Insight into the Trophic Ecology of Yellowfin Tuna, *Thunnus albacares*, from Compound-Specific Nitrogen Isotope Analysis of Proteinaceous Amino Acids

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1. Introduction

There is widespread concern and debate on the extent that commercial fisheries are altering the structure and function of marine ecosystems (Pauly et al. 1998; NRC 1999; Myers and Worm 2003; Hampton et al. 2005; Sibert et al. 2006). Selective removal of large predatory fishes from food webs can impart changes in trophic structure and stability via trophic cascades, defined as inverse patterns in abundance or biomass across more than one trophic level in a food web (Carpenter, Kitchell and Hodgson 1985; Pace et al. 1999). Recent calls for policy makers to adopt an ecologically-based approach to fisheries management (Botsford, Castilla and Peterson 1997; Pikitch et al. 2004) places renewed emphasis on achieving accurate depictions of trophic links and biomass flows through the food web in exploited systems. Such an approach would take into consideration the indirect effects of fishing, such as declines in diversity, changes in the species composition of the prey community, and changes in trophic-level structure (e.g., aggregate removals at various trophic levels) (Gislason et al. 2000).

There is general agreement of the importance of measuring changes in trophic structure as a means to evaluate fishery impacts on ecosystems, and ecosystem indicators that take trophic level into consideration are desirable (Gislason et al. 2000; Murawski 2000; Rice 2000). Monitoring the trophic level of key food web components and functional groups instead of the mean trophic level of the fisheries catch (Pauly et al. 2001) serves as a useful fisheries-independent metric of ecosystem change and sustainability because it integrates an array of biological and ecological relationships and processes. In addition to adopting ecosystem metrics, ecosystem-based fisheries management is facilitated through the development of multi-species models that represent indirect ecological interactions among species or guilds (Latour, Brush and Bonzek 2003). Among these models, mass-balance models of food webs (e.g., Cox et al. 2002; Olson and Watters 2003) explicitly represent trophic links between biomass pools based upon diet relations determined from stomach contents analysis.
Stable isotope ratios have been used extensively in ecosystems research and are a valuable compliment to traditional methods used to study food webs (Peterson and Fry 1987). In particular, nitrogen isotopic ratios have been frequently used to examine trophic dynamics (Peterson and Fry 1987; Lajtha and Michener 1994). At each trophic level, an increase of ~3‰ has been observed in the bulk tissue $\delta^{15}$N values of many consumers (Deniro and Epstein 1981; Minagawa and Wada 1984; Post 2002). However, the $\delta^{15}$N value of any consumer is predominantly a function of both the trophic level of that consumer and the $\delta^{15}$N value of the primary producers at the base of the food web. In marine environments, the microalgae that support marine food webs typically have $\delta^{15}$N values that change spatially and seasonally due to incomplete utilization of nitrogenous nutrients (e.g., Altabet 2001; Lourey, Trull and Sigman 2003), uptake of partly denitrified nitrate (e.g., Cline and Kaplan 1975; Voss, Dippner and Montoya 2001; Sigman et al. 2005), and because primary producers can use different sources of nitrogen (nitrate, ammonium, N$_2$) in different areas and seasons (e.g., Dugdale and Goering 1967; Owens 1987; Dugdale and Wilkerson 1991, Dore et al. 2002). When determining the relative trophic level of top predators, characterizing the $\delta^{15}$N values of the base of marine food webs can be challenging because marine microalge have very short life spans and can be difficult to isolate from other organic suspended particulate material. An alternative approach is to use primary consumers (e.g., zooplankton or bivalve mollusks), which may integrate short-term and spatial variability in the $\delta^{15}$N values of their diet, to represent trophic level 2 or slightly higher (e.g., Post 2002; Jennings et al. 2002). Unfortunately, zooplankton are also not ideal for this purpose, since they too have short life spans and many are omnivorous (Rolff 2000).

Compound-specific isotopic analyses (CSIA) can compliment whole-tissue or whole-animal isotopic results and can distinguish metabolic and trophic-level relationships in a food web from changes in isotopic compositions at the base of the food web (Uhle et al. 1997; Fantle et al. 1999; McClelland and Montoya 2002). For example, Uhle et al. (1997) used the $\delta^{13}$C of
individual fatty and amino acids to elucidate the sources of metabolic carbon used for synthesis of these compounds in foraminifera. Previous research has also shown that $\delta^{13}C$ values of essential and nonessential amino acids distinguished between the basal carbon sources and diet of a consumer (Fantle et al. 1999). These researchers showed that essential amino acids (EAA), which are produced only by plants and bacteria, were not heavily fractionated by juvenile blue crabs whereas nonessential amino acids (NAA) were fractionated to a greater extent. More recently, Jim et al. (2006) using laboratory rats grown on diets of isotopically- and nutritionally-manipulated purified C$_3$ and/or C$_4$ macronutrients, found that EAA and conditionally-indispensable amino acids were routed from diet to collagen with little isotopic fractionation, whereas NAA differed by up to 20‰. The essential and nonessential amino acid designation is based on the flow of carbon through biochemical systems, and although it is a convenient way to organize our thoughts, it unfortunately does not necessarily provide an accurate picture of the origins of amino nitrogen. For example, McClelland and Montoya (2002) indicated that a mixture of EAA and NAA were incorporated with little alteration in $\delta^{15}N$ values from dietary sources into herbivorous zooplankton fed a known algal diet. These authors found $\delta^{15}N$ values of glycine, lysine, phenylalanine, serine, threonine and tyrosine were nearly identical in producer and consumer. Of these amino acids, only lysine, phenylalanine, and threonine are considered EAA. On the other hand, the amino acids alanine, aspartic acid, glutamic acid, isoleucine, leucine, proline and valine were enriched in $^{15}N$ by ~5-7‰ in the consumer relative to those in the producer. Leucine, isoleucine and valine are considered EAA. The work of McClelland and Montoya (2002) clearly showed that the classic essential and nonessential grouping of amino acids did not correlate well with either $\delta^{15}N$ values or trophic position in their simple laboratory food web study. However, the principle finding of McClelland and Montoya (2002) for applications of isotope ecology is that the $\delta^{15}N$ values of some amino acids in consumers apparently can provide accurate determination of the isotopic composition of the base of the food web. We consider this group of compounds the “source” amino acids. On the other hand, other amino acids are either synthesized by animal consumers de novo or undergo significant
transamination and deamination reaction, are enriched in $^{15}$N by ~5-7‰ relative to the first group of amino acids, and appear to reflect the trophic level of the consumer (McClelland and Montoya 2002). We consider these the “trophic” amino acids.

