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Food-web inferences of stable isotope spatial patterns in copepods and yellowfin tuna in the pelagic eastern Pacific Ocean

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ABSTRACT

Evaluating the impacts of climate and fishing on oceanic ecosystems requires an improved understanding of the trophodynamics of pelagic food webs. Our approach was to examine broad-scale spatial relationships among the stable N isotope values of copepods and yellowfin tuna (*Thunnus albacares*), and to quantify yellowfin tuna trophic status in the food web based on stable-isotope and stomach-contents analyses. Using a generalized additive model fitted to abundance-weighted-average $\delta^{15}N$ values of several omnivorous copepod species, we examined isotopic spatial relationships among yellowfin tuna and copepods. We found a broad-scale, uniform gradient in $\delta^{15}N$ values of copepods increasing from south to north in a region encompassing the eastern Pacific warm pool and parts of several current systems. Over the same region, a similar trend was observed for the $\delta^{15}N$ values in the white muscle of yellowfin tuna caught by the purse-seine fishery, implying limited movement behavior. Assuming the omnivorous copepods represent a proxy for the $\delta^{15}N$ values at the base of the food web, the isotopic difference between these two taxa, "$\Delta_{YFT\text{-}COP}$," was interpreted as a trophic-position offset. Yellowfin tuna trophic-position estimates based on their bulk $\delta^{15}N$ values were not significantly different than independent estimates based on stomach contents, but are sensitive to errors in the trophic enrichment factor and the trophic position of copepods. An apparent inshore–offshore, east to west gradient in yellowfin tuna trophic position was corroborated using compound-specific isotope analysis of amino acids conducted on a subset of samples. The gradient was not explained by the distribution of yellowfin tuna of different sizes, by seasonal variability at the base of the food web, or by known ambit distances (i.e. movements). Yellowfin tuna stomach contents did not show a regular inshore–offshore gradient in trophic position during 2003–2005, but the trophic-position estimates based on both methods had similar scales of variability. We conclude that trophic status of yellowfin tuna increased significantly from east to west over the study area based on the spatial pattern of $\Delta_{YFT\text{-}COP}$ values and the difference between the $\delta^{15}N$ values of glutamic acid and glycine, "trophic" and "source" amino acids, respectively. These results provide improved depictions of trophic links and biomass flows for food-web models, effective tools to evaluate climate and fishing effects on exploited ecosystems.

1. Introduction

Marine ecologists are challenged by questions concerning the ecological implications expected from climate-induced environmental alterations (Stenseth et al., 2002; Edwards and Richardson, 2004). Concurrent with climate effects, selective removal of large predatory fishes from marine food webs can impart top-down changes in trophic structure and stability via trophic cascades (Carpenter et al., 1985; Pace et al., 1999; McClanahan and Arthur, 2001; Worm and Myers, 2003; Essington and Hansson, 2004; Frank et al., 2005). Lagging focus on open-ocean marine ecosystems and top predators has motivated an international effort by Global Ocean Ecosystem Dynamics (GLOBEC) via the Climate Impacts on Oceanic

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Top Predators (CLIOTOP) program to identify, characterize, and model the key processes involved in the dynamics of oceanic pelagic ecosystems in a context of climate variability and change, and intensive fishing of top predators (Maury and Lehodey, 2005).

The ecological effects of climate variability and fishing both transmit through the food web (Watters et al., 2003). The structure of the food web and the interactions among its components have a demonstrable role in determining the dynamics, productivity, and stability of ecosystems (Carpenter et al., 1985; Pace et al., 1999; Essington and Hansson, 2004; Bascompte et al., 2005; Frank et al., 2005). To anticipate future climate-induced changes in marine populations and the potential effects of fishing over the backdrop of variable physical processes requires a greater understanding of ecosystem processes and the extant variability in food webs. Furthermore, increasing worldwide interest in adopting an ecologically based approach to fisheries management (Pikitch et al., 2004; Marasco et al., 2007) has placed renewed emphasis on achieving accurate depictions of trophic links and biomass flows in exploited ecosystems.

