin tuna collected from the eastern tropical Pacific (ETP) to demonstrate that the variations recorded in top predator bulk $\delta^{15}N$ values were driven by isotope shifts at the base of the food web. Specifically, adult yellowfin tuna $\delta^{15}N$ values increased by 5.2‰ over a N-S distance of approximately 3600km in the ETP, from 8°S to 20°N (Popp et al. 2007). If tuna migrated extensively throughout this region, isotopic variation apparent at the base of the food web would be integrated into the tuna tissues. Popp et al. (2007) also showed that bulk $\delta^{15}N$ values of mesozooplankton exhibited the same geographical trend as the $\delta^{15}N$ values of bulk WMT of yellowfin tuna in this region.

Mesozooplankton, which are short-lived organisms at low trophic levels, are more likely to track spatial and temporal changes in nutrient dynamics than long-lived, highly-mobile predators. The similar spatial pattern in tuna and mesozooplankton bulk $\delta^{15}N$ values provides further evidence that the $\delta^{15}N$ values of tuna in this region reflect isotopic variations at the base of the food web (Popp et al. 2007). Thus, the nitrogen isotope gradient in tuna suggest that although yellowfin are capable of basin-wide migrations, in the ETP they must have a relatively high level of regional residency such that long-distant N-S movements within this region must be restricted to less than the time required for the tissues to reach a steady state isotopic value with the local isotopic baseline.

With the tissue turnover rates determined from this study, we can begin to place constraints on the maximum net directional movement that would be required to maintain the nitrogen isotope gradient observed in the ETP. Assuming an overall WMT turnover rate averaging 0.02 N day^{-1} (range 0.006-0.022 N day^{-1}), the length of time to shift (i.e. 3 half-lives) to the observed ETP gradient of 5.2‰ would be approximately 260 days. From these simple distance and time estimates, a net N-S meridonal movement estimate can be calculated that would maintain the observed N-S spatial $\delta^{15}N$ gradient (i.e. 3600 km/260 days = 14 km day^{-1} or a range of 11 to 21 km day^{-1}). Combining tissue turnover rates determined here with the observed gradient in yellowfin tuna $\delta^{15}N$ values discussed in Popp et al. (2007) suggests that tuna could not have exceeded a net meridonal movement of approximately 14 km day^{-1} in the eastern tropical Pacific. This net N-S movement estimate is conservative for at least 3 reasons. First, in this illustrative example, E-W movements are not considered although they could play an important component in yellowfin movements, especially if a gradient in prey availability occurs

along that axis. Data from acoustic tags placed on adult yellowfin in the ETP demonstrate that the majority of net movements were in the meridonal (N-S) axis and E-W net movements were much more restricted (e.g. Carey and Olson 1983). Second, the diet of yellowfin would change gradually as it migrates in the pelagic environment and not instantaneously or rapidly as was the case in the experimental diet shift of this study. Finally, the tissue turnover rates estimated in both the natural and experimental settings were for juvenile yellowfin tuna. Juveniles generally have higher tissue turnover rates than adults mainly because of differences in their growth rates (e.g. Fry and Arnold 1982; Bosley et al. 2002; Trueman et al. 2005). Therefore, to maintain the observed spatial patterns in yellowfin tuna $\delta^{15}N$ values in the eastern tropical Pacific, the maximum net (directional) N-S movement might be significantly less than our estimate of 14 km day^{-1}.

Tuna movements derived from bulk isotopic values and tissue turnover rates can be assessed by comparison with results obtained from individual-based sonic and electronic tagging studies. Carey and Olson (1983) measured average movement rates of yellowfin tuna in the ETP as 1 m s^{-1}, which is equivalent to a straight-line distance of approximately 86 km day^{-1}. Schaefer et al. (2007) estimated mean speeds for yellowfin carrying archival tags off Baja California of 76 ± 58 km day^{-1} (mean $\pm$ 95%CI), where average accuracy of the tag-estimated recapture position was 108 km. If these mean swimming speed estimates were directional and maintained over the region, no gradient would have been observed in yellowfin $\delta^{15}N$ values in the ETP because tuna tissue turnover rates are rapid enough to average that isotopic variation over this spatial scale. Thus, when spatial isotope gradients exist at the base of the food web (Graham et al. 2006), the baseline values are propagated up the food web to top predators (Popp et al.

2007) and given the tissue turnover rates that we observed, stable isotope techniques can potentially set limits information on the net movements of tropical tunas. Movements determined by these isotope tags could then be included in present fisheries models (Hampton and Fournier 2001; Lehodey et al. 2003) to strengthen population-level models of movements of tropical tunas in the Pacific Ocean. Collecting tropical tuna samples for bulk $\delta^{15}N$ analysis during different time periods (e.g. seasonal, interannual, during an El Niño phase, etc.) could also provide information on changing patterns of net movement over varying temporal scales or in relation to large-scale changes in the oceanography of the equatorial Pacific Ocean.

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3.7 References

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Table 3.1 Calculated tissue turnover rates for captive and wild diet shifts (Eqn 1 and 3, respectively). Tissue turnover rates (b) represents the calculated exponential fit of the model (N day^{-1}) and are expressed as half-lives (HL) in days (Eqn 2), Y_0 is $\delta^{15}N$ value (‰) at the start of the diet switch, and Y_n is the expected $\delta^{15}N$ value (‰) at the asymptote. LS represent the least-squares best estimate and 95% CI represent 95% confidence intervals.

Captive Diet Shift								
	B		***Y_0***		***Y_n***		***HL***	
	LS	95% CI	LS	95% CI	LS	95% CI	LS	Range
WMT	0.011	*0.006 - 0.022*	6.59	*6.39 - 6.74*	14.61	*12.32 - 19.30*	62.9	*30.9 - 123.8*
RMT	0.018	*0.009 - 0.030*	7.72	*7.45 - 7.95*	15.03	*13.59 - 18.64*	39.4	*23.1 - 81.5*
LVR	0.057	*0.044 - 0.720*	5.45	*5.13 - 5.74*	13.34	*12.67 - 13.84*	12.2	*1.0 - 15.8*
GLF	0.035	*0.026 - 0.051*	6.05	*5.82 - 6.22*	12.72	*12.28 - 13.17*	19.9	*13.6 - 26.7*
Wild Diet Shift								
	b		***Y_0***		***a***		***HL***	
	LS	95% CI	LS	95% CI	LS	95% CI	LS	Range
WMT	0.035	*0.024 - 0.058*	6.23	*5.88 - 6.45*	0.0066	*0.0001 - 0.0538*	19.7	*11.9 - 29.0*
LVR	0.026	*0.010 - 0.046*	5.17	*3.37 - 5.78*	0.0343	*0.0007 - 0.7110*	27.1	*15.2 - 71.1*

Table 3.2 Estimated trophic enrichment factors (TEF), or the difference between $\delta^{15}N_{organism}$ and $\delta^{15}N_{diet}$ from the captive and wild diet shift datasets. The average natural diet is based on an 85% characterization of juvenile yellowfin tuna diet, resulting in a TEF field estimate of 2.1 for white muscle tissue (Graham et al. 2007). The captive tissue $\delta^{15}N$ values are the $\delta^{15}N$ values estimated for the end of the experimental period or Y_0 (‰), the modeled $\delta^{15}N$ asymptote.

Natural Diet Shift Average $diet_{field}$ = 4.4 ± 0.5 ‰			**Experimental Diet Shift** Average $diet_{exp}$ = 13.4 ± 0.7 ‰		
Tissue	$\delta^{15}N$, ‰	TEF	Tissue	$\delta^{15}N$, ‰ (Y_0)	TEF
LVR	5.7 ± 0.7	1.3	LVR	13.3	-0.1
WMT	6.5 ± 0.3	2.1	WMT	14.6	1.2
GLF	5.8 ± 0.6	1.4	GLF	12.7	-0.7

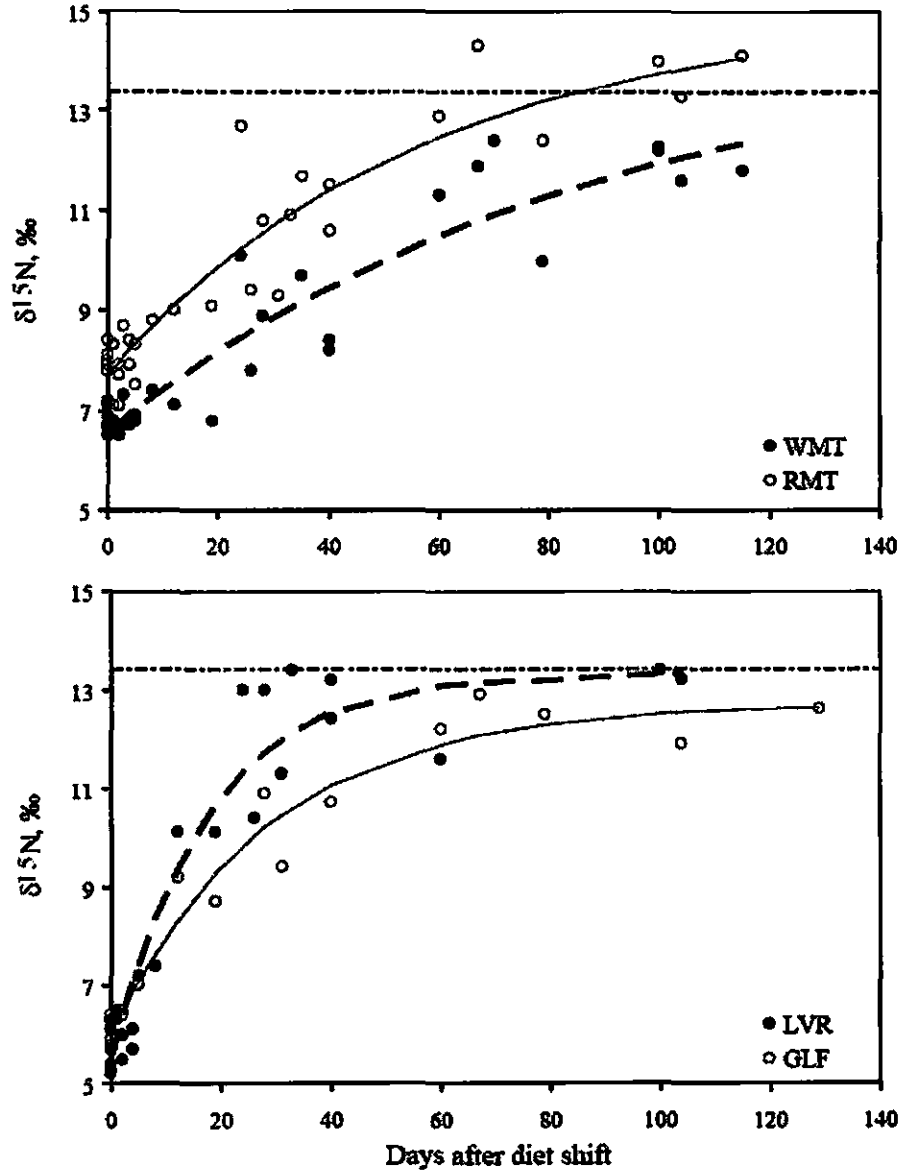

Figure 3.1 The $\delta^{15}N$ of captive yellowfin tuna plotted as a function of the time since the onset of the diet shift. The lines are the exponential model fits (Eqn 1) for the different tissues analyzed. The dashed line at 13.4‰ represents the $\delta^{15}N$ value of the captive diet. *Top panel*: white muscle tissue (WMT) and red muscle tissue (RMT). *Bottom panel*: liver tissue (LVR) and gill filaments (GLF). These results indicate that the time required

for 88% equilibration (i.e. 3 half-lives) with the diet is ~190 days for WMT, ~120 days for RMT, 57 days for GLF, and 36 days for LVR.

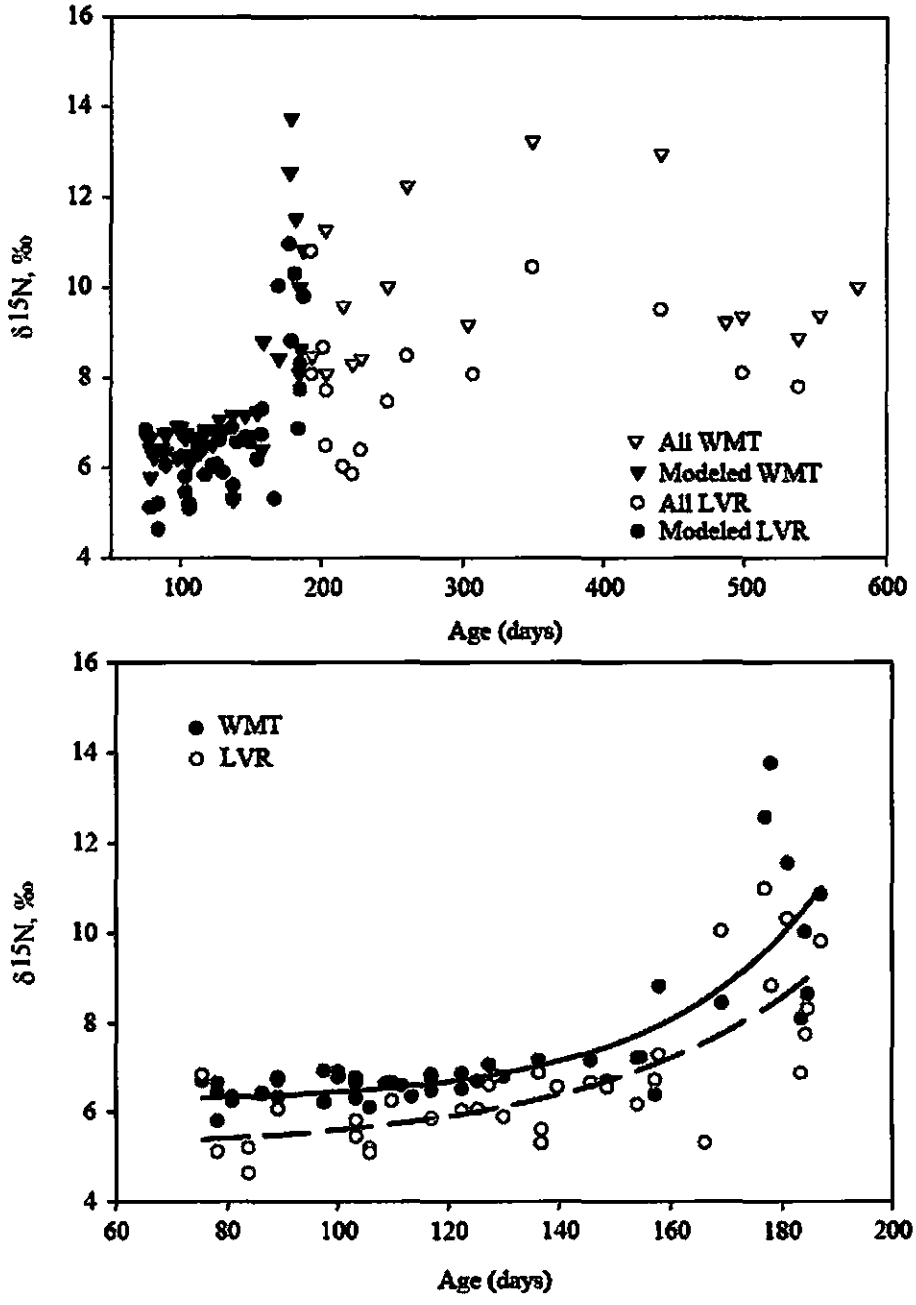

Figure 3.2 Natural diet shift in juvenile yellowfin tuna using the results of Graham et al. (2007). *Top panel*: $\delta^{15}N$ values of yellowfin tuna white muscle (WMT) and liver (LVR) tissues versus estimated tuna age in days (Lehodey and Leroy 1999). Black symbols represent the sample data used was used to calculate tissue turnover rates and is shown in

the bottom panel. *Bottom panel*: Exponential model fit (Eqn 3) to the natural diet shift observed in juvenile yellowfin tuna WMT and LVR tissues.

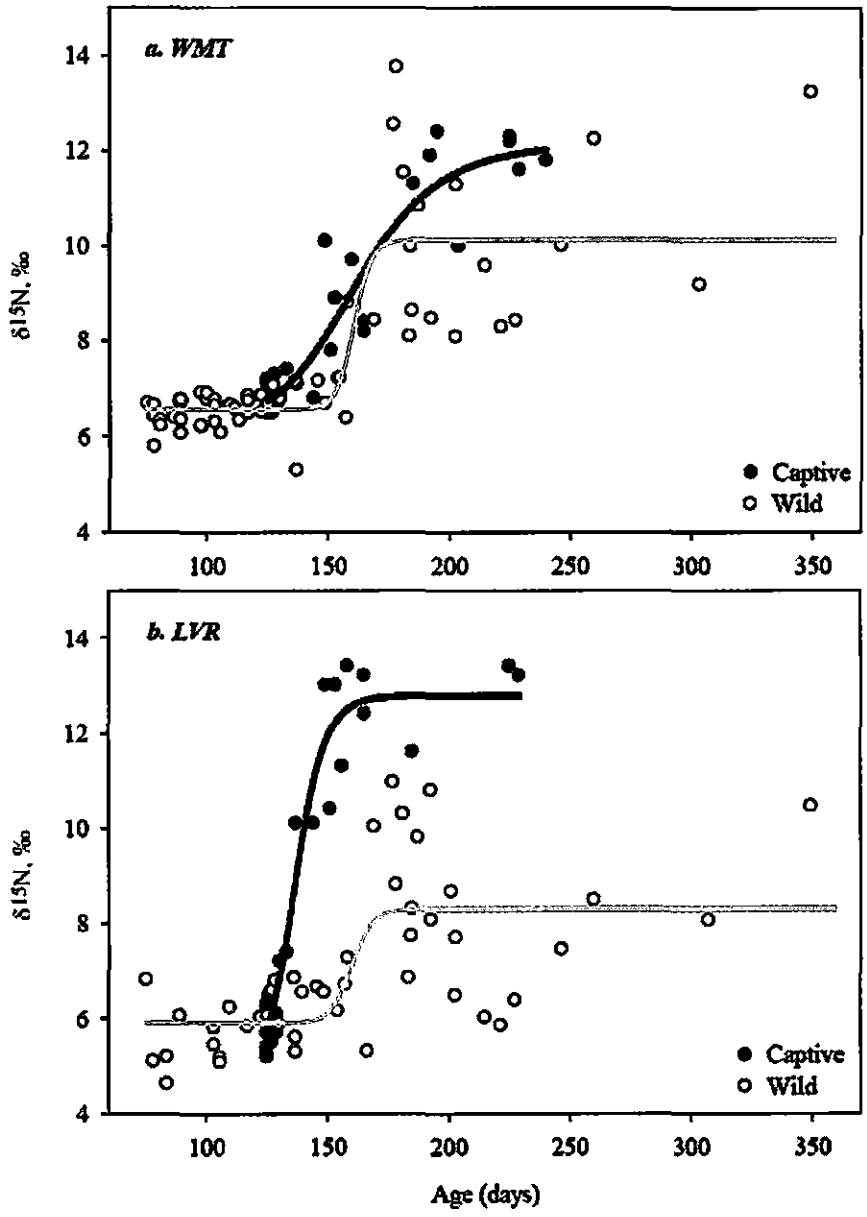

Figure 3.3 A comparison of captive and wild diet shifts in juvenile yellowfin tuna along a common temporal axis. The age of captive tuna was based on the average length of all captive tuna being 37cm ± 2cm and this length corresponds to an age of approximately 125 days (i.e., 1 day residence time in the tank was set at 125 days of age; Lehodey and Leroy 1999). The models presented for (a) white muscle tissue and (b) liver measured in

wild and captive tuna are the same models presented in Graham et al. (2007) and in this study, respectively.

CHAPTER 4

Bulk $\delta^{15}N$ cartography: a novel approach to examining directed movements of tropical tunas in the equatorial Pacific Ocean.

4.1 Abstract

The stable isotopic compositions of animal tissues can serve as internal chemical recorders or tags that provide information about their movements. By mapping the $\delta^{15}N$ and $\delta^{13}C$ values of three top-level pelagic predators, yellowfin (*Thunnus albacares*), bigeye (*T. obesus*), and skipjack (*Katsuwonus pelamis*) tuna throughout the equatorial Pacific Ocean (eqPac), I demonstrate systematic geographic isotopic variation and that this "isotope cartography" approach can begin to characterize regional residency and directional movement patterns in tropical tunas. If tropical tuna were highly migratory or a uniformly mixed population, then little geographical isotopic variation would be observed because regional isotopic differences would be integrated over space and time. Tropical tunas exhibited high spatial variability in their $\delta^{15}N$ values (~12‰), which strongly suggests these species exhibit a large degree of regional residency or site fidelity in the eqPac. Combining tuna $\delta^{15}N$ values with tissue turnover rates measured in yellowfin tuna and then comparing these data to the $\delta^{15}N$ values at the base of the food web , I estimated directed or straight-line movements (km day^{-1}) for the three different species in two regions of the equatorial Pacific Ocean. Bigeye tuna demonstrated higher (north-south) directed movements in the central eqPac, whereas yellowfin had the lowest

directed movement rates along both N-S transects in the central and eastern eqPac and demonstrated the greatest regional residency. Skipjack tuna had intermediate movements relative to bigeye and yellowfin tuna. The movement estimates for skipjack and yellowfin tuna were higher in the ETP than the CTP EqPac. I discuss the potential mechanisms occurring at the base of the food web that could produce the spatial variability observed in tropical tuna $\delta^{15}N$ values. Overall, isotope cartography of tropical tunas suggests restricted (directed) movements at the population-level and that its occurrence is a common feature throughout the equatorial Pacific.

4.2 Introduction

Industrial fisheries have removed fifty million tons of tuna from the Pacific Ocean since the 1950s (Sibert et al. 2006). The majority of this exploited biomass has been tropical tunas removed from the equatorial Pacific Ocean (i.e., 20°N to 20°S). In order to develop the most successful fisheries management strategies and determine population dynamics, movement patterns for these highly mobile pelagic predators must be accurately determined. The inability to directly observe migratory predators and the massive extent of their pelagic habitat has hindered our understanding of their movements. Recently these challenges have spurred the development of new technologies and novel methods to remotely examine animal movements, especially by tracking fish that carry acoustic (Carey and Olson 1982; Holland et al. 1990; Brill et al. 1999; Dagorn et al. 2000) and sophisticated satellite tags (Holland and Sibert 1994; Block et al. 1998; Lutcavage et al. 1999). These electronic tagging studies are generally limited

to studying individual movements, but the launch of extensive electronic tagging programs (e.g., Block et al. 2005) and advancements in tag-to-tag communication (Holland *personal communication*) will circumvent the difficulties of interpreting movement patterns based on only a small number of individuals.

Some species of tropical tunas associate to fixed (e.g., seamounts, fish aggregating devices) and dynamic (e.g., fronts, meso-scale eddies) oceanographic features (Holland et al. 1990; Holland et al. 1999; Polovina et al. 2001; Howell et al. 2006). However, tuna movements between these sometimes distant oceanic features are not well understood. On a basin-wide scale, there is some evidence from conventional tag and recapture programs of restricted mixing between the eastern Pacific Ocean (EPO) and the western and central Pacific Ocean (WCPO) (e.g., Hampton 2002a; Hoyle and Maunder 2006; Maunder and Hoyle 2006). Hence, integrated stock assessments of tropical tunas in the Pacific Ocean are currently developed for the entire tropical Pacific basin (Sibert et al. 2006) or the EPO and WCPO management regions (Hampton 2002a; Hoyle and Maunder 2006; Maunder and Hoyle 2006). If tropical tuna movements are further restricted within these large oceanic regions, where commercial fishing effort is highly variable then current exploitation rates may be locally unsustainable. Several conventional and electronic tagging studies have recently demonstrated restricted movements in unassociated bigeye tuna (*Thunnus obesus*) and yellowfin tuna (*T. albacares*) tracked around the Hawaii Islands (Adams et al. 2003; Sibert et al. 2003), bigeye tracked in the Coral Sea (Gunn et al. 2005), and yellowfin tracked in the north EPO near Baja California, Mexico (Schaefer et al. 2007). If higher spatial fidelity is not recognized, then managing tropical tuna fisheries on a basin-wide or sub-basin scale

could lead to serious localized depletions in these regions where tuna have restricted movements. Thus, to produce the most successful stock assessments and maintain sustainable tuna fisheries, tuna movements throughout the equatorial Pacific must be determined and incorporated into spatially-structured, integrated stock assessment models.

Stable isotope analysis has been extensively applied to examining short and long-distance animal migration in terrestrial, freshwater, and coastal ecosystems (Chamberlain et al. 1997; Kelly and Finch 1998; Fry et al. 2003; Gillanders et al. 2003). The general concept is to use the stable isotope value of an organism as a "natural tag" to trace their movements through ecosystems (Fry 1981). While foraging in specific environments the tissues of individuals and populations acquire the isotopic compositions or natural tags of the local prey that then can be used to locate migration pathways, locate breeding regions, and map species dispersals (Wolf and Martínez del Rio 2000; Hobson et al. 1999; Rubsenstein and Hobson 2004; Hobson et al. 2005; Cherel and Hobson 2007). For example, in coastal ecosystems, stable isotope techniques were applied to estimate patterns of shrimp residency and movements (Fry et al. 2003). Residents had $\delta^{13}C$ and $\delta^{15}N$ values similar to their local environment, whereas the values of migratory individuals were distinctly different. Determining habitat use by resident and migrant shrimps provided information on which habitats were most essential for sustaining coastal shrimp fisheries (Fry et al. 2003). Thus, by determining an animal's isotopic composition and relating it to the baseline isotope values observed in the local environment, animals can be linked to particular regions and, in turn, this new

understanding in their habitat utilization can aid in their conservation and management (Hobson 1999; Fry et al. 2003; Hobson et al. 2005; Cherel et al. 2006).

Applying a similar chemical tag approach to study tropical tuna movements in the equatorial Pacific requires considerable systematic variation in the tracer of interest within the environment. Carbon isotopic compositions of consumers often reflect that of marine algae, which generally have high $\delta^{13}C$ values associated with rapid growth rates of large phytoplankton and low values associated with slow growth rates in small phytoplankton (Cifuentes et al. 1988; Fry and Wainright 1991; Goericke et al. 1994; Popp et al. 1998). In the equatorial Pacific, variations in nutrient limitation and hydrological regimes (e.g., upwelling vs. water column stratification) can lead to variations in the relative growth rates of marine phytoplankton, and, in turn, variations in baseline $\delta^{13}C$ values (Bidigare et al. 1999). A much larger isotopic variation in baseline $\delta^{15}N$ values is expected because of variations in the isotope value of the nitrogen sources (e.g., N_2 vs. NO_3^-) and the isotopic fractionation associated with the biologically-mediated transformations of these nitrogen sources (e.g., NO_3^- assimilation or denitrification). $\delta^{15}N$ values of the base of the food web, as indicated by samples of surface particulate nitrogen (PN), sinking PN, and deep-sea sediments, exhibit large variations and coherent geographical patterns in the equatorial Pacific Ocean and have been related to changes in the $\delta^{15}N$ values of the nitrate assimilated by phytoplankton (e.g., Altabet 1988; Altabet and François 1994; Farrell et al. 1995; Altabet 2001). For example, Altabet (2001) showed 10‰ variation in the $\delta^{15}N$ values of PN from 12°S to 12°N, along a meridional transect in the central Pacific. As marine animals forage in these isotopically-distinct environments, the baseline variations should propagate up the

food web in a systematic manner and be reflected in their tissues if the animals are long-term residents of these environments.

During assimilation, discrimination of ^{15}N and ^{13}C occurs at each successive trophic level (e.g., Minagawa and Wada 1984). This isotopic discrimination between a consumer and its prey, referred to as the trophic enrichment factor or TEF, has been shown to be on average 3.4‰ and 0.7‰ increase in $\delta^{15}N$ and $\delta^{13}C$ values, respectively (Deniro and Epstein 1978, 1981; Post 2002). A lower TEF has been observed in marine predators and tropical tuna that excrete NH_4^+ as their primary nitrogen waste product (Adams and Sterner 2000; Vander Zanden and Rasmussen 2001; Vanderklift and Possard 2003; Graham et al. 2007; Chapters 1 and 3). Therefore, an animal's $\delta^{15}N$ value depends upon the $\delta^{15}N$ value of phytoplankton and the predator's trophic level. If there are gradients in the $\delta^{15}N$ values of phytoplankton in the pelagic ecosystem and the trophic level of a predator remained relatively constant over habitats, then the $\delta^{15}N$ value of a resident fish would reflect this baseline $\delta^{15}N$ value plus the accumulated TEF. Alternatively, the $\delta^{15}N$ value of a migratory fish would reflect its accumulated TEF plus the average baseline $\delta^{15}N$ value of all habitats it had foraged within and the value would be anomalous from the local residents in a given location.

The main objective of the present study was to determine the $\delta^{15}N$ values of tropical tuna and use these values as natural tags to examine population-level habitat utilization and movements in the equatorial Pacific Ocean. This isotope cartography approach could provide an opportunity to geographically identify tunas to specific regions within the equatorial Pacific. Furthermore, estimating directed movement rates of tropical tunas with stable isotope analysis provides an important step towards

comparative analysis between biogeochemical data, tagging datasets, and fisheries models with the ultimate goal of maintaining sustainable tropical tuna fisheries.

4.3 Material and Methods

4.3.1 Study Site

The equatorial Pacific is a temporally and spatially dynamic system that is dominated by two large biogeochemical provinces, an oligotrophic region in the west and a high nutrient-low chlorophyll (HNLC) region in the east (e.g., LeBorgne et al. 2002). Trade winds generally blow to the west across the equatorial Pacific Ocean, piling up warm surface water and depressing the thermocline to create a warm pool in the western equatorial Pacific (Wyrtki 1975; McPhaden 1999). In the eastern and central equatorial Pacific the trade winds drive a divergence of surface water at the equator, which is then advected poleward by Ekman transport. These forces set up a strong, persistent upwelling zone that extends from the coast of South America to the eastern boundary of the warm pool. The cold tongue region of the equatorial Pacific is characterized as an HNLC region because limited micronutrients (e.g., iron), effective microzooplankton grazing, or a combination of both restricts the maximal utilization of macronutrients by marine phytoplankton in the euphotic zone (Martin et al. 1990; Coale et al. 1996; Landry et al. 1997). In contrast to the cold tongue, the warm pool is comparable to the oligotrophic subtropical gyres where biological production is lower and can be controlled by macronutrient injection into the surface mixed layer (Le Borgne et al. 2002). The spatial extent of both the warm pool and cold tongue can vary spatially and temporally,

especially in relation to El Nino/Southern Oscillation (ENSO) events (Wyrtki 1975; McPhaden 1999).

4.3.2 Sample Collection

We report $\delta^{15}N$ and $\delta^{13}C$ values for three top pelagic predators, yellowfin (YFT), bigeye (BET), and skipjack (SKJ) tuna collected throughout the equatorial Pacific Ocean. In the eastern tropical Pacific, YFT, BET, and SKJ were captured by purse-seine fishing vessels between 16 August 2003 and 6 December 2004, and were sampled on board the vessels by observers of the Inter-American Tropical Tuna Commission (IATTC 2004). In the western and central tropical Pacific, these tropical tunas were captured by long-line and by purse-seine fishing vessels and were sampled on board the vessels by observers from the national observer programs of the region (i.e., Papua New Guinea, Federated States of Micronesia, Solomon Islands, Fiji, Marshall Islands, New Caledonia, French Polynesia, Wallis & Futuna). Longline fish were captured between 12 March 2001 and 29 September 2005 in a large oceanic region (8°58N-21°41S and 145°46E-146°05W) and these fish measured between 44 and 155cm (bulk of the distribution 90-120cm, average length 107.8cm). Purse-seine fish were caught between 8 May 2001 and 28 August 2005 mainly in the equatorial area (4°20N-7°30S and 138°54E-176°39W) and these fish measured between 27 and 158cm (bulk of the distribution 40-55cm, average length 56.9cm).

With each collection event, the geographical location was recorded (latitude and longitude), the fish were measured fork length (FL, cm), samples of white muscle tissue (WMT) were removed from the dorsal region adjacent to the second dorsal fin, and the

tissue samples were stored at -20°C until processed further. In the western and central Pacific long-line samples, WMT samples were removed from the anal region (to avoid damage to these high-value fish). Stomachs and liver samples were also collected, but the data reported here are only for WMT. In the eastern tropical Pacific (ETP), subsamples of WMT from up to 6 individuals per purse-seine set and size class (<90 and ≥90 cm FL) were combined into one sample for stable isotope analysis. Due to the sporadic sampling in the western and central tropical Pacific (WTP and CTP, respectively), WMT samples from individuals were analyzed for isotope analysis. After collection, ETP, CTP, and WTP tuna were transported in freezers to shore-based laboratories and stored at -20°C. At the shore-based laboratories tuna WMT samples were lyophilized or oven dried (60°C 24-48hrs) and then shipped to the University of Hawaii's Isotope Biogeochemistry Laboratory for stable isotope analysis.