In this paper, we tested the premise that a single sample from an upper trophic level pelagic predator fish could provide information on both the trophic level of the fish and the $\delta^{15}$N value at the base of the food web. We analyzed the nitrogen isotopic composition of individual amino acids in white muscle tissue (WMT) of yellowfin tuna (Thunnus albacares) from the eastern tropical Pacific (ETP) to determine if the observed variations in the $\delta^{15}$N values of WMT are primarily controlled by the nitrogen isotopic composition at the base of the food web or the trophic level of the tuna. We used the difference between the $\delta^{15}$N values of source and trophic amino acids to estimate the trophic level of yellowfin, and we compare this to estimates based on two independent methods, stomach content analysis and the isotopic difference between the $\delta^{15}$N values of mesozooplankton and yellowfin tuna in the ETP. We found that nitrogen isotopic analyses of individual amino acids in tuna can be used to distinguish between nutrient and trophic dynamics in pelagic ecosystems and we discuss implications of these results for investigating the long-term impact of commercial fishing on the food web structure of marine ecosystems.

2. Oceanographic Setting

The ETP contains some of the most biologically-productive waters of the world’s oceans. In the ETP, the northeast and southeast trade winds converge north of the equator along the intertropical convergence zone. The northeast trade winds drive the north equatorial current to the west at ~10°N and the southeast trade winds drive the south equatorial current to the west at ~3°S (Wyrtki 1966). The equatorial countercurrent flows eastward between the north and south equatorial currents in the region where the trade winds are the weakest. This circulation pattern
results in a band of cold, nutrient-rich water near the equator that extends west from South America far into the central equatorial Pacific. The intensity and spatial extent of this “cold tongue” can vary seasonally and interannually (Chelton et al. 2001). Ekman drift in the most eastern part of the ETP carries these nutrient-rich waters polewards along the coasts of Baja California and of Ecuador and Peru (Philander, Hurlin and Seigel 1987).

Coastal upwelling along the eastern boundary of the ETP and oceanic upwelling along offshore divergences bring new macronutrients (nitrogen, phosphorous and silicon) to the euphotic zone (e.g., Wyrtki 1981), and can account for the high biological productivity of this region (Fiedler, Philbrick and Chavez 1991). Oxic respiration associated with the sinking of organic matter produced by high biological productivity can result in oxygen conditions low enough (~0.1 mL L\(^{-1}\)) that bacteria can use nitrate as an alternative electron acceptor in the respiratory process (i.e., denitrification). Although these low-oxygen oceanic regions represent only 0.1% of the total ocean volume, half of the global denitrification occurs in pelagic oxygen minimum zones with \(O_2\) levels less than 0.05 mL L\(^{-1}\) (Codispoti and Christensen 1985). The importance of pelagic denitrification in the ETP has been recognized for many years on the basis of N-O-P stoichiometric relationships, the existence of a nitrite maximum within the \(O_2\) minimum zone (e.g., Brandhorst 1959; Thomas 1966; Cline and Richards 1972), and observations of apparent \(N_2O\) consumption in the \(O_2\) minimum zone (Cohen and Gordon 1978). The ETP is the largest region of the world’s oceans with low oxygen and high rates of water column denitrification, which results in ~35-45% of global pelagic denitrification (e.g., Cline and Richards 1972; Codispoti and Richards 1976).

Denitrification preferentially consumes \(^{14}\)NO\(_3^-\), which leads to a marked increase in nitrate \(\delta^{15}N\) values in oceanic regions with strong oxygen minimum zones (Cline and Kaplan 1975; Liu and Kaplan 1989; Voss, Dippner and Montoya 2001). Nitrogen isotopic compositions of nitrate coupled with consideration of the nitrate deficit in the water column indicate that denitrification
in the ETP strongly affects the $\delta^{15}$N values of NO$_3^-$ (Sigman et al. 2005), as well that of suspended and sinking particulate matter (Voss, Dippner and Montoya 2001). Consequently, variations in the $\delta^{15}$N value of primary producers, and in turn, consumers in the ETP can be affected by the spatial and temporal intensity of algal production and denitrification.

3. Sample Collection and Analytical Methods

3.1 Samples – Yellowfin tuna were captured by purse-seine fishing vessels in the eastern Pacific Ocean 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). The fish were measured (fork length (FL), mm) and the sex determined if the fish were mature enough to do so. Samples of white muscle tissue (WMT) were removed from the dorsal region adjacent to the second dorsal fin and stored at -20°C until processed further. Stomachs and liver samples were also collected, but the data reported here are only for white muscle. Subsamples of WMT from up to 6 individuals per purse-seine set and size class (<900 and $\geq$900 mm FL) were combined into one sample for stable isotope analysis. Purse-seine set locations are shown in Figure 1.1.

Samples of zooplankton were collected by a 0.6 m diameter bongo net (Smith and Richardson 1977), on board the NOAA research ships *David Starr Jordan* and *McArthur II* in the ETP from 5 August to 5 December 2003 (Figure 1.1). The bongo net frame with two 333-μm mesh cylindrical-conical nets was towed obliquely from 200 m for 15 minutes and the material collected by the inboard net was stored at -20°C until processed further. A flowmeter was used on the outboard net, where an average of about 400 m$^3$ of water was filtered per tow. In the laboratory, the zooplankton samples were thawed slowly, sorted for copepods (to species level), amphipods (to order), euphausiids (to order), and chaetognaths (to phylum) using a stereoscopic microscope, and refrozen. Collectively, we define these taxa as components of the mesozooplankton guild, after Chai et al. (2002) and Olson and Watters (2003).
Samples of WMT for CSIA were chosen from similar-size yellowfin tuna along a broad latitudinal gradient in the $\delta^{15}$N values. The latitudinal gradient was defined based on nitrogen isotopic analysis of 95 composite samples of tuna bulk WMT. The samples selected for CSIA were from fish that ranged from about 600 to 800 mm (Table 1.1). Although our sample from the equatorial region was chosen from fish caught further to the west than the other four samples (Figure 1.1), the $\delta^{15}$N value of bulk WMT is representative of yellowfin tuna caught along the equator in the ETP (Figure 1.2).