The tropical and subtropical Pacific Ocean is the site of major fisheries for tunas and other pelagic fishes. Current understanding of trophodynamics in the eastern tropical Pacific (ETP) is limited mostly to commercially important fishes and sensitive species at upper-trophic levels (Alverson, 1963; Roger and Grandperrin, 1976; Olson and Boggs, 1986; Galván-Magaña et al., 1989; Abitia-Cardenas et al., 1997, 1999; Robertson and Chivers, 1997; Markaida and Sosa-Nishizaki, 1998, 2003; Cortés, 1999; Ballance et al., 2001; Spear et al., 2001, 2007; Olson and Galván-Magaña, 2002), and general patterns of phytoplankton and zooplankton trophic interactions (e.g. Chai et al., 2002; Fernández-Álamo and Färber-Lorda, 2006). Little is known about the biomass, productivity, and trophodynamics of animals responsible for transferring secondary production through the intermediate trophic levels in pelagic regions. Uncertain food-web complexity diminishes the utility of ecosystem models (e.g. Cox et al., 2002; Olson and Watters, 2003) for evaluating the top-down effects of fishing and the bottom-up effects of the physical environment.

Stable-isotope analysis is a useful tool for delineating the complex structure of marine food webs. Nitrogen isotope compositions, in particular, are well-suited for examining trophic dynamics (Peterson and Fry, 1987; Lajtha and Michener, 1994) and the effects of climate and fishing pressure (Wainright et al., 1993; Becker and Beissinger, 2006; Christensen and Richardson, 2008). At each discrete 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). The $\delta^{15}N$ value of any consumer, however, is a function of both the consumer's trophic position and the $\delta^{15}N$ value of the primary producers at the base of the food web. The $\delta^{15}N$ values of marine primary producers can vary spatially and seasonally owing to a variety of reasons (Dugdale and Goering, 1967; Cline and Kaplan, 1975; Altabet, 2001; reviewed by Popp et al. (2007)). Characterizing the $\delta^{15}N$ values at the base of marine food webs can be challenging because primary producers have short life spans, they respond quickly to fluctuations in biogeochemical and physical forces, and they can be difficult to isolate from other organic suspended particulate material. An alternative approach is to use a primary consumer (e.g. zooplankton) as the isotopic reference, i.e. a proxy for the base of the food web, representing trophic position 2 or slightly higher (e.g. Post, 2002). Primary consumers integrate the isotopic signal from the phytoplankton over a longer term, reducing the uncertainty in trophic position estimation of consumers higher in the food web (Vander Zanden and Rasmussen, 2001; O'Reilly et al., 2002; Post, 2002).

Mesozooplankton represent the dominant component of secondary production in marine ecosystems, and copepods often comprise the principal biomass of mesozooplankton assemblages (e.g. Escribano et al., 2007). In upwelling areas, animals that feed at upper-trophic levels depend on food webs that pass energy primarily from small diatoms to higher consumers via copepods (Cushing, 1989). The marine environment and plankton dynamics are tightly coupled, and zooplankton's response to climate-induced variability propagates upward to exploited fish stocks (Hays et al., 2005).

Our approach was to examine broad-scale spatial relationships among the $\delta^{15}N$ values of copepods and yellowfin tuna (*Thunnus albacares*), both dominant components of the ecosystem, and to quantify the tuna's trophic status in the food web using stable-isotope and stomach-contents analyses. Complimentary to whole-tissue or whole-animal ("bulk") isotope analysis, we used compound-specific isotope analysis (CSIA) of amino acids on a focused subset of samples to interpret bulk isotopic trends. In samples of consumer tissues, "source" amino acids (e.g. phenylalanine, glycine) retained the isotopic values at the base of the food web, and "trophic" amino acids (e.g. glutamic acid) became enriched in ^{15}N by about 7‰ relative to the baseline (McClelland and Montoya, 2002; McCarthy et al., 2007; Popp et al., 2007; Hannides et al., 2009). In CSIA, predator tissues alone are adequate for trophic-position estimates, and separate analysis of the isotopic composition of the base of the food web is not necessary.