To begin to assess net tuna movements in the eqPac, $\delta^{15}N$ values of particulate nitrogen (PN) collected in the equatorial Pacific Ocean were compared to the $\delta^{15}N$ values of tuna. The first set of PN samples were collected during a Tropical Atmosphere Ocean (TAO) project cruise aboard the R/V Kaimimoana (cruise GP1-05-KA) from 18 Feb to 25 Mar 2005. Near-surface water was collected with an uncontaminated seawater line from approximately 9°S to 11°N, along the 125°W meridian transect. PN samples were collected by filtering the near-surface seawater onto pre-combusted 25mm Whatman GF/F Filters, with the filtrate volume determined by the timing of filter saturation (1.5-8L). The second PN $\delta^{15}N$ dataset was taken from Altabet (2001). This dataset was collected in August 1992 for the U.S. JGOFS EqPac program and corresponded to a period of relatively normal conditions in the equatorial Pacific (i.e., the S2 transect or

non-El Niño cruise). The Altabet (2001) PN dataset was collected along a latitudinal transect at 145°W in the central tropical Pacific and the collection methods were comparable to those used to collect the PN datasets aboard the R/V Kaimimoana.

4.3.3 SIA Analysis

Lipids were not removed from tuna WMT samples, because the C/N (mol%) of tuna WMT was close to protein (average C/N = 3.5) (Graham et al. 2007). Prior to isotope analysis, all tuna tissues were homogenized with mortar and pestle and POM samples (i.e., GF/F filters) were dried, cut in half, and encapsulated in tinfoil cups. Sample nitrogen and carbon isotope values were determined using an on-line CN analyzer (Carlo Erba NC2500) coupled (ThermoFinnigan ConFlo II) to an isotope ratio mass spectrometer (ThermoFinnigan Delta S). Isotope values are reported in standard δ-notation relative to atmospheric N_2. A sample of glycine in which the $\delta^{13}C$ and $\delta^{15}N$ values were well characterized was measured after every 10 samples as a measure of the accuracy of isotope measurements. Several samples were also measured in duplicate or triplicate and the analytical error associated with all of these measurements was typically $\leq 0.2‰$.

4.3.4 Isotope Cartography

The spatial distribution of $\delta^{15}N$ values of tuna or the "isotope maps" were constructed with MATLAB v. 7.0 (Mathworks, Natick, Massachusetts, USA). The latitude and longitude of the sample locations were transformed into uniform surface arrays that were then used to fit a surface, Z, where Z is the isotope value interpolated for

a location. Data were gridded using a triangle-based linear interpolation and the Z surface always bisected the measured data points. Because the spatial coverage of BET samples was limited and the $\delta^{13}C$ spatial variability low (relative to the other 2 tuna species), only YFT and SKJ $\delta^{13}C$ values and C/N ratios were mapped for the entire equatorial Pacific.

4.3.5 Tropical Tuna Movements

To estimate movements of tropical tunas in the equatorial Pacific based on isotope cartography, a temporal scale on which to interpret the observed bulk $\delta^{15}N$ patterns is required. The average turnover rate of white muscle tissue in juvenile wild and captive yellowfin is 0.02 N day^{-1} and the half-life is approximately 35 days (half-life = ln(0.5)/turnover rate) (Chapter 3). For tuna to reflect the $\delta^{15}N$ value of the base of the food web we assume the majority of their mass must reach a steady state with the local $\delta^{15}N$ value. Therefore, after 3 to 4 months (3 half-lives or 88% mass turnover), tuna $\delta^{15}N$ values should reflect the local baseline $\delta^{15}N$ value. The $\delta^{15}N$ values of these resident tuna would then be:

$$\delta^{15}N_{TUNA} = \delta^{15}N_{BASELINE} + ((TL_{TUNA} - 1) \times TEF) \,, \qquad (1)$$

where the trophic level (TL) of yellowfin tuna in the ETP has been estimated as approximately 4.6 (Olson and Watters 2003; Popp et al. 2007), the field-based trophic enrichment factor (TEF) for yellowfin tuna is 2.1‰ (Graham et al. 2007), and the $\delta^{15}N$ values of PN is assumed to be the baseline and at TL = 1. Particulate nitrogen collected on a filter might not best represent the available nitrogen to the food web because it does not consist entirely of phytoplankton and could include components that are not actively

grazed upon by primary consumers. An alternative to PN samples is to sample a primary consumer, such as zooplankton (e.g., Kline 1993). However, the collection of zooplankton samples is time intensive and unless net samples are sorted to the species level, the sample also includes a range of trophic guilds. Accordingly, to estimate directional movement rates of tunas, I compared the $\delta^{15}N$ values of tunas to the values of the base of the food web, or PN, with the assumption that resident tuna have $\delta^{15}N$ values ~7.6‰ higher than the local isotopic baseline.

In Chapter 3, the N-S net movement of YFT was determined by calculating the directional swimming speed that could not be exceeded to maintain the observed latitudinal gradient in the $\delta^{15}N$ values of YFT at 135°W in the ETP. This approach does not consider whether the observed variation in tuna $\delta^{15}N$ values reflects the baseline $\delta^{15}N$ values. Animals are residents if their $\delta^{15}N$ values approach the $\delta^{15}N$ value of the local environment taking into account the TEF (e.g., Fry et al. 2003). To further examine the degree of residency demonstrated by tropical tuna, isotope samples of (a) yellowfin, bigeye, and skipjack collected from the eastern tropical Pacific (ETP; 125°W ± 15° longitude) were compared to the $\delta^{15}N$ values of PN samples collected along the same meridional transect and (b) yellowfin and skipjack collected from the central tropical Pacific (CTP; 140°W ± 15° longitude) were compared to the $\delta^{15}N$ values PN samples collected by Altabet (2001) along a 140°W meridional transect. Because BET samples were very limited around 140°W their movements were not examined in this region. North-south, directed movements (km day^{-1}) of tuna were estimated at the two different transects by calculating the maximum movement rate that could not be exceeded to maintain the observed nitrogen isotopic gradient or isogradient (i.e., an observed linear

change in the isotope values of the base of the food web or PN). The time required for the $\delta^{15}N$ value of a tuna to reflect the isotope gradient is simply the isogradient (in ‰) divided by the white muscle tissue (WMT) turnover rate (b) (Chapter 3). I choose an average WMT turnover rate based on the natural and experimental diet shift experiments of 0.02 N day^{-1}. Therefore, a simple approximate of the directed movement is the time required to reflect the isogradient divided by the straight-line distance of the two endpoints. In order to provide a reference point for movement rates, $\delta^{15}N$ values of "resident" tuna were calculated and are based on Eqn 1, where the $\delta^{15}N$ value of a resident tuna should reflect the isotopic baseline and the accumulated trophic enrichment factor.

This approach assumes that tuna movements are only uni-directional and does not consider random movements, which are important components of tuna movements in the pelagic ecosystem (e.g., Carey and Olson 1982; Sibert et al. 1999; Schaefer et al. 2007). Examining these isotopic datasets with the addition of an advection-diffusion model would greatly improve the population-level estimates of movements for tunas in the equatorial Pacific.

4.3.6 Inter-Species Geographical Anomalies

To allow for comparison between species, the $\delta^{15}N$ values of individual tuna species were normalized for the entire basin. A matrix of normalized $\delta^{15}N$ values ($\delta^{15}N^*$) or z-scores were calculated for each tuna species as

$$\delta^{15}N^*_{tuna\,x} = \frac{\delta^{15}N_{tuna\,x} - avg\,\delta^{15}N_{tuna\,x}}{stdev\,\delta^{15}N_{tuna\,x}} \quad (5)$$

where $\delta^{15}N^*$ is the basin-wide normalized value for a specific location, $\delta^{15}N$ is the isotope value at each matrix location, $avg\delta^{15}N$ is the basin-wide average of all $\delta^{15}N$ values for $tuna_x$, $SD\delta^{15}N$ is the basin-wide standard deviation of all $\delta^{15}N$ values for $tuna_x$, and $tuna_x$ is YFT, SKJ, or BET. $\delta^{15}N^*$ represents the distance between the nominal $\delta^{15}N$ values of the tuna and the population mean in units of the standard deviation. Inter-species isotope anomaly maps were then calculated by simply subtracting the normalized or z-score matrices of two different species of tuna (e.g., $tuna_a$ and $tuna_b$).

4.4 Results

4.4.1 Isotope Cartography

Sample coverage across the cold-tongue warm pool system was widespread for yellowfin and skipjack tuna, but coverage for bigeye tuna was restricted to an equatorial band between 10°N to 10°S (Fig. 4.1). A total of 2064 individual tropical tunas were included in the bulk carbon and nitrogen isotope analysis of this study. Areas within the maps where there is little data coverage should be viewed cautiously as the isotope values in these regions are based only on linear interpolation. Table 4.1 includes a summary and descriptive statistics of the data incorporated into the isotope maps for the 3 different species of tropical tunas. Ontogenetic diet shifts can lead to large $\delta^{15}N$ differences between juveniles and adult tunas. For example, Graham et al. (2007) found a distinct difference (~5‰) between yellowfin tuna <45 cm FL and yellowfin >50 cm FL collected from Hawaiian waters. Although previous isotopic research suggests that spatial changes in tuna $\delta^{15}N$ values is not correlated to trophic changes (Popp et al. 2007, see also

Chapters 3 and 5), all tuna $\delta^{15}N$ values were plotted against forklength in the western, central, and eastern Pacific regions to determine if their were trophic effects over a large range of size (Fig. 4.2). No distinct ontogenetic $\delta^{15}N$ shifts were observed in these 3 regions, but some of the very smallest tunas had lower $\delta^{15}N$ values than larger tuna. These small tuna with low $\delta^{15}N$ values were collected from the region directly around the equator in the central and eastern Pacific, suggesting that diet shifts do not appear to affect the $\delta^{15}N$ values of tuna as much as location of capture. Accordingly, all tuna samples collected in the eqPac were included in the construction of the tuna isotope maps.

The $\delta^{15}N$ values of tuna display striking geographical variability of 13.7‰, 11.4‰, and 12.5‰ for YFT, SKJ, and BET, respectively (Fig. 4.3). The variability is relatively coherent between species, with similar geographical areas of high and low $\delta^{15}N$ values (Fig. 4.3). The highest $\delta^{15}N$ values of YFT, SKJ and BET were observed in a region west, south-west (W-SW) of the Marquesas Islands (French Polynesia) and W-SW of the Gilbert Islands (Kiribati) (Table 4.1, Fig. 4.3), whereas, the lowest $\delta^{15}N$ values of YFT, SKJ, and BET were observed in the equatorial upwelling zone within the cold tongue region of the CTP and ETP (Table 4.1, Fig. 4.3). Another broad region of higher $\delta^{15}N$ values was observed in the north ETP (Fig. 4.1).

Much less variation was present in the $\delta^{13}C$ values of tropical tunas collected from the equatorial Pacific basin; 3.9‰, 2.9‰, and 3.7‰ for YFT, SKJ, and BET, respectively (Table 4.1). Similar to the $\delta^{15}N$ tuna maps, $\delta^{13}C$ values of YFT and SKJ were highest in a region west, south-west (W-SW) of the Marquesas Islands and W-SW of the Gilbert Islands (Table 4.1, Fig. 4.4), whereas, the lowest $\delta^{13}C$ values of the 3

species of tuna were in the equatorial upwelling zone within the cold tongue region of the CTP and ETP (Table 4.1, Fig. 4.4). Because lipids were not removed from the WMT tissues prior to isotope analysis, these $\delta^{13}C$ variations could reflect changes in lipid content of the tuna samples. Lipids in fish are depleted in ^{13}C relative to the bulk tissue (e.g., Doucett et al. 1999). However, YFT and SKJ C/N ratios demonstrated little variation and were around the C/N ratio of protein (i.e., 3.5 mole%) (Fig. 4.4). There were only a few regions (e.g., near Marquesas Islands) where C/N increased above 4.0, indicating a possible increase in the percentage of lipids, but these areas did not correspond to areas with lower $\delta^{13}C$ values of YFT and SKJ. On the contrary, the $\delta^{13}C$ values of tuna were higher in the areas with elevated C/N ratios (Fig. 4.4).

4.4.2 Tropical Tuna Movements

At 125°W the $\delta^{15}N$ values of YFT and SKJ show a similar latitudinal variation as the $\delta^{15}N$ values of PN (Fig. 4.5). Low $\delta^{15}N$ values occur between 5°S and the equator and increase at higher latitudes. The variations in the $\delta^{15}N$ values of BET are less variable and relatively consistent over the range of 20 degrees latitude (Fig. 4.6). At 140°W the $\delta^{15}N$ values of PN and YFT and SKJ tuna show a similar latitudinal variation as the 125°W transect. Based on the relationship between $\delta^{15}N$ values of PN and tuna and WMT tissue turnover rates observed in yellowfin tuna, directed movement rates were calculated for the three tuna species at the two meridional transects (Table 4.2). BET exhibited the greatest N-S movement rates at 125°W within the range examined (10°S to 8°N), whereas YFT exhibited the most restricted movements at both transects (Table 4.2, Fig. 4.5, Fig. 4.6). SKJ net movement rates were greater than YFT, but less than BET at

125°W (Table 4.2, Fig. 4.7, Fig. 4.6). YFT and SKJ net N-S movement rates were similar at the 140°W transect, approximately 4 and 5 km day^{-1}, respectively (Fig. 4.7). These maximum net movement estimates are dependent upon the TL_{tuna} and accumulated TEF. The TEF value was based on wild juvenile YFT in Hawaii and larger YFT in ETP (Graham et al. 2007; Popp et al. 2007). TEF and TL could vary between tuna species because of differences in physiology, nitrogen efficiency rates, and growth rates (see review by Gannes et al. 1997). Furthermore, in an ECOPATH model developed for the ETP, Olson and Watters (2003) estimated the TL of BET to be greater than YFT and SKJ. Therefore, there should be variation in both the trophic level (TL ≈ 4.6 ± 0.5) and trophic enrichment factor (TEF ≈ 2.1 ± 1.0), but I assume that these parameters should vary in a similar fashion across the N-S transects. In other words, the predicted $\delta^{15}N$ values of resident tuna would vary relative to changes in TEF and TL but the movement estimate would not vary; the movement estimate depends upon the isogradient and tissue turnover rate.

4.4.3 Inter-Species Geographical Anomalies

Although the spatial variability in the $\delta^{15}N$ values of tropical tuna was high, the average $\delta^{15}N$ value of BET was still statistically higher than the average YFT and SKJ $\delta^{15}N$ values (c.a. 1.5‰; t-test, both $p_{\delta 15N\ values} < 0.01$) (Table 4.1). The average YFT and SKJ $\delta^{15}N$ values were not statistically different (t-test, $p_{\delta 15N} = 0.27$). YFT and BET average $\delta^{13}C$ values were not statistically different (t-test, $p_{\delta 13C}$ value = 0.23), but YFT and BET average $\delta^{13}C$ values were significantly lower than the average $\delta^{13}C$ value of SKJ (t-test, $p_{\delta 13C}$ values = << 0.01 and 0.01, respectively). However, this difference in

tuna $\delta^{13}C$ values is approximately 0.2‰, and therefore may be of limited ecological significance. Once each tuna $\delta^{15}N$ dataset was normalized (eqn. 5) and a species's dataset subtracted from another, spatial anomalies in the $\delta^{15}N$ patterns between species were apparent (Fig. 4.8). In comparing YFT and BET isotope maps, $\delta^{15}N$ anomalies occur in the equatorial upwelling in the central and eastern tropical Pacific, where YFT have greater $\delta^{15}N$ values than BET (Fig. 4.8). There are a few regions south of 10°S where BET have $\delta^{15}N$ values ~2‰ higher than YFT. The spatial $\delta^{15}N$ anomalies between YFT and SKJ are less than between YFT and BET, and YFT generally have higher $\delta^{15}N$ values than SKJ (Fig. 4.8).

4.5 Discussion

4.5.1 Isotope Cartography

Spatial and temporal variability in $\delta^{15}N$ values of freshwater and marine animals can be erroneously interpreted exclusively as differences in foraging strategies or trophic level (TL) (e.g., Domi et al. 2005). If shifts in trophic levels were the primary factor producing the 11-14‰ spatial variation observed in $\delta^{15}N$ values of tropical tunas, then there would be regional variations of approximately 4 to 5 TLs in tropical tunas. Not only is a TL variation of this magnitude highly unrealistic in marine ecosystems that generally have a total of 4 to 5 TLs (Fry 1988; Hobson and Welch 1992; Olson and Watters 2003), but stomach content studies conducted in the Pacific Ocean have demonstrated little foraging specialization in tropical tunas (e.g., Alverson 1963; Reintjes and King 1953). Because consumer $\delta^{15}N$ values are a function of both their trophic level

and the $\delta^{15}N$ values at the base of the food web, the isotope variations in tuna across the equatorial Pacific (Fig. 4.3) could result from differences in the sources of nitrogenous nutrients or from variability in nutrient dynamics.

The $\delta^{15}N$ values of surface particulate nitrogen (PN), sinking PN, and seafloor sediments demonstrate strong geographical patterns (e.g., Saino and Hattori 1987; Farrell et al. 1995) and have been used as a proxy to estimate nitrate utilization by phytoplankton in oceanic surface waters (Altabet and François 1994; Sigman et al. 1999; Altabet 2001). In the equatorial Pacific the pattern is low $\delta^{15}N$ values near the equatorial upwelling zone (cold tongue) and increasing $\delta^{15}N$ values as the surface water is advected poleward to higher latitudes (Altabet and François 1994; Farrell et al. 1995; Altabet 2001). The spatial pattern in the $\delta^{15}N$ values of tuna demonstrates a similar geographical pattern to the one observed in surface and sinking PN and seafloor sediments (Fig. 4.3). The isotopic variability in tuna could then relate to variations in the nutrient dynamics of the pelagic food web. Popp et al. (2007) used compound-specific nitrogen isotope analysis of amino acids isolated from tissues of five yellowfin tuna to show that spatial variations in their bulk $\delta^{15}N$ values in the eastern tropical Pacific Ocean are controlled by variations in the $\delta^{15}N$ values at the base of the food web and not variations in TL. Thus, the spatial variation observed in the $\delta^{15}N$ values of tropical tunas could be mainly related to variations observed in the source and isotopic composition of biologically-fixed nitrogen.

4.5.2 Tropical Tuna Movements

If a predator migrated extensively in the eqPac, then little geographical isotopic variation would be expected in that predator's tissues because regional differences in

baseline $\delta^{15}N$ values would be integrated over time. Tropical tuna $\delta^{15}N$ values demonstrate highly variable (~12‰), but spatially coherent structure in the eqPac, which suggests tuna must reside and forage within a region for a long enough period to reflect the baseline isotope value. Thus, this natural tag approach reveals that tropical tunas, especially yellowfin and skipjack tuna are not 'highly migratory' and suggest a high degree of regional residency in the eqPac. Furthermore, as the directed movements for the tropical tunas were low, random movements must play an important role in the overall mixing of the population over time and space. These isotope-based conclusions corroborates recent electronic tagging studies that demonstrate restricted movements in individual BET and YFT tracked in three different regions within the subtropical Pacific Basin (Adam et al. 2003; Sibert et al. 2003; Sibert and Hampton 2003; Gunn et al. 2005; Schaefer et al. 2007), but also demonstrates that this residency behavior exists for yellowfin and skipjack over a much larger spatial scale than previously documented.

The $\delta^{15}N$ values of tropical tunas indicate inter-specific differences in movement rates. Yellowfin tuna demonstrated the greatest degree of site fidelity among the three species, especially in the central equatorial Pacific were the estimated movement rate was similar to the predicted "resident" tuna. This net N-S movement rate is based on approximately 375 individual YFT collected in the region and, thus, provides an initial estimate of population-level directed movements. Within the cold-tongue ETP region, BET demonstrated movement rates greater than YFT and SKJ. At the 140°W transect the average $\delta^{15}N$ value of all PN sample (3.5 ± 4.0‰) plus the accumulated TEF (7.6‰) was approximately 11.3 ± 4.0‰ and this value would indicate the $\delta^{15}N$ value of a migratory tuna. The average $\delta^{15}N$ value of BET from this region was similar to this average

baseline value plus the accumulated TEF and equaled 11.8 ± 1.3‰. These migratory and restricted movements observed in the three species of tuna, especially the resident behavior observed in the isotope cartography of the $\delta^{15}N$ value of yellowfin and skipjack, should be incorporated into stock assessment models, because managing tropical tuna fisheries on a basin-wide or sub-basin scale could lead to localized depletions in the regions where tuna demonstrate the greatest site fidelity.

As discussed in Chapter 3, these directed movement estimates are simplistic and conservative for several reasons. First, random movements are not included in these movement calculations. Including a random movement component (e.g., advection-diffusion model) in the analysis would provide a much more realistic estimate of tuna movement in the eqPac (e.g., Sibert et al. 1999). In addition, east-west directed movements are not considered, mainly because over much of the eqPac there were not strong E-W gradients in $\delta^{15}N$ and $\delta^{13}C$ values. On a regional scale, data from acoustic tags placed on adult yellowfin in the ETP showed that the majority of net movements were in the meridonal (N-S) axis and E-W net movements were much more restricted (Schaefer et al. 2007). The only consistent E-W gradient observed in tuna isotope values was between the cold tongue-warm pool transition zone in the central Pacific, which suggests little mixing between the two eco-regions. A second important factor involved in estimating movements based on isotope cartography is that the diet of yellowfin tuna would change gradually as they migrate in the pelagic environment and not rapidly as was the case in the experimental diet shift study where the tissue turnover rates were determined. Finally, the tissue turnover rates estimated in both the natural and experimental settings were for juvenile yellowfin tuna and standard errors associated to

the estimates were considerable (Chapter 3). Juveniles generally have higher tissue turnover rates than adults, mainly due to differences in growth rates (e.g. Fry and Arnold 1982; Bosley et al. 2002; Trueman et al. 2005). Furthermore, tissue turnover rates could vary between individuals and schools and over time, because they relate to an organism's overall nitrogen balance and metabolism. Due to all of these factors the N-S, directional movement for BET, YFT, and SKJ is a conservative estimate. Even considering the limitations of this initial approach, isotope cartography suggests that tropical tunas, especially yellowfin tuna, are not a uniformly mixed population in the sub-basin regions on the scale of several months. Instead, these three species of tropical tunas demonstrate a large degree of regional residency in the equatorial Pacific.

4.5.3 Inter-Species Geographical Anomalies

On a basin-wide scale, the average $\delta^{15}N$ value of BET is statistically higher than the average $\delta^{15}N$ value of YFT. However, in the region of the central and eastern equatorial Pacific or the cold tongue, YFT $\delta^{15}N$ values are ~2‰ higher than BET $\delta^{15}N$ values. Unless YFT are foraging at a higher trophic level in the eastern equatorial Pacific compared to the average basin, then this isotopic difference could represent deviations in BET and YFT movement patterns and habitat utilization. If BET forage more exclusively in the area directly surrounding the equator, then their $\delta^{15}N$ values would reflect the low $\delta^{15}N$ values observed at the base of the food web along the equator (e.g., Farrell et al. 1995; Altabet 2001). Calculated BET net N-S movement rates were much higher than YFT between 10°N and 10°S suggesting that BET move much more extensively throughout this equatorial area. Conversely, if YFT spent more time foraging

at higher latitudes where there are higher baseline $\delta^{15}N$ values, then the difference between the $\delta^{15}N$ values of the two species reflects their residency at the equator. Schaefer et al. (2007) suggested that large (reproductive) YFT in the eastern Pacific make seasonal N-S movements to the equator during spawning periods and smaller or non-reproductive individuals remain at the higher latitudes. Accordingly, YFT could make N-S excursions into the equatorial region from higher latitudes (high $\delta^{15}N$ values) to utilize the warm surface waters for spawning periods in the central Pacific, whereas BET migrate extensively within the equatorial region (low $\delta^{15}N$ values and high net movement rates) and forage within this region during spawning and non-spawning periods. Adult or reproductively mature YFT and BET might show population-level niche separation, whereby tropical tunas share habitat along the equator during spawning, but otherwise separate their main foraging regions.

4.5.4 Biogeochemical Provinces Identified using Isotope Cartography

$\delta^{13}C$ Spatial Trends:

High YFT and SKJ C/N ratios did not correlate to lower $\delta^{13}C$ values, indicating factors other than lipid content must be controlling the observed carbon isotopic patterns of tuna in the equatorial Pacific. In high latitudes, variations in the $\delta^{13}C$ values of primary consumers have been shown to vary in relation to the $\delta^{13}C$ values of primary producers (Schell et al. 1989; Burton and Koch 1999). By using maps of $\delta^{13}C$ values or isotope cartography in the high-latitude seas of the North Pacific, Schell et al. (1998)

showed that zooplankton foraging in regions of high primary productivity generally had higher $\delta^{13}C$ values than zooplankton foraging in high-nutrient, low-chlorophyll (HNLC) regions. These baseline variations in $\delta^{13}C$ values (range, 3-5‰) were then used successfully as chemical tags to track resident and migratory marine mammals (Schell et al. 1989; Burton and Koch 1999; Lee et al. 2005). By analogy, 3-4‰ variation in $\delta^{13}C$ values of tropical tuna could therefore reflect the $\delta^{13}C$ variations at the base of the food web in the equatorial Pacific Ocean.

The key factors that affect the $\delta^{13}C$ values of marine phytoplankton are (1) the $\delta^{13}C$ value and concentration of dissolved CO_2 ($[CO_2]_{aq}$) (Goericke and Fry 1994; Laws et al. 1995; Popp et al. 1998), (2) the community composition and growth rate of phytoplankton (Fry and Wainwright 1991; Laws et al. 1995; Popp et al. 1999), and (3) phytoplankton cell morphology (Popp et al. 1998, 1999). In a comprehensive study examining the controls on the carbon isotopic compositions of marine phytoplankton in the Southern Ocean, Popp et al. (1999) showed that $[CO_2]_{aq}$, community composition and cell morphology all appeared to affect the spatial patterns of phytoplankton $\delta^{13}C$ values. In one 145°W transect from 45°S to 65°S, growth rate appeared to have little to no effect on the $\delta^{13}C$ value of phytoplankton (Popp et al. 1999). However, the Southern Ocean is a HNLC region. Thus, phytoplankton in this region could exhibit less than maximal growth rates, because of micronutrient limitation (Martin et al. 1990; Coale et al. 1996).

Because Fe limitation may affect phytoplankton growth rates (Martin et al. 1990) and affect community composition by selecting for phytoplankton species better adapted to low Fe concentrations, the $\delta^{13}C$ values of marine phytoplankton can be correlated to Fe availability in the euphotic zone. The carbon isotopic composition of a biomarker of

phytoplankton (phytol) increased by 7‰ after an iron-addition experiment in the equatorial Pacific (Bidigare et al. 1999). Iron-enrichment enhances phytoplankton growth, and, in turn, the isotope fractionation associated with photosynthetic carbon fixation (ε_p *sensu* Freeman and Hayes 1992) decreases. As such, regions with Fe-addition should exhibit higher $\delta^{13}C$ values at the base of the food web. These $\delta^{13}C$ values can then be propagated up to the higher trophic levels (e.g., Schell et al. 1989; Burton and Koch 1999; Lee et al. 2005). Tropical tuna $\delta^{13}C$ values partially support this hypothesis. YFT and SKJ $\delta^{13}C$ values are lowest in the equatorial upwelling region, which is another HNLC region. Similar to the Southern Ocean, these $\delta^{13}C$ values could be low because Fe is limiting growth rates in phytoplankton. Alternatively, low $\delta^{13}C$ values of DIC, a result of remineralization at depth, are upwelled at the equator and could explain the low $\delta^{13}C$ values observed in the cold tongue region (Kroopnick 1985). However, the $\delta^{13}C$ values of shallow- and deepwater DIC only vary by ~1.0‰ (e.g., Lerperger et al. 2000; Zhang and Quay 1997) and cannot exclusively explain the observed 3-4‰ variation in $\delta^{13}C$ values of tuna. High $\delta^{13}C$ values of tropical tunas are found west of the Marquesas and Gilbert Islands, which could suggest that these areas support higher growth rates in marine phytoplankton relative to the equatorial Pacific HNLC region. However, before we can conclude that semi-resident tuna $\delta^{13}C$ values are elevated in regions with enhanced phytoplankton growth rates, we should examine the spatial patterns in the $\delta^{15}N$ values of tropical tunas in the equatorial Pacific.

$\delta^{15}N$ Spatial Trends:

Tuna $\delta^{15}N$ spatial patterns in the equatorial Pacific appear to be driven, in large part, by the isotope fractionation associated to nitrate utilization by marine phytoplankton in the euphotic zone (see below). However, there are several other factors that can affect the spatial patterns observed in the $\delta^{15}N$ values of tuna. To aid in the discussion of these $\delta^{15}N$ spatial patterns, I separated the equatorial Pacific basin into isotope-based biogeochemical provinces (Fig. 4.6). In the following sections I will discuss the predominant nutrient dynamics, and, in turn, the factors potentially affecting the $\delta^{15}N$ value at the base of the food web in these regions. Unlike other isotopic studies examining nitrogen dynamics in the eqPac (e.g., Saino and Hattori 1987; Altabet and François 1994; Altabet 2001; Voss et al. 2001), this is a unique top-down approach using tuna $\delta^{15}N$ values as a starting point. Consequently, temporal and spatial patterns in baseline $\delta^{15}N$ values will be averaged and only broad patterns will persist up the food web to the top marine predators.

Iso-Biogeochemal Province 1: Equatorial Pacific Ocean

In the cold tongue region of the equatorial Pacific Ocean, a high nutrient-low chlorophyll (HNLC) region, new nutrients are delivered to the euphotic zone by upwelling at the equator (Fig. 4.6) (e.g., Martin et al. 1994). These nutrient-rich waters advect poleward by Ekman transport and are continuously consumed by marine phytoplankton. As nitrate in the surface waters is consumed, fractionation associated with nitrate assimilation results in progressive enrichment in ^{15}N in the remaining NO_3- pool becomes and produces a subsequent increase in the $\delta^{15}N$ value of the newly formed PN (e.g., Altabet 2001). Thus, the relationship between the concentration of NO_3- and

δ^{15}N-PN values near upwelling zones is mainly related to variations in relative NO_3- utilization (Altabet and Francois 1994; Farrell et al. 1995; Altabet 2001). The isotope fractionation (ε) associated with nitrate assimilation by phytoplankton is approximately 5‰ (Wada and Hattori 1978; Waser et al. 1998; Granger et al. 2004), but can vary considerably and depends on the rate of NO_3- efflux from the cell to the rate of nitrate reduction within the cell (Granger et al. 2004; Needoba et al. 2005). The fact that field-based studies have shown ε values consistently near 5‰ suggests that nitrate efflux must be relatively constant and minimal in oceanic surface waters. Assuming ε is 5‰, the effect of nutrient utilization on the δ^{15}N values of the nitrate and PN can be modeled using simple Rayleigh isotopic fractionation principles (e.g. Altabet 2001):

$$\delta^{15}N\text{-}NO_3^- = \delta^{15}N\text{-}NO_3^-{}_{intial} - \varepsilon \times \ln\left(\frac{[NO_3\text{-}]_{obs}}{[NO_3\text{-}]_{intial}}\right) \qquad (6)$$

where $\delta^{15}N\text{-}NO_3^-{}_{intial}$ is the δ^{15}N value of NO_3^- in deep, upwelled ocean waters and has been measure in the field as 5 to 7‰ (Sigman et al. 1999; Sigman et al. 2005; P. Rafter *unpublished*), ε equals the average kinetic isotopic effect associated with NO_3^- uptake by phytoplankton measured in the open ocean (~5‰, see above), and $[NO_3^-]_{intial}$ is the NO_3^- concentration delivered to the surface by equatorial upwelling in the ETP and is approximately 18.4 μM l^{-1} (World Ocean Atlas 1998 dataset). Thus, as the nitrate pool in the surface water is continuously drawn down by biological uptake (i.e. as $\left(\frac{[NO_3\text{-}]_{obs}}{[NO_3\text{-}]_{intial}}\right)$ decreases), the δ^{15}N value of the remaining NO_3^- pool and newly-formed PN will increase (e.g., Altabet 2001). This nitrate utilization pattern appears to propagate up the food web to upper-trophic level predators. Tropical tuna δ^{15}N values are the lowest at the

equator, where the $\left(\frac{[NO_3^-]_{obs}}{[NO_3^-]_{initial}}\right)$ is the greatest, and increase away from the equator, in both the southern and northern directions, as nitrate is assimilated and fractionated by marine phytoplankton (Fig. 4.2, Fig 4.6) in a pattern similar to that observed in suspended particulate nitrogen (Farrell et al. 1995). The similarity between the $\delta^{15}N$ spatial patterns at the base of the food web and of tropical tunas in the central and eastern equatorial Pacific suggests that the isotopic baseline is relatively conserved in the food web and that tuna $\delta^{15}N$ values reflect these baseline shifts.