3.2 Bulk Isotope Analyses – The tuna tissue samples were lyophilized or oven dried (60°C, ~24 h) and homogenized to a fine powder using a mortar and pestle. An average of about 70 individuals per species (for the copepods), per order (for the amphipods and euphausiids), or per phylum (for the chaetognaths) per sample was combined into a single sample for stable isotope analysis. One-hundred forty-nine mesozooplankton samples were analyzed. Bulk carbon and nitrogen isotopic compositions of tuna and mesozooplankton 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 $\delta$-notation relative to the international V-PDB and atmospheric N$_2$ for carbon and nitrogen, respectively. A glycine standard was analyzed approximately every 10 samples 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 $\leq 0.2\%$.

3.3 Acid Hydrolysis – The samples were prepared for compound specific nitrogen isotope analysis of amino acids by acid hydrolysis followed by derivatization to produce trifluoroacetic amino acid esters (Macko et al. 1997). Approximately 10 mg homogenized sample was hydrolyzed following procedures modified from Cowie and Hedges (1992). The homogenized sample and norleucine (0.43 nmol mg$^{-1}$ sample) were transferred to 5 mL reaction vials (Reacti-
Vial, Pierce Scientific), 1 mL 6N HCl added (Sequanal Grade, Pierce Scientific), the vial flushed with N₂ and capped using a Teflon/silicone liner (18 mm Tuf-Bond, Pierce Scientific) before heating at 150°C for 70 minutes. Norleucine was used as an internal recovery standard. The hydrolysate was evaporated to dryness at 55°C under a gentle stream of N₂ and the residue redissolved in 1 mL 0.01N HCl. This solution was purified by filtration (0.22 µm Millex-GP, Millipore Corporation) followed by a rinse with 1 mL 0.01N HCl. The solution was further purified by cation-exchange chromatography (Dowex 50W8-400, Sigma-Aldrich) following the method of Metges, Petzke and Hennig (1996). Briefly, a 5-cm column of cation-exchange resin was prepared in a Pasteur pipette and the amino acids eluted with 4 mL 2N ammonium hydroxide. The eluant was evaporated to dryness under a stream of N₂ at 80°C. Prior to derivatization, samples were re-acidified with 1 mL 0.01N HCl and then evaporated to dryness under a stream of N₂ at 55°C. The samples were split and one-half was archived.

3.4 Derivatization – The samples were first reacted with acidified isopropanol to esterify the carboxyl terminus of the amino acids. Approximately 2 mL of 4:1 isopropanol:acetyl chloride were added to each sample, the vial was flushed with N₂ and sealed with a Teflon-lined cap, and then the sample was heated (110°C) for 60 min. The samples were then dried under a stream of N₂ at 60°C. The resultant amino acid esters were acylated by the addition of 1 mL 3:1 methylene chloride: trifluoroacetic anhydride (TFAA, 99+%, Pierce Scientific). The vials were flushed with N₂, sealed with Teflon-lined caps and heated (100°C) for 15 minutes. Samples were then further purified using solvent extraction (Ueda et al. 1989). The acylated amino acid esters were evaporated at room temperature under a stream of N₂ and then re-dissolved in 3 mL 1:2 chloroform:P-buffer (KH₂PO₄ + Na₂HPO₄ in Milli-Q water, pH 7). Vigorous shaking caused the acylated amino acid esters to partition into the chloroform and high boiling point contamination ended up in the buffer. The solvents were separated by centrifugation (10 min at 600g) the chloroform was transferred to a clean vial, and the solvent extraction process repeated. Veugter et al. (2005) showed full recovery of the acylated amino acid esters using this technique. Finally, to
insure complete derivatization, the acylation step was repeated. The samples were stored in 3:1 methylene chloride:trifluoroacetic anhydride at 4°C and analyzed within 1 month.

3.5 CSIA – The nitrogen isotopic composition of TFA derivatives of amino acids were analyzed by isotope ratio monitoring gas chromatography-mass spectrometry using a Finnigan MAT 252 mass spectrometer interfaced to a Trace GC gas chromatograph through a GC-C III combustion furnace (980°C), reduction furnace (650°C), and liquid nitrogen cold trap. L-2-amino adipic acid for which the $\delta^{15}$N value had been previously independently determined was co-injected as an internal standard. Prior to analysis, the TFA derivatives were dried at room temperature under a stream of N$_2$ and re-dissolved in 100 μL of ethyl acetate. The samples (1 μL) and L-2-amino adipic acid (1 μL, ~20 nmole) 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 for 2 min at 52°C and increased, in stages, to temperatures of 190°C at a rate of 8.0°C min$^{-1}$, and 300°C at 10.0°C min$^{-1}$, then finally held at 300°C for 8 min. The samples were analyzed in triplicate, and the measured nitrogen isotope compositions were corrected relative to the $\delta^{15}$N value of the amino acid internal standard. Reproducibility associated with these isotopic measurements averaged 1.4‰ and ranged from 0.1 to 4.4‰.

3.6 Tuna-Mesozooplankon Comparisons – Bulk $\delta^{15}$N values of yellowfin tuna and mesozooplankton were compared to derive independent estimates of trophic level over a range of latitudes in the ETP. The data were compared in fifteen 5x5-degree areas (Figure 1.1) where samples of both taxa were collected. The trophic level of the yellowfin tuna (TLYFT) sampled from each purse-seine set in the 5x5-degree areas was calculated as:

$$TL_{YFT} = \frac{\delta^{15}N_{YFT} - \delta^{15}N_{Mesozoo} + TL_{Mesozoo}}{TEF}$$  (1)
Tuna Trophic Dynamics

where \( TL_{Mesozoo} \) is the estimated trophic level of mesozooplankton in the ETP, \( \delta^{15}N_{Mesozoo} \) is the mean \( \delta^{15}N \) value of mesozooplankton in each 5-degree area, and \( TEF \) represents the trophic enrichment factor between a consumer and its diet. \( TL_{Mesozoo} \) was estimated as 2.7 by Olson and Watters (2003), based on the nutrient-phytoplankton-zooplankton-detritus model of Chai et al. (2002) for the eastern equatorial Pacific. The \( TL_{Mesozoo} \) depends on the relative proportions of mesozooplankton predation on microzooplankton and grazing on diatoms, derived from the nitrogen balance of Chai et al. (2002). The trophic level of a food web component is 1.0 plus the weighted average of the trophic levels of its prey. That is,