Stomach-contents analysis has for decades been the conventional method to study food webs. The stomach contents of a consumer, however, represent only the most recent prey ingested, and for opportunistic predators with a broad forage base, defining the principal trophic links and correlates with environmental variation requires large numbers of stomach samples and a comprehensive sampling design. A balanced sampling design is not possible for tunas because commercial fisheries are the only feasible means of comprehensive sampling. The stable isotopes of a consumer's tissues, on the other hand, integrate information about the entire assimilated diet over time. Simultaneous stable-isotope and stomach-contents analyses are an effective complement to determine trophic interactions and to identify the taxonomic composition of the assimilated diet (Ruiz-Cooley et al., 2006; Graham et al., 2007; Sarà and Sarà, 2007).

The objectives of this study were to (1) describe and compare the spatial variability of the $\delta^{15}N$ values of omnivorous copepods and yellowfin tuna in the ETP and (2) to estimate the trophic status of yellowfin tuna in the food web from the relative spatial patterns of yellowfin tuna and copepod $\delta^{15}N$ values, from CSIA of yellowfin tuna, and from yellowfin tuna stomach contents. This study represents a unique application of stable-isotope analyses across multiple trophic levels and over a large spatial scale in a pelagic marine ecosystem, and demonstrates that copepods provide a reasonable proxy for the isotopic variation at the base of the food web. Spatial depictions of trophic status provide a quantitative schematic for characterizing the diet of tuna and other exploited predator populations from stomach contents, which is essential for effective ecosystem models. Furthermore, this approach supports additional analyses to investigate the effects of climate variability (e.g. El Niño Southern Oscillation) and long-term climate change on trophic pathways.

2. Materials and methods

2.1. Study area

Our study area comprised a large portion of the eastern tropical Pacific Ocean, a region located between the subtropical gyres of the North and South Pacific in Tropical Surface Water (TSW). The TSW water mass is defined by surface temperatures >25 °C and salinity <34 (Fiedler and Talley, 2006). Sampling locations encompassed parts of the eastern Pacific warm pool, the North Equatorial Coun-

tercurrent, the North Equatorial Current, and the southern terminus of the California Current (Fiedler and Talley, 2006). The region has diverse oceanography, and is extremely productive (see reviews by Fernández-Álamo and Färber-Lorda (2006), Fiedler and Talley (2006), Kessler (2006) and Wang and Fiedler (2006)). Coastal and offshore upwelling of nutrients and large oceanic oxygen minimum zones (regions of denitrification) influence the stable-isotope values of the biota in the study area (Cline and Richards, 1972; Liu and Kaplan, 1989; Voss et al., 2001).

2.2. Sample collection

Samples of zooplankton were collected on board two ships, R/V David Starr Jordan and R/V McArthur II, of the US National Oceanic and Atmospheric Administration (NOAA) in the ETP from 5 August to 5 December 2003 (Table 1). This sampling was a component of the *Stenella* Abundance Research (STAR) Project conducted by the Southwest Fisheries Science Center, La Jolla, California, USA; the cruise tracklines were designed to sample the known range of spotted (*Stenella attenuata*) and spinner dolphins (*S. longirostris*). The zooplankton samples were collected using a 0.6-m diameter bongo net (Smith and Richardson, 1977), with two 333-μm mesh cylindrical-conical nets, towed obliquely from 200 m for 15 min at about 19:30–23:30 local time. The material collected by the inboard net was stored at −20 °C. A flowmeter was used on the outboard net, and an average of 438 m^3 of water was filtered per tow. In the laboratory, the zooplankton samples were thawed slowly, and counts by species were made in 20-mL aliquots taken with a Stempel pipette. The remaining sample was sorted for copepods, by species, using a stereoscopic microscope, and the copepods were refrozen for subsequent stable-isotope analysis. The sampling locations are shown in Fig. 1, and sample dates, locations, plankton volumes, and copepod species composition are presented in Table 1. The copepods were collected during months characterized as normal or tending slightly to La Niña conditions, based on the Southern Oscillation Index (http://www.cdc.noaa.gov/enso/enso.mei_index.html).