Altabet (2001) documented an almost doubling of NO_3- concentrations during sampling in the HNLC region of the equatorial Pacific between times of El Nino and La Nina events but saw no change in the $\delta^{15}N$ values of PN. These observations suggest that the relative utilization of NO_3- in the eqPac is relatively constant and possibly controlled by Fe limitation (e.g., Martin et al. 1994; Gordon et al. 1997; Landry et al. 2000). Tuna $\delta^{13}C$ values are lowest in this HNLC region suggesting that growth rates of phytoplankton in this region could be limited (Bidigare et al. 1999). A large-scale Fe-addition field study in the Southern Ocean produced a 3-4‰ increase in the $\delta^{15}N_{PON}$ relative to the $\delta^{15}N_{PON}$ in the control site (Karsh et al. 2003). Favorable growth conditions in the euphotic zone could support enhanced NO_3^- utilization and then influence $\delta^{15}N$ values of marine biota. Therefore, if Fe was present in higher concentrations (even additions 0.01 nM levels), nutrient utilization would not be Fe-limited, and then the subsequent increase in NO_3- drawdown would drive an even greater increase in the $\delta^{15}N$ (Karsh et al. 2004) and $\delta^{13}C$ values (Bidigare et al. 1999). Remarkably, the spatial patterns observed in the $\delta^{15}N$ values of mobile tropical tunas

corroborate previous findings on the general patterns of nitrate utilization by marine phytoplankton in the equatorial Pacific (Altabet and Francois 1994; Farrell et al. 1995; Altabet 2001).

Iso-Biogeochemical Province 2: Eastern Tropical Pacific OMZ

Water column denitrification, or the bacterial reduction of NO_3- to N_2, occurs in localized areas of high surface production and low oxygen source waters or oxygen minimum zones (OMZs). The eastern tropical north Pacific (ETNP) is the largest OMZ and exhibits high rates of water column denitrification, which is a process that preferentially consumes $^{14}NO_3$- and leads to an increase in the $\delta^{15}N$ value of the remaining NO_3- pool (Cline and Richards 1972; Cline and Kaplan 1975; Voss et al. 2001). Nitrogen isotopic fractionation (ε) associated with denitrification is large and has been calculated from the results of field studies to range between 20 to 30‰ (Cline and Kaplan 1975; Altabet et al. 1999; Voss et al. 2001). Based on water column $\delta^{15}N$-NO_3- values, the southern boundary of the ETNP OMZ begins at approximately 8°N and occurs between the depths of 300 to 600m (Voss et al. 2001; P. Rafter *unpublished data*). Voss et al. (2001) showed that the $\delta^{15}N$ of PN in the ETNP ranged from ~9‰ at 14°N to 11.2‰ at 24°N and results from the phytoplankton uptake of partly denitrified, ^{15}N-enriched nitrate. It appears that the isotopic signal of this water-column denitrification in the ETNP is propagated up the food web to tuna (e.g., Popp et al. 2007). Bulk tissue $\delta^{15}N$ values of hundreds of yellowfin tuna and mesozooplankton examined in that study increased by ~5‰ over a N-S distance of approximately 3600km in the ETP, from 8°S to 20°N (Popp et al., 2007). Thus, the high $\delta^{15}N$ values in the yellowfin tuna and

zooplankton in the ETNP (>8°N) could be associated with the predominance of water column denitrification (Voss et al., 2001), whereas the spatial patterns in tuna $\delta^{15}N$ values at the lower latitudes in the ETP are consistent with nitrate utilization by phytoplankton (Altabet and François 1994; Farrell et al. 1995; Altabet 2001).

Iso-Biogeochemal Province 3: Marquesas Islands (French Polynesia)

When isolated oceanic islands experience strong and steady currents a doming of isopycnals and turbulent mixing can occur in the lee of the islands (e.g., Heywood et al. 1990; Signorini et al. 1999). In addition to affecting the hydrological regime downstream, islands can be a source of macro and micro-nutrients to the surrounding waters (e.g., Martin et al. 1994; Blain et al. 2001). This 'island mass effect' can therefore, potentially inject deep, nutrient rich waters into the euphotic zone, deliver limiting nutrients, and, in turn, increase primary production in the surrounding area. SeaWiFS chlorophyll *a* observations have shown large and persistent phytoplankton blooms in the lee of the Marquesas Islands in the South Pacific Ocean that occur with shifts between El Niño and La Niña conditions (Signorini et al. 1999; Martinez and Maamaatuaiahutapu 2004). Signorini et al. (1999) suggested that by intersecting the Marqieasas Islands, the westward flowing South Equatorial Current (SEC) creates an island mass effect and promotes the upwelling of waters from below the thermocline. Similar to equatorial upwelling, small scale or mesoscale upwelling events can deliver macronutrients to the euphotic zone. However, the Marquesas Islands are at the leading edge of the cold-tongue, HNLC region where there are sufficient macronutrients available for phytoplankton growth. Thus, the doming of isopycnals and turbulent

mixing may not be the sole mechanism accounting for an increase in primary production downstream from the islands.

Iron-enrichment could be required to support enhanced primary production in HNLC regions (e.g., Martin et al. 1994; Coale et al. 1996). Weathering processes occurring on the Marquesas Islands could provide a terrigenous source of Fe to the surrounding oceanic waters, because these young, step volcanic islands do not have any boundaries that would obstruct the delivery of river drainage to oceanic waters (e.g., Signorini et al. 1999; Blain et al. 2001). Thus, island topography, a terrigenous Fe-source, and the SEC delivery of these micronutrients downstream of the Marquesas Islands could work in concert to enhance primary production in the region (Signorini et al. 1999; Martinez and Maamaatuaiahutapu 2004). Legeckis et al. (2004) proposed an alternative hypothesis to an 'island-Fe source' and stated that the delivery of Fe-rich waters to the Marquesas Islands was by tropical instability waves (TIW) produced by the eastward flowing equatorial undercurrent (EUC). The sources waters of the EUC are from the western Coral Sea, and these waters travel northward through the Vitaiz and Solomon straits and then past the Sepik River in Papua New Guinea and other river drainages in northern New Ireland, respectively (e.g., Lindstrom et al. 1987). These river drainages deliver bio-available forms of Fe to the surrounding coastal and oceanic regions, and, in turn, contribute Fe to the source waters of the EUC (e.g., Martin et al. 1994; Coale et al. 1996; Mackey et al. 2002). During the upwelling of the EUC in the central and eastern equatorial Pacific, westward propagating TIWs form and the southern boundaries of these waves can deliver the EUC Fe-rich waters to higher latitudes (e.g. the Marquesas Islands; Legeckis et al. 2004). Once the EUC-produced TIWs intersect and

interact with the islands, the Fe-rich waters are delivered westward (downstream) by the SEC. Phytoplankton blooms to the west of the Galápagos Islands have been shown to be produced by the interaction of the EUC with the archipelago and the advection of these Fe-rich waters to the west by the SEC (Martin et al. 1994; Gordon et al. 1998; Palacios 2002). Most recently, Blain et al. (2008) showed a subsurface increase in dissolved Fe near the Marquesas Islands that could be associated to the leading edge of the eastern Pacific OMZ. Furthermore, these authors suggest that the phytoplankton blooms west of the islands is a result of the vertical transport of these subsurface waters by mesocale activity. The importance of wind-driven mesoscale activity on the delivery of nutrients to surface waters and the subsequent effect on biological productivity has been recently demonstrated in the oligotrophic HNLC regions (e.g., Benitz-Nelson et al. 2007, Mcgillicuddy et al. 2007) and provide a compelling explanation for the observed phytoplankton blooms around the Marqueasas Islands.

Whether the enhanced primary production in the lee of the Marquesas Islands is triggered by (a) Fe from a terrigenous source, (b) the delivery of Fe-rich waters by EUC-produced TIWs, (c) mesoscale activity advecting subsurface waters, or some combination of both, the nutrient and iron-rich waters could explain the enhanced growth by marine phytoplankton observed in the region (Signorini et al. 1999). Furthermore, a release in iron limitation could trigger greater nitrate assimilation by phytoplankton and the subsequent drawdown of NO_3- could be the mechanism behind the high $\delta^{15}N$ values observed in this region (Fig. 4.6) (Karsh et al. 2004). An increase in phytoplankton growth rates in this region could also produce higher $\delta^{13}C$ values (Bidigare et al. 1999).

In other words, the elevated isotope values observed in tuna collected downstream from the Marquesas Islands could result from the release of iron limitation.

Iso-Biogeochemal Province 4: Gilbert Islands (Kiribati)

Another area that experienced large scale phytoplankton blooms during the transition between the 1998 El Niño and La Niña conditions was along the equator near 170°E, west of the Gilbert Islands (Ryan et al. 2002). These low coral atolls rise up from the abyssal floor directly at the equator and, therefore, obstruct the eastward flowing EUC in the western Pacific Ocean. Similar to the Marquesas Islands, the intersection of the EUC with the Gilbert Islands produces thermocline uplift and vertical mixing in the lee (west) of the islands (Ryan et al. 2002; Messie et al. 2006). As such, deep, nutrient-rich water are injected into the euphotic zone west of the islands where the blooms have been detected with SeaWIFs (Ryan et al. 2002). However, the island mass effect can not maintain enhanced primary production in the region without a supply of iron (e.g., Martin et al. 1994; Coale et al. 1996). Messie et al. (2006) suggested that the localized EUC shoaling east of the islands could provide iron to the euphotic zone to the Gilbert Islands and produce the observed phytoplankton bloom. Again, a combination of macro- and micronutrients delivery to the euphotic zone creates ideal growth conditions for marine phytoplankton.

An increase in nitrate assimilation and the subsequent drawdown of the remaining N pool would result in high $\delta^{15}N_{PON}$ values as described by Rayleigh isotope fractionation principles (Eqn. 3). As noted earlier, Karsh et al. (2003) showed in the large-scale iron enrichment experiment that δ^{15}N-PON values, on average, were 3-4‰

higher in the iron-enrichment sites relative to control sites. Tuna collected from the areas surrounding the Marquesas and Gilbert Islands show the highest $\delta^{15}N$ values in the equatorial Pacific (Fig. 4.6) and the values were approximately 3-4‰ higher than the other elevated $\delta^{15}N$ regions (e.g., ENTP OMZ). Therefore, isotope cartography suggests that iron delivery to the euphotic zone could be driving the elevated $\delta^{15}N$, by supporting greater NO_3- assimilation, and elevated $\delta^{13}C$ values because of the enhance growth rates. These baseline isotope gradients are then propagated up to the top predators and these signals can then be used to examine their movements. For example, anomalously high $\delta^{15}N$ regions such as the Marquesas and Gilbert Islands could be used as "natural" tagging sites and the dissolution of the isotope signal in the predator would provide individual attrition rates from the site.

4.6 Conclusions

The main advantage of examining tuna movements with stable isotope techniques is that marine animals are tagged naturally, without physical handling. Therefore, large spatial scales can be examined with this method and a population approach to estimating net movement rates can be accomplished. The main disadvantage of the "natural tag" method is the poor resolution of fine-scale movements. A mult-isotope and multi-element approach (e.g., trace metal and element concentrations, compound-specific isotope datasets, fatty acid profiles) could help further resolve regional and local scale movement patterns. Overall, when the research objective is to examine population-level movements patterns of pelagic predators or movements over extensive spatial or temporal

scales in the marine environment, stable isotope techniques can be a cost-effective (i.e., <1% of the cost of electronic tagging pelagic fish) compliment to conventional tracking techniques. In this study, the natural tag method has revealed that tropical tunas display limited direct movements in the equatorial Pacific Ocean. To maintain sustainable tuna fisheries, we should further incorporate restricted movements into spatially-structured, integrated stock assessment models and potentially develop management strategies for specific regions or even island nations in the equatorial Pacific.

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Table 4.1 A summary and descriptive statistics for the $\delta^{15}N$ and $\delta^{15}C$ values (‰) of 3 tropical tuna species collected in the equatorial Pacific. Basin-wide averages include all isotope sample values for a given species included in the isotope $\delta^{15}N$ and $\delta^{15}C$ maps (SD=standard deviation).

	YELLOWFIN	SKIPJACK	BIGEYE
Number of samples	387	223	204
Number of individuals	956	550	558
$\delta^{15}N$ values, ‰			
Highest,	21.5,	18.9,	21.6,
Location	*SW Marquesas Is., W Gilbert Is.*	*W Gilbert Is.*	*SW Marquesas Is., W Gilbert Is.*
Lowest,	7.8,	7.5,	9.1,
Location	*3°S in CTP & ETP*	*New Caledonia, 3°S in CTP*	*3°S in CTP & ETP*
Basin-wide Avg ± SD	**13.0 ± 2.7**	**12.8 ± 2.6**	**14.5 ± 2.9‡**
$\delta^{13}C$ values, ‰			
Highest,	-13.9,	-14.0,	-14.3,
Location	*W Gilbert Is.*	*W Gilbert Is.*	*SW Gilbert Is.*
Lowest,	-17.8,	-16.9,	-18.0,
Location	*CTP & ETP Eq.*	*120°W at the Eq.*	*CTP & ETP Eq.*
Basin-wide Avg ± SD	**-15.8 ± 0.5**	**-15.6 ± 0.5†**	**-15.8 ± 0.6**
C/N range	3.1-5.2	3.4-8.9	3.5-5.8

‡ BET is significantly higher than YFT and SKJ

† SKJ is significantly lower than YFT and BET

Table 4.2 North-south, directed movements (km day^{-1}) estimated for tropical tunas at two different transects in the eastern tropical Pacific (ETP) and central tropical Pacific (CTP). These are movement rates required to maintain the observed isogradient (i.e., an observed linear change in the $\delta^{15}N$ values of the base of the food web or PN). The movement rate is the time required to reflect the isogradient divided by the straight-line distance along the transect. The $\delta^{15}N$ values of "resident" tuna reflect the $\delta^{15}N$ isotopic baseline and the accumulated trophic enrichment factor (Eqn 1).

	PN	Predicted Resident	YFT	SKJ	BET
ETP (125°W)					
$\delta^{15}N$ values at 7.0°N (‰)	9.2	16.8	13.7	13.2	13.2
$\delta^{15}N$ values at -3.6°S (‰)	0.8	8.4	8.9	9.5	10.2
Isogradient (‰)	8.4	8.4	4.8	3.7	3.0
†Time (days), *given b = 0.02*		*420*	*240*	*185*	*150*
Max N-S, directed movement (km day^{-1})		**2.8**	**4.9**	**6.4**	**7.8**
CTP (140°W)					
$\delta^{15}N$ values at 7.4°N (‰)	9.0	16.6	15.9	14.1	-
$\delta^{15}N$ values at -4.0°N (‰)	2.4	10.0	9.2	9.2	-
Isogradient (‰)	6.6	6.6	6.7	4.9	-
†Time (days), *given b = 0.02*		*330*	*335*	*245*	
Max N-S, directed movement (km day^{-1})		**3.8**	**3.8**	**5.2**	**na**

† The time required for the $\delta^{15}N$ value of a tuna to reflect the isotope gradient is simply the isogradient (in ‰) divided by the white muscle tissue (WMT) turnover rate (b) (Chapter 3). The estimates are based an average WMT turnover of 0.02 N day^{-1}.

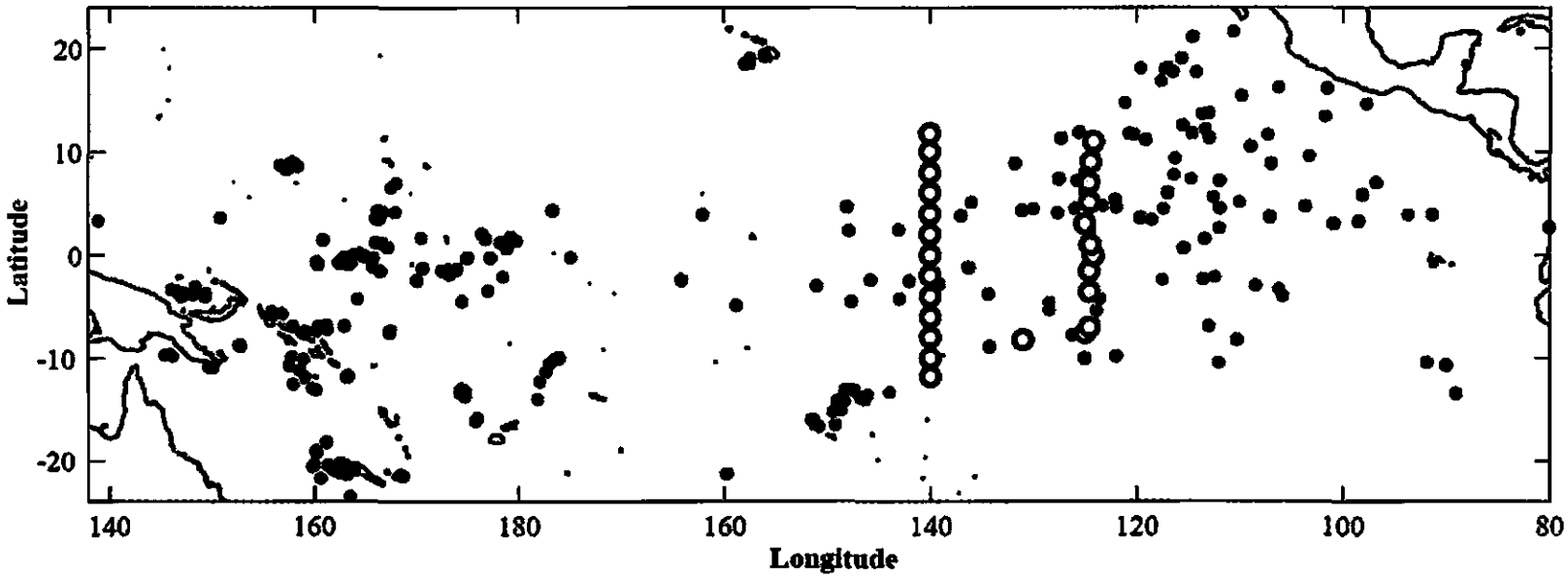

Fig. 4.1 Study region. Yellowfin, bigeye, and skipjack tuna (●) and POM (◘) sample locations within the equatorial Pacific Ocean. Tuna samples overlap throughout the map and in the ETP are composites of many individuals, whereas, in the WTP, tuna samples consist of single individuals.

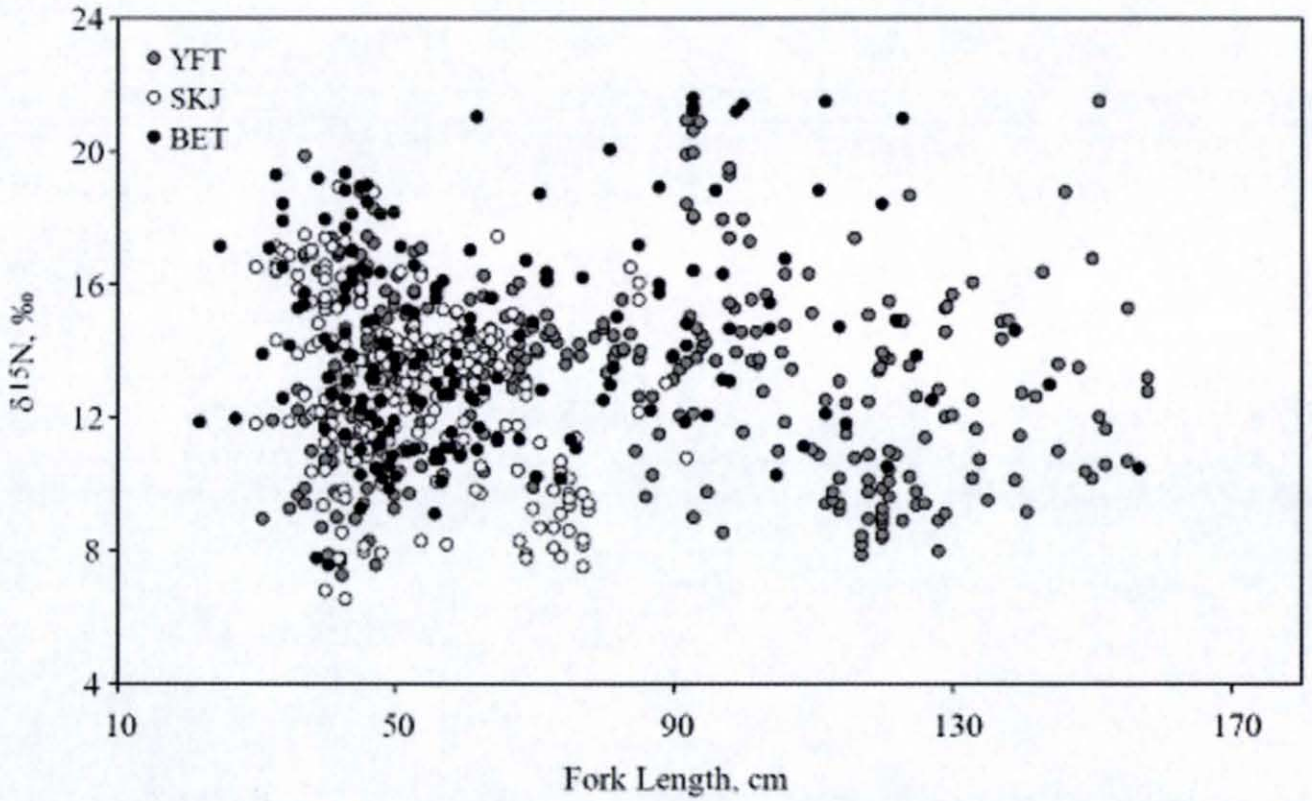

Fig. 4.2 Variations in yellowfin tuna (YFT), skipjack (SKJ), and bigeye tuna (BET) $\delta^{15}N$ values with fork length (cm) collected from the Equatorial Pacific Ocean.

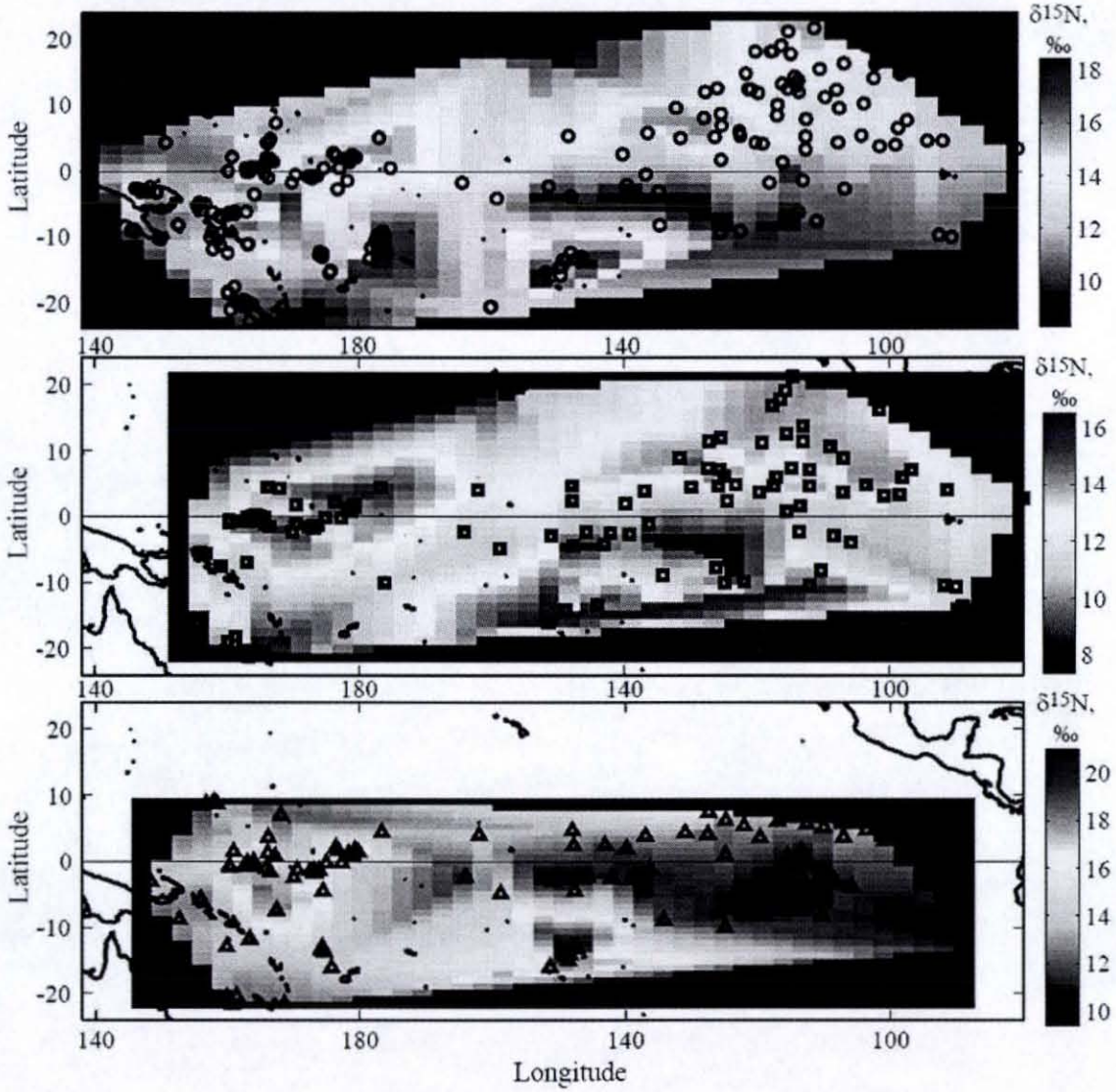
δ15N, ‰
18
16
14
12
10
δ15N, ‰
16
14
12
10
8
δ15N, ‰
20
18
16
14
12
10
Latitude
20
10
0
-10
-20
140
180
140
100
Longitude

Fig. 4.3 Geographical variation in the $\delta^{15}N$ values (‰) of yellowfin (*Top panel),* skipjack (*Middle panel*), and bigeye tuna (*Bottom panel*). The scales are different for each tuna species. Fig. 4.3. is best viewed in color - contact the author for a color copy.

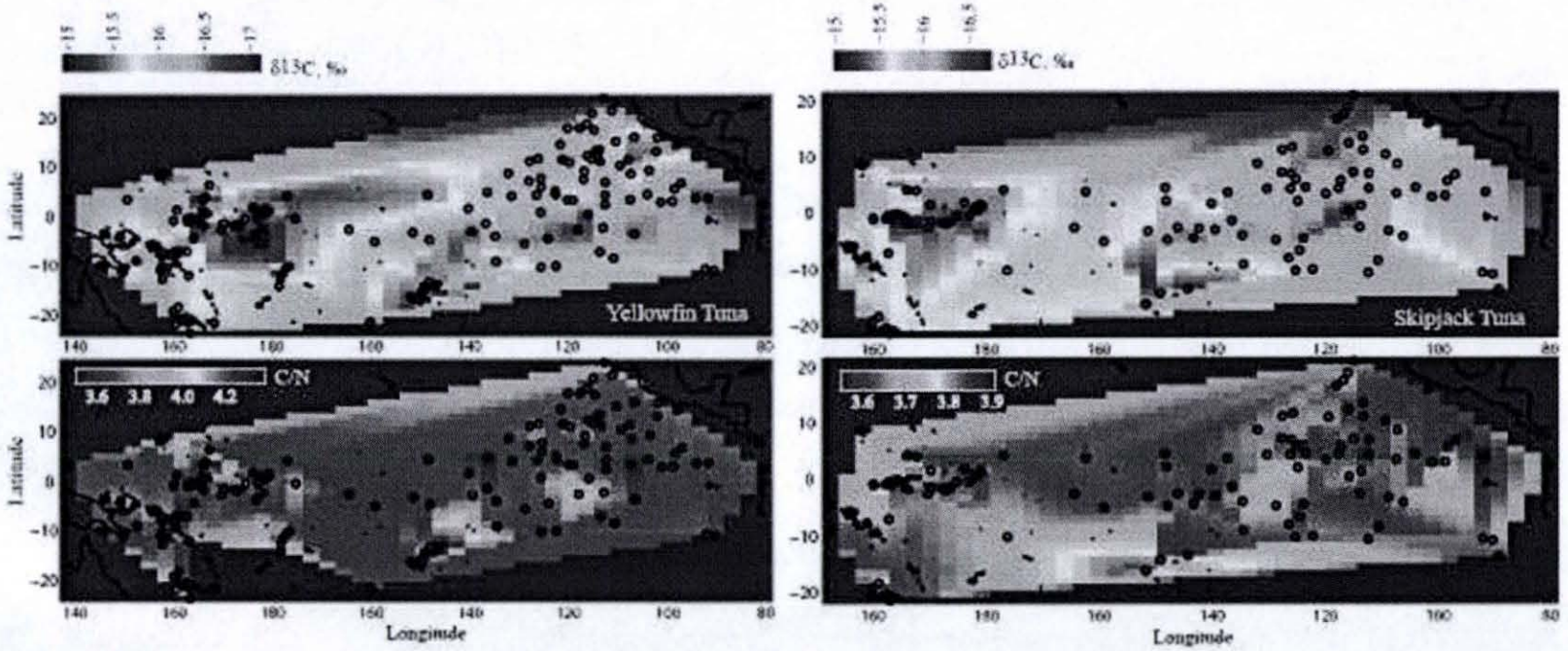

Fig. 4.4 Geographical patterns of $\delta^{13}C$ values (*top panels*) and C/N ratios (*bottom panels*) for yellowfin tuna (*left panels*) and skipjack tuna (*right panels*) collected in the equatorial Pacific Ocean. Fig. 4.4. is best viewed in color - contact the author for a color copy.