\[
TL = 1.0 + \sum_i (P_i \times TL_i)
\]

where \( P_i \) is the diet proportion of the \( i \)th prey group and \( TL_i \) is the trophic level of the \( i \)th prey group. Several workers have adopted an average \( TEF \) value of 3.4‰ for many taxa (Minawaga and Wada 1984; Vander Zanden and Rasmussen 2001; Post 2002), however there is acknowledged variance in \( TEF \) values (e.g., Gannes, O’Brian and Martinez del Rio 1997). For example, compilations of data for laboratory-grown ammonotelic fish show lower \( TEF \) values (e.g. 2.0‰ Vanderklift and Ponsard 2003; 2.3‰ McCutchan et al. 2003). Recent field studies of the nitrogen isotopic compositions of juvenile yellowfin tuna and their prey from Hawaii yielded an average \( TEF \) of 2.1‰ based on ~85% characterization of the tuna diet (Graham et al. 2007).

4. Results and Discussion

4.1 Variation in \( \delta^{15}N \) Values of White Muscle Tissue – The bulk WMT nitrogen isotopic composition of the five yellowfin tuna samples chosen for CSIA varied from 10.4‰ at ~10°S to 15.6‰ at ~25°N near the tip of Baja California (Table 1.1). Variation in \( \delta^{15}N \) values with latitude in this small number of samples is representative of the variation in the \( \delta^{15}N \) values of WMT in our much larger dataset for the ETP (Figure 1.2). This consistent spatial variation in the \( \delta^{15}N \) values of an upper-level predator could be a result of several factors, including variation in the organism’s trophic level due to dietary differences, in the organism’s physiology, or in the
nutrient dynamics at the base of the food web. Assuming a TEF of 3‰ for each trophic level in this ecosystem, if the observed gradient in δ\textsuperscript{15}N values with latitude were due to dietary differences alone, then it would represent a gradient of ~1.7 trophic levels occupied by yellowfin tuna. This observation is not explained by an ontogenetic gradient in foraging behavior or food habits (M. Bocanegra-Castillo and F. Galván-Magaña, unpublished data) or by correlation between tuna size and δ\textsuperscript{15}N values of bulk WMT (Table 1.1, and unpublished data for the ETP). Additional factors can affect the δ\textsuperscript{15}N values of organisms such as diet quality and quantity and even protein catabolism can affect a consumer’s δ\textsuperscript{15}N value (see review by Gannes, O’Brian and Martinez del Rio 1997). However, the diets of yellowfin tuna are protein-rich and we have observed no obvious relationship between δ\textsuperscript{15}N values and stomach fullness.

As described above, changes in the intensity of denitrification can affect the nitrogen isotopic composition of the nitrate pool in the ETP. For example, Voss, Dippner and Montoya (2001) showed that the δ\textsuperscript{15}N values of sinking particles in the ETP, which most likely includes organic matter derived from algae, bacteria and zooplankton, change from ~9‰ at 14°N to 11.2‰ at 24°N. If the gradient in δ\textsuperscript{15}N values with latitude originates at the base of the food web in the ETP, and that signal is propagated up the food web to the upper-level pelagic predators, it provides another viable explanation for the observed latitudinal shift in the δ\textsuperscript{15}N values of bulk WMT. However, the degree to which isotopic variability at the base of the food web affect a consumer’s δ\textsuperscript{15}N value depends upon the animal’s movement patterns, life span, tissue turnover rates and foraging behavior. Highly mobile organisms will integrate the δ\textsuperscript{15}N values of the prey consumed over large spatial scales, whereas less mobile consumers will reflect local spatial and shorter temporal trends in nutrient dynamics at the base of the food web.

4.2. Variation in the δ\textsuperscript{15}N Values of Amino Acids – To help interpret the coherent spatial variation in the δ\textsuperscript{15}N values of bulk WMT, we analyzed the isotopic composition of individual amino acids in ETP yellowfin tuna. The nitrogen isotopic composition of eight individual amino
acids and two combinations of chemically-related amino acids were determined (Table 1.2). However, before considering the results of the CSIA, it is informative to review sources of amino nitrogen for metabolism. Nitrogen in the body and the diet of tunas is predominantly protein and the amino acids from which protein is synthesized. Proteins are synthesized from ~20 common amino acids, which are divided into two classes, essential and nonessential amino acids (e.g., Young and El-Khoury 1995). Essentiality of amino acids is not the same in all organisms, but is often species-specific (e.g., NRC 1994; NRC 1995) and as discussed above does not provide an accurate picture of the origins of amino nitrogen. Protein ingested by higher organisms is denatured in the stomach and hydrolyzed to amino acids and short polypeptides. Most of these compounds are absorbed and transported to the liver, where about 75% of the amino acids are incorporated into the organism (NRC 1994; NRC 1995). The excess amino acids are catabolized producing ammonia, which is eliminated mainly through the gills as ammonium. The amino acids incorporated via the liver may undergo deamination and transamination to provide the precursors for gluconeogenesis, lipogenesis and protein synthesis (Smutna, Vorlova and Srovobodova 2002). Transamination reactions do not occur in some amino acids, whereas in others there is complete equilibration and these reactions can change the $^{15}$N content of the amino acids (e.g., Hare and Estep 1983; Macko et al. 1987; Hare et al. 1991). For example, Hare and Estep (1983) found a 19‰ range in $\delta^{15}$N values in amino acids from bovine tendon collagen. Tissues can vary in their protein composition and therefore in their amino acid distribution (Wilson and Poe 1985; Gunasekera, Shim and Lam 1997), which in turn, can affect the bulk tissue $\delta^{15}$N values.

The distribution of $\delta^{15}$N values of individual amino acids in yellowfin tuna caught in the ETP are strongly bimodal, comprising a group of “high” $\delta^{15}$N amino acids and a group of “low” $\delta^{15}$N amino acids (Table 1.2). Enrichment in $^{15}$N is not related to the class of amino acid essentiality but rather follows the patterns originally observed by McClelland and Montoya (2002). For example, within the high $\delta^{15}$N group (Table 1.2), alanine, aspartic and glutamic acid are NAA...
whereas leucine and isoleucine are considered EAA (e.g., Schepartz 1973). It should be noted that leucine and isoleucine have similar metabolic origins and belong to the pyruvate family of amino acids (e.g., Stryer 1988). In the low δ\textsuperscript{15}N group (Table 1.2), phenylalanine is an EAA, whereas glycine is usually considered to be a NAA (e.g., Schepartz 1973).