Yellowfin tuna were captured by purse-seine fishing vessels in the ETP between 16 August 2003 and 16 November 2005. They were sampled on board the vessels by observers of the Inter-American Tropical Tuna Commission (IATTC, 2004). Fifteen fish, caught in the same school, were randomly sampled from purse-seine sets immediately after capture, and the date, time, position, and sea surface temperature (SST) were recorded for each set that yielded samples. On board the vessels, the observers measured the fork length (FL) of the fish (mm), removed samples of white muscle from the dorsal musculature adjacent to the second dorsal fin, and stored them at about −20 °C until processed further. The observers also excised the stomach from each fish and immediately froze it, with its contents inside, at about −20 °C. The yellowfin tuna samples used for stable-isotope analysis in this study originated from 50 purse-seine sets on 16 fishing trips during August 2003–September 2004 and these set locations overlapped with the copepod study area (Fig. 2a, Table 2). These months are characterized as normal or tending slightly to La Niña conditions, based on the Southern Oscillation Index.

2.3. Analytical methods

Approximately 50–200 individual copepods per species were sorted from each bongo haul, combined into a single sample, and lyophilized for stable-isotope analysis. Subsamples of white muscle from up to six individual yellowfin tuna per purse-seine set and size class (<90 and ⩾90 cm FL) were combined into one composite sample for stable-isotope analysis. Thus, there were 50 composite samples of 231 fish. We used composite samples of several individuals because our focus was on broad-scale isotopic patterns. Nine of the 50 composite yellowfin tuna samples (samples 10, 11, 16, 23, 27, 29, 30, 34, and 41; Table 2) were further analyzed for within-composite (i.e. within-school) isotopic variability. The white muscle samples for each yellowfin tuna ($n = 47$) that comprised the nine composites were analyzed separately for stable-isotope values. All muscle samples were lyophilized or oven dried (60 °C, ∼24 h) and homogenized to a fine powder using a mortar and pestle.

Bulk carbon and nitrogen isotopic compositions of the copepods and yellowfin tuna were determined without pretreatment 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 the international V-PDB and atmospheric N_2 for carbon and nitrogen, respectively. A glycine reference compound for which the isotopic compositions were known was analyzed periodically to ensure accuracy of all isotope measurements. Several samples were measured in duplicate or triplicate, and the analytical error associated with these measurements was typically ⩽0.2‰.

An additional six of the composite yellowfin tuna muscle samples from an onshore–offshore transect were analyzed for compound-specific isotope analysis (CSIA) of amino acids. The locations of the samples taken in September and October 2003 are shown by triangles and those from May and June 2004 are shown by squares in Fig. 2a. The six composite samples (samples 10, 13, 16, 31, 33, and 34; Table 2) were from 19 fish. Sample preparation and CSIA are described by Popp et al. (2007) and Hannides et al. (2009).

Stomach-contents analysis was performed using standard methods (Chipps and Garvey, 2007). The stomach contents were identified to the lowest taxon possible, weighed to the nearest gram, and enumerated when individuals were recognizable.

2.4. Data analyses

Our inferences about the food web are derived from a working hypothesis that omnivorous copepods represent a proxy for the base of the food web. We used generalized additive models (GAMs) to model the spatial structure of the copepod $\delta^{15}N$ values. GAMs are suitable owing to the non-linear aspects of open-ocean ecosystems (e.g. Vilchis et al., 2006). A bivariate surface in latitude and longitude was estimated using thin plate regression splines with the *mgcv* library (Wood, 2006) of the statistical computing software *R* (R Development Core Team, 2007). The amount of smoothing was estimated from the data by generalized cross validation. The weighted-average $\delta^{15}N$ values (weighted by abundance) of copepods at each sample station was the response variable. We derived this relationship for only omnivorous copepods, classified by species according to an analysis of site-specific $\delta^{13}C$ and $\delta^{15}N$ values (López-Ibarra, 2008).

To compare spatial patterns of the yellowfin tuna $\delta^{15}N$ values in relation to the $\delta^{15}N$ values of our proxy for the base of the food web, we used the above GAM to predict the $\delta^{15}N$ of omnivorous copepods at the exact latitude and longitude where the yellowfin tuna were sampled. For each yellowfin tuna sample, the $\delta^{15}N$ value of omnivorous copepods predicted from the GAM surface was subtracted from the $\delta^{15}N$ value measured from the yellowfin tuna (hereafter "$\Delta_{YFT\text{-}COP}$") to afford us an estimate of the relative trophic position of the tuna (Eq. (1)). We follow Post et al.'s (2000) terminology and consider "trophic position" a continuous measure based on stable isotopes and food-web models, as opposed to discrete trophic levels.