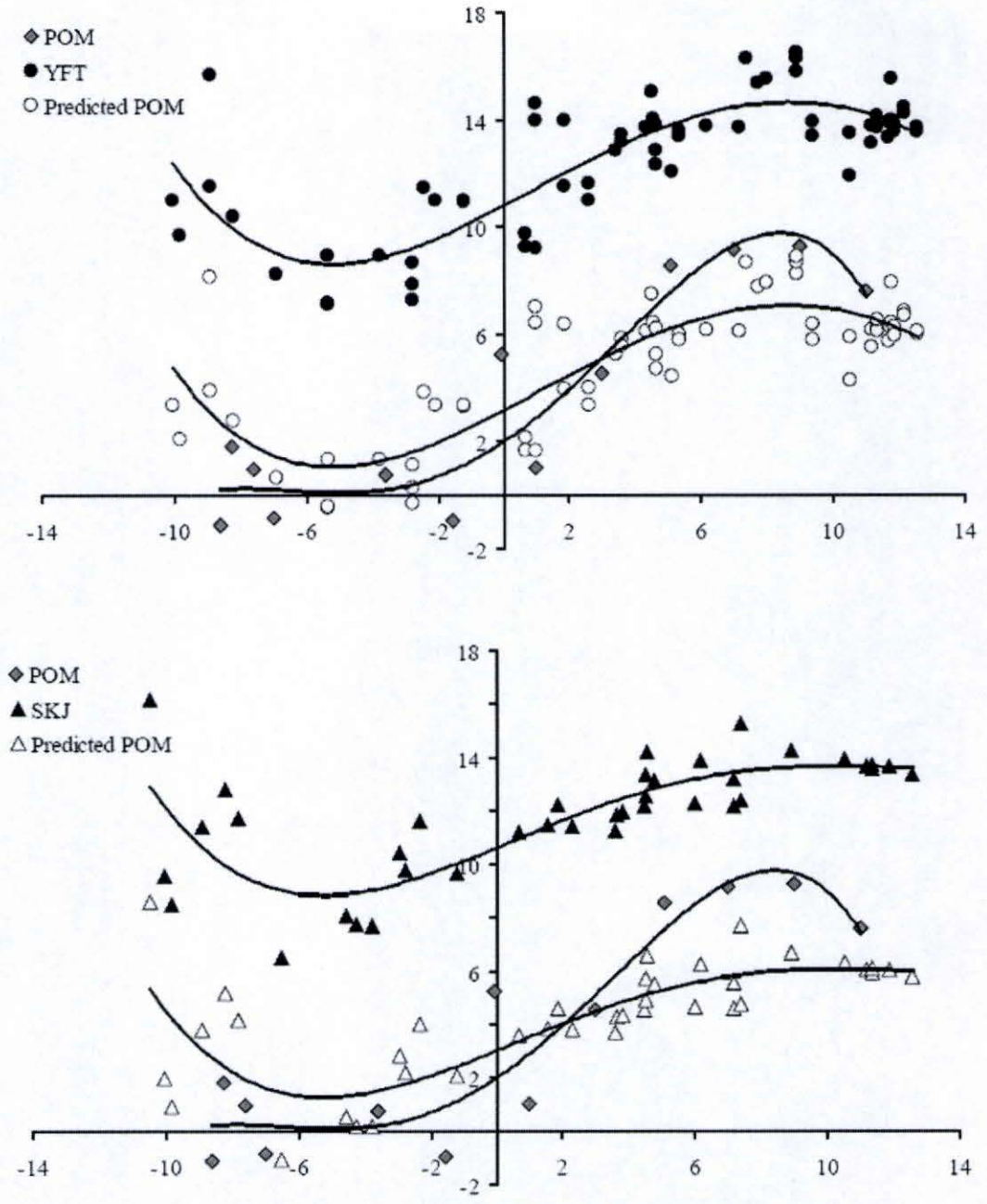

Fig. 4.5 Yellowfin tuna (top panel), skipjack tuna (bottom panel), and POM $\delta^{15}N$ values versus latitude for an equatorial transect at 125°W in the eastern Pacific Ocean. Predicted POM $\delta^{15}N$ values were calculated using the tuna $\delta^{15}N$ values, and is simply the tuna $\delta^{15}N$

value minus $TL_{POM\text{-}TUNA}$*(TEF) or 7.6‰ (Eqn 1). Deviations of the predicted values from the measured POM $\delta^{15}N$ values indicate net meridonal movements.

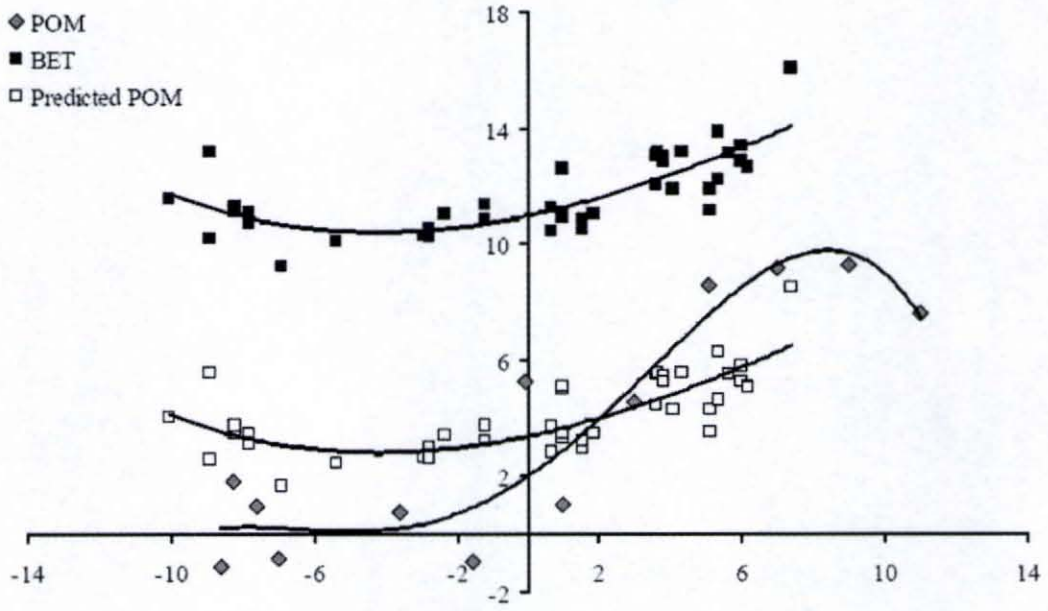

Fig. 4.6 Bigeye tuna and POM $\delta^{15}N$ values versus latitude for an equatorial transect at 125°W in the eastern Pacific Ocean. Predicted POM $\delta^{15}N$ values were calculated using the tuna $\delta^{15}N$ values, and is simply the tuna $\delta^{15}N$ value minus $TL_{POM\text{-}TUNA}$*(TEF) or 7.6‰ (Eqn 1). Deviations of the predicted values from the measured POM $\delta^{15}N$ values indicate net meridonal movements.

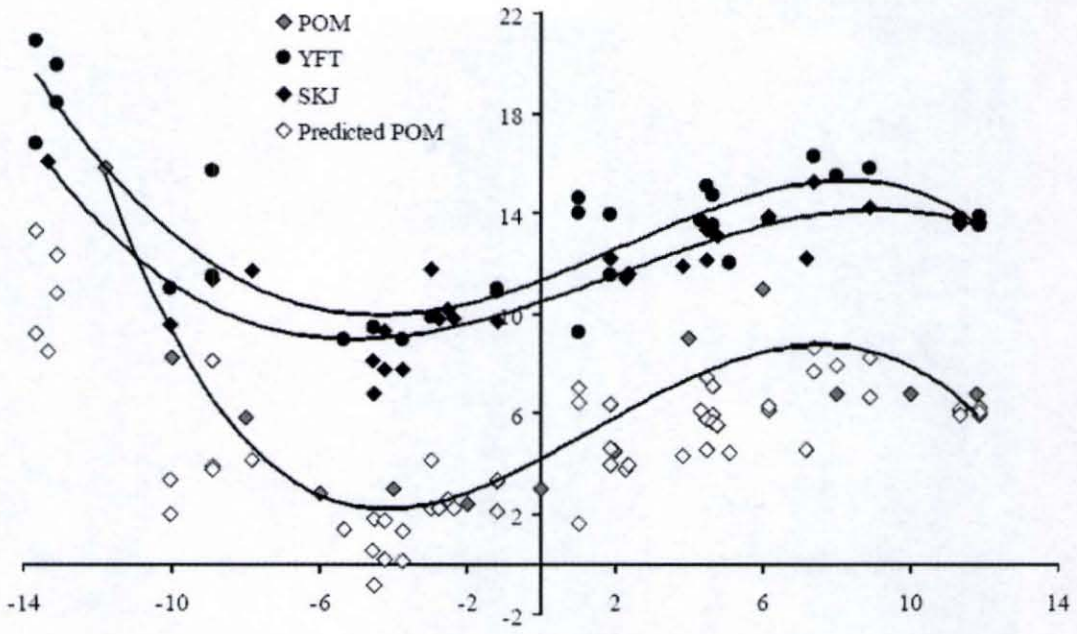

Fig. 4.7 Yellowfin tuna, skipjack tuna, and POM $\delta^{15}N$ values versus latitude for an equatorial transect at 140°W in the central Pacific Ocean. Predicted POM $\delta^{15}N$ values were calculated using the measured tuna $\delta^{15}N$ values, and are the tuna $\delta^{15}N$ value minus $TL_{POM\text{-}TUNA}$*(TEF) or 7.6‰ (Eqn 1). Similarities Deviations of the predicted POM values from the measured POM $\delta^{15}N$ values indicate net meridonal movements by tuna.

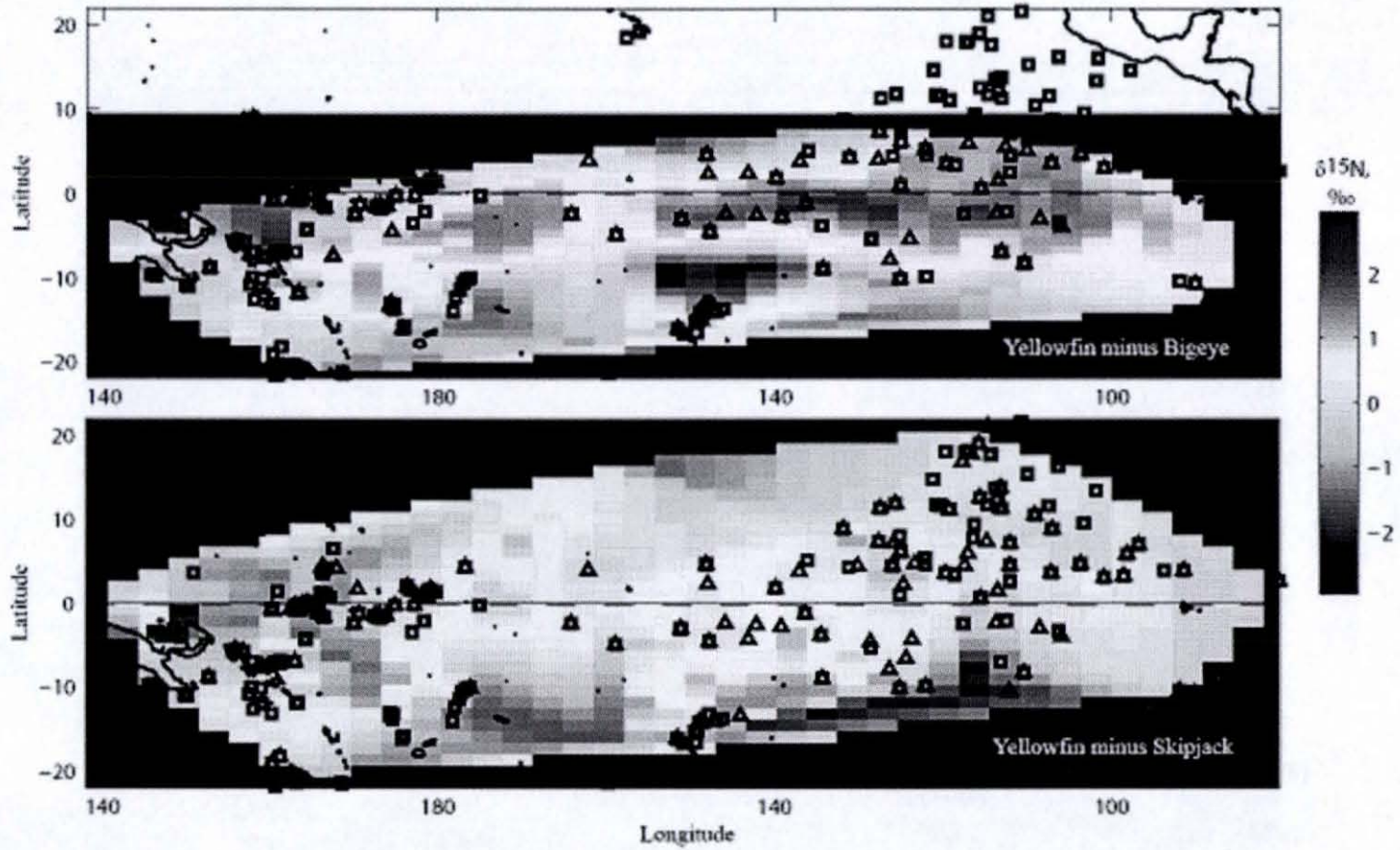
Yellowfin minus Bigeye
Yellowfin minus Skipjack
Latitude
Longitude
20
10
0
−10
−20
140
180
140
100
δ15N,
‰
2
1
0
−1
−2

Fig. 4.8 Inter-species geographical differences in $\delta^{15}N$ values. *Top panel*: the difference between yellowfin and bigeye tuna $\delta^{15}N$ values, once the values have been normalized to the basin average. *Bottom panel*: the difference between yellowfin and skipjack tuna. Fig. 4.8. is best viewed in color - contact the author for a color copy.

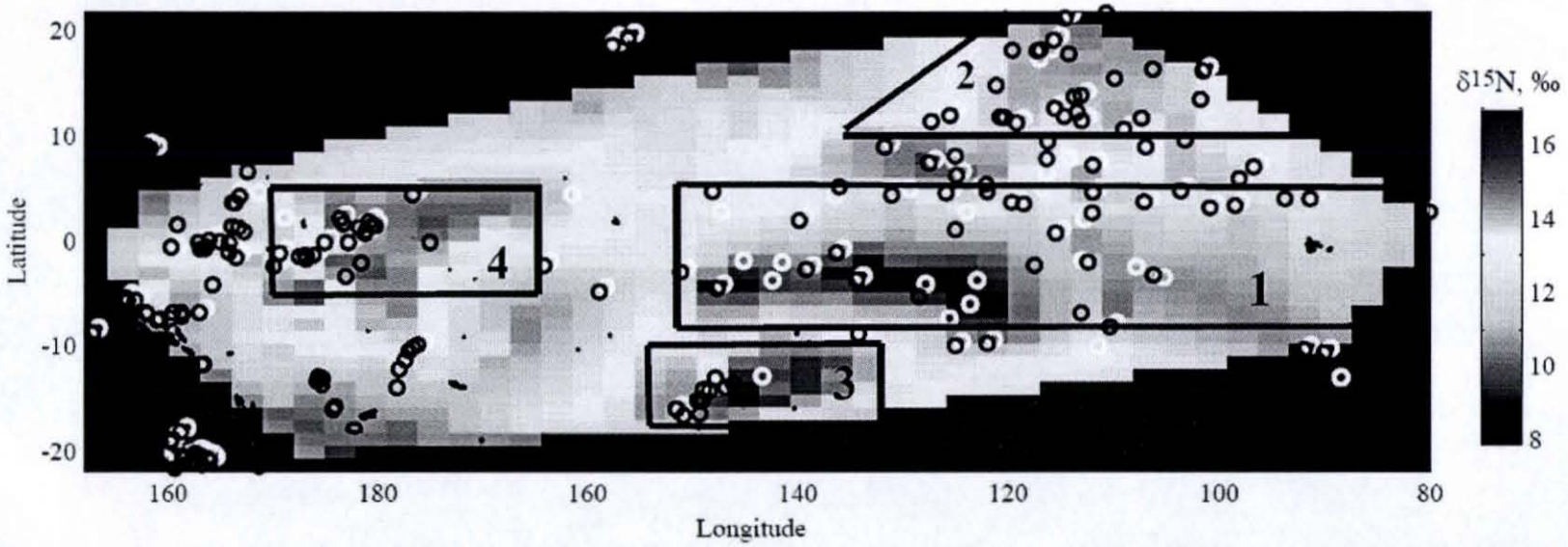

Fig. 4.9 A map of distinct iso-biogeochemical provinces. Yellowfin and skipjack data combined. 1 = equatorial upwelling zone, HNLC region. 2 = northern ETP OMZ region. 3 = Marquesas Islands. 4 = Gilbert Island. Fig. 4.9. is best viewed in color - contact the author for a color copy.

CHAPTER 5

Trophic dynamics of yellowfin tuna in the tropical Pacific Ocean inferred from bulk tissue and compound-specific nitrogen isotope analysis.

5.1 Abstract

This study combines bulk tissue nitrogen isotope results of yellowfin tuna collected across the tropical Pacific Ocean with compound-specific nitrogen isotope analysis of individual amino acids to determine the mechanisms producing variations in bulk tissue $\delta^{15}N$ values. Bulk $\delta^{15}N$ values of animals have been used to examine foraging strategies, and, in general, marine food web structure across varying spatial and temporal scales. However, variations in a predator's bulk tissue $\delta^{15}N$ value can be erroneously interpreted as shifts in foraging behavior or trophic level if these isotopic variations actually reflect spatial and temporal changes in nutrient dynamics and their affect on the $\delta^{15}N$ values of primary producers at the base of the food web. Recent evidence suggests that compound-specific nitrogen isotopic analyses (CSIA) of individual amino acids can be used to distinguish the competing influences of trophic level and changing $\delta^{15}N$ value of primary producers on the bulk tissue $\delta^{15}N$ values of marine predators.

On a basin-wide scale, CSIA indicates that the observed 12‰ variation in yellowfin tuna bulk $\delta^{15}N$ values across the equatorial Pacific reflected changes in the isotopic compositions of phytoplankton at the base of the food web and that the trophic level of yellowfin tuna did not vary significantly. On a regional scale, CSIA indicates that the trophic

level between small and large size classes of yellowfin from Hawaii did not differ. Instead, an observed rapid ontogenetic shift in the $\delta^{15}N$ values of white muscle tissue reflected a change in vertical foraging habitat, with juveniles confined to the surface mixed layer and large size classes foraging over a greater vertical range. Bulk and amino acid isotope results of larger yellowfin suggest there are at least two general foraging strategies within this regional population, with one group foraging at greater depths than the other group. Thus, yellowfin tuna in Hawaii may be demonstrating physiological and behavioral vertical niche separation. Over all spatial scales, compound-specific isotope results reveal that tuna $\delta^{15}N$ values reflect processes that occur at the base of the food web and that these CSIA data can be used to examine vertical and horizontal foraging patterns in the pelagic environment.

5.2 Introduction

Commercial fishing and whaling has selectively removed large quantities of top predators from marine food webs since the early 20th century, which has lead to a scientific debate on the affect of industrialized fisheries and whaling in altering marine ecosystems (Pauly et al. 1998; Springer et al. 2000; Myers and Worm 2003; Sibert et al. 2006). Selective removal of large predators from food webs can impart changes to the food web structure through trophic cascades (Carpenter et al. 1985; Estes et al. 1998; Pace et al. 1999; Pauly et al. 2000; Springer et al. 2000; Myers et al. 2007). If the majority of top predator biomass is reduced, then there could be a release in predation and within-trophic guild competition (e.g., Springer et al. 2000). These changes could then lead to niche expansions and retractions in other functional groups within the pelagic food web, and, ultimately, nutrient and biomass

redistribution (Estes et al. 1998; Springer et al. 2000). For example, it has been suggested that the recent range expansion and increase in the biomass of humboldt squid (*Dosdicus gigas*) in the eastern tropical Pacific relates to the reduction of top-down predation because their primary predators, large tuna and billfish, have been removed by industrialized fishing (Zeidberg and Robison 2007). Complex trophic cascades are often difficult to detect in marine ecosystems, and even more difficult to predict, especially when our current knowledge on the trophic status of top marine predators is relatively limited and based mainly on fisheries-dependent data. Before we can begin to assess the effects of industrialized fishing on the trophic dynamics of top predators, and, in general, on the pelagic food web, the trophic status of these predators must be established.

Stable isotope analysis (SIA) has been shown to be a potentially powerful tool for past and present diet reconstructions of individuals and populations (e.g., Fry 1988; Hobson et al. 2004; Cherel and Hobson 2007). The ratio of ^{15}N to ^{14}N ($\delta^{15}N$ in ‰ notation) exhibits a stepwise enrichment at each trophic level (Minagawa and Wada 1984). $\delta^{15}N$ values of consumers increase by 2-4‰ relative to their diet and this value is commonly referred to as the trophic enrichment factor (TEF) (DeNiro & Epstein 1981; Vander Zanden and Rasmussen 2001; Vanderklift and Ponsard 2003; Chapters 2 and 3). In addition to the stepwise trophic enrichment, a consumer's $\delta^{15}N$ value is also a function of the $\delta^{15}N$ value of the primary producers at the base of the food web or the isotope baseline (e.g., Shell et al. 1998). In the tropical Pacific Ocean, $\delta^{15}N$ values of marine phytoplankton can change temporally and spatially due to variations in the $\delta^{15}N$ values of nitrogen sources, the degree of nitrate utilization by phytoplankton in the surface mixed layer, and uptake of partly denitrified nitrate in the oxygen minimum zone of the eastern tropical Pacific. Therefore,

before inferring trophic level (TL) from the bulk $\delta^{15}N$ values of top predators, the isotopic composition at the base of the food web or the isotopic baseline needs to be characterized. Otherwise, the affects of spatial and temporal variations in nutrient dynamics on the $\delta^{15}N$ values (or TL estimates) of marine predators might be erroneously interpreted as shifts in foraging behavior.

The $\delta^{15}N$ value of marine phytoplankton is commonly inferred by analyzing suspended particulate nitrogen (PN) collected in surface waters (e.g., Fry 1988; Kaehler et al. 2000; Hill et al. 2006). However, these samples might not be representative of phytoplankton or the available nitrogen to the base of the food web because they may not consist entirely of phytoplankton cells and can contain consumers (microzooplankton) and refractory components that are not actively grazed upon by consumers. In addition, the $\delta^{15}N$ values of isolated phytoplankton can respond to very short-term temporal fluctuations at the base of the food web (e.g., O'Reily et al. 2002; Jennings et al. 2002), and, therefore, might be decoupled from top predators because of temporal differences in their biological responses to environmental changes. Alternatively, a primary consumer, such as herbivorous zooplankton, could be sampled to assess the spatial and temporal variability at the base of the food web (Schell et al. 1998; Kline 1999; Jennings et al. 2002; Jennings and Warr 2003). Similar to PN samples, the collection of zooplankton is time and labor intensive and unless net samples are sorted to the species level, these samples can include a range of trophic guilds and non-zooplankton organic matter. Although $\delta^{15}N$ values of predators can be used to examine foraging behavior and food web structure across varying spatial and temporal scales in the marine ecosystem (Fry 1988; Schell et al. 1998; Kline et al. 1999; Jennings et al. 2002; Hobson et al. 2004; Cherel and Hobson 2007), these predator $\delta^{15}N$ values must be

coupled with ancillary biological, chemical and isotopic data to evaluate the potential affects of a fluctuating isotopic baseline.

Compound-specific $\delta^{15}N$ analysis of specific amino acids isolated from consumers' tissues can distinguish between changes in trophic dynamics and spatial shifts in the $\delta^{15}N$ values at the base of the food web (McCelland and Montoya 2002; McCelland et al. 2003; Schmidt et al. 2004; Pakhmov et al. 2004; Popp et al. 2007). McCelland and Montoya (2002) were the first to show that a group of amino acids (alanine, leucine, isoleucine, aspartic acid, and glutamic acid) strongly fractionate ^{15}N with each trophic level, whereas another group of amino acids (glycine and phenylalanine) do not fractionate from one trophic level to the next. These authors also demonstrated that a stepwise TEF between the fractionating and conservative group of amino acids was between 5-7‰. Accordingly, an organism's trophic level and the $\delta^{15}N$ value of the nitrogen incorporated into the base of the food web can potentially be determined by comparing $\delta^{15}N$ values of trophic transfer (fractionating) and source (conservative) amino acids extracted from a consumer's tissue sample (e.g., Schmidt et al. 2004; Pakhomov et al. 2004; Popp et al. 2007; Hannides 2007). Past CSIA studies have shown that this method can be used to determine the trophic level of a diverse group of marine organisms (e.g., phytoplankton, composite and isolated zooplankton samples, gastropods, tuna) in both the open ocean and coastal ecosystems (McCelland and Montoya 2002; McCelland et al. 2003; Schmidt et al. 2004; Pakhomov et al. 2004; Popp et al. 2007; Hannides 2007). A major advantage of compound-specific isotope analysis (CSIA), relative to characterizing the isotopic baseline and the $\delta^{15}N$ value of the consumer with bulk SIA, is that the TL of a consumer and the $\delta^{15}N$ values at the base of the food web can be simultaneously determined with a single sample of the consumer.

In the equatorial Pacific, bulk tissue $\delta^{15}N$ values of tropical tunas are highly variable but show coherent spatial patterns (Chapter 4). Although the spatial patterns in the $\delta^{15}N$ values of tuna are similar to observed patterns in the $\delta^{15}N$ values at the base of the food web in the Pacific Ocean, as revealed by $\delta^{15}N$ analyses of PN (e.g., Altabet et al. 2001; Voss et al. 2001; Dore et al. 2002) and sediment (e.g., Farrell et al. 1995) samples, the variations could also relate to changes in the TL of tuna. By measuring the $\delta^{15}N$ of amino acids isolated from yellowfin tuna collected in the eastern tropical Pacific (ETP), Popp et al. (2007) showed that a latitudinal $\delta^{15}N$ trend observed in their bulk $\delta^{15}N$ values was due to changes in $\delta^{15}N$ values at the base of the food web and not differences in the TL of yellowfin tuna. These authors found lower $\delta^{15}N$ values of tuna near the equator that presumably reflected isotopic fractionation associated with minimal phytoplankton nitrate assimilation of the new upwelled NO_3- (see also Chapter 4), and higher $\delta^{15}N$ values of tuna at higher latitudes that probably reflect a combination of increased nitrate utilization and the biological incorporation of partly denitrified NO_3- present in the ETP oxygen minimum zone (e.g., Cline and Kaplan 1975; Voss et al. 2001). At these different latitudes in the ETP, a TL of 4.5±0.1 (±1SD) for yellowfin tuna was estimated from the difference between the $\delta^{15}N$ of a trophic transfer (glutamic acid) and source (glycine) amino acids (Popp et al. 2007). This CSIA-based TL estimate matched well an estimate of 4.6-4.7 based on stomach content analysis (Olson and Watters 2003) and a TL estimate of 4.2±0.4 (±1SD) based on the difference between the $\delta^{15}N$ values of hundreds of mesozooplankton and yellowfin tuna in the ETP (see Popp et al. 2007). The convergence of these independent TL estimates provides additional evidence that the CSIA TEF of 7‰ between glutamic acid and a source amino acid is robust. The framework now appears to be set to use compound-specific isotope analysis of amino acids to separate

the potentially confounding influences of TL and baseline variations on bulk tissue $\delta^{15}N$ values and, in turn, resolve the trophic status of yellowfin tuna in the tropical Pacific.

The aim of this study was two-fold. My first objective was to extend the work of Popp et al. (2007) from a regional to basin-wide CSIA investigation of the trophic status of yellowfin tuna. Specifically, I examined if trophic dynamics or shifts in the $\delta^{15}N$ values at the base of the food web were responsible for large (~12‰) geographical variations observed in bulk tissue $\delta^{15}N$ values of yellowfin tuna in the equatorial Pacific Ocean (see Chapter 4). Second, I applied CSIA to investigate if the distinct ontogenetic shift observed in juvenile yellowfin tuna from Hawaii was produced by a change in trophic level or a change in foraging habitat. Graham et al. (2007) showed that the $\delta^{15}N$ values of yellowfin tuna collected around Hawaii increased by 5-6‰ rapidly between 45-50 cm forklength (c.a., 1.5 kg) suggesting a rapid change in diet. Stomach content analysis corroborated this finding and demonstrated that the smallest size classes of juvenile yellowfin fed predominatly on planktonic organisms inhabiting the shallow mixed layer, whereas larger individuals fed heavily on teleosts and a vertically migrating mesopelagic species of shrimp (Graham et al. 2007). These authors hypothesized that the observed ontogenetic diet shift in yellowfin tuna was triggered by the onset of functional endothermy whereby a threshold mass was reached and juvenile tunas could then penetrate below the thermocline and access additional prey. Two hypotheses could explain the distinct ontogenetic shift in tuna bulk tissue $\delta^{15}N$ values. First, a shift in trophic level occurred because juvenile tuna confined to the surface mixed layer were foraging on prey that were at lower trophic levels than the larger size classes of yellowfin. Alternatively, the ontogenetic shift was produced by a difference in the $\delta^{15}N$ value of nitrogen source(s) present in the surface mixed layer and waters below the

thermocline. By analyzing samples of small and large size classes of yellowfin tuna from around Hawaii for CSIA, I determine if the ontogenetic $\delta^{15}N$ shift is a result of a shift in trophic level, foraging habitat, or a combination of both factors.

5.3 Material and Methods

5.3.1 Sample Collection

Detailed descriptions of collection of yellowfin (YFT) and bigeye (BET) tuna from Hawaii and from the western and central tropical Pacific can be found in Graham et al. (2007) and Chapter 4, respectively. I present here only a brief description of methods used to collect tuna from the equatorial Pacific. The majority of bulk tissue and all of CSIA $\delta^{15}N$ values presented for YFT from the eastern tropical Pacific Ocean (ETP) were reported by Popp et al. (2007). In the western and central tropical Pacific, yellowfin tuna were captured by long-line and purse-seine fishing vessels between March 2001 and September 2005 and were sampled on board the vessels by observers from the national observer programs of the region (Papua New Guinea, Federated States of Micronesia, Solomon Islands, Fiji, Marshall Islands, New Caledonia, French Polynesia, Wallis & Futuna). With each collection event, the geographical location was recorded (latitude and longitude), the fish were measured for fork length (FL, cm), white muscle tissue collected for stable isotope analyses, and samples were then stored at -20°C until processed further. CSIA samples were selected from regions where the bulk $\delta^{15}N$ values of yellowfin were anomalously high (i.e., Marquesas Islands and Gilbert Islands) or low (i.e., Samoan Islands) to determine if these values were produced by variations in trophic dynamics, nutrient dynamics, or a combination of both.

Six adult tuna samples collected from Hawaii were selected for CSIA. Five yellowfin tuna and 1 bigeye tuna white muscle tissue samples were collected from a nearshore FAD (MM FAD; 21.4°N, 157.3°W) during July 2004. Also, muscle tissue from two juvenile yellowfin tuna selected for CSIA that were collected from 2 nearshore FADs (S and L FAD; 21.2-21.4°N, 157.6-158.1°W) during November 2002. Two major prey items of juvenile and adult tuna were also selected for CSIA from the stomachs of tuna. Larval stomatopoda (*Odontodactylus spp.*) occur in the surface mixed layer and comprise 39% (mean precent volume) of the diet of juvenile tuna (<45cm FL) (Graham et al. 2007). The CSIA stomatopod sample was a composite of many larvae (>12 individuals) and was collected in October 2003. A vertically migrating mesopelagic species of shrimp, *Oplophorus gracilirostris* comprises 26% (mean precent volume) of the diet of large tuna (>50cm FL) and the composite sample (6 individuals) was collected in June 2005. Both prey items were collected at nearshore FADS on the windward side of Oahu.

5.3.2 Sample Preparation for Compound-specific Stable Isotope Analyses

Preparation of samples for compound-specific stable isotope analysis followed the protocols summarized in Popp et al. (2007). Only an overview is presented here and I refer the reader to Popp et al. (2007) for the specific details on the methods and materials. During sample preparation sample vials were (a) always capped using a Teflon-faced lid, (b) prior to heating, flushed with N_2, and (c) dried or evaporated with a stream of N_2. To hydrolyzed samples, approximately 4-6 mg of homogenized muscle tissue were transferred to high-temperature reaction vials, ~1 mL 6N HCl added, heated to 150°C for 70 minutes, and cooled to room temperature with an ice bath. These hydrolysates were evaporated and the residue

redissolved in 1 mL 0.01N HCl. This solution was purified by filtration (0.45 μm hydrophilic filter) followed by a 1 mL 0.01N HCl rinse. The solution was further purified using a cation-exchange column and the amino acids were eluted with 4 mL 2N ammonium hydroxide and dried (see Metges et al. 1996). Prior to the derivatization, samples were re-acidified with 1 mL 0.01N HCl.

Amino acid derivatization included esterification of the carboxyl terminus followed by trifluoracetylation of the amine group. To derivatize the samples, 2 mL of 4:1 isopropanol:acetyl chloride were added to dried samples, the vials capped, and heated to 110°C for 60 min. Samples were then dried, acylated by the addition of 1 mL 3:1 methylene chloride: trifluoroacetic anhydride (TFAA), heated to 100°C for 15 minutes, and then dried at room temperature. Samples were further purified using the method of Ueda et al. (1989). Briefly, 3 mL of 1:2 chloroform:P-buffer (KH_2PO_4 + Na_2HPO_4 in Milli-Q water, pH 7) were added to the samples, samples were sonicated for 1-5 minutes, and the separate chloroform portion transferred to a clean vial. The purification process was repeated twice. Finally, to insure complete derivatization of the samples, the TFAA acylation step was repeated. The amino acid samples or TFA derivatives were stored in 3:1 methylene chloride:trifluoroacetic anhydride at 4°C. Prior to stable isotope analysis, samples were evaporated and 100 to 1000 μl of ethyl acetate was added.