The δ\textsuperscript{15}N values of individual amino acids have not commonly been measured in marine organisms (e.g., Schmidt et al. 2004). However, nitrogen isotopic compositions of individual amino acids have been determined for laboratory cultures of rotifers (McClelland and Montoya 2002), size-fractionated zooplankton from the tropical Atlantic (McClelland, Holl and Montoya 2003) and postlarval euphausiids from the Southern Ocean (Schmidt et al. 2004). These authors found that alanine, leucine, isoleucine, aspartic acid and glutamic acid were strongly fractionated in food-web relationships, whereas the \textsuperscript{15}N content of glycine, lysine, phenylalanine, serine and tyrosine did not appear to change within the food web. These results imply that the δ\textsuperscript{15}N values of some amino acids such as alanine, aspartic acid and glutamic acid should reflect the relative trophic position of yellowfin tuna in the food web and that the δ\textsuperscript{15}N values of glycine and phenylalanine should record the δ\textsuperscript{15}N value of the source of nitrogen-supporting production. Although glycine is not typically considered an EAA, results suggest that its carbon skeleton is derived from an EAA without alternation of the \textsuperscript{15}N content of the amino nitrogen in the molecule, or that in the marine organisms studied, glycine is derived from dietary sources (McClelland and Montoya 2002; McClelland, Holl and Montoya 2003; Schmidt et al. 2004). Glycine is thought to derive mainly from serine, which is produced from 3-phosphoglycerate, an intermediate in glycolysis (Stryer 1988). It is possible that glycine is a conditionally essential amino acid (e.g., see Reeds 2000) in yellowfin tuna. The degree to which glycine may be regarded as essential or indispensable could be a function of the quantity of serine in the diet of pelagic marine organisms. McClelland and Montoya (2002) also noted little \textsuperscript{15}N enrichment of these amino acids in a consumer relative to its controlled diet, suggesting that the δ\textsuperscript{15}N value of glycine and serine appear to record the δ\textsuperscript{15}N value of the source of nitrogen-supporting
production. One scenario that would give a conserved $\delta^{15}N$ value of glycine would be that glycine travels through the food web primarily as part of an amino acid dimer, with the other part of the dimer being a rare amino acid. In this scenario, the glycine-containing dimers would be conserved and shunted from prey protein into predator protein and not undergo expected metabolic fractionations associated with deaminations and transaminations. Of course, this is speculation at this time. In reality, we do not know why the nitrogen isotopic composition of glycine reflects the $\delta^{15}N$ value at the base of the food web, but existing laboratory (McClelland and Montoya 2002) and field research (McClelland, Holl and Montoya 2003; Schmidt et al. 2004) suggests that glycine is conservative and records the $\delta^{15}N$ values at the base of the food web in large and small marine organism. On the other hand, the amino acids alanine, aspartic acid and glutamic acid are mainly derived from intermediates in the citric acid cycle (Stryer 1988) and all show $^{15}N$ enrichment in consumer organisms relative to glycine, lysine, phenylalanine, serine and tyrosine in the same sample (McClelland and Montoya 2002; McClelland, Holl and Montoya 2003; Schmidt et al. 2004).

Given the observed isotopic behavior of source and trophic amino acids, two general scenarios can explain an increase in the $\delta^{15}N$ values of yellowfin tuna with latitude in the ETP. If the trophic level of yellowfin tuna increased with latitude, then one would expect the $\delta^{15}N$ values of source amino acids (glycine and phenylalanine) to remain constant at all latitudes, and differences between the $\delta^{15}N$ values of source and trophic (alanine, aspartic acid or glutamic acid) amino acids to increase as the trophic level increases to the north (Figure 1.3a). If the trophic level of the yellowfin tuna remained constant over the region, then the $\delta^{15}N$ values the source amino acids should parallel those of bulk WMT and the $\delta^{15}N$ values of the trophic amino acids (Figure 1.3b). Our results reveal that the $\delta^{15}N$ values of glycine, phenylalanine, alanine, aspartic acid, and glutamic acid in tuna show latitudinal trends similar to the $\delta^{15}N$ values of bulk WMT (Figure 1.4), indicating that the $^{15}N$ enrichment in the north is due to changes in $\delta^{15}N$ values at the base of the food web. Increasing $\delta^{15}N$ values to the north is consistent with the
effects of denitrification on the $\delta^{15}N$ values of nitrate and the transfer of this isotopic composition to phytoplankton (see also Fig. 7A in Voss, Dippner and Montoya 2001). It is remarkable that the $\delta^{15}N$ values of a highly-mobile and metabolically-active predator would track processes at the base of the food web and our results suggest that even though yellowfin tuna are capable of basin-wide migrations, in the ETP they may have a relatively high level of regional residency.

4.3. Trophic Level of ETP Yellowfin Tuna – We used the difference between the $\delta^{15}N$ values of glutamic acid and glycine to estimate the trophic level of yellowfin tuna in the ETP, assuming the difference between the $\delta^{15}N$ values of glutamic acid and glycine is 7‰ per trophic level. We chose 7‰ based on the work of McClelland and Montoya (2002). These authors cultured the marine rotifer Brachionus plicatilis on a diet of the alga Tetraselmis suecica to examine changes in the nitrogen isotopic composition of individual amino acids between a plankton consumer and their food source. They found a ~2‰ increase in bulk $\delta^{15}N$ value with trophic position, which resulted from averaging large increases in the $\delta^{15}N$ values of some amino acids and little or no change in the $\delta^{15}N$ values of others. McClelland and Montoya (2002) proposed that the amino acids showing consistently large increases in $\delta^{15}N$ values provided a more robust estimate of the trophic level of a consumer than bulk tissue. In particular, glutamic acid was enriched in $^{15}N$ by ~7‰ in the consumer relative to the food (McClelland and Montoya 2002). The trophic level estimated from the weighted mean difference between the $\delta^{15}N$ values of glutamic acid and glycine assuming an amino acid TEF of 7‰ is 4.5±0.1 (1 SD). Weighting was based on the analytical uncertainty in the $\delta^{15}N$ values of the amino acids (Table 1.2). In contrast, the trophic level calculated from the weighted mean difference between the $\delta^{15}N$ values of glutamic acid and phenylalanine is 4.2±0.1 (1 SD). We prefer to use the $\delta^{15}N$ value of glycine, rather than phenylalanine for trophic level estimates because phenylalanine can be used in large amounts to form tyrosine if the latter is not adequately supplied in the diet (Schepartz 1973) and using our CSIA methods, the $\delta^{15}N$ value of glycine was easier to determine relative to phenylalanine.
because close elution between phenylalanine and glutamic acid made quantification of the δ^{15}N value of phenylalanine more difficult. For comparison, we also calculated trophic level based on the average difference between trophic (alanine + aspartic acid + glutamic acid) and source (glycine + phenylalanine) amino acids. The trophic level calculated from the weighted mean difference between the δ^{15}N values of the trophic and source amino acids assuming a TEF of 7‰ is 4.6±0.1 (1 SD) (Table 1.2).