Given that intraspecific variation in $\delta^{15}N$ values is often correlated with consumer size (Fry and Quiñones, 1994; Jennings et al., 2002), we used multiple linear regression to explore the rela-

Table 1
Characteristics of the bongo-net hauls, copepod species sampled for stable-isotope analysis, and abundance-weighted average carbon and nitrogen isotope values of omnivorous copepods in the samples.

Sample date	Latitude	Longitude	Plankton volume mean m^3 1000 m^{-3} strained	Copepod species[a]	Copepod abundance mean indiv 1000 m^{-3}	$\delta^{13}C$ (‰) mean	$\delta^{15}N$ (‰) mean
5-Aug-2003	24.47°N	115.43°W	42	5,6,7	725	−22.6	7.7
7-Aug-2003	24.21°N	111.73°W	68	2	976	−23.3	11.9
8-Aug-2003	22.33°N	111.25°W	172	2	2066	−19.9	11.0
9-Aug-2003	22.30°N	109.61°W	91	8,9	2090	−19.8	11.4
10-Aug-2003	24.54°N	109.65°W	144	3,9	1054	−20.6	11.3
11-Aug-2003	26.69°N	111.04°W	116	2	5420	−19.5	11.4
12-Aug-2003	25.18°N	109.04°W	136	2,3	2722	−20.4	10.8
14-Aug-2003	21.83°N	106.60°W	86	2,8,9	4757	−20.2	11.0
15-Aug-2003	20.70°N	108.01°W	66	2,3,7	1947	−21.6	10.3
16-Aug-2003	19.48°N	105.24°W	154	3,8	2133	−21.2	10.0
20-Aug-2003	18.11°N	103.68°W	114	3,8	1227	−20.1	9.4
21-Aug-2003	17.42°N	106.18°W	104	2,3,9	1022	−20.8	10.8
22-Aug-2003	16.65°N	108.97°W	116	3,8,9	2264	−21.3	10.3
23-Aug-2003	15.82°N	111.96°W	54	3	1730	−21.3	9.2
24-Aug-2003	14.95°N	115.11°W	31	1,3,5,7	4857	−22.1	8.5
25-Aug-2003	14.08°N	118.09°W	44	3	546	−21.3	9.8
26-Aug-2003	13.24°N	121.19°W	31	3,9	646	−21.6	9.0
27-Aug-2003	12.08°N	123.79°W	51	3,5,8	2484	−22.8	7.0
29-Aug-2003	10.75°N	121.19°W	44	3	1217	−21.3	8.8
30-Aug-2003	11.65°N	118.67°W	78	3,9	999	−22.3	7.4
3-Sep-2003	15.83°N	107.00°W	51	3,8	1328	−21.6	9.6
4-Sep-2003	16.56°N	104.77°W	60	3,8	1436	−20.5	9.8
17-Sep-2003	16.53°N	99.41°W	73	2,3,8	1029	−20.6	8.1
19-Sep-2003	14.05°N	100.34°W	43	3	1778	−20.4	8.9
20-Sep-2003	13.00°N	101.12°W	143	6,9	1282	−22.3	9.9
21-Sep-2003	13.24°N	98.31°W	72	2,3,8	1045	−21.2	8.1
22-Sep-2003	13.48°N	95.47°W	170	8	5022	−23.0	7.5
24-Sep-2003	13.13°N	90.29°W	200	2,8,9	2586	−20.8	8.7
25-Sep-2003	10.95°N	89.64°W	148	2,8	760	−21.3	7.0
26-Sep-2003	4.95°N	96.02°W	72	6,7,8,9	1886	−22.0	8.1
26-Sep-2003	11.60°N	88.58°W	159	9	2378	−20.8	5.7
29-Sep-2003	9.49°N	85.19°W	108	2,6,8	1501	−20.1	7.8
7-Oct-2003	9.48°N	84.97°W	71	2,6,8	2305	−19.9	8.0
8-Oct-2003	7.61°N	86.74°W	116	1,9	1233	−22.8	6.7
9-Oct-2003	5.94°N	88.54°W	108	9	568	−22.5	7.0
10-Oct-2003	6.14°N	90.98°W	136	6,8,9	757	−22.1	7.8
11-Oct-2003	6.29°N	93.34°W	118	8	2361	−23.9	6.1
12-Oct-2003	6.46°N	95.74°W	133	6,9	938	−23.2	7.2
13-Oct-2003	6.34°N	98.36°W	98	6,7,8	1499	−22.1	9.2
14-Oct-2003	6.24°N	101.12°W	58	3,6,9	1004	−22.1	10.6
15-Oct-2003	6.11°N	104.03°W	90	6,7,8	1018	−22.5	8.5
16-Oct-2003	6.02°N	107.21°W	85	8	2729	−23.0	8.5
17-Oct-2003	7.52°N	106.90°W	60	1,2,9	1159	−22.2	7.1
19-Oct-2003	11.33°N	102.58°W	98	3,4,9	2500	−21.6	10.1
21-Oct-2003	11.60°N	97.57°W	140	8	415	−21.9	8.7
28-Oct-2003	13.89°N	92.19°W	56	1,2,9	4979	−20.6	6.8
8-Nov-2003	6.52°N	120.33°W	70	4,9	851	−21.5	6.1
9-Nov-2003	7.94°N	120.05°W	36	1,6,8	1134	−22.3	8.5
10-Nov-2003	9.35°N	117.23°W	40	3,9	1094	−22.8	7.4
13-Nov-2003	13.36°N	108.82°W	73	3,8	1482	−21.3	9.6
14-Nov-2003	14.73°N	105.93°W	93	3,4,9	1251	−20.9	9.9
15-Nov-2003	16.04°N	103.25°W	86	8	1458	−22.0	8.9
16-Nov-2003	17.36°N	101.58°W	82	3,9	1024	−21.1	9.4
17-Nov-2003	18.26°N	103.48°W	158	2,8	2676	−20.8	9.0
22-Nov-2003	19.03°N	105.72°W	83	2,3,8,9	4343	−21.0	9.7
23-Nov-2003	18.36°N	108.78°W	59	3,8	3285	−20.1	11.2
24-Nov-2003	17.65°N	111.91°W	61	8	11,062	−21.7	10.8
25-Nov-2003	16.84°N	114.80°W	18	3	1566	−20.6	10.5
26-Nov-2003	16.25°N	118.16°W	20	7	2550	−22.0	9.2
27-Nov-2003	15.58°N	121.47°W	15	3	2194	−20.8	8.9
28-Nov-2003	9.54°N	102.74°W	148	9	1254	−18.6	8.1
28-Nov-2003	17.89°N	121.52°W	18	6	1212	−21.0	7.5
29-Nov-2003	18.55°N	118.69°W	16	6	1999	−20.9	8.8
30-Nov-2003	19.16°N	115.39°W	19	6,7	1031	−21.4	7.8
2-Dec-2003	19.82°N	110.95°W	34	3,6,8	2551	−20.8	11.1
3-Dec-2003	21.83°N	112.61°W	35	7,8	1595	−21.7	11.6
4-Dec-2003	21.29°N	117.19°W	26	7	1070	−21.9	9.9
5-Dec-2003	23.17°N	116.41°W	31	9	1067	−21.5	11.6