Amino acids from a few tuna muscle tissues were identified using a Hewlett-Packard 6890 gas chromatograph coupled to a Hewlett-Packard 5973 mass selective detector (GC-MS). Before analysis, TFA derivatives were evaporated at room temperature under N_2 and re-dissolved in 1000 μl ethyl acetate. Samples (1-2 μl) were injected (split/splitless, 50:1 split ratio) onto a 30 m HP-5ms column (0.25 mm i.d., 0.25 mm film thickness) at an injector

temperature of 180°C and a constant helium flow rate of 1 mL min^{-1}. The column oven was initially held at 50°C for 1 min, ramped to 185°C at 5°C min^{-1} and then to 300°C at 10°C min^{-1}, and finally held at 300°C for 10 min. The mass inlet temperature was 300°C. Amino acid composition of each sample was determined by comparison with the 1998 NIST library. GC-MS and CSIA measured alanine, glycine, threonine, valine, serine, leucine, isoleucine, proline, aspartic acid (incl. asparagine), glutamic acid (incl. glutamine), phenylalanine, histidine, tyrosine, lysine, and arginine. Clear, isolated peaks for threonine, valine, serine, tyrosine, lysine and arginine were not produced with our analytical methods and, therefore, isotopic measurements were not recorded for these specific amino acids.

5.3.4 Compound-specific Stable Isotope Analyses

The $\delta^{15}N$ values of specific amino acids were analyzed by isotope ratio monitoring gas chromatography-mass spectrometry (irmGCMS) using a ThermoFinnigan Delta-Plus XP mass spectrometer interfaced to a Trace GC gas chromatograph through a GC-C III combustion furnace (980°C), reduction furnace (680°C), and liquid nitrogen cold trap. L-2-aminoadipic acid (AAA), for which the $\delta^{15}N$ value was known, was co-injected as an internal reference. Samples (1-3 µl), plus the AAA reference, were injected (split/splitless, 5:1 split ratio) onto a 50 m HP Ultra-2 column (0.32 mm i.d., 0.5 µm film thickness) at an injector temperature of 180°C and a constant helium flow rate of 2 mL min^{-1}. The column oven was initially held at 50°C for 2 min, ramped to 190°C at 8°C min^{-1} and then to 280°C at 10°C min^{-1}, and finally held at 280°C for 10 min. Samples were analyzed at least in triplicate and the measured $\delta^{15}N$ values were normalized to the $\delta^{15}N$ of the aminoadipic acid reference

standard. Reproducibility associated with these isotopic measurements averaged 0.9 ‰ and ranged from 0.1 and 2.3‰.

5.3.5 Trophic Level Calculations

Trophic level of yellowfin tuna was determined by comparing the $\delta^{15}N$ values of source and trophic transfer amino acids:

$$TL_{Glu-Gly} = \left[\frac{\delta^{15}N_{Glu} - \delta^{15}N_{Gly}}{\text{TEF}}\right] + 1 \quad \text{and} \quad TL_{TT-S} = \left[\frac{Avg\delta^{15}N_{TT} - Avg\delta^{15}N_{S}}{\text{TEF}}\right] + 1 \qquad (1)$$

where TEF represents the trophic enrichment value between a consumer and its diet and has been estimated as 7‰ for glutamic acid and a source amino acid (McClelland and Montoya 2002). Glycine was used as the primary source amino acid, rather than phenyalanine, because with the irmGCMS method used the measured reproducibility of glycine was greater than phenylalanine. In addition to a single-amino acid source and trophic transfer comparison, I also calculated trophic level based on the average difference between trophic transfer (TT) (alanine, leucine, isoleucine, and glutamic acid) and source (S) (glycine and phenylalanine) amino acids.

5.4 Results

The distribution in the $\delta^{15}N$ values of trophic transfer and source amino acids measured in adult yellowfin tuna was similar to that observed in earlier CSIA studies (McCelland and

Montoya 2002; Schmidt et al. 2003; Popp et al. 2007; McCarthy et al. 2007), except $\delta^{15}N$ values of phenylalanine (Phe) were consistently greater than Glycine (Gly) (Table 5.1, 5.2; Fig. 5.2). Alanine (Ala), leucine (Leu), isoleucine (IsoLeu), and glutamic acid (Glu) represented the group of enriched, trophic transfer amino acids whereas Gly and Phe represent the conservative, source amino acids (Table 5.1, Fig. 5.2). A very large range was observed in average source (-7.4 to 13.8‰) and trophic transfer (17.6 to 31.2‰) amino acid $\delta^{15}N$ values among the yellowfin collected from the tropical Pacific (Table 5.1, 5.2).

5.4.1 Basin-wide Trophic Dynamics of Yellowfin Tuna

For all adult YFT in the tropical Pacific, the trophic level (TL) estimate based on Glu and Gly ($TL_{Glu\text{-}Gly}$) was consistently higher than TL estimated by an average of trophic transfer and source amino acids ($TL_{TT\text{-}S}$) (Eqn 1). This discrepancy results from $TL_{S\text{-}TT}$ including the $\delta^{15}N$ values of Phe, which are consistently higher than Gly (Fig. 5.2). The average $TL_{TT\text{-}S}$ and $TL_{Glu\text{-}Gly}$ estimates for all YFT were relatively consistent over the tropical Pacific at 4.1 ±0.3‰ and 4.4 ±0.2‰, respectively. A CSIA-based TL estimate should, however, be considered as a relative measure for comparative purposes and not as an absolute measure of TL until studies are conducted to determine the controls on the $\delta^{15}N$ values of source AA and the error associated with the CSIA trophic enrichment factor.

For the reminder of the discussion, I will report the TL estimate as $TL_{TT\text{-}S}$ to aid in comparisons with previous studies (i.e. Popp et al. 2007; McCarthy et al. 2007; Hannides 2007). There was only one individual YFT (FL= 63cm, bulk $\delta^{15}N$ value = 10.4‰) that had a significantly higher $TL_{TT\text{-}S}$ estimate than this basin-wide average (t-test, p-value < 0.01; Table 5.1, Fig. 5.2). Popp et al. (2007) reported CSIA data for this individual and it was a

composite sample collected at 8.1°S and 110.3°W in the ETP. When comparing only the $TL_{TT\text{-}S}$ of YFT between regions, yellowfin tuna collected in the ETP (reported by Popp et al. (2007)) had significantly higher $TL_{TT\text{-}S}$ estimates than yellowfin collected in the western tropical Pacific and Hawaii (t-test, both p-values <0.01). However, this regional difference in $TL_{TT\text{-}S}$ needs to be viewed cautiously because the error associated to the CSIA-TEF of 7‰ has not be systematically examined and this comparison only represents 16 samples of YFT.

A positive correlation exists between the $\delta^{15}N$ values of the YFT source amino acids and the $\delta^{15}N$ values of bulk (Fig. 5.3). The $\delta^{15}N$ values of the YFT trophic transfer (Glu and Ala) and source (Gly) amino acids had a relatively constant offset (i.e. demonstrated by the consistent YFT $TL_{TT\text{-}S}$) of approximately 25‰ (Table 5.1). The slopes (and standard error) of the linear regressions for Glu, and Ala, and Gly were 0.93±0.17, 0.87±0.11, and 0.80 ±0.14, respectively. Source amino acid $\delta^{15}N$ values measured for 3 YFT collected in the ETP fell above ±1SE of the model estimated from the average source $\delta^{15}N$ value observed for all YFT (Fig. 5.3). Two YFT samples, collected in the Samoan Islands and Hawaii had source amino acid values that fell below ±1SE linear model (Fig. 5.3).

5.4.2 Regional Trophic Dynamics of Yellowfin Tuna

The average $TL_{TT\text{-}S}$ estimates for juvenile and adult YFT were 3.8 ±0.1 and 3.9 ±0.1, respectively, and were not statistically different (t-test, p-value > 0.01) (Table 5.1, Fig. 5.4). Bigeye tuna did have a higher $TL_{TT\text{-}S}$ estimate (4.2 ± 0.1) than the YFT, but inferences are limited because there was only a single sample. Stomatopod and oplophorid shrimp, major prey items of juvenile and adult tuna, had similar but lower $TL_{TT\text{-}S}$ estimates than tuna and were 3.1 ±0.1 and 3.0 ±0.1, respectively (Table 5.1).

The $\delta^{15}N$ value of a source amino acid, $\delta^{15}N_{Gly}$, was significantly less in juvenile than adult tuna YFT (t-test, p-value < 0.01) (Table 5.3, Fig. 5.4). Among the adult YFT, the $\delta^{15}N_{Gly}$ values of two YFT were significantly higher than for the 3 other adult YFT (t-test, p-value < 0.01) and were -3.7 ±0.2‰ and 0.0 ±0.1‰, respectively (Fig. 5.4). The $\delta^{15}N_{Gly}$ value of BET was similar to the group of YFT that had lower $\delta^{15}N_{Gly}$ values (Table 5.2). Stomatopod larvae had a lower $\delta^{15}N_{Gly}$ value than ophloporid shrimp (Table 5.2). For both tuna and prey, the other source amino acid, $\delta^{15}N_{Phe}$, demonstrates a similar pattern as $\delta^{15}N_{Gly}$ values, but all values were higher (Table 5.2, Table 5.3, Fig. 5.4).

5.5 Discussion

Yellowfin tuna play a key role in the pelagic environment as an upper-trophic level predator (e.g., Cox et al. 2002; Olson et al. 2003) that can access an extended vertical habitat range because of their unique endothermic capabilities (Carey 1973; Stevens and Carey 1983). To assess their trophic status and foraging behavior, stomach contents of yellowfin tuna have been described from several locations in the tropical Pacific Ocean. These studies generally concluded that yellowfin tuna are opportunistic predators that feed on very diverse prey base (e.g., Alverson 1963; Reintjes & King 1953; Brock 1985; Buckley and Miller 1994; Bertrand et al. 2002). However, these studies are conducted in discrete regions throughout the Pacific basin and until these findings are compiled and synthesized, insights into the trophic status of yellowfin across larger spatial scales will be limited (R. Olson/J. Young *personal communication*). This lack of understanding of the foraging behavior of tropical tuna over a broad scale is of special concern, because they are targeted by commercial fisheries across

the equatorial Pacific. In order to examine the potential trophic-cascading effects associated with the removal of tropical tuna biomass, the foraging habits of tropical tuna must be known over a spatial scale that is equivalent to the area covered by industrialized fisheries.

5.5.1 Basin-wide Trophic Dynamics of Yellowfin Tuna

Trophic Dynamics:

The trophic level of yellowfin tuna, inferred from compound-specific isotope analyses of source and trophic transfer amino acids was remarkably constant across the tropical Pacific Ocean. A trophic level estimate does not provide information on prey preference, but if individuals or populations have different trophic levels than there must be a divergence in foraging strategies. A consistency in the TL of yellowfin tuna is notable because the tropical Pacific Ocean is comprised of two conspicuously different biogeochemical provinces. The western warm pool and cold tongue in the eastern and central Pacific have different hydrological regimes, nutrient sources and concentrations, primary production rates, and community compositions (e.g., Martin et al. 1994; Longhurst 1998; Chavez et al. 1999; McPhaden 1999; Landry and Kirchman 2002; Mackey et al. 2002; LeBorgne et al 2002). Growing evidence suggests yellowfin tuna have restricted net movements in the (sub)tropical Pacific Ocean (Adams et al. 2003; Schaefer et al. 2007; Chapter 4) and restricted long-distant movements between warm pool and cold tongue regions (Hampton 2002). As such, semi-resident tuna could have developed divergent or region-specific foraging strategies for the warm pool and cold tongue ecosystems. CSIA

indicates that there is no large-scale differences in yellowfin TL and corroborates previous stomach content studies that show tuna are highly opportunistic predators (Alverson 1963; Reintjes & King 1953; Brock 1985; Buckley and Miller 1994; Bertrand et al. 2002). The prevalence of this opportunistic foraging strategy, inferred by a constant trophic level, suggests this strategy must be successful in the temporally and spatially dynamic tropical Pacific Ocean.

Isotopic Baseline:

With little difference observed in the TL of tuna based on the difference between the $\delta^{15}N$ values of source and trophic transfer amino acids implies that the variations in the bulk $\delta^{15}N$ values of tuna must be produced by changes in the $\delta^{15}N$ values of phytoplankton at the base of the food web (Fig. 5.3). This finding extends the initial work of Popp et al. (2007) that changes in nutrient dynamics at the base of the food web (i.e., isotopic baseline) produce variations in the bulk $\delta^{15}N$ values of tuna in the ETP to across the equatorial Pacific Ocean. It is remarkable that the 12‰ variation observed in bulk $\delta^{15}N$ values of yellowfin tuna reflect changes in the isotopic baseline. This relationship between the base of the food web and predator could provide general information on large scale patterns of biological fixed nitrogen in the pelagic environment. For example, deviations in the $\delta^{15}N$ values of source amino acids from the observed trend with $\delta^{15}N$ values of bulk tissue (see Fig. 5.3) could indicate the influence of alternate N sources on the isotopic baseline, or processes other than NO_3- utilization by phytoplankton (see Ch 4). For example, the higher $\delta^{15}N_{Gly}$ values observed for 3 of the ETP YFT, relative to the $\delta^{15}N_{Gly}$ values of the other EqPac tuna, could

indicate the influence of partly denitrified NO_3- originating from the ENTP oxygen minimum zone on the isotopic baseline (e.g., Voss et al. 2001; Popp et al. 2007), whereas the lower $\delta^{15}N_{Gly}$ values could indicate the contributions of N_2-fixation (Dore et al. 2002; Hannides 2007) (Fig. 5.4). Thus, variations in the bulk $\delta^{15}N$ values of tuna are not produced by changes in trophic level but rather reflect sources and processes affecting biologically-fixed nitrogen. This finding has important implications for other isotope ecology studies examining the foraging behavior of marine predators. Interpreting variations in the $\delta^{15}N$ values of marine animals solely as changes in TL or foraging behavior (e.g., Domi et al. 2005) would neglect the importance of nutrient sources and processes in controlling the isotopic baseline. Compound-specific isotope analysis of amino acids provides one direct method to potentially separate the competing influences of TL and isotopic baseline variations on the bulk tissue $\delta^{15}N$ values of marine predators and could perhaps hold for a variety of non-marine environments. Once these variables are separated the source amino acids $\delta^{15}N$ values could be used to examine the movements of tunas between isotopically-distinct environments much like bulk $\delta^{15}N$ values (Chapter 4).

5.5.2 Regional Trophic Dynamics of Yellowfin Tuna

Graham et al. (2007) proposed that a mass threshold was achieved by yellowfin tuna at ca. 45 cm FL that provided sufficient endothermic capability to allow tuna to dive into colder, deeper waters and access deeper-dwelling prey. Preliminary data from acoustic tags placed on FAD-associated tuna in two size groups, <40 cm FL and >65 cm FL, indicate to some degree that the smallest individuals show less extensive vertical movements than larger specimens coexisting at the same FADs around Oahu, Hawaii (K. Holland, *unpublished*

data). Further evidence that supports a physiological niche segregation by YFT, and suggests this phenomena is more universal, is that the % lipid measured in the muscle tissue of YFT collected from waters around New Caledonia showed a distinct and significant increased around 45-50cm FL, with larger tuna having higher lipid content than the smallest size classes (V. Allain, *unpublished data*). This preliminary data suggests that the expansion in vertical habitat range and the commensurate increase in prey resources could promote an increase in energy consumption and subsequently an increase in lipid reserves. Future research should examine if the high natural mortality rate (M) observed in the 20-45 cm FL size class of YFT around the Hawaiian Islands (Adams et al. 2003) relates to a restricted vertical habitat range reducing their foraging success or some other factor (e.g., size-related predation). In addition to the potential effect on foraging success or the fitness of an individual, the confinement of small size classes of YFT to the SML might also increase their interaction with artesianal and commercial fishing gear (e.g., Hampton 2000).

Trophic Dynamics:

Two hypotheses could explain the rapid increase in bulk $\delta^{15}N$ values at ~45cm FL in YFT from Hawaii. First, the shift could be produced by the surface-confined juveniles foraging on prey at lower trophic levels than the larger size classes of yellowfin. However, smaller and large size classes of YFT collected at FADs around Hawaii had a similar CSIA-based TL and this TL was comparable to the TL of YFT analyzed from other regions within the tropical Pacific Ocean. If TL did not vary between YFT <45cm FL and YFT >60cm FL, then the distinct ontogenetic shift must have been produced by a difference in the $\delta^{15}N$ value

of the nitrogen source in the surface mixed layer compared to the waters below the thermocline. The $\delta^{15}N$ values of the source (or conservative) amino acids were significantly different between juvenile YFT (i.e. <45cm FL) and larger YFT (>60cm FL). Juvenile $\delta^{15}N_{Gly}$ and $\delta^{15}N_{Phe}$ values were 2.5 and 5.4‰ lower than large YFT tuna, respectively. Thus, the ontogenetic shift observed in YFT around ~45 cm FL was not driven by a change in trophic level, but instead, the shift reflected a vertical gradient in the $\delta^{15}N$ values at the base of the food web (Rau et al. 1989; Graham et al. 2007).

In the tuna tissues analyzed in this study, $\delta^{15}N_{Gly}$ values were always less than the $\delta^{15}N_{Phe}$ values (an average difference of 8.6‰). This relationship was not observed in the two crustacean samples analyzed in this study. Furthermore, previous CSIA research conducted on a range of marine organisms showed that the $\delta^{15}N_{Gly}$ values and $\delta^{15}N_{Phe}$ were similar and that gly functioned as a source amino acid that tracked the nitrogen supporting the base of the food web (McClelland and Montoya 2002; McClelland, Holl and Montoya 2003; Schmidt et al. 2004; Popp et al. 2007; McCarthy et al. 2007). In general, gly does not undergo transamination as rapidly as many other amino acids, including the indepensible amino acid phe. Instead, gly is largely catabolized by deamination only (T. Preston *personal communication*). Glycine could become a conditionally essential amino acid if the metabolic demand for gly is greater than the amount that is biosynthesized. In crustacean and fish tissues gly is very abundant, with crustaceans having nearly 5 to 10 times the amount of gly as fish tissues (Carr et al. 1996). However, if gly was conditionally dispensable in tuna muscle tissues then we would expect to see higher $\delta^{15}N_{Gly}$ than $\delta^{15}N_{Phe}$ values. The contradictory relationship observed between gly and phe in tuna tissues might suggest that the transamination process leads to the greatest isotopic fractionation, resulting in a ^{15}N

enriched phe pool. Continuing with this reasoning, gly would best track the baseline isotope value and show less isotopic alteration. However, the $\delta^{15}N_{Gly}$ values are much lower than we would predict for the isotopic baseline in offshore waters around Oahu (Hannides 2007) and previous research shows a strong similarity between $\delta^{15}N_{Gly}$ than $\delta^{15}N_{Phe}$ values (McClelland and Montoya 2002; McClelland, Holl and Montoya 2003; Schmidt et al. 2004; McCarthy et al. 2007). Another explaination for the observed trend could be that there is some partitioning of gly between the metabolic pool and gly bound in the protein of the muscles, and the outputs (i.e., excretion) of gly is conserved or limited. Overall, the observed gly anomaly in the tuna CSIA dataset has produced more questions on amino acid metabolism and associated ^{15}N fractionation than answers.

Isotopic Baseline:

Biological N_2 fixation contributes significantly to new production in the surface mixed layer (SML) in the nitrogen-limited and stratified waters north of Oahu (Karl et al. 1997). In oceanic regions where N_2 fixation is important the $\delta^{15}N$ value at the base of the food web should be low (-2 to 1‰; Carpenter et al. 1997), because the $\delta^{15}N$ of atmospheric N_2 is 0‰ and there is little isotopic fractionation associated to the biological fixation of N_2 (Wada and Hattori 1976; Saino and Hattori 1987; Carpenter et al. 1997). The other source of new nitrogen to the SML is the injection of deep-water NO_3-, which has a $\delta^{15}N$ value of approximately 5-7‰ (Sigman et al. 1999; Sigman et al. 2005; P. Rafter *unpublished data*). This difference in the $\delta^{15}N$ values in nitrogen sources provides a framework for assessing the importance of N_2-fixation and NO_3- injection in to SML. For example, bulk $\delta^{15}N$ values of

suspended particulate nitrogen (PN) collected from the surface waters north of Oahu were low (-1 to 1‰) and indicated a significant input of N_2 fixation to the base of the food web in the SML (Dore et al. 2002).

Nitrogen isotopic values of the source amino acids should reflect baseline variations. By analyzing individual amino acids isolated from zooplankton and a N_2-fixing cyanobacterium (*Trichodesium spp.*), McCelland et al. (2003) showed that low $\delta^{15}N$ values of source amino acids observed in mixed zooplankton samples reflected N_2 fixation in the surface waters of the tropical North Atlantic. More recently, Hannides (2007) showed that bulk and source amino acid $\delta^{15}N$ values of several individual species of zooplankton collected north of Oahu varied extensively over time and were lower in the summer when water column stratification was the strongest and higher in the winter during wind-induced mixing of the water column. During periods of water column stratification the injection of NO_3- to the SML is reduced, which can provide a competitive advantage to N_2-fixers (Karl et al. 1997). Bulk and source $\delta^{15}N$ values are the lowest in waters north of Oahu during these periods and interpreted as the relative importance of N_2 fixation to SML production (Dore et al. 2002; Hannides 2007). From a top-down perspective, the low source amino acid $\delta^{15}N$ values observed in juvenile yellowfin collected around Oahu suggest that while juvenile tuna are confined to the SML, N_2-fixation may play a more important role in supporting the secondary production or their prey production. Juvenile yellowfin $\delta^{15}N_{Gly}$ and $\delta^{15}N_{Phe}$ or source amino acid values were 3 and 5‰ less than those of large size classes of YFT. After the onset of endothermy, yellowfin can then dive below the SML and access food resources that rely more heavily on nitrate production, which could explain why their source amino acid $\delta^{15}N$ values are higher than the surface-dwelling juveniles. Compound-specific isotope

analysis of the two major prey items of tuna also supports that there is a vertical $\delta^{15}N$ gradient in the waters around Hawaii, with lower source amino acid $\delta^{15}N$ values in the SML than below the thermocline. Larval stomatopods, which occur in the SML and comprise a major component of the juvenile tuna (<45cm FL) diet, have the same trophic level (i.e. ~3.0) but a lower $\delta^{15}N_{Gly}$ or source amino acid $\delta^{15}N$ value than the vertically migrating mesopelagic species of shrimp that is an important component of the diet of larger tuna. However, before we can suggest the $\delta^{15}N$ values of the source amino acids isolated from two major tuna prey relates to the depth at which they feed, more samples would need to be analyzed in both space and time to examine the influence of vertical depth and seasonal stratification of the water column on source amino acid $\delta^{15}N$ values. In summary, compound-specific isotope analysis shows that the observed ontogenetic shift in tuna bulk $\delta^{15}N$ values is driven mainly by changes at the base of the food web and suggests that N_2-fixation is more important in supporting biological production in the SML than at depth in the nearshore waters north of Oahu.

Among the larger yellowfin tuna (YFT) collected in Hawaii, two individuals had significantly higher $\delta^{15}N_{Gly}$ values than the other 3 individual yellowfin and 1 bigeye tuna (Fig. 5.4). These distinct source amino acid values could indicate that these individuals recently migrated from another region with a higher $\delta^{15}N$ values at the base of the food web (e.g., ETP). Both of these individuals were captured in the summer time, which is the time YFT spawn in Hawaii waters and a time when there is an influx of large (reproductive-aged) YFT to the region (Itano 2002). However, these individuals were smaller than the minimum length of maturity for YFT in Hawaii (81cm FL; Itano 2002) and their small size indicates that they are not yet a component of the spawning population. Alternatively, these tuna with

different $\delta^{15}N_{Gly}$ values could represent yellowfin tuna residents with different foraging strategies.

A tag and recapture study conducted in Hawaii revealed that large numbers of yellowfin tuna reside in the region for several years (Adam et al. 2003). In addition to the $\delta^{15}N_{Gly}$ high and low group, bulk $\delta^{15}N$ of YFT ≥50 cm FL from Hawaii could be roughly grouped into two categories, with a group with higher $\delta^{15}N$ values (~ 13‰) and lower $\delta^{15}N$ values (~9.5-10‰) (Graham et al. 2007; Fig. 2.2). These two $\delta^{15}N$ groups, reflected in bulk and compound-specific isotope analysis, could result from different foraging modes within the population, where a fraction of the resident adult population forages at depth more frequently (higher values) and the others forage more commonly within the SML (lower values). Neither bulk nor compound-specific isotope results can clearly indicate the temporal and spatial plasticity of this behavioral vertical niche partitioning, but with the resolution of electronic and acoustic tags the extent and frequency these foraging modes could be addressed. In the eastern tropical North Pacific, Shaeffer et al (2007) demonstrated, with acoustic tagging, two unique modes of diving behavior by yellowfin tuna. One group spent the majority of its time in the upper 100m and second group made regular diving excursions down to 300m depth (Schaefer et al. 2007). In regions where a vertical isotopic gradient is present, such as in the stratified waters around Hawaii (e.g., Dore et al. 2002), measuring bulk $\delta^{15}N$ and $\delta^{15}N_{Gly}$ values of predators could provide an additional and complimentary method to examine physiological and behavioral vertical habitat segregation by top marine predators.

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Table 5.1 Average trophic level estimates and $\delta^{15}N$ values (±1SD) of bulk tissue and amino acids of yellowfin tuna from the equatorial Pacific. Source amino acids are indicated by bold print. † Data taken from Popp et al. (2007). In the western and central Equatorial Pacific, the location abbreviations are FP = French Polynesia, SI = Samoan Islands, GI = Gilbert Islands, and CEP = central equatorial Pacific. * indicates a YFT analyzed by Popp et al. (2007) that I classify as collected in the central equatorial Pacific.

				Amino Acid $\delta^{15}N$ values, ‰								Trophic Level Estimates		
		Lat, Lon	Bulk $\delta^{15}N$	Ala	Glu	Leu	IsoLeu	Pro	Asp	Gly	Phe	$TL_{Glu\text{-}Gly}$	$TL_{TT\text{-}S}$	Avg TL
Western and Central Equatorial Pacific														
FL = 92	FP	14.0°S, 146.8°W	20.9	*30.3* ±0.3	*28.7* ±1.1	*28.5* ±0.8	*29.3* ±1.3	*24.8* ±0.8	*30.7* ±0.7	*4.8* ±0.6	*13.5* ±0.5	4.4 ±0.2	3.9 ±0.1	**4.2** ±0.3
FL = 94	FP	13.7°S, 146.1°W	20.9	*31.2* ±0.9	*30.6* ±1.0	*30.0* ±0.5	*30.0* ±0.9	*26.7* ±0.3	*33.1* ±0.7	*6.7* ±0.6	*13.8* ±1.8	4.4 ±0.1	4.0 ±0.1	**4.2** ±0.3
FL = 125	SI	10.2°S, 176.6°W	9.7	*20.6* ± 1.8	*17.6* ±1.6	*18.9* ±1.7	*19.1* ±2.1	*15.5* ±2.0	*20.1* ±2.3	*-7.4* ±1.1	*5.3* ±1.4	4.6 ±0.1	3.9 ± 0.1	**4.2** ±0.4
FL = 53	GI	2.1°N, 176.4°W	17.0	*30.2* ±0.7	*27.5* ±0.9	*27.8* ±1.4	*28.3* ±1.4	*23.8* ±0.7	*29.6* ±1.3	*4.9* ±1.0	*11.4* ±1.9	4.2 ± 0.1	4.0 ± 0.1	**4.1** ±0.2
FL = 129	GI	0.2°S, 175.0°W	14.6	*25.6* ±0.2	*23.0* ±0.9	*23.8* ±1.0	*24.3* ±1.4	*20.1* ±1.7	*25.8* ±1.1	*-2.1* ±0.9	*11.6* ±1.1	4.6 ±0.2	4.1 ±0.5	**4.4** ±0.5
FL = 48	CEP	4.9°S, 158.8°W	11.7	*23.1* ±0.1	*22.7* ±0.2	*22.1* ±0.6	*23.4* ±0.8	*19.7* ±0.1	*24.3* ±0.1	*-2.4* ±0.3	*4.3* ±0.1	4.5 ±0.1	4.1 ±0.1	**4.3** ±0.2
Eastern Equatorial Pacific†														
Avg. FL = 63		8.1°S, 110.3°W	10.4	*25.4* ±0.2	*27.3* ±0.7	*29.5* ±1.3		*19.8* ±0.3	*29.7* ±0.1	*1.7* ±0.7	*2.7* ±1.6	4.7 ±0.2	4.8 ±0.1	
*Avg. FL = 60		1.1°S, 136°W	10.9	*18.0* ±1.7	*23.4* ±0.3	*25.3* ±0.7		*17.8* ±0.6	*27.7* ±0.5	*-2.3* ±1.5	*2.7* ±1.0	4.7 ±0.3	4.3 ±0.2	
Avg. FL = 68		10.3°N, 109.0°W	13.5	*24.6* ±2.6	*24.7* ±1.1	*29.0* ±2.0		*22.3* ±4.4	*29.2* ±1.3	*2.8* ±1.5	*3.2* ±3.1	4.1 ±0.4	4.4 ±0.3	
Avg. FL = 80		13.5°N,	14.7	*28.5*	*29.2*	*29.7* ±1.8		*22.0*	*31.1*	*7.2*	*5.3*	4.2 ±0.4	4.4 ±0.3	

	113.1°W		±2.0	±1.2		±3.8	±2.1	±1.8	±0.9		
Avg. FL = 64	24.2°N,	15.6	*29.3*	*30.6*	*33.5* ±2.9	*26.5*	*32.5*	*7.5*	*7.5*	4.3 ±0.4	4.3 ±0.2
	112.3°W		±1.9	±2.2		±0.7	±2.8	±0.3	±1.4		

Table 5.2 Average trophic level estimates and $\delta^{15}N$ values (±1SD) of bulk tissue and amino acids of tuna and their prey from Hawaii. Source amino acids are indicated by bold print. Juvenile tuna are <45cm FL and adult tuna are ≥50 cm FL. Larval stomatopods (i.e. *Odontodactylus spp.*) comprises 39% (mean precent volume) of diet of juvenile YFT tuna and adult oplophorid shrimp (i.e. *Oplophorus gracilirostris*) comprise 26% (mean precent volume) of the diet of larger YFT.

		Amino Acid $\delta^{15}N$ values, ‰								Trophic Level Estimates		
	Bulk $\delta^{15}N$	Ala	Glu	Leu	IsoLeu	Pro	Asp	Gly	Phe	$TL_{Glu\text{-}Gly}$	$TL_{TT\text{-}S}$	Avg TL
YFT Prey Items												
Stomatapods	4.2	*16.6* ±0.4	*16.5* ±0.9	*12.0* ±0.1	*12.2* ±0.9	*7.6* ±0.2	*12.6* ±0.4	*-1.0* ±0.2	*-0.2* ±0.4	3.5 ±0.2	3.1 ±0.1	**3.3** ±0.2
Oplophorids	5.5	*18.5* ±1.0	*15.4* ±0.5	*16.6* ±0.2	*16.3* ±1.1	*10.5* ±0.9	*12.6* ±0.6	*2.5* ±0.8	*0.9* ±0.5	2.8 ±0.1	3.0 ±0.1	**2.9** ±0.1
Juvenile YFT												
FL = 33	6.3	*17.1* ±2.4	*14.7* ±1.6	*15.0* ±1.5	*15.4* ±1.5	*11.4* ±1.0	*15.3* ±1.6	*-7.1* ±1.8	*-1.6* ±1.5	4.1 ±0.1	3.8 ±0.1	**4.0** ±0.2
FL = 34	6.1	*19.3* ±0.2	*18.1* ±1.2	*16.8* ±0.5	*18.0* ±0.1	*13.7* ±0.5	*19.2* ±1.4	*-5.3* ±0.1	*2.4* ±0.5	4.3 ±0.2	3.8 ±0.1	**4.1** ±0.3
Adult YFT												
FL = 50	11.3	*23.9* ±0.1	*23.9* ±0.7	*22.6* ±0.4	*23.8* ±0.7	*19.4* ±0.8	*25.0* ±0.2	*0.1* ±0.7	*6.2* ±1.5	4.4 ± 0.2	4.0 ± 0.1	**4.2** ± 0.3
FL = 54	8.4	*20.9* ±0.6	*19.8* ±0.3	*19.6* ±0.3	*20.5* ±0.5	*15.7* ±0.3	*21.2* ±0.5	*-3.2* ±0.4	*4.7* ±0.3	4.3 ± 0.1	3.8 ± 0.1	**4.0** ± 0.3
FL = 72	13.2	*25.1* ±1.4	*23.8* ±1.2	*23.7* ±0.3	*23.5* ±1.2	*21.1* ±0.9	*na*	*-0.1* ±1.4	*9.4* ±2.3	4.4 ± 0.1	4.0 ±0.5	**4.1** ± 0.4
FL = 91	9.4	*21.4* ±0.9	*21.3* ±0.5	*21.5* ±0.7	*22.7* ±2.1	*17.5* ±1.4	*22.8* ±0.8	*-3.7* ±0.7	*6.0* ±1.5	4.6 ± 0.1	3.9 ± 0.2	**4.2** ± 0.4
FL=100.0	10.0	*22.6* ±0.7	*21.7* ±0.7	*20.7* ±0.3	*19.8* ±0.8	*17.0* ±0.2	*23.5* ±1.0	*-4.1* ±0.9	*7.2* ±1.2	4.7 ± 0.2	3.9 ± 0.1	**4.3** ± 0.5
Adult BET												

FL = 52.0	10.9	*23.5* ±0.6	*22.1* ±0.7	*21.6* ±0.7	*23.1* ±1.6	*20.1* ±0.7	*24.1* ±0.2	*-3.4* ±0.1	*3.9* ±0.6	4.6 ± 0.1	4.2 ±0.1	4.4 ±0.2

Table 5.3 Average $\delta^{15}N$ values (±1SD) of bulk, source amino acids (Glycine & Phenylalanine; bold print) and trophic amino acids (Alanine and Glutamic acid), and trophic level estimates of yellowfin tuna (YFT) from Hawaii. $\Delta^{15}N$ represents the difference between the $\delta^{15}N$ values of juvenile and adult YFT.