Bulk δ^{15}N values of mesozooplankton exhibited the same geographical trend as the bulk WMT and amino acid δ^{15}N values of yellowfin tuna in the ETP, increasing from ~10°S to ~25°N (Figure 1.2). These short-lived organisms at low trophic levels are more likely to track spatial and temporal changes in nutrient dynamics than long-lived, highly-active predators. The similar spatial pattern in tuna and mesozooplankton bulk δ^{15}N values provides further evidence that the ^{15}N enrichment in the north is due to changes in the δ^{15}N values at the base of the food web.

Applying equation 1 to the δ^{15}N values of both taxa (Figure 1.2, filled symbols) in the fifteen 5x5 degree areas where both taxa were collected (Figure 1.1) yielded a yellowfin tuna trophic level estimate ranging from 4.1 (±0.3 SD) assuming a TEF value of 3.4‰ to 4.9 (±0.5 SD) assuming the field-based TEF value of 2.1‰. These estimates are in close agreement with the trophic level estimates derived from the compound specific stable isotope data. In addition, trophic level estimates for yellowfin tuna using diet data in a mass balance ecosystem model for the ETP were 4.6-4.7 (Olson and Watters 2003). Thus, results of CSIA of amino acids in yellowfin tuna appear to be a sensitive indicator of the trophic level of this upper-level pelagic predator, a conclusion in broad agreement with that of McClelland and Montoya (2002).

5. Implications

Our results set the stage for the application of compound-specific stable isotope techniques to support ecosystem-based approaches for the management of pelagic tuna fisheries. Fisheries that
target specific components of the food web act as potential agents of ecological change, in some cases profoundly restructuring marine food webs (Estes et al. 1998; Jackson et al. 2001; Worm and Myers 2003). One way that fishing may alter exploited ecosystems is termed “fishing down the food web” (Pauly et al. 1998). The commonly-held interpretation of fishing down the food web is a gradual reduction in the mean trophic level of fisheries landings caused by serial depletion of high-trophic-level species and replacement by lower-trophic-level species, although there is another more-tenable interpretation (Essington, Beaudreau and Wiedenmann 2006).

High-seas purse-seine and longline fisheries target tuna and billfish species that are dominant, high-level predators in pelagic ecosystems. A decrease in the biomass of top predators could impart a top-down trophic cascade, which could affect the overall structure and function of the ecosystem (Carpenter, Kitchell and Hodgson 1985; Pace et al. 1999). Fisheries-induced restructuring of food webs has not been demonstrated empirically for high-seas pelagic ecosystems of the Pacific Ocean, although modeling studies of the central north Pacific (Cox et al. 2002) and the ETP (Olson and Watters 2003) have shown strong evidence for top-down effects of harvesting predators on the productivity of their prey (Walters et al. 2005). A recent analysis of all available data from Pacific tuna fisheries for 1950-2004 indicated substantial, though not catastrophic, impacts of fisheries on top-level predators and minor impacts on the ecosystem in the Pacific Ocean (Sibert et al. 2006). We propose that trophic level estimates derived from amino acid nitrogen isotopic analysis of archived samples of pelagic fishes will provide valuable insight into the historic effects of fishing on pelagic marine ecosystems.

To examine the potential historical effects of commercial fishing on pelagic fisheries, using stable isotope data, both the trophic level and isotope baseline of a single archived sample must be estimated. Previous workers (Thompson, Furness and Lewis 1995; Bearhop et al. 2001; Jennings et al. 2002; Becker and Beissinger 2006) have used bulk isotopic compositions to examine short- and long-term effects of commercial fishing on the trophic level of marine predators. These studies required extensive characterization of isotopic variability at the base of
Tuna Trophic Dynamics

marine food webs, which as we noted above can be notoriously difficult. The principle advantage of δ¹⁵N analyses of individual amino acids is that using only a sample of the consumer, the trophic level of the consumer and the δ¹⁵N value at the base of the food web can be estimated. That is, additional sampling of basal food resources and prey is not required, since predator trophic levels and the basal isotope values can be obtained simply by comparing of δ¹⁵N values of trophic and source amino acids extracted from the tissues of the predator. Time series of trophic-level and baseline isotope estimates for a given predator, using CSIA studies, could provide insight into the relative importance of fisheries and physical forcing in structuring marine ecosystems.

6. Summary and Future Research

Bulk δ¹⁵N values of yellowfin tuna white muscle tissue increased by ~5‰ from 10°S to 25°N, and followed spatial trends documented for hundreds of other δ¹⁵N analyses of tuna and mesozooplankton. We observed parallel latitudinal trends in the δ¹⁵N values of bulk WMT, source and trophic amino acids indicating that the ¹⁵N enrichment in the north was due to changes in the δ¹⁵N values at the base of the food web. The increase in δ¹⁵N values to the north was consistent with the effects of denitrification on the δ¹⁵N value of nitrate and the transfer of this isotopic composition to phytoplankton.