[a] Species 1 = *Acartia danae*, 2 = *Centropages furcatus*, 3 = *Euchaeta indica*, 4 = *Euchaeta marina*, 5 = *Lucicutia flavicornis*, 6 = *Pleuromamma abdominalis*, 7 = *Pleuromamma gracilis*, 8 = *Subeucalanus subcrassus*, 9 = *Subeucalanus subtenuis*.

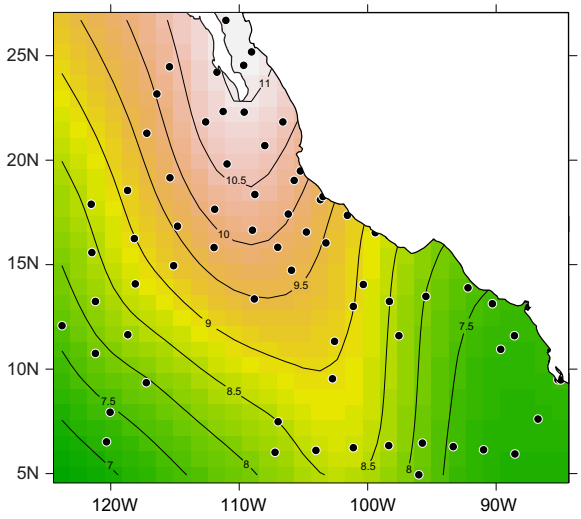

Fig. 1. Contour plot of the bivariate surface for $\delta^{15}N$ values (‰) of omnivorous copepods estimated from the GAM model. The black dots are 68 sampling stations where omnivorous copepods were sampled by bongo net.