	Juvenile YFT (n = 2)	Adult YFT (n = 3)	$\Delta^{15}N$
Forklength (cm)	33, 33.5	82 ± 24	
Bulk WMT $\delta^{15}N$	**6.2** ± 0.1	**9.3** ± 0.8	
	Amino Acids $\delta^{15}N$ values (‰)		
Alanine	18.4 ± 1.7	21.6 ± 0.9	3.2
Glutamic acid	16.5 ± 2.2	20.9 ± 1.0	4.4
Glycine	-6.2 ± 1.5	-3.7 ± 0.5	2.5
Phenylalanine	0.6 ± 2.2	6.0 ± 1.3	5.4
	Trophic Level		
$TL_{Glu\text{-}Gly}$	4.2 ± 0.2	4.5 ± 0.2	
$TL_{TT\text{-}S}$	3.8 ± 0.1	3.9 ± 0.1	

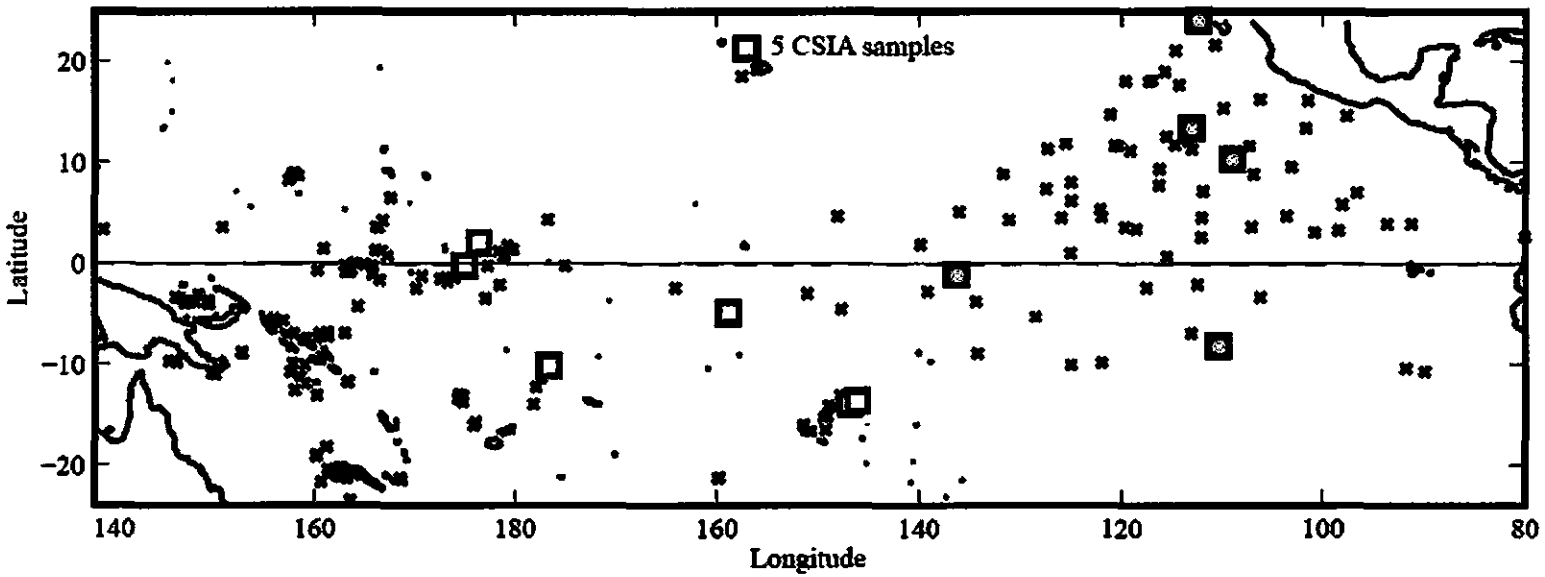

Fig. 5.1 Location of bulk (×) and amino acid (□) $\delta^{15}N$ values of adult yellowfin tuna analyzed from the equatorial Pacific Ocean. Darker squares in the ETP are sample locations of yellowfin tuna reported by Popp et al. (2007). Samples analyzed for CSIA from Hawaii include 2 juvenile yellowfin tuna, 5 adult yellowfin tuna, and 1 adult bigeye tuna.

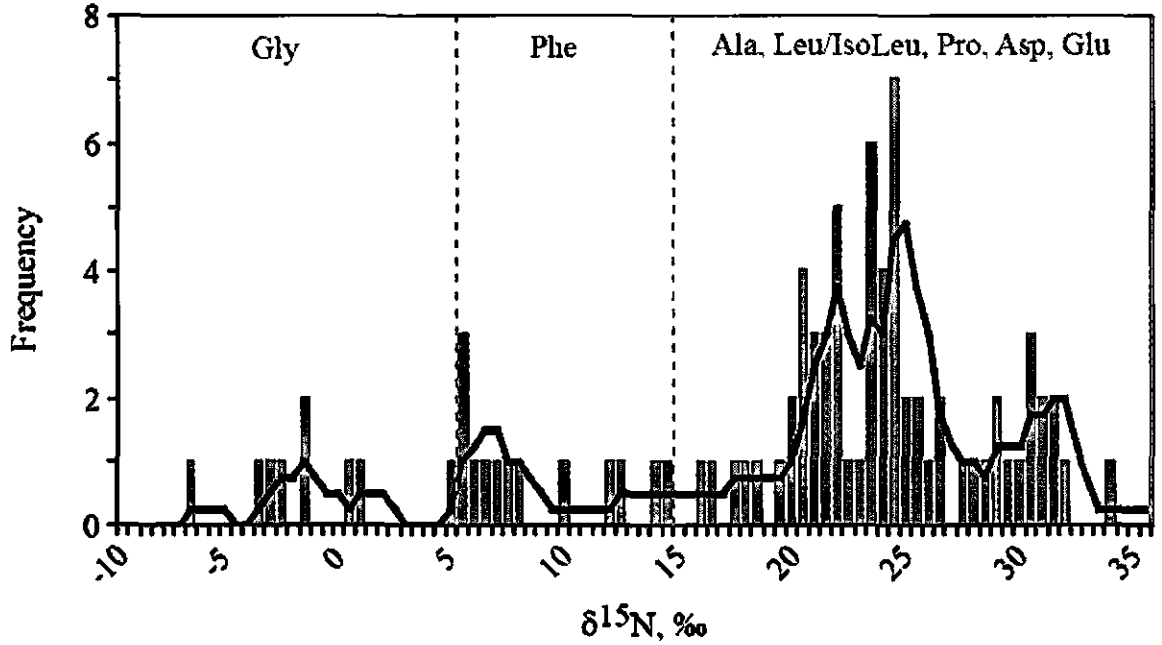

Fig. 5.2 Frequency of the $\delta^{15}N$ values of amino acids isolated from all adult YFT muscle samples (n=16). The histogram includes source amino (Gly and Phe) and trophic transfer (Ala, Leu, IsoLeu, Pro, Asp, Glu) amino acids. $\delta^{15}N$ values of amino acids were rounded to the nearest 0.5‰. The black line represents a running 4-sample average.

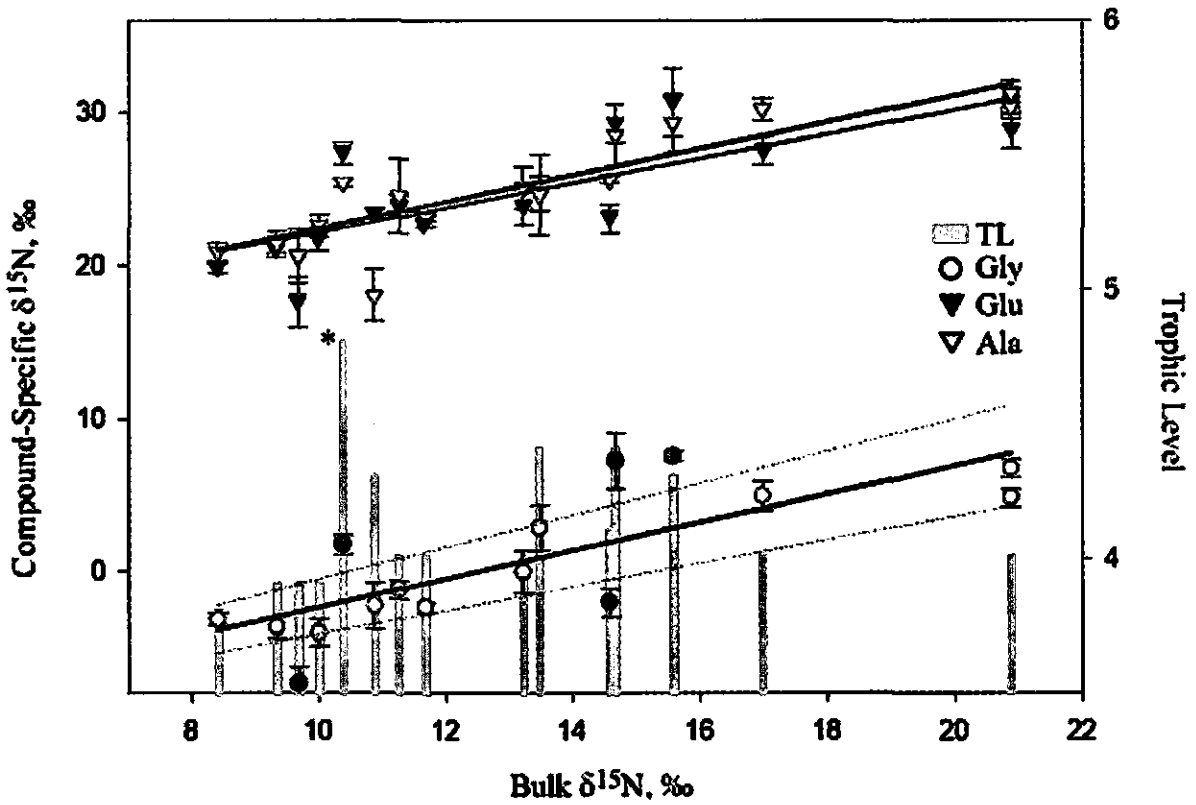

Fig. 5.3 Average compound-specific $\delta^{15}N$ values (±1SD) and $TL_{TT\text{-}S}$ for yellowfin tuna collected in the EqPac. The $TL_{TT\text{-}S}$ was determined with the average source (Gly+Phe) and trophic transfer (Glu+Ala+Iso+IsoLeu+Asp) amino acid $\delta^{15}N$ values (Eqn. 1). Source values that are dark gray and black indicate YFT collected from ETP and WTP that fell below and above the observed

relationship, respectively. * Represents a significant difference in the individual's $TL_{TT\text{-}S}$ from the average $TL_{TT\text{-}S}$ of all YFT in the tropical Pacific (4.1 ± 0.3). Models: $Y_{Gly} = -11.7 + 0.93x$ (R^2=0.63), $Y_{Glu} = 13.7 + 0.87x$ (R^2=0.64), $Y_{Ala} = 14.2 + 0.80x$ (R^2=0.77).

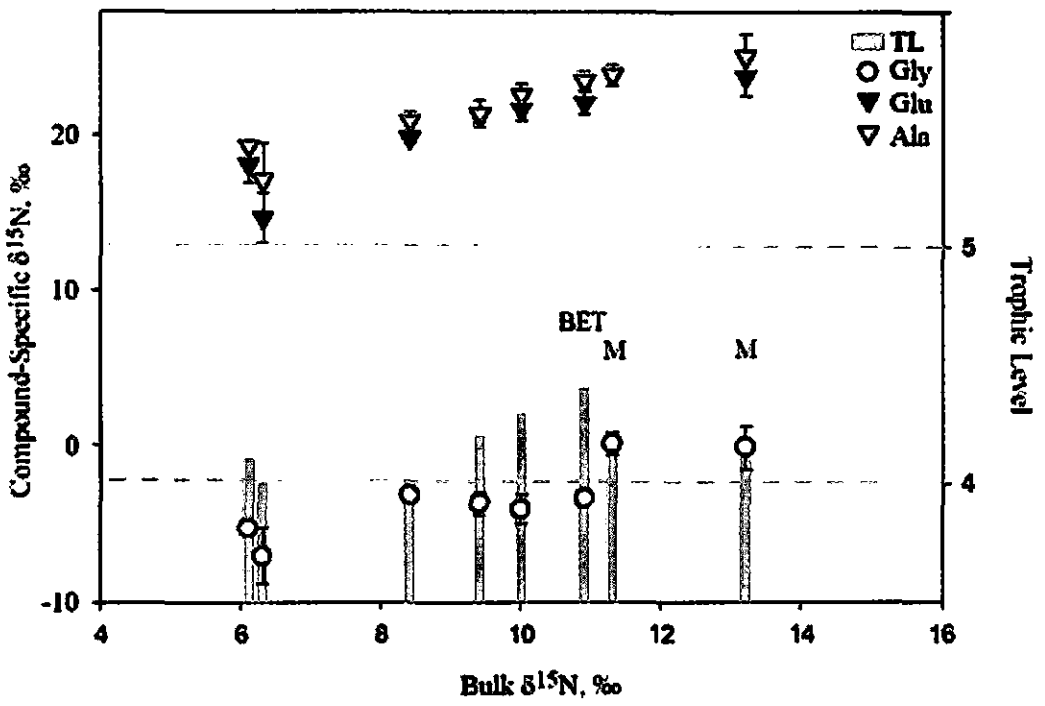

Fig. 5.4 Average bulk $\delta^{15}N$ values (±1SD), compound-specific $\delta^{15}N$ values (±1SD), and trophic levels (±1SD) of yellowfin tuna collected from FADs around Hawaii. One bigeye tuna (BET) is included for an interspecies comparison. Glutamic acid (Glu) and alanine (Ala) are trophic transfer amino acids and glycine (Gly) is a source amino acid. The trophic level estimates are an average of the trophic levels based on Glu-Gly and Trophic Transfer-Source amino acids (Eqns 2 and 3). M represents adult YFT that have significantly higher $\delta^{15}N_{Gly}$ values and could represent either migrants or individuals with a different foraging strategy.

CHAPTER 6

Summary and Directions for Future Research

6.1 Research Summary

The aim of this study was to apply stable isotope analysis to examine the trophic dynamics and movements patterns of tuna in the tropical Pacific Ocean. On a regional scale, the results revealed evidence of physiological vertical niche partitioning between small and large size classes of yellowfin tuna (Chapter 2). Over broader scales, the datasets suggest that the trophic level of yellowfin is relatively constant across the equatorial Pacific (Chapter 5). Consistency in trophic level suggests that tuna may have a similar foraging strategy over large spatial scales and corroborates the findings of previous stomach content studies that tunas are opportunistic feeders that forage on a diverse prey base. Although yellowfin tuna appear to exhibit a similar foraging strategy, resuls of bulk and compound-specific isotope analyses provide initial evidence of behavioral trophic niche partitioning in the pelagic ecosystem by larger-size classes of yellowfin (Chapter 5). Schaeffer et al. (2007) recently showed, with acoustic tagging methods that distinctly different modes of vertical diving behavior exist in yellowfin tuna from the eastern tropical Pacific. Thus, the opportunistic foraging strategy of tuna could be similar across the ocean basin, but tuna may segregate their vertical foraging habitats. Interspecific vertical niche partitioning has been hypothesized and demonstrated between bigeye and yellowfin tuna (review by Brill and Lutcuvage 2001), but less is known about intraspecific niche partitioning and more importantly, whether the separate vertical diving

ranges translate into trophic niche segregation. Determining the extent, temporal, and spatial plasticity of trophic niche partitioning, and its relative importance to intra- and interspecific trophic dynamics will be important directions for future research, with the ultimate goal to determine if tunas' trophic niche is produced by the competitive exclusion principle (Connell 1983; Tilman 1994) or physiological constraints (Hutchinson 1961). Finally, establishing the current trophic position of yellowfin tuna across a large spatial scale provides a reference point to examine historical and future temporal changes in tuna trophic dynamics and examine potential trophic cascades produced by commercial fisheries (see Popp et al. 2007).

By examining isotope values as not only an indicator of trophic dynamics but as natural tags that can be used to trace movements patterns, I placed for the first time the distribution of $\delta^{15}N$ values of top predators in a geographical, basin-wide framework (Chapter 4). This isotope cartography of tropical tunas revealed large and coherent spatial structure, which indicates that tropical tunas must exhibit some degree of residency behavior in the equatorial Pacific. Furthermore, these isotope maps, coupled with experimentally-determined tissue turnover rates (Chapter 3), also highlighted interspecies differences in the degree of regional residency, with yellowfin and skipjack displaying the lowest net movement rates relative to bigeye tuna (Chapter 4). To maintain sustainable tuna fisheries, we may need to continue to deviate from basin-wide and sub-basin management programs and instead, incorporate population net movements into integrated stock assessment models that apply to specific regions or even island nations in the equatorial Pacific. In summary, this study established that the $\delta^{15}N$ values

of tropical tunas are effective chemical tags or indicators of their foraging behavior, habitat utilization, and movements at various spatial scales in the Pacific Ocean.

6.2 Significance and Implications

The findings of this research have significant implications for the field of isotope ecology and the evaluation of spatial and temporal changes in structure and function of marine ecosystems. Due to the goals and extensive sampling approach of this study, we now understand that the isotopic baseline and its flucuations are propagated up to and reflected in top marine predators. In previous research and literature, comparative studies on trophic dynamics have measured the isotope values of animals and species from distant marine environments. This study reveals that before large-scale or historical trophic analysis can be completed, a careful examination of the isotopic baseline and nutrient dynamics in the study area must be conducted. In other words, the isotope value at the base of the food web needs to play a central role in the interpretation of stable isotopic datasets.

Compound-specific isotopic analyses (CSIA) can distinguish an animal's trophic level from flucuations in the $\delta^{15}N$ values at the base of the food web (e.g., McClelland and Montoya 2002). In this research and the work of Popp et al. (2007), we extended this CSIA approach to marine fisheries and established that the difference between the $\delta^{15}N$ values of source (or conservative) and trophic transfer amino acids can be used to determine the isotopic baseline and the trophic position of the tuna. These CSIA results (a) extend the findings of bulk SIA by conclusively demonstrating that tuna $\delta^{15}N$ values

are mainly a reflection of isotopic baseline flucuations and (b) have important implications for investigating the long-term impact of commercial fishing on the food web structure of marine ecosystems. Using simple ECOSIM models, hypotheses can be developed to determine which top predators will be most affected by fisheries removal (e.g., Essington et al. 2006 and references therein). Once model-derived hypotheses are developed on potential trophic cascades in the pelagic ecosystem and targeted species determined, archived samples from universities and museums can be collected and CSIA applied to determine if commercial fisheries have had a significant effect on the trophic structure of marine food webs. One timely application of this approach is determination of the trophic level of humboldt squid (*Dosdicus gigas*) prior to and during its recent range expansion and increase in biomass in the eastern tropical Pacific (Zeidberg and Robison 2007). If the range expansion and increase in *Dosdicus* biomass relates to the reduction of predation by their primary predators, large tuna and billfish, by industrialized fishing, then the TL of similar-sized individuals may have changed as well. In other words, with the reduction in the number and maximum size of their predators (e.g., Sibert et al. 2006), the adaptable *Dosdicus* could have expanded into the trophic niche of these top vertebrate predators. Finally, in addition to examining potential shifts in predator-prey and trophic relationships, CSIA of a time series of marine organisms could distinguish natural flucuations at the base of the food web that can occur with climate and regime shifts.

Another method to assess the impacts of fisheries and climate on the structure and function of food webs is a community-based approach that couples body-size spectra and trophic position (Jennings et al. 2002a, Jennings et al. 2002b, Jennings et al. 2002c). This

emerging method provides information on the energy and nitrogen flow through the food web, predator-prey body-mass ratios, and estimates of trophic transfer efficiencies between trophic levels (Jennings et al. 2002a, Jennings et al. 2002b, Jennings et al. 2002c). Not only has this research provided connections between marine community structure and ecosystem function, but these size spectra and $\delta^{15}N$ values have now been incorporated into complex ecosystem models with the goal of assessing the impacts of climate change on top predators in marine food webs (e.g., O. Maury's APECOSM model). To date this novel approach has been developed for only one marine benthic community or assemblage in the north east Atlantic Ocean. Isotopic data from this research and the information gathered from the stomach content analysis of the larger PFRP-funded project could be used to determine if this emerging theory and method applies to an open ocean, basin-wide scale. In other words, the datasets developed in this research could provide important information on the universality or applicability of this community-based theory to marine ecosystems.

6.3 Future Research Directions

6.3.1 Fish physiology

To ensure long-term survival and reproductive success juvenile fish must meet energetic requirements while avoiding predation. Because of their high metabolic rates (e.g., Dewar and Graham 1994), tuna might be even more sensitive to resource availability in the pelagic ecosystem. Food limitation can initially affect an animal's behavior by changing movement patterns or habitat use (e.g., Sogard and Olla 1996). However, if (a)

food limitation continues and internal energy stores are fully utilized or (b) the reduction in energy stores further increases the risk of predation or disease, fish succumb to natural mortality. In fisheries stock assessment and ecosystem models, natural and fishing mortality rates are used to parameterize population dynamics, and, in turn, assess the effects of commercial fishing on the biomass and status of fish stocks (e.g., Hampton 2000; Adam et al. 2003). The effects of starvation on fish with high standard metabolic rates have been examined in laboratory studies, but these studies have mainly focused on the effects of food deprivation on metabolic rates (Boggs and Kitchell 1993; O'Connor 2000). Estimating the frequency of nutritional stress in pelagic fish in the field is especially difficult given the size of the population and environment. Furthermore, pelagic predators are likely to be more successful in "sampling" nutritionally stressed fish than scientists. In addition to biochemical and enzymatic indicators (Moyes et al. 2006), stable isotope techniques could potentially be an additional method to investigate nutritional stress in top predators in the pelagic ecosystem.

In their seminal paper, Hobson et al. (1993) examined the effects of fasting that occurred with breeding on the $\delta^{15}N$ values of a species of arctic goose (*Chen rossii*) and documented that ^{15}N enrichment occurs when animals experience nutritional stress. The mechanism is analogous to the ^{15}N trophic enrichment. When organisms breakdown their own tissues during starvation (i.e., protein catabolism), a trophic-level increase occurs in the $\delta^{15}N$ value of the remaining tissue (Hobson et al. 1993). Bulk tissue $\delta^{15}N$ values of a fasting or starved individual are therefore, higher than the same animals in good condition (Cherel et al. 2005). During the 2 year trophic ecology study of yellowfin tuna described in Chapter 2, three samples of small juveniles (<40cm FL) collected at the

same time and presumably from the same school had significantly higher $\delta^{15}N$ values (t-test, p-value <<0.01) relative to all other yellowfin tuna of comparable size (Fig. 6.1). As discussed in Chapter 4, elevated isotope values could also indicate individuals who emigrated from a region with a higher isotopic baseline. However, as discussed in Chapter 5, results of tagging studies suggest that yellowfin tuna are residents to the Hawaiian Archipelago for several years, especially in the case of smaller size classes of tuna (Adam et al. 2003).

To further investigate the 3 anomalously high individuals and to determine if CSIA could be used as an indicator of nutritional stress in fish, the $\delta^{15}N$ values of individual amino acids isolated from the bulk white muscle tissues from these high $\delta^{15}N$ value-juveniles were determined. Similar to all other yellowfin tuna samples analyzed for CSIA, the $\delta^{15}N$ values of the amino acids in these 3 juvenile exhibited ^{15}N enrichment pattern consistent with the trophic transfer (fractionating) and source (conservative) amino acids grouping (see Chapter 5). More importantly, the $\delta^{15}N$ values of both individual trophic transfer and source amino acids were highly elevated in the juveniles with anomalously high bulk $\delta^{15}N$ values than comparable-sized juveniles (Fig. 6.2). If these remarkably high bulk tissue $\delta^{15}N$ values reflect the onset of food deprivation, then the amino acid CSIA results suggest no differential catabolism of select amino acid groups or individual amino acids. Rather, muscle tissue appears to have been catabolized during starvation with no preferential loss of specific amino acids, which would lead to elevated $\delta^{15}N$ values for all amino acids (Fig. 6.2). The enrichment pattern in the $\delta^{15}N$ values of individual amino acids of the elevated-juveniles is similar to the pattern observed in the larger size classes (i.e., >50cm FL) of yellowfin tuna. The TL of the

juvenile and adults are similar (Chapter 5), but the elevated-juveniles have a higher estimated TL. In addition, the source amino acids (phe and gly) are more similar between large size classes and the juveniles with elevated $\delta^{15}N$ values than the remaining juveniles. The source $\delta^{15}N$ values of prey are not similar with any group of tunas. Thus, CSIA of tuna tissues can not currently provide a conclusive demonstration of starvation. High $\delta^{15}N$ values in elevated-yellowfin could indicate (a) starvation or (b) these individuals reached their functional endothermic capabilities at a smaller size than all other juvenile tuna (Chapters 2, 5).

Before CSIA can be effectively used as an indicator of starvation in animal ecology, controlled or natural experiments need to be performed. Furthermore, to establish connections between the observed trends in an animals' $\delta^{15}N$ values of individual amino acids to their dynamic physiology under varying environmental conditions, I suggest future CSIA investigations capitalize on natural fasting or starvation periods observed in animals (e.g. king penguin; Cherel et al. 2005). Once the response of individual amino acids to nutritional stress are established, bulk tissue $\delta^{15}N$ values in pelagic marine fish could be an initial indicator of nutritionally stressed fish and then CSIA could be applied to examine the affect of nutritional stress on protein catabolism in individual animals. Both bulk and compound-specific isotope analyses could be applied to investigate natural mortality rates over different spatial and temporal scales. For example, some pelagic predators associate with fish aggregating devices in large numbers, but these sites may provide limited food resources to support the upper-trophic level biomass (e.g., Menard et al. 2000; Dagorn et al. 2007). This ecological-trap theory (Marsac et al. 2000) could be explored by determining the frequency of starvation, as

indicated by bulk and compound-specific isotope analyses and % lipid measurements, in areas with a high density of FADs relative to regions with out FADs (e.g., Secretariat of the Pacific Community's Regional Tuna Tagging Programme).

6.3.2 Smaller-scale movement patterns

Horizontal movements:

Characterizing individual and population movements between different environments can reveal key breeding and foraging habitats and elucidate population conductivity (e.g., Chamberlain et al. 1997; Rubenstein et al. 2002; Briers et al. 2003). A qualitative method for examining movements within the pelagic environment would be to compare the isotope values of the same predator between two regions that have distinctly different isotopic baselines. If the predator migrates extensively between the two regions, then the isotopic difference between regions would be less than a predator who displayed a greater degree of residency. For example, the isotopic baseline in French Polynesia (i.e., west of the Marquesas Islands) is remarkably high (Chapter 4, 5). By comparing the same predator from French Polynesia to a region in the equatorial Pacific where the isotopic baseline is low, the difference between values can provide an indication of the relative rate of regional migration (Fig. 6.3). It would be most valuable to compare predator $\delta^{15}N$ values between region that are considered important for foraging and/or reproduction.

Another method that could be applied to examining smaller scale movements is statistically identifying isotopic outliers within a region (e.g., principal component analysis; Stewart et al. 2003). A recent arrival to a region will have different isotopic

values than the individuals that have foraged in that region (Fig. 6.4). To increase the statistical power of this approach, the addition of other natural tags (e.g., trace metals and elements in tissues and otoliths) and all these datasets could then be used to distinguish immigrants from residents within a region. A multi-element, multi-isotope approach could be especially valuable in the warm pool in the western Pacific where a high number of islands, archipelagos, and seamounts could deliver a potentially unique suite of biogeochemical markers to the surrounding coastal and oceanic regions. With such a suite of biogeochemical markers, key habitats of tropical tunas and the movements between these habitats could be identified.

Vertical Movements:

A nitrogen isotopic vertical gradient occurs in the stratified waters surrounding the Hawaiian Islands (Chapter 2, 5). In general, $\delta^{15}N$ values at the base of the food web are lower in the surface mixed layer than below the thermocline. Predators and prey that are confined to the SML (e.g., juvenile yellowfin tuna <45cm FL) will have lower bulk and source amino acid $\delta^{15}N$ values than predators that forage at depth. Shifts in resident predator bulk tissue and amino acid $\delta^{15}N$ values could define the average foraging utilization or vertical habitat range of predators and prey. Similar to nitrogen isotope values at the base of the food web, ambient mercury (Hg) concentrations vary with depth in the marine environment.

In the surface oceans of the tropical Pacific, methylmercury (MHg) concentrations are near detection limits and increase with depth and reach the highest

values in the oxygen minimum layer (Mason and Fitzgerald 1991; Mason and Fitzgerald 1993). Ambient MHg enters marine food webs via microbes at the base of the food web. Due to the affinity of MHg to bind with proteins, MHg increases with an animal's size and demonstrates stepwise trophic level bioaccumulation and is, therefore, tightly coupled to the consumer's trophic level (e.g., Bearhop et al. 2000). Total mercury (THg) is the common measurement of Hg in food web studies, because MHg is the predominant form of mercury in fish (Bloom 1992; Kraepiel et al. 2003). Monteiro et al. (1996) demonstrated that mesopelagic prey in the waters surrounding the Azores had significantly higher THg concentrations than prey that foraged in the surface mixed layer. This vertical THg gradient propagates up to top marine predators and has been used to examine the foraging behavior of seabirds (Monteiro et al. 1998; Arcos et al. 2002). Accordingly, THg concentrations can be used as indicators of the vertical foraging range and vertical migration behaviors of predators and prey in the pelagic ecosystem.

Top predators collected from both the eastern tropical Pacific and Hawaii demonstrated variations in average THg values that indicate differences in trophic level and average foraging depths (Fig. 6.5). In chapter 5, the CSIA dataset suggests that small and large size classes of yellowfin collected around Hawaii had similar trophic level. Therefore, their THg concentrations are mainly a function of mass and foraging depth. In chapter 2, I hypothesized that the rapid ontogenetic diet shift in the $\delta^{15}N$ values and stomach content compositions of yellowfin tuna was triggered by the onset of functional endothermy whereby a threshold mass was reached and juvenile tunas could then penetrate below the thermocline and access additional mesopelagic prey. Accordingly, the vertical gradient in MHg should lead to the THg concentrations of yellowfin ~45-

50cm FL being higher than the surface-confined individuals. Although more yellowfin samples covering a large range of mass need to be analyzed for THg, initial data indicate that yellowfin tuna that undergo the rapid ontogenetic shift (~45cm FL) do indeed have higher Hg values (Fig. 6.6). Furthermore, the THg concentrations of two major prey items also supports that there is a vertical $\delta^{15}N$ gradient in the waters around Hawaii. Larval stomatopods, which occur in the SML and comprise a major component of the juvenile tuna (<45cm FL) diet, have lower THg concentrations that a vertically migrating mesopelagic species of shrimp that is an important component of the diet of larger tuna (Fig. 6.6). By coupling $\delta^{15}N$ and THg measurements, the vertical habitat utilization could be determined for marine pelagic predators. One application of this multi-tag approach would be to determine if there are interspecies differences in the endothermic capability of small size classes of bigeye and yellowfin tuna and if the differences are related to or independent of environmental conditions.