We used the difference between the δ¹⁵N values of trophic (alanine, aspartic acid and glutamic acid) and source (glycine and phenylalanine) amino acids to estimate the trophic level of ETP yellowfin tuna. Assuming the difference between the δ¹⁵N values of source and trophic amino acids changed by 7‰ per trophic level (McClelland and Montoya 2002), we estimated that the trophic level of ETP tuna ranges from 4.2 to 4.6. This amino acid-derived estimate matched well the estimate of 4.6 to 4.7 derived from diet analysis (Olson and Watters 2003) and an estimate of 4.1-4.9 calculated from a model based on the difference between the δ¹⁵N values of bulk
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mesozooplankton and yellowfin tuna in the ETP. The implication of our results is that \( \delta^{15}N \) analyses of individual amino acids in tuna can be used to estimate the \( \delta^{15}N \) values at the base of the food web and their trophic level in a single sample. Other results of compound-specific nitrogen isotopic analysis of amino acids in marine food webs suggest that this generalization holds for marine organisms at a variety of trophic levels (McClelland and Montoya 2002; McClelland, Holl and Montoya 2003; Schmidt et al. 2004). Compound-specific nitrogen isotopic analysis of amino acids can offer a unique opportunity to elucidate the nitrogen dynamics in a variety of food webs if these generalizations apply to other ecosystems.

We propose that differences between the \( \delta^{15}N \) values of source and trophic amino acids can be used to examine possible historical changes in the trophic level of archived samples of fishes to investigate potential effects of fisheries removal on the trophic dynamics of pelagic ecosystems. However, before CSIA of amino acids is broadly applied to ecological studies, we must first determine tissue-specific turnover rates of amino acids and test several critical assumptions. The primary assumption that must be evaluated is the constancy and the mechanisms underlying the 7‰ per trophic level difference between the \( \delta^{15}N \) values of the source and trophic amino acids. The agreement between trophic level estimates based on diet analysis (4.6-4.7) and those from differences between the \( \delta^{15}N \) values of glutamic acid -glycine (4.5±0.1) and \( \Sigma \)trophic-\( \Sigma \)source (4.6±0.1) amino acids provides some level of confidence in the TEF of 7‰, however this TEF value must be further evaluated using laboratory and additional field studies. Second, we need to better investigate the origins and metabolic cycling of amino nitrogen in the amino acids in organisms and if these origins and metabolic cycling change at the ecosystem level, for example, as a response to nitrogen availability. Third, it is known that EAA are produced by plants and bacteria. However, currently little is known about controls on the \( \delta^{15}N \) values of bacterially-produced amino acids (e.g., Veuger et al. 2005). We must evaluate amino acid production and cycling in the microbial-loop (sensu Azam et al. 1983) on the \( \delta^{15}N \) values at the base of the food web and of marine organisms that feed especially in mesopelagic environments.
To examine historical changes in trophic level, we must also consider the effects of preservation on archived specimens. Archived samples of fish are typically preserved with alcohol or formalin. Preservation of fish tissues with alcohol and formalin can have a small, uniform affect on bulk nitrogen stable isotope values (Arrington and Winemiller 2002). Bulk nitrogen isotope values generally increase by less than 1‰ in animal tissues treated with preservatives, which is small relative to the observed shifts in δ¹⁵N values associated with trophic dynamics (Hobson, Gibbs and Gloutney 1997; Bosley and Wainright 1999). Preliminary results on CSIA of amino acids in subtropical Pacific zooplankton (Hannides and Popp, unpublished data) indicated little change in the δ¹⁵N values of amino acids in samples preserved in formalin as compared with similar frozen samples. We therefore suspect that affects of preservation of animal tissues in alcohol or formalin to be small for individual amino acids, but this supposition must be thoroughly tested. Consequently, several assumptions should be critically evaluated before the δ¹⁵N values of individual amino acids in organisms can be used to examine animal physiology, foraging behavior, movement patterns, and trophic level estimates in archived specimens to examine natural and anthropogenic changes to ecosystem structure and function. If these assumptions can be evaluated the future application of CSIA of amino acids may have great potential for many biological fields, from animal physiology to conservation biology.

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8. References


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Table 1.1. Sample location in the eastern tropical Pacific, number of individuals included in the composite sample, mean fork length (± standard deviation), C/N ratio and stable isotopic data for bulk white muscle tissue from the yellowfin tuna used for amino acid compound specific isotope analysis.

<table>
<thead>
<tr>
<th>Sample Location</th>
<th>Number of Individuals</th>
<th>Mean Fork Length, mm</th>
<th>C/N</th>
<th>δ(^{13})C, ‰</th>
<th>δ(^{15})N, ‰</th>
</tr>
</thead>
<tbody>
<tr>
<td>8° 13.8’S</td>
<td>6</td>
<td>630 (±86)</td>
<td>3.6</td>
<td>-15.9</td>
<td>10.4</td>
</tr>
<tr>
<td>1° 10.8’S</td>
<td>6</td>
<td>595 (±13)</td>
<td>3.6</td>
<td>-16.0</td>
<td>10.9</td>
</tr>
<tr>
<td>10° 31.8’N</td>
<td>6</td>
<td>682 (±80)</td>
<td>3.7</td>
<td>-16.0</td>
<td>13.5</td>
</tr>
<tr>
<td>13° 45.0’N</td>
<td>6</td>
<td>801 (±148)</td>
<td>3.8</td>
<td>-16.0</td>
<td>14.7</td>
</tr>
<tr>
<td>24° 15.0’N</td>
<td>2</td>
<td>643 (±5)</td>
<td>3.8</td>
<td>-16.7</td>
<td>15.6</td>
</tr>
</tbody>
</table>
Table 1.2. Bulk and amino acid $\delta^{15}$N values from the white muscle tissue (WMT) and estimated trophic level of yellowfin tuna as a function of latitude in the eastern tropical Pacific.