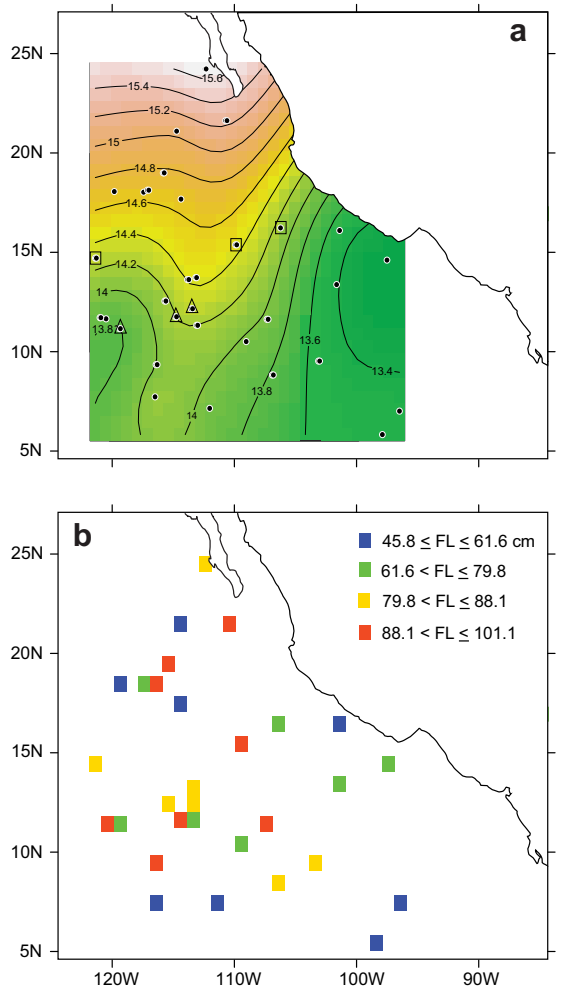

Fig. 2. (a) Contour plot of $\delta^{15}N$ values (‰) of yellowfin tuna sampled for this study. The black dots are the locations where the fish were caught in purse-seine sets. The sample locations for CSIA of amino acids taken in September–October 2003 are bordered by triangles, and those from May–June 2004 are bordered by squares. (b) Fork length (FL, cm) of yellowfin tuna sampled for stable-isotope analysis. Color levels show quartiles.

tionship between the yellowfin tuna $\delta^{15}N$ values (response variable), and the GAM-predicted $\delta^{15}N$ values of omnivorous copepods (treating them as known quantities) and fish length.

We applied the $\Delta_{YFT\text{-}COP}$ values (i.e. $\delta^{15}N_{YFT}-\delta^{15}N_{COP}$) to derive estimates of yellowfin trophic position (TP) as:

$$TP_{YFTj} = \frac{\delta^{15}N_{YFTj} - \delta^{15}N_{COPj}}{TEF} + TP_{COP}, \quad (1)$$

where TP_{YFTj} is the trophic position estimate for yellowfin tuna at the site of sample j, $\delta^{15}N_{YFTj}$ is the $\delta^{15}N$ value of yellowfin tuna at the site of sample j, $\delta^{15}N_{COPj}$ is the GAM-estimated $\delta^{15}N$ value of omnivorous copepods at the site of sample j, and TP_{COP} is an estimate of the average TP of omnivorous copepods in the ETP. The denominator of Eq. (1), *TEF*, is the trophic enrichment factor, and represents our best estimate of isotopic enrichment between yellowfin tuna and its diet. We adopt a *TEF* of 2.4‰, the mean *TEF* for marine fishes based on Vanderklift and Ponsard's (2003) review of an extensive body of literature that reported consumer-diet ^{15}N enrichment (see also Caut et al., 2009). We used a first approximation of 2.5 for TP_{COP}, assuming equal proportions of predation and grazing by omnivorous copepods, and subsequently explored the effect of TP_{COP} on the TP_{YFT} estimates. Assuming that *TEF* and TP_{COP} are constants, and disregarding any correlation between predicted values of $\delta^{15}N_{COP}$, we approximated the standard error of the average trophic position (averaged across samples) as the square root of [the variance of the average $\delta^{15}N_{YFT}$, plus one over the squared sample size, multiplied by the sum of the squared standard errors of the $\delta^{15}N_{COP}$ values obtained from the GAM model], all divided by *TEF*.