6.4 References

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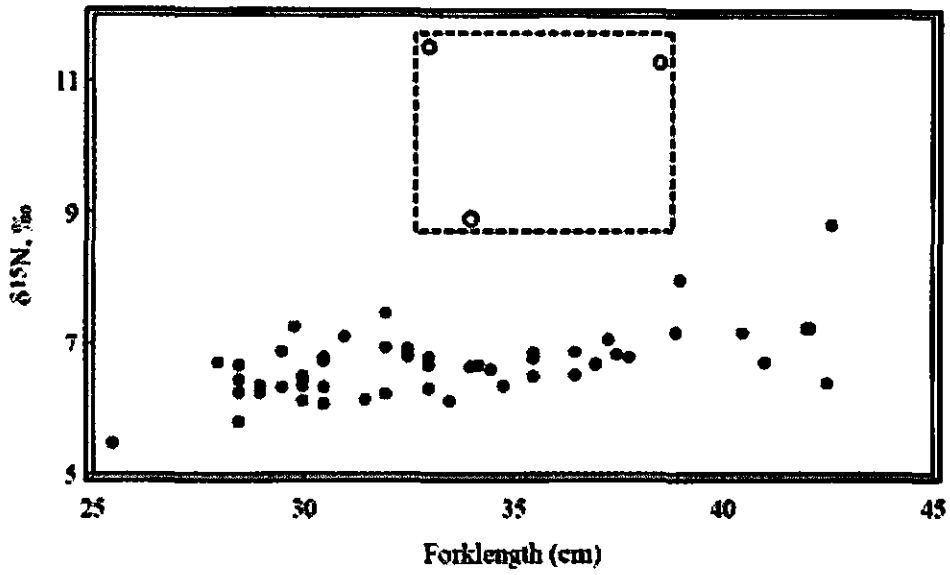

Fig. 6.1 Bulk $\delta^{15}N$ values of yellowfin tuna collected from nearshore FADs around Hawaii. In this population, a distinct ontogenetic shift in foraging range occurred around 45cm FL. The (o) data points within the box indicate three individual tuna with significantly higher bulk $\delta^{15}N$ values that I hypothesized experienced food deprivation.

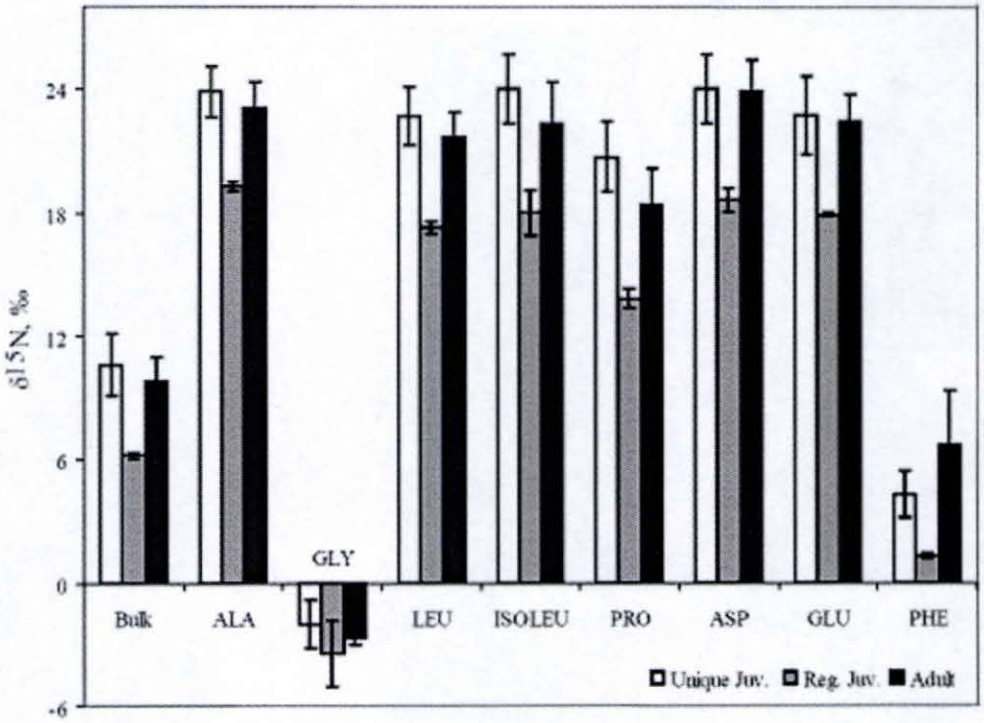

Fig. 6.2 Average (± SD) bulk and individual amino acid $\delta^{15}N$ values for (i) three juvenile tuna with anomalously high $\delta^{15}N$ values ("Unique Juv"), (ii) two juveniles that had isotope values typical of the remaining juveniles analyzed over a 2yr study ("Reg Juv"), and (iii) four larger yellowfin ("Adult"). All samples were collected from nearshore fish aggregating devices around Oahu, Hawaii. $\delta^{15}N$ values of amino acid values can be separated into two general groups: source amino (Gly and Phe) and trophic transfer (Ala, Leu, IsoLeu, Pro, Asp, Glu) amino acids. Both source and trophic transfer amino acid $\delta^{15}N$ values were highly enriched for the (potentially) nutritionally stressed juvenile tuna.

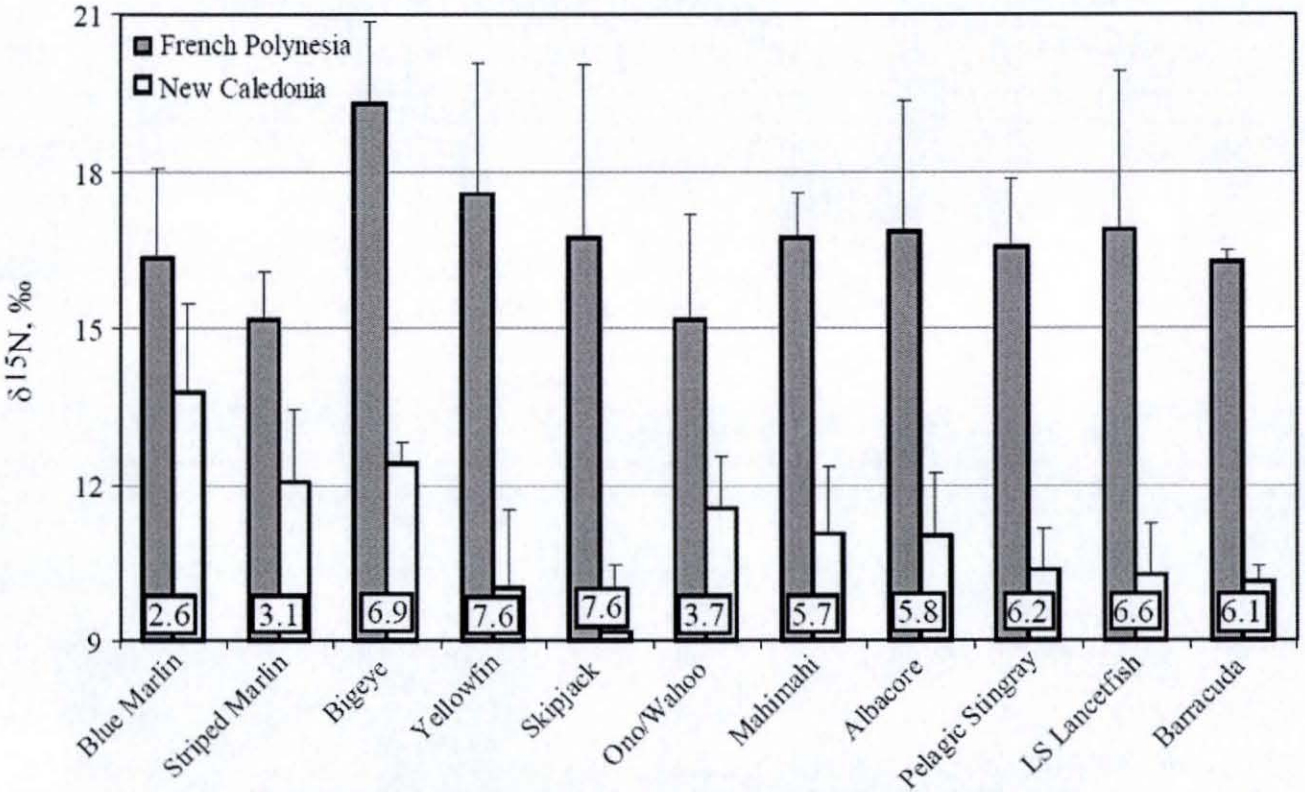

Fig. 6.3 $\delta^{15}N$ values of higher-trophic level predators from French Polynesia and New Caledonia. Values in the boxes indicated the difference between the $\delta^{15}N$ values of the predator collected in French Polynesia and New Caledonia. Lower values indicated greater movement rates and higher values indicated a greater degree of residency behavior. LS Lancetfish is the longsnouted lancetfish.

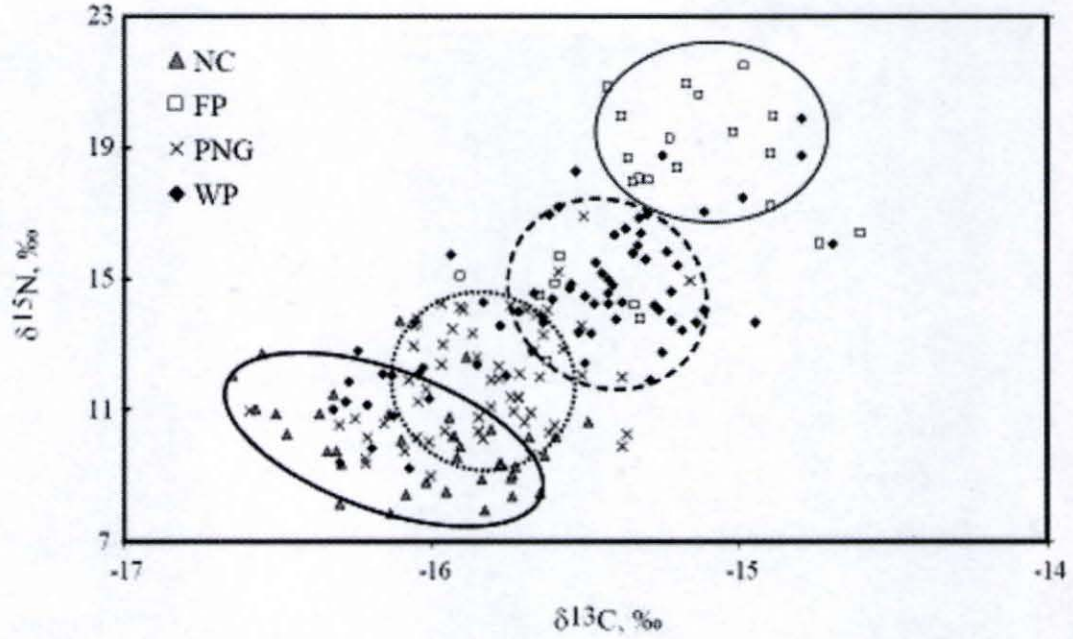

Fig. 6.4 $\delta^{15}N$ and $\delta^{13}C$ values yellowfin tuna from New Caledonia (NC), French Polynesia (FP), Papua New Guinea (PNG), and the warm pool of the western tropical Pacific (WP). The circles indicate the qualitative groupings of the majority of yellowfin analyzed from the different regions and can aid in the "sourcing" of tuna to particular regions. Outliers in the generalized groupings are an initial indicator of individuals that have recently immigrated into the region.

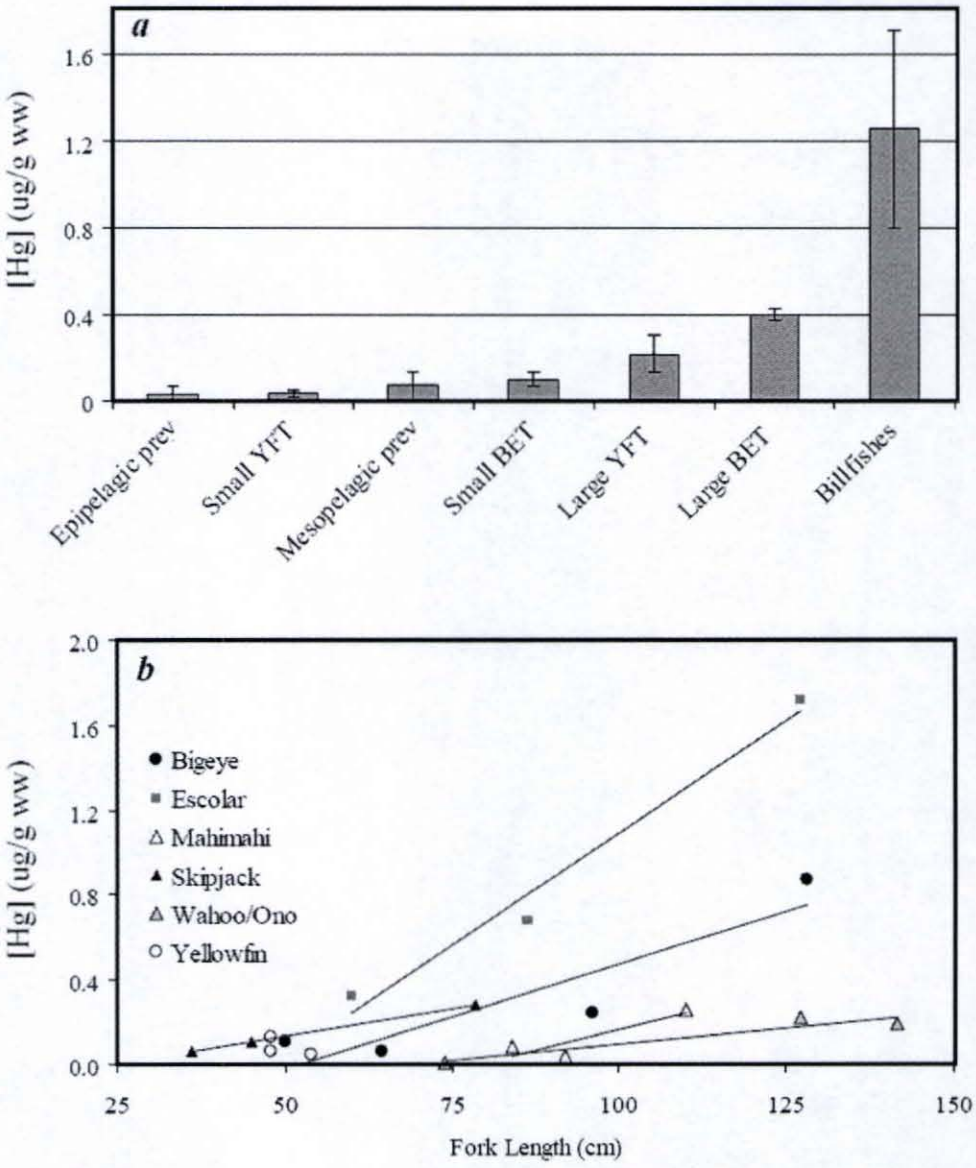

Fig 6.5 Total mercury concentrations (ug/g) wet weight for a range of predators collected from the (a) eastern tropical Pacific (ETP) and (b) Hawaii. The difference between large and small size classes of tuna in the ETP could reflect bioaccumulation of Hg associated to an increase in mass or reflect differences in foraging depth. Future research could remove these confounding factors by normalizing the THg concentration

by mass or length. The THg analysis was performed by Dr. Robert Gambrell at Louisiana State University. Reproducibility of the analyses was ±0.1 ug/g wet weight.

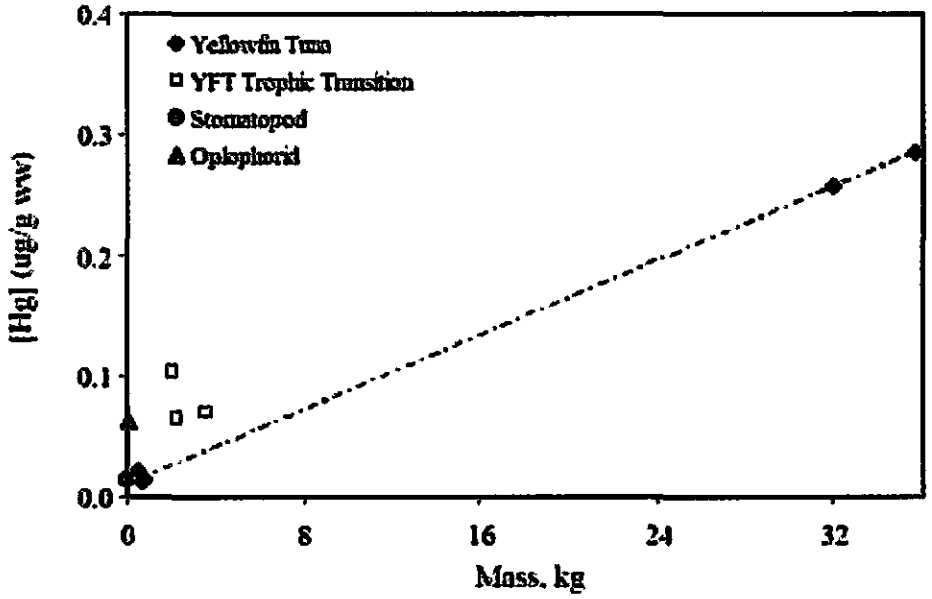

Fig. 6.6 Total mercury concentrations (ug/g) wet weight of yellowfin (YFT) muscle tissue and two prey groups collected from nearshore FADs around Oahu, Hawaii. Yellowfin at the "trophic transition" were between 45-50 cm FL and appear to be higher than would be predict based solely on mass. Future research could use this clear example of a sudden shift in foraging depth in yellowfin tuna to determine the accuracy of THg as an indicator of vertical foraging behavior in top predators. The THg analysis was performed by Dr. Matthew Chumchal (Texas Christian University). THg concentrations were analyzed with a direct mercury analyzer that used thermal decomposition, gold amalgamation, and atomic absorption spectrometry. Recovery was near 100% and the duplicate samples analyzed had a mean relative percent difference of 1.71%.

APPENDICIES

APPENDIX 1: *RAW DATA FROM CHAPTER 2 - HAWAIIAN TUNA & PREY DATA.* **SIA/UH ID** is the ID code assigned to each sample and would be the code to use in future sample retrieval. Sample Date is the date the fish or prey item was caught or collected. The information in the **Details** column indicates the type of tissue collected from the predator or the prey item description (indicated as "P/..."). The abbreviations are as follows, WMT is white muscle tissue, RMT is red muscle tissue, LVR is liver tissue, HRT is heart tissue, SPLN is spleen tissue, GLP is a sample of gill plates, STM is a sample of the stomach tissue, LNS is the lense in the eye, PFIN is a sample of the pectoral fin, DFIN is a sample of the dorsal fin, and FINT is a sample of the finnetes located on the anterior side. For the prey items the abbreviations are as follows, P/Shrp is 'shrimp', P/MShp is mantis shrimp, P/Stp is stomatopods, P/Omm is Ommastrephidae squid, P/HOmm is Hyaloteuthis Ommastrephidae squid, P/Oplo is Oplophoridae, P/M is a sample of megalopae/larval decapod, P/Lys is Lyssiosquilloidae, P/srf is a small reef fish, P/Squ is Squilloidea, P/Dac is Dactoylopteriodae, P/Ost is Ostraciliade, P/Mul is Mulidae, and P/Ff is flying fish. **LC** is the location of capture of the fish or sample. All letters represent the alphabetic code of the nearshore buoy (Fig. 2.1), except SMT, which represents fish caught by commercial fishers at or near Cross Seamount. **MT** is the method of capture used, where 'T' represents trawling, 'G' represents gigging, and 'LL' represents commercial long-lining by fishers. **SPE** is the species code of the fish. 'YFT' is yellowfin tuna and 'BET' is bigeye tuna. The species of fish where prey were collected from their stomachs is also indicated with the same abbreviations. **FL** is the fork length, in cm, of

the fish or prey item. Both nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) stable isotopic compositions are reported in traditional delta notation (‰). C/N is the carbon to nitrogen ratio of the predator's tissues or prey items. NLE and LE represent non-lipid and lipid extracted samples, respectively. Carbon and nitrogen sotope values indicated in bold and italics indicate values that concern the author and should not be included in future analyses.

SIA/ UH ID	Sample Date	Details†	LC	MT	SPE	FL (cm)	LE $\delta^{15}N$, ‰	NLE $\delta^{15}N$, ‰	LE $\delta^{13}C$, ‰	NLE $\delta^{13}C$, ‰	LE C/N	NLE C/N
2	10/3/02	WMT	S	T	YFT	38.5	11.3		-16.3			
3	10/3/02	RMT	S	T	YFT	38.5	11.9		-17.2			
6	10/3/02	RMT	S	T	YFT	34.0	9.7		-17.2			
7	10/3/02	WMT	S	T	YFT	34.0	8.9		-17.0			
8	10/3/02	RMT	S	T	YFT	33.0	12.0		-17.1			
9	10/3/02	WMT	S	T	YFT	33.0	11.5		-16.0			
12	10/18/02	SPLN	S	T	YFT	33.0	6.3		-17.0			
13	10/18/02	LNS	S	T	YFT	33.0	6.0		-17.0			
14	10/18/02	GLP	S	T	YFT	33.0	5.9		-16.2			
15	10/18/02	P/Shp	S	T	YFT	33.0	3.8		-17.7			
16	10/18/02	HRT	S	T	YFT	33.0	7.9		-16.6			
18	10/18/02	HRT	S	T	YFT	39.0	8.4		-16.9			
19	10/18/02	RMT	S	T	YFT	39.0	7.7		-17.0			
20	10/18/02	P/Shp	S	T	YFT	39.0	3.1		-17.9			
21	10/18/02	LVR	S	T	YFT	39.0	5.6		-16.9			
22	10/18/02	SPLN	S	T	YFT	39.0	5.7		-15.5			
23	10/18/02	GLP	S	T	YFT	39.0	6.4		-16.0			
24	10/18/02	WMT	S	T	YFT	33.0	6.3		-16.0			
25	10/18/02	WMT	S	T	YFT	39.0	*5.3*		*-16.8*			

27	11/6/02	WMT	LL	T	YFT	33.5	6.1		-16.0			
28	11/6/02	RMT	LL	T	YFT	33.5	7.4		-16.8			
29	11/6/02	LVR	LL	T	YFT	33.5	5.2		-16.9			
31	11/6/02	SPLN	LL	T	YFT	33.5	5.8		-16.7			
32	11/6/02	HRT	LL	T	YFT	33.5	8.2		-17.2			
34	11/6/02	WMT	LL	T	YFT	28.5	5.8		-16.4			
35	11/6/02	RMT	LL	T	YFT	28.5	7.0		-16.8			
36	11/6/02	GLP	LL	T	YFT	28.5	5.1		-15.7			
37	11/6/02	LVR	LL	T	YFT	28.5	5.1		-17.3			
38	11/6/02	HRT	LL	T	YFT	28.5	6.3		-16.2			
76	12/8/02	RMT	S	T	YFT	29.5	7.6		-17.2		4.2	
77	12/8/02	LVR	S	T	YFT	29.5	5.2		-17.2		3.9	
79	12/8/02	RMT	S	T	YFT	30.0		8.1		-18.1		5.0
82	12/8/02	RMT	S	T	YFT	33.5	7.6	7.8	-18.0	-17.3	4.8	4.7
83	12/8/02	LVR	S	T	YFT	33.5		5.1		-17.2		4.5
85	12/8/02	RMT	S	T	YFT	30.0		7.8		-17.7		4.7
88	12/8/02	RMT	S	T	YFT	31.0		7.7		-17.6		4.6
90	11/25/02	WMT	R	T	YFT	30.5	6.7	6.7	-16.2	-16.2	3.9	3.9
92	11/25/02	LVR	R	T	YFT	30.5	6.1	6.0	-17.3	-17.7	4.7	4.8
93	11/25/02	WMT	R	T	YFT	30.5		6.8		-16.2		3.6
94	11/25/02	RMT	R	T	YFT	30.5		7.8		-17.9		4.6
96	11/25/02	WMT	R	T	YFT	28.0		6.7		-16.4		3.6
99	11/25/02	P/Msp	R	T	YFT	28.0	4.3		-18.3		6.2	
101	11/25/02	P/Ff	R	T	YFT	30.5	3.7	3.9	-18.4	-18.5	7.1	4.7
107	11/25/02	P/Msp	R	T	YFT	31.0	4.3	3.9	-18.4	-18.1	7.2	4.5
108	11/25/02	P/Ff	R	T	YFT	31.0	3.6	3.3	-18.1	-19.0	3.9	4.9
109	11/25/02	WMT	R	T	YFT	31.0	7.1		-15.8			
110	11/25/02	RMT	R	T	YFT	31.0	7.2		-17.1			4.1
112	11/25/02	P/Msp	R	T	YFT	27.5	3.8	3.6	-18.6	-19.1	6.6	8.1
113	11/25/02	WMT	R	T	YFT	32.0		6.2		-15.9		3.6
114	11/25/02	RMT	R	T	YFT	32.0		7.4		-17.3		4.4

116	11/25/02	P/Msp	R	T	YFT	31.0	4.4	4.9	-18.0	-19.2	4.7	7.8
117	11/25/02	WMT	R	T	YFT	32.0	6.3	6.2	-16.0	-15.8	4.3	3.5
118	11/25/02	RMT	R	T	YFT	32.0		7.6		-17.5		4.6
120	11/25/02	WMT	R	T	YFT	29.5	6.9		-15.9			
121	11/25/02	RMT	R	T	YFT	29.5	7.3		-16.9		4.0	
122	11/25/02	LVR	R	T	YFT	29.5	4.6		-17.8		4.2	
124	11/25/02	RMT	R	T	YFT	29.0		8.2		-17.9		4.5
129	11/25/02	WMT	R	T	YFT	29.0		6.3		-16.1		3.6
132	11/25/02	WMT	R	T	YFT	29.0		6.2		-16.2		3.5
133	11/25/02	RMT	R	T	YFT	29.0		7.9		-17.8		4.4
138	11/25/02	WMT	R	T	YFT	30.0		6.4		-16.0		3.6
141	11/25/02	WMT	R	T	YFT	30.5	6.4	6.3	-15.7	-15.3	4.2	3.5
149	11/25/02	WMT	R	T	YFT	32.5		6.8		-16.4		3.6
151	11/25/02	WMT	R	T	YFT	28.5		6.7		-16.2		3.6
155	11/25/02	WMT	R	T	YFT	30.5		6.1		-15.8		3.6
161	11/25/02	WMT	R	T	YFT	30.0	6.4	6.3	-16.2	-15.6	4.4	3.5
170	1/23/03	LVR	CO	G	YFT	44.0	5.3		-16.9		4.0	
172	1/23/03	RMT	CO	G	YFT	44.0	8.2		-16.4		4.1	
174	1/23/03	WMT	CO	G	YFT	35.5	6.9		-15.9		3.5	
177	1/23/03	WMT	CO	G	YFT	35.5	6.5		-16.2		3.5	
178	1/23/03	RMT	CO	G	YFT	35.5	7.9		-17.3		4.2	
180	1/23/03	WMT	CO	G	YFT	36.5	6.5		-16.2		3.5	
181	1/23/03	RMT	CO	G	YFT	36.5	7.9	7.8	-17.5	-17.5	4.3	4.6
189	1/23/03	WMT	CO	G	YFT	34.0		6.6		-16.0		3.7
192	1/21/03	WMT	SMT	LL	YFT	64.0	11.1		-15.6			
193	1/21/03	WMT	SMT	LL	YFT	83.0		11.1		-15.6		3.9
196	1/21/03	LVR	SMT	LL	BET	95.0	9.2		-15.5			
198	1/21/03	WMT	SMT	LL	YFT	83.0		12.2		-16.3		3.9
199	1/21/03	LVR	SMT	LL	YFT	77.0		12.0		-15.7		3.8
200	1/21/03	WMT	SMT	LL	YFT	77.0	12.2		-15.4			
201	1/21/03	LVR	SMT	LL	BET	79.0	10.0		-17.3			

202	1/21/03	WMT	SMT	LL	BET	79.0	11.2		-17.0		3.6	
208	1/21/03	WMT	SMT	LL	BET	104.0	11.0		-15.6		3.6	
210	12/31/02	LVR	BO	T	YFT	33.0	5.5		-17.3		3.8	
214	12/31/02	WMT	BO	T	YFT	33.0	6.8		-16.0		3.5	
215	12/31/02	RMT	BO	T	YFT	33.0	8.3		-17.0		4.0	
216	12/31/02	LVR	BO	T	YFT	32.0	6.5		-17.1			
217	12/31/02	WMT	BO	T	YFT	32.0	7.5		-16.1			
219	12/31/02	LVR	BO	T	YFT	33.0	5.8		-17.4		4.0	
220	12/31/02	WMT	BO	T	YFT	33.0	6.7		-16.1		3.5	
221	12/31/02	RMT	BO	T	YFT	33.0	7.8		-17.7		4.3	
223	12/31/02	WMT	BO	T	YFT	32.0	6.9		-16.0		3.5	
224	12/31/02	RMT	BO	T	YFT	32.0	8.1		-17.9		4.6	
226	12/31/02	WMT	BO	T	YFT	30.5	6.8		-16.1		3.5	
227	12/31/02	RMT	BO	T	YFT	30.5	8.4		-17.3		4.1	
229	12/31/02	WMT	BO	T	YFT	34.5	6.6		-15.8		3.5	
230	12/31/02	RMT	BO	T	YFT	34.5	8.3		-17.2		4.3	
250	12/31/02	LVR	U	T	YFT	25.5	4.9		-18.0			
251	12/31/02	WMT	U	T	YFT	25.5	5.5		-16.1			
252	12/31/02	RMT	U	T	YFT	25.5	7.6		-17.9			
254	12/31/02	WMT	U	T	YFT	30.0	6.5		-16.5			
255	12/31/02	RMT	U	T	YFT	30.0	8.3		-17.6			
256	12/31/02	PFIN	U	T	YFT	30.0	5.1		-14.5		3.4	
257	12/31/02	DFIN	U	T	YFT	30.0	5.2	4.9	-15.3	-15.3	3.5	3.9
258	12/31/02	LVR	T	T	YFT	30.0	5.3		-17.1			
259	12/31/02	WMT	T	T	YFT	30.0	6.1		-16.0			
260	12/31/02	RMT	T	T	YFT	30.0	7.4		-17.0		4.3	
262	12/31/02	LVR	T	T	YFT	31.5	5.4		-17.5			
263	12/31/02	WMT	T	T	YFT	31.5	6.1		-16.0			
264	12/31/02	RMT	T	T	YFT	31.5	8.1		-16.8			
265	12/31/02	FINT	T	T	YFT	31.5	5.0		-14.1		3.4	
266	12/31/02	LVR	U	T	YFT	28.5	6.4		-17.6			

267	12/31/02	WMT	U	T	YFT	28.5	6.2		-17.4			
268	12/31/02	RMT	U	T	YFT	28.5	8.8		-16.6			
270	12/31/02	LVR	T	T	YFT	29.5	5.3		-17.6			
271	12/31/02	WMT	T	T	YFT	29.5	6.3		-16.5			
272	12/31/02	RMT	T	T	YFT	29.5	6.8		-16.8		3.7	
273	12/31/02	FINT	T	T	YFT	29.5	5.1		-15.3		3.4	
275	3/21/03	WMT	HH	T	BET	47.0	11.4		-15.6		3.7	
276	3/21/03	STM	HH	T	BET	47.0		10.7		-16.1		3.7
277	3/21/03	LVR	HH	T	BET	47.0	8.9		-17.5		4.1	
278	3/10/03	WMT	HH	T	BET	53.7	11.9		-15.6		3.7	
279	3/10/03	STM	HH	T	BET	53.7	10.4		-16.1		4.2	
280	3/10/03	LVR	HH	T	BET	53.7	9.6		-16.8		4.0	
281	3/21/03	WMT	HH	T	YFT	39.0	8.0		-16.6		3.6	
282	3/21/03	STM	HH	T	YFT	39.0		8.6		-16.1		3.8
283	3/21/03	LVR	HH	T	YFT	39.0	8.2		-17.2		4.3	
286	3/10/03	LVR	HH	T	BET	53.4		8.0		-17.5		4.2
287	3/10/03	WMT	HH	T	BET	58.0	12.5		-16.3		3.7	
288	3/10/03	STM	HH	T	BET	58.0	11.4		-15.8		4.1	
289	3/10/03	LVR	HH	T	BET	58.0	9.5		-16.3		4.1	
290	3/13/03	WMT	HH	T	BET	49.6	12.0		-16.7		3.7	
291	3/13/03	STM	HH	T	BET	49.6	11.4		-16.2		4.2	
292	3/13/03	LVR	HH	T	BET	49.6	10.0		-16.7		4.9	
294	3/13/03	STM	HH	T	BET	62.0	10.6		-16.4		4.4	
296	3/21/03	WMT	HH	T	YFT	84.0	13.0		-15.4		3.7	
297	3/21/03	STM	HH	T	YFT	84.0		10.9		-15.6		4.1
298	3/21/03	LVR	HH	T	YFT	84.0	9.5		-17.9		4.6	
299	3/10/03	WMT	HH	T	YFT	42.0	7.2		-16.8		3.6	
300	3/10/03	STM	HH	T	YFT	42.0	7.0		-16.6		4.1	
301	3/10/03	LVR	HH	T	YFT	42.0	6.2		-18.1		4.1	
303	3/13/03	STM	HH	T	BET	48.0	10.8		-16.4		4.2	
304	3/13/03	LVR	HH	T	BET	48.0	8.4		-17.8		4.2	