<table>
<thead>
<tr>
<th></th>
<th>8° 13.8' S</th>
<th>1° 10.8' S</th>
<th>10° 31.8' N</th>
<th>13° 45.0' N</th>
<th>24° 15.0' N</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bulk WMT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.4</td>
<td>10.9</td>
<td>13.5</td>
<td>14.7</td>
<td>15.6</td>
</tr>
<tr>
<td><strong>Amino Acids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>25.4 $\pm$0.2</td>
<td>18.0 $\pm$1.7</td>
<td>24.6 $\pm$2.6</td>
<td>28.5 $\pm$2.0</td>
<td>29.3 $\pm$1.9</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.7 $\pm$0.7</td>
<td>-2.3 $\pm$1.5</td>
<td>2.8 $\pm$1.5</td>
<td>7.2 $\pm$1.8</td>
<td>7.5 $\pm$0.3</td>
</tr>
<tr>
<td>Leucine + isoleucine</td>
<td>29.5 $\pm$1.3</td>
<td>25.3 $\pm$0.7</td>
<td>29.0 $\pm$2.0</td>
<td>29.7 $\pm$1.8</td>
<td>33.5 $\pm$2.9</td>
</tr>
<tr>
<td>Proline</td>
<td>19.8 $\pm$0.3</td>
<td>17.8 $\pm$0.6</td>
<td>22.3 $\pm$4.4</td>
<td>22.0 $\pm$3.8</td>
<td>26.5 $\pm$0.7</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>29.7 $\pm$0.1</td>
<td>27.7 $\pm$0.5</td>
<td>29.2 $\pm$1.3</td>
<td>31.1 $\pm$2.1</td>
<td>32.5 $\pm$2.8</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>27.3 $\pm$0.7</td>
<td>23.4 $\pm$0.3</td>
<td>24.7 $\pm$1.1</td>
<td>29.2 $\pm$1.2</td>
<td>30.6 $\pm$2.2</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2.7 $\pm$1.6</td>
<td>2.7 $\pm$1.0</td>
<td>3.2 $\pm$3.1</td>
<td>5.3 $\pm$0.9</td>
<td>7.5 $\pm$1.4</td>
</tr>
<tr>
<td>Tyrosine + lysine</td>
<td>8.3 $\pm$0.4</td>
<td>0.3 $\pm$0.4</td>
<td>5.0 $\pm$1.1</td>
<td>8.8 $\pm$1.5</td>
<td>11.9 $\pm$0.6</td>
</tr>
<tr>
<td>Argine</td>
<td>2.2 $\pm$1.5</td>
<td>-1.1 $\pm$0.3</td>
<td>2.1 $\pm$1.4</td>
<td></td>
<td>6.6 $\pm$1.0</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.9 $\pm$0.9</td>
<td>-2.4 $\pm$1.4</td>
<td>2.1 $\pm$2.3</td>
<td>5.7 $\pm$0.5</td>
<td>6.8 $\pm$1.1</td>
</tr>
<tr>
<td><strong>Trophic Level</strong></td>
<td>4.7 $\pm$0.2</td>
<td>4.7 $\pm$0.3</td>
<td>4.1 $\pm$0.4</td>
<td>4.2 $\pm$0.4</td>
<td>4.3 $\pm$0.4</td>
</tr>
<tr>
<td><strong>Trophic Level</strong></td>
<td>4.8 $\pm$0.1</td>
<td>4.3 $\pm$0.2</td>
<td>4.4 $\pm$0.3</td>
<td>4.4 $\pm$0.3</td>
<td>4.3 $\pm$0.2</td>
</tr>
</tbody>
</table>

*Values in parentheses are 1 SD.
†Trophic level = 1 + ($\delta^{15}$N$_{Glutamic acid}$ – $\delta^{15}$N$_{Glycine}$)/7
*Trophic level = 1 + ($\delta^{15}$N$_{\Sigma trophic}$ – $\delta^{15}$N$_{\Sigma source}$)/7
**Figure 1.1.** Purse-seine set locations (squares) and zooplankton collection sites (triangles) for yellowfin tuna and mesozooplankton samples, respectively, used for bulk stable isotope analysis. Circles are locations of the yellowfin tuna samples used for compound specific stable isotope analysis. Fifteen 5x5 degree areas used for trophic level estimates based on comparisons of yellowfin and mesozooplankton $\delta^{15}$N are indicated by shading.
**Figure 1.2.** Bulk $\delta^{15}$N values for white muscle tissue of yellowfin tuna (squares) and for whole mesozooplankton (triangles) versus latitude in the eastern tropical Pacific. The samples used to estimate yellowfin trophic level by comparison with mesozooplankton bulk $\delta^{15}$N values are represented by filled squares and triangles. The five yellowfin tuna samples used for CSIA determinations are shown by circles. Equations describing these lines are: $\delta^{15}$N$_{bulk} = 0.17 (\pm 0.03)*$Latitude $+ 11.9 (\pm 0.3), \delta^{15}$N$_{mesozoo} = 0.20 (\pm 0.03)*$Latitude $+ 6.8 (\pm 0.5)$. The slopes of the regressions are not different at the 95% confidence interval.
Figure 1.3. Conceptual diagrams showing changes with latitude in the $\delta^{15}$N values of eastern tropical Pacific yellowfin tuna bulk white muscle tissue (●, solid line) and of source (dotted line) and trophic (dashed line) amino acids with (a) no change in the $\delta^{15}$N value at the base of the food web and a gradient with latitude in the trophic level of tuna and (b) change with latitude in the $\delta^{15}$N values of phytoplankton at the base of the food web and no gradient in tuna trophic level. The filled circles are the bulk $\delta^{15}$N values for white muscle tissue of the yellowfin tuna used for compound specific isotope analysis. $\text{TLYFT} = 1 + ((\delta^{15}\text{N}_{\text{trophic}} - \delta^{15}\text{N}_{\text{source}})/7)$
Figure 1.4. $\delta^{15}N$ values as a function of latitude for bulk white muscle tissue (●) and the source (glycine – ■, phenylalanine – ○) and trophic (alanine – ▼, aspartic acid – ▲ and glutamic acid – □, dashed line) amino acids from the white muscle tissue of eastern tropical Pacific yellowfin tuna. The parallel trends in the $\delta^{15}N$ values of source and trophic indicate that the $^{15}N$ enrichment in the north is due to a trend in $\delta^{15}N$ values at the base of the food web. Equations describing these lines are: $\delta^{15}N_{\text{bulk}} = 0.18 \pm 0.07 \times \text{Latitude} + 11.6 \pm 0.1$, $\delta^{15}N_{\text{glycine}} = 0.26 \pm 0.35 \times \text{Latitude} + 1.4 \pm 0.7$, $\delta^{15}N_{\text{phenylalanine}} = 0.15 \pm 0.14 \times \text{Latitude} + 3.1 \pm 0.3$, $\delta^{15}N_{\text{alanine}} = 0.23 \pm 0.49 \times \text{Latitude} + 23.4 \pm 0.9$, $\delta^{15}N_{\text{aspartic}} = 0.11 \pm 0.17 \times \text{Latitude} + 29.2 \pm 0.3$, $\delta^{15}N_{\text{glutamic}} = 0.14 \pm 0.34 \times \text{Latitude} + 25.9 \pm 0.6$. The slopes of all regressions are not different at the 95% confidence interval.