306	3/10/03	STM	HH	T	BET	49.3		11.1		-15.4		3.6
350A	6/30/03	LVR	SMT	LL	BET	77.0		10.7		-17.1		6.0
350B	6/30/03	WMT	SMT	LL	BET	77.0	11.8		-16.3		3.8	
351A	6/30/03	LVR	SMT	LL	BET	85.0	11.0		-17.2		5.0	
352A	6/30/03	WMT	SMT	LL	BET	74.0	11.4	12.0	-16.2	-15.9	3.6	3.6
353A	6/30/03	LVR	SMT	LL	BET	87.0	11.1		-17.1		4.8	
353B	6/30/03	WMT	SMT	LL	BET	87.0	13.5	14.0	-16.3	-16.6	3.6	3.8
355A	6/30/03	LVR	SMT	LL	BET	86.0		10.3		-17.1		4.7
355B	6/30/03	WMT	SMT	LL	BET	86.0	11.7	12.1	-16.4	-16.0	3.6	3.7
356A	6/30/03	WMT	SMT	LL	BET	66.0	11.9	12.0	-16.2	-15.4	4.3	3.6
357B	6/30/03	LVR	SMT	LL	BET	77.0		9.7		-17.4		5.0
358A	6/30/03	WMT	SMT	LL	BET	71.0	11.2	11.1	-17.1	-16.0	3.7	3.7
358B	6/30/03	LVR	SMT	LL	BET	71.0	10.5	10.3	-17.1	-16.6	6.2	4.3
359A	6/30/03	WMT	SMT	LL	BET	85.0	12.3		-16.3		3.8	
359B	6/30/03	LVR	SMT	LL	BET	85.0	11.2	11.1	-16.9	-16.8	5.8	4.4
360B	6/30/03	WMT	SMT	LL	BET	68.0	10.3		-16.3		3.8	
361A	6/30/03	WMT	SMT	LL	BET	74.0	10.5		-16.3		3.9	
361B	6/30/03	LVR	SMT	LL	BET	74.0	10.1		-17.1		4.6	
362A	6/30/03	LVR	SMT	LL	BET	75.0	10.4		-16.9		4.9	
362B	6/30/03	WMT	SMT	LL	BET	75.0	11.5	11.5	-16.2	-16.0	4.4	3.5
363A	6/30/03	WMT	SMT	LL	BET	67.0	12.0	12.4	-16.3	-16.0	3.6	3.8
363B	6/30/03	LVR	SMT	LL	BET	67.0	10.6		-17.1		4.7	
364A	6/30/03	WMT	SMT	LL	BET	61.0	11.1	11.2	-16.0	-16.0	3.5	3.6
364B	6/30/03	LVR	SMT	LL	BET	61.0	10.0		-17.2		4.7	
366A	6/30/03	LVR	SMT	LL	YFT	128.0		11.0		-16.8		4.5
366B	6/30/03	WMT	SMT	LL	YFT	128.0	13.3	13.1	-16.5	-16.0	4.5	3.6
367A	6/30/03	LVR	SMT	LL	YFT	48.0	9.3		-17.5		5.9	
368A	6/30/03	WMT	SMT	LL	YFT	48.0	9.6		-16.2		3.8	
369	11/7/02	WMT	LL	T	BET	96.0		9.9		-16.4		3.7
375	11/8/02	WMT	LL	T	BET	126.0		9.8		-16.6		3.9
381	11/8/02	WMT	LL	T	BET	60.0		9.7		-16.2		3.7

388	11/8/02	WMT	LL	T	YFT	65.5		9.2		-15.7		3.9
400	4/22/03	WMT	SMT	LL	BET	115.0	12.8		-16.6		3.6	
401	4/22/03	WMT	SMT	LL	BET	103.0	10.6		-15.9		3.5	
402	4/22/03	WMT	SMT	LL	BET	108.0	9.9		-16.3		3.6	
403	4/22/03	WMT	SMT	LL	BET	111.0	11.0		***-21.2***		3.5	
404	4/22/03	WMT	SMT	LL	BET	113.0	11.2		-16.3		3.7	
405	4/22/03	WMT	SMT	LL	BET	101.0	11.0		-16.2		3.6	
406	4/22/03	WMT	SMT	LL	BET	99.0	10.7		***-19.6***		3.6	
407	4/22/03	WMT	SMT	LL	BET	104.0	10.0		-16.1		3.6	
408	4/22/03	WMT	SMT	LL	YFT	114.0	9.7		-15.4		3.7	
409	4/22/03	WMT	SMT	LL	BET	98.0	12.0		-16.1		3.6	
410	4/22/03	WMT	SMT	LL	BET	83.0	10.6		***-19.9***		3.7	
411	4/22/03	WMT	SMT	LL	BET	107.0	11.5		***-20.4***		3.7	
412	4/22/03	WMT	SMT	LL	BET	103.0	10.3		-16.7		3.6	
413	4/22/03	WMT	SMT	LL	BET	96.0	10.8		-16.0		3.5	
414	4/22/03	WMT	SMT	LL	BET	109.0	10.6		-16.0		3.6	
415	4/22/03	WMT	SMT	LL	YFT	115.0	9.3		-16.7		3.9	
416	4/22/03	WMT	SMT	LL	BET	104.0	10.1		-16.2		3.6	
417	4/22/03	WMT	SMT	LL	YFT	93.0	10.9		***-20.1***		3.8	
418	4/22/03	WMT	SMT	LL	BET	117.0	9.1		-16.1		3.6	
419	4/22/03	WMT	SMT	LL	BET	112.0	10.6		-15.9		3.6	
420	4/22/03	WMT	SMT	LL	BET	117.0	12.6		-17.2		3.7	
421	4/22/03	WMT	SMT	LL	BET	99.0	11.8		-16.6		3.7	
435	10/3/03	WMT	U	T	YFT	29.8		7.2		-16.5		3.7
437	10/3/03	WMT	U	T	YFT	34.8		6.3		-16.0		3.7
449	10/21/03	***W/RMT?***	BO	T	YFT	26.8		7.2		-17.4		4.0
450	10/21/03	RMT	BO	T	YFT	26.8	7.8		-17.4		4.1	
451	10/21/03	LVR	BO	T	YFT	26.8	6.8		-16.8		4.1	
452	10/27/03	WMT	U	T	YFT	41.0		6.7		-16.3		3.6
453	10/27/03	RMT	U	T	YFT	41.0	8.2		-17.2		4.3	
454	10/27/03	LVR	U	T	YFT	41.0	6.6		-16.7		4.3	

455	10/21/03	WMT	HH	T	YFT	37.5		6.8		-16.2		3.8
456	10/21/03	RMT	HH	T	YFT	37.5	7.9		-17.0		4.2	
457	10/21/03	LVR	HH	T	YFT	37.5	6.8		-17.3		4.7	
458	10/18/03	WMT	II	T	YFT	37.8		6.8		-16.0		3.7
459	10/18/03	RMT	II	T	YFT	37.8	7.4		-16.9		4.3	
460	10/18/03	LVR	II	T	YFT	37.8	5.9		-17.8		5.4	
461	10/18/03	WMT	R	T	YFT	32.5		6.8		-15.8		3.6
462	10/18/03	RMT	R	T	YFT	32.5	7.9		-16.9		4.2	
463	10/19/03	WMT	II	T	YFT	37.3		7.1		-15.8		3.7
464	10/19/03	RMT	II	T	YFT	37.3	7.8		-17.0		4.9	
465	10/19/03	LVR	II	T	YFT	37.3	6.6		-16.9		5.3	
466	10/27/03	WMT	U	T	YFT	35.5		6.8		-16.1		3.7
467	10/27/03	RMT	U	T	YFT	35.5	7.9		-17.1		4.2	
468	10/27/03	LVR	U	T	YFT	35.5	5.8		-16.7		4.2	
469	10/27/03	P/Stpd	U	T	YFT	35.5	4.4		-17.7		4.5	
470	12/18/02	WMT	R	T	YFT	42.5		6.4		-15.8		3.6
471	12/18/02	RMT	R	T	YFT	42.5	7.2		-17.0		4.4	
472	12/18/02	LVR	R	T	YFT	42.5	6.7		-16.9		4.5	
473	10/19/03	WMT	II	T	YFT	40.5		7.2		-15.8		3.8
474	10/19/03	RMT	II	T	YFT	40.5		7.8		-18.0		5.3
475	10/19/03	LVR	II	T	YFT	40.5		6.7		-16.3		4.5
476	1/25/03	LVR	LL	T	YFT	39.0	5.4	5.2	-17.3	-17.9	3.9	4.2
477	1/25/03	LVR	LL	T	YFT	39.5		6.6		-16.7		4.5
478	1/25/03	LVR	LL	T	YFT	66.0		8.1		-16.7		5.1
479	1/25/03	LVR	LL	T	YFT	95.5		7.8				
480	1/25/03	LVR	LL	T	YFT			9.0		-16.3		4.7
481	1/25/03	WMT	LL	T	YFT	95.5		8.9		-15.4		3.6
482	1/25/03	RMT	LL	T	YFT	95.5		9.5		-16.0		4.3
483	1/16/04	P/Mul	U	T	YFT	36.5	5.3	5.0	-17.8	-19.3	3.8	4.7
484	1/16/04	P/Mul	U	T	YFT	37.0	5.6	5.1	-17.7	-18.8	3.9	5.1
485	1/16/04	P/Mul	U	T	YFT	39.2	5.4	4.9	-17.4	-18.5	3.8	4.3

487	6/27/03	WMT	U	T	YFT	97.2	9.4	-16.6	3.7
496	8/5/03	LVR	MM	T	YFT	49.7	8.7	-17.0	5.7
497	1/16/04	WMT	U	T	YFT	36.5	6.9	-16.5	3.6
499	1/16/04	LVR	U	T	YFT	36.5	6.0	-17.7	4.8
501	1/16/04	RMT	U	T	YFT	37.0	6.3	-16.3	3.5
502	1/16/04	LVR	U	T	YFT	37.0	6.1	-17.8	4.5
503	1/16/04	WMT	U	T	YFT	34.2	6.7	-16.1	3.5
505	1/16/04	LVR	U	T	YFT	34.2	6.2	-17.3	4.4
506	1/20/04	WMT	LL	T	BET	46.0	10.0	-16.4	3.7
507	1/20/04	RMT	LL	T	BET	46.0	10.4	-17.8	4.9
509	1/20/04	WMT	LL	T	YFT	42.1	7.2	-16.3	3.7
510	1/20/04	RMT	LL	T	YFT	42.1	8.0	-17.7	5.2
512	6/22/03	WMT	U	T	YFT	89.6	9.2	-16.4	3.7
515	11/25/02	na	R	T	YFT	30.0	9.0	-17.0	3.7
517	8/5/03	RMT	MM	T	YFT	48.9	8.8	-17.5	4.3
519	8/5/03	LVR	MM	T	YFT	47.5	9.8	-18.1	5.9
520	8/5/03	RMT	MM	T	YFT	47.5	10.3	-19.3	6.5
521	8/5/03	WMT	MM	T	YFT	47.5	10.8	-16.7	3.7
523	8/5/03	RMT	MM	T	YFT	48.4	11.2	-17.4	4.5
524	8/5/03	LVR	MM	T	YFT	48.4	10.8	-16.9	4.4
525	8/5/03	WMT	MM	T	YFT	100.2	10.0	-16.7	3.9
530	4/23/03	WMT	SMT	LL	BET	84.0	10.9	-15.7	3.7
531	4/23/03	WMT	SMT	LL	YFT	150.0	10.9	-18.7	5.5
532	4/23/03	WMT	SMT	LL	YFT	112.0	10.9	-16.2	3.6
533	4/23/03	WMT	SMT	LL	YFT	77.0	10.2	-15.5	3.6
534	4/23/03	WMT	SMT	LL	BET	119.0	9.8	-17.0	3.8
535	4/23/03	WMT	SMT	LL	BET	112.0	10.3	-16.7	3.7
536	4/23/03	WMT	SMT	LL	BET	118.0	9.7	-16.8	3.6
537	4/23/03	WMT	SMT	LL	BET	86.0	15.0	-15.6	3.7
538	3/31/04	WMT	MM	T	YFT	44.5	8.4	-16.1	3.7
539	3/31/04	LVR	MM	T	YFT	44.5	10.0	-18.4	6.7

540	4/1/04	WMT	T	T	YFT	45.8	12.5	-16.6	3.7
541	4/1/04	LVR	T	T	YFT	45.8	11.0	-17.0	4.7
542	11/25/02	WMT	R	T	YFT	32.5	6.9	-15.8	3.6
543	4/1/04	WMT	T	T	YFT	38.9	7.2	-16.7	3.7
544	4/1/04	LVR	T	T	YFT	38.9	6.9	-17.9	4.7
545	3/31/04	WMT	MM	T	YFT	46.5	11.5	-16.6	3.7
546	3/31/04	LVR	MM	T	YFT	46.5	10.3	-17.2	4.8
547	na	P/Stpd		T	YFT	34.0	3.8	-19.2	5.8
548	6/27/03	WMT	U	T	YFT	42.6	8.8	-17.0	3.8
549	6/27/03	LVR	U	T	YFT	42.6	7.3	-18.6	5.3
550	11/25/02	WMT	R	T	YFT	37.0	6.7	-15.8	3.6
551	7/25/03	WMT	MM	T	YFT	47.1	8.6	-16.7	3.8
552	7/25/03	LVR	MM	T	YFT	47.1	8.3	-20.8	8.3
553	7/25/03	WMT	MM	T	YFT	48.4	8.5	-16.7	3.8
554	7/25/03	LVR	MM	T	YFT	48.4	8.1	-18.2	6.2
555	6/27/03	WMT	U	T	YFT	46.9	8.1	-17.0	3.8
556	6/27/03	LVR	U	T	YFT	46.9	6.9	-19.1	5.7
557	11/25/02	WMT	R	T	YFT	28.5	6.4	-16.2	3.6
558	7/29/04	WMT	MM	T	YFT	53.0	8.3	-16.4	3.7
559	7/29/04	LVR	MM	T	YFT	53.0	5.9	-18.5	5.2
560	7/29/04	WMT	MM	T	YFT	57.0	10.0	-16.4	3.7
562	7/29/04	LVR	MM	T	YFT	46.0	8.8	-17.6	4.6
563	7/29/04	WMT	MM	T	YFT	46.0	13.8	-16.6	3.7
564	7/29/04	LVR	MM	T	BET	52.0	8.1	-18.6	6.4
565	7/29/04	WMT	MM	T	BET	52.0	10.9	-16.6	3.8
566	7/29/04	WMT	MM	T	YFT	54.0	8.4	-16.6	3.8
567	7/29/04	LVR	MM	T	YFT	54.0	6.4	-18.0	4.9
568	7/29/04	WMT	MM	T	YFT	50.0	11.3	-16.6	3.8
569	7/29/04	LVR	MM	T	YFT	50.0	7.7	-17.8	4.8
570	7/29/04	WMT	MM	T	YFT	50.0	8.1	-16.8	3.8
571	7/29/04	LVR	MM	T	YFT	50.0	6.5	-19.4	6.3

572	7/29/04	RMT	MM	T	YFT	50.0	6.4	-18.2	4.9
573	7/29/04	WMT	MM	T	YFT	52.0	9.6	-16.6	3.8
574	7/29/04	LVR	MM	T	YFT	52.0	6.0	-18.7	5.3
575	7/29/04	RMT?	MM	T	YFT	52.0	6.6	-17.4	4.3
576	7/29/04	WMT	MM	T	YFT	59.0	12.2	-16.3	3.7
577	7/29/04	LVR	MM	T	YFT	59.0	8.5	-17.4	4.7
578	7/29/04	RMT	MM	T	YFT	59.0	8.7	-17.4	4.4
579	7/29/04	WMT	MM	T	YFT	47.0	10.0	-16.7	3.7
580	7/29/04	LVR	MM	T	YFT	47.0	7.7	-18.2	5.1
581	7/29/04	WMT	MM	T	YFT	91.0	9.4	-17.1	4.3
582	7/29/04	LVR	MM	T	YFT	91.0	8.1	-17.9	5.0
583	7/29/04	WMT	MM	T	YFT	72.0	13.2	-16.6	3.7
585	Jun-05	P/Omm			YFT		6.6	-18.7	4.4
586	Jun-05	P/HOmm			YFT		5.8	-18.9	4.4
587	Jun-05	P/Oplo, 1A			YFT		5.5	-19.2	5.8
588	Jun-05	P/Oplo, 1A			YFT		5.3	-18.3	5.0
589	Jun-05	P/M 1FL			YFT		3.6	-19.8	6.4
590	Jun-05	P/Lys			YFT		5.4	-20.0	5.9
591	Jun-05	P/Stpd			YFT		5.3	-19.8	5.7
592	Jun-05	P/srf			YFT		5.6	-18.3	4.1
593	Jun-05	P/Squ			YFT		5.2	-20.0	5.9
594	3/11/04	LVR	MM	T	YFT	122.0	8.4	-17.2	4.9
595	3/11/04	WMT	MM	T	YFT	122.0	9.5	-16.1	4.0
596	3/11/04	RMT	MM	T	YFT	122.0	10.4	-16.4	4.4
597	3/11/04	WMT	MM	T	YFT	126.7	9.4	-15.5	3.8
598	3/11/04	LVR	MM	T	YFT	126.7	8.0	-17.0	4.7
599	3/11/04	RMT	MM	T	YFT	126.7	10.1	-16.5	4.5
600	8/1/05	P/Dac			YFT	na	4.7	-18.9	4.5
602	8/1/05	P/Ost; wmt			YFT	3.5	5.4	-18.8	3.9
603	8/1/05	P/Ost; whl			YFT	1.3	3.2	-18.2	4.0

APPENDIX 2: *RAW DATA FROM CHAPTER 3 - CAPTIVE DIET SWITCH.* **SIA Sample ID** is the ID code assigned to each sample and would be the code to use in future sample retrieval. Tank Residence time is the number of days an individual tuna spent in the tank system, with day 1 representing the animal's introduction to the tank. **'Mortality'** is an individual tuna that was found after death but that was in good condition and **'Sacrificed'** is an individual tuna capture and killed. **Tissue Type** is as follows, WMT is white muscle tissue, RMT is red muscle tissue, LVR is liver tissue, DGD is digestive gland tissue, SPLN is spleen tissue, HRT is heart tissue, STM is stomach tissue, STMC is stomach contents (i.e., experimental diet sample), GLF is gill filaments from a gill plate, PFIN is pectoral fins, DFIN is dorsal fins, FINN is finnettes, WBLD is whole blood, TBLD is spun blood, top portion, BBLD is spun blood, bottom portion, EPAR is ecto-parasites on GLF. Both nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) stable isotopic compositions are reported in traditional delta notation (‰). **C/N** is the carbon to nitrogen ratio of the predator's tissues or prey items. "No tag" is a fish that was collected that had shed its ID tag.

SIA Sample ID	Fish #	Tank Residence Time (days)	Mortality (M) or Sacrificed (S)	Initial Fork Length (cm)	Final Fork Length (cm)	Final Mass (g)	Tissue Type†	$\delta^{15}N$, ‰	$\delta^{13}C$, ‰	C/N
T04	1	79	M	-	35.5	-	WMT	10.0	-16.0	3.6
T05							RMT	12.4	-17.4	5.0

T06							PFIN	11.5	-14.2	3.3
T07							STM	12.2	-15.8	3.9
T09							GLF	12.5	-15.8	3.9
T10							FINN	9.7	-14.0	3.4
T11	2	67	M	-	37.5	-	WMT	11.8	-16.3	3.7
T12							RMT	14.3	-17.5	4.8
T13							PFIN	13.5	-14.4	3.4
T14							STM	13.4	-16.6	4.0
T16							GLF	12.9	-16.3	3.9
T18	3	104	M	-	38.9	1126	WMT	11.6	-15.7	3.7
T19							RMT	13.3	-19.0	6.7
T20							STM	13.0	-16.0	4.1
T21							LVR	13.2	-16.7	4.4
T22							GLF	11.9	-15.8	4.1
T23							FINN	12.7	-14.4	3.6
T24							PFIN	12.0	-15.7	4.0
T25							DFIN	12.5	-15.8	4.3
T26	4	35	M	-	33.0	599.6	WMT	9.7	-15.8	3.8
T27							RMT	11.7	-16.9	4.7
T28							PFIN	10.0	-14.5	3.6
T29							FINN	11.5	-18.4	5.0
T30	5	0	S	37.0	37.0	903.3	WMT	6.9	-16.3	3.6
T31							RMT	7.9	-17.6	4.1
T32							WBLD	6.6	-17.1	4.0
T33							LVR	5.3	-18.4	4.6
T34							PFIN	5.5	-16.1	3.6
T35							FINN	5.9	-16.6	4.1
T36	6	0	S	38.0	38.0	899.2	WMT	6.5	-16.2	3.6
T37							RMT	7.8	-17.8	4.4
T38							WBLD	6.7	-17.2	4.0
T39							PFIN	5.7	-15.7	3.5

T40							LVR	5.2	-19.2	5.3
T41							FINN	6.1	-16.1	3.8
T42							WBLD	6.6	-17.0	3.9
T43							RMT	8.0	-17.9	4.7
T44	7	0	S	37.5	37.5	894	WMT	6.6	-16.0	3.6
T45							PFIN	6.1	-15.7	3.5
T46							LVR	5.4	-18.0	4.4
T47							FINN	6.0	-16.8	4.0
T48	8	2	S	~36	36.5	910	WBLD	6.6	-16.9	3.8
T49							WMT	6.6	-16.1	3.6
T50							RMT	7.1	-17.3	4.0
T51							LVR	5.5	-17.6	4.1
T52							PFIN	5.6	-14.8	3.1
T53							STM	6.8	-16.9	4.0
T54							FINN	6.4	-16.7	4.0
T55							GLF	6.5	-17.0	4.0
T56	9	2	S	~38	38.5	926	WBLD	6.4	-16.5	3.9
T57							WMT	6.5	-15.7	3.6
T58							RMT	7.7	-17.7	4.4
T59							LVR	6.0	-17.8	4.5
T60							STM	6.9	-17.1	4.0
T61	10	2	S	~35	35.5	708	WBLD	6.5	-17.0	3.9
T62							WMT	6.6	-16.3	3.6
T63							RMT	7.9	-18.0	4.3
T64							LVR	6.0	-17.7	4.2
T65							STM	6.3	-17.4	4.0
T66							PFIN	5.5	-14.0	3.1
T67							GLF	6.4	-16.9	3.8
T68	11	60	M	-	38.5	1189	WMT	11.3	-16.0	3.7
T69							RMT	12.9	-18.9	6.6
T70							PFIN	9.7	-13.9	3.3

T71							GLF	12.2	-16.6	4.2
T72							FINN	12.9	-17.7	5.1
T73							STM	12.2	-16.7	4.4
T74							LVR	11.6	-15.9	3.8
T75	12	4	M	-	34.5	678.8	WMT	6.7	-16.3	3.6
T76							RMT	7.9	-18.1	4.5
T77							LVR	5.7	-18.4	4.6
T78							STM	7.7	-17.2	4.0
T79	13	4	M	-	38.0	918.9	WMT	6.8	-16.2	3.6
T80							RMT	8.4	-17.9	4.5
T81							LVR	6.1	-18.0	4.5
T82							STM	6.5	-16.8	4.0
T83	14	no tag	M	-	38.0	950	WMT	7.9	-18.2	4.9
T84							RMT	6.9	-15.9	3.6
T85							LVR	6.1	-18.1	4.4
T86							STM	6.8	-16.8	4.0
T89	15	5	S	~37	37.0	863.8	WBLD	7.2	-17.1	4.0
T90							WMT	6.9	-16.0	3.6
T91							RMT	8.3	-17.3	4.2
T92							GLP	7.0	-16.6	3.7
T93							DGD	7.3	-18.0	4.5
T94							SPL	7.0	-16.8	3.9
T95							LVR	7.2	-17.4	4.5
T96							PFIN	5.8	-14.7	3.0
T97							FINN	7.9	-16.0	3.8
T98							HRT	9.4	-17.5	4.4
T99							STM	7.5	-17.0	4.0
T102	16	19	S	~36	36.0	770.6	WBLD	7.4	-17.0	3.9
T103							TBLD	9.8	-17.3	4.4
T104							GLF	8.7	-17.0	3.9
T105							WMT	6.8	-16.3	3.6

T106							RMT	9.1	-17.7	4.5
T107							LVR	10.1	-17.5	4.2
T108							DGD	9.9	-17.8	4.2
T109							FINN	7.3	-15.9	3.9
T110							SPLN	7.9	-17.0	3.9
T111							HRT	10.2	-17.5	4.2
T112							PFIN	6.2	-14.2	3.2
T113							EPAR	10.6	-17.0	4.3
T114							STM	8.7	-17.0	4.1
T115							DFIN	5.1	-14.0	3.3
T116	17	28	S	~35	40.0	1225	WBLD	8.8	-17.0	3.9
T117							TBLD	11.5	-17.9	4.6
T118							BBLD	8.6	-16.9	3.8
T120							RMT	10.8	-17.9	5.2
T121							PFIN	9.9	-15.5	3.5
T124							GLF	10.9	-17.0	4.1
T125							SPLN	9.8	-16.3	3.6
T126							DGD	12.0	-18.2	4.6
T127							LVR	13.0	-17.4	4.9
T128							HRT	12.2	-17.5	4.3
T129							EPAR	12.3	-17.4	4.4
T130							STM	10.9	-17.3	4.2
T131							STMC	13.6	-19.9	4.7
T133	18	1	M	~35	35.8	704.2	WMT	6.6	-16.5	3.5
T134							RMT	7.1	-17.1	3.8
T136							HRT	9.1	-17.6	4.1
T137							STM	7.0	-17.3	4.0
T138							LVR	6.3	-17.9	4.4
T139							DGD	5.9	-17.9	4.3
T140	19	1	M	~37	37.3	872.7	WMT	6.8	-16.3	3.6
T141							RMT	8.3	-18.0	4.6

T142							DGD	6.2	-17.6	4.1
T143							LVR	6.5	-18.2	4.6
T144							PFIN	5.7	-15.1	3.4
T146							HRT	8.6	-17.5	4.2
T147							STM	7.0	-17.2	4.0
T148	20	26	M	~36	36.0	830.3	WMT	7.8	-16.3	3.5
T149							RMT	9.4	-17.0	3.9
T150							PFIN	10.9	-17.2	4.1
T151							HRT	10.6	-16.9	4.1
T153							STM	8.8	-16.7	4.0
T154							LVR	10.4	-17.2	4.4
T155							DGD	7.3	-17.7	4.4
T156	21	5	M	-	38.0	765.4	WMT	6.8	-16.5	3.6
T157							RMT	7.5	-18.0	4.8
T158							PFIN	8.6	-14.4	4.1
T159	22	8	M	~38	37.5	744	WMT	7.4	-16.4	3.6
T160							PFIN	5.0	-14.0	4.1
T161							RMT	8.8	-17.1	4.2
T162							LVR	7.4	-18.1	5.1
T163	23	40	S	~38	38.0	933.7	WBLD	8.8	-17.0	4.0
T164							TBLD	13.5	-18.1	5.0
T165							BBLD	8.4	-16.8	3.9
T166							WMT	8.2	-16.2	3.6
T167							RMT	10.6	-17.9	4.6
T168							FINN	9.2	-15.8	3.9
T170							GLF	10.7	-17.3	4.0
T171							LVR	12.4	-17.8	4.5
T172							DGD	11.7	-18.4	4.4
T173							SPLN	9.3	-17.2	3.9
T174							STMC	13.9	-23.7	4.5
T175							STM	9.9	-17.3	4.0

T176							HRT	12.2	-17.5	4.2
T180	24	90	M	-	43.5	1455	PFIN	13.7	-14.3	3.3
T181							WMT	12.3	-16.1	3.6
T182							WMT	12.2	-16.1	3.6
T183							RMT	14.0	-18.5	5.7
T184							LVR	13.4	-17.3	4.4
T185							DGD	13.6	-17.7	4.8
T186							SPLN	12.3	-16.8	4.2
T188							HRT	15.4	-17.0	4.4
T190	25	12	S	-	38.5	1031	WMT	7.1	-16.2	3.6
T177							BLD	7.6	-17.1	3.9
T178							TBLD	11.5	-18.6	5.1
T179							BBLD	7.1	-17.2	3.9
T191							RMT	9.0	-18.4	5.1
T192							FINN	9.0	-15.9	3.8
T194							GLF	9.2	-17.0	4.0
T195							LVR	10.1	-17.7	5.2
T196							DGD	10.4	-19.5	4.9
T197							SPLN	7.6	-17.0	4.0
T198							STMC	13.3	-19.7	4.6
T199							HRT	10.4	-17.7	4.3
T200							STM	8.8	-17.4	4.0
T201	26	0	S	-	37.4	955.7	WMT	7.1	-16.4	3.6
T202							RMT	8.4	-18.2	4.7
T203							LVR	6.3	-17.7	4.9
T204							WMT	7.2	-16.5	3.7
T205							RMT	7.8	-17.8	4.6
T206							LVR	6.3	-17.6	5.4
T207							BLD	7.0	-17.5	4.2
T208							WMT	6.7	-16.2	3.6
T209							RMT	8.1	-18.3	5.1

T210							LVR	7.5	-18.5	6.9
T211							BLD	7.0	-17.4	4.1
T216	27	33	S	-	40.0	1136	RMT	10.9	-18.5	5.4
T217							LVR	13.4	-17.9	6.3
T218							DGD	12.9	-18.1	4.6
T221							STM	11.4	-17.2	4.2
T222							STMC	13.9	-16.6	4.5
T223	28	16	S	~38	37.5	716.9	BLD	7.7	-17.0	3.9
T229							SPLN	7.4	-16.9	4.0
T232	29	72	S	40	-	-	SPLN	11.2	-17.0	4.0
T233							WMT	10.0?	-16.2	3.6
T234							DGD	12.7	-18.2	5.0
T235							PFIN	15.6	-15.5	3.8
T236							HRT	16.2	-17.4	4.3
T237							BLD	12.9	-16.8	4.0
T238							GLF	15.1	-16.6	4.1
T239							STMC	13.3	-18.5	5.0
T240							LVR	17.1	-17.0	4.6
T244	30	no tag	M	-	38.8	1232	LVR	13.2	-17.7	4.6
T245							SPLN	11.5	-17.5	4.2
T249	31	129	M	-	41.0	1365	GLF	12.6	-16.9	4.4
T266	32	no tag	M	-	37.5	1001*	HRT	12.1	-18.1	4.5
T267	33	70	M	34.0	39.5	1325	WMT	12.4	-16.6	3.7
T268	34	31	M	37.0	36.5	808	RMT	9.3	-17.9	4.8
T270							GLF	9.4	-16.9	4.3
T271							LVR	11.3	-17.5	5.2
T272							DGD	11.1	-17.9	4.5
T273							HRT	11.1	-17.2	4.2
T274							PFIN	7.9	-15.4	3.8
T275							SPLN	9.8	-17.2	4.2

T276	35	3	M	-	35.5*	642*	WMT	7.3	-16.5	3.6
T277							RMT	8.7	-17.8	4.4
T278	36	24	S	36.0	37.0*	907*	WMT	10.1	-16.6	3.6
T279							RMT	12.7	-17.9	4.4
T280							HRT	13.9	-17.8	4.2
T281							DGD	12.6	-17.6	4.3
T282							SPLN	11.7	-17.5	4.1
T283							LVR	13.0	-18.0	4.5
T284							PFIN	10.2	-14.9	3.8
T285	37	115	M	-	37.9	959.2	WMT	11.8	-16.6	3.7
T286		115					RMT	14.1	-17.5	4.3
T287	38	40	M	-			WMT	8.4	-16.1	3.7
T288		40					RMT	11.5	-17.8	4.7