We analyzed stomach-contents data from yellowfin tuna of the same size range as those analyzed for stable isotopes (45.8–129.3 cm) as the proportional composition by weight of each prey type in each individual tuna and averaged for each prey type over all fish with food remains in the stomachs (Chipps and Garvey, 2007) as:

$$MW_i = \frac{1}{P}\sum_{j=1}^{P}\left(\frac{W_{ij}}{\sum_{i=1}^{Q} W_{ij}}\right), \quad (2)$$

where MW_i is mean proportion by weight for prey item i, W_{ij} is the weight of prey item i in fish j, P is the number of fish with food in their stomachs, and Q is the number of prey types in the sample. Graham et al. (2007) illustrated how the MW_i avoids biases in the traditional approach of calculating percent weight of each prey taxon by dividing the total weight of a given taxon by the total weight of all prey in all stomachs pooled. The weights of residual hard parts (cephalopod mandibles and fish otoliths) were disregarded because of the likelihood that they accumulate in the stomachs from previous meals. The prey mean proportions were averaged by prey class for each purse-seine set, and treated as replicates to avoid pseudoreplication. In addition to the gravimetric data analysis, we estimated a weighted-average TP (weighted by mean proportional contribution in prey weight) of the prey in the stomach contents over 5-deg area strata. Nominal TP values of the prey were based on a trophic mass-balance model for the ETP in 1992–1994 (Olson and Watters, 2003).

We also estimated yellowfin TP from the CSIA, based on the relative $\delta^{15}N$ values of glutamic acid and glycine as:

$$TP_{GLU\text{-}GLYj} = \frac{\delta^{15}N_{GLUj} - \delta^{15}N_{GLYj}}{7‰} + 1, \quad (3)$$

where $TP_{GLU\text{-}GLYj}$ is the trophic position estimate for yellowfin tuna at the site of sample j, $\delta^{15}N_{GLUj}$ is the $\delta^{15}N$ value of glutamic acid at site j, and $\delta^{15}N_{GLYj}$ is the $\delta^{15}N$ value of glycine at site j. Although other forms of Eq. (3) exist (McCarthy et al., 2007; Hannides et al., 2009), for consistency with previous amino acid research on tuna in the ETP, we use the form of the equation in Popp et al. (2007).

Table 2
Purse-seine set characteristics, composite sample characteristics, and carbon and nitrogen stable-isotope values for yellowfin tuna sampled for this study. The values to the right of the commas in the $\delta^{15}N$ (‰) column are mean (SE) values for the individual fish in the composite samples (47 fish total).

Sample number	Set date	Latitude	Longitude	Number of individuals	Mean (SD) FL (cm)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
1	16-Aug-2003	7.03°N	96.73°W	1	50.7	−16.5	13.8
2	9-Sep-2003	24.25°N	112.32°W	2	64.3 (0.49)	−16.7	15.6
3	9-Sep-2003	24.25°N	112.32°W	6	109.6 (5.67)	−17.8	16.3
4	23-Sep-2003	12.57°N	115.55°W	6	74.6 (10.18)	−16.3	13.6
5	23-Sep-2003	12.57°N	115.55°W	3	101.2 (13.48)	−15.9	13.7
6	24-Sep-2003	13.65°N	113.73°W	6	76.6 (10.09)	−16.0	14.2
7	24-Sep-2003	13.65°N	113.73°W	4	92.5 (2.52)	−16.3	15.0
8	29-Sep-2003	5.85°N	98.10°W	6	58.4 (3.28)	−16.2	13.4
9	29-Sep-2003	12.18°N	113.43°W	6	81.5 (3.06)	−15.9	14.5
10[a,b]	29-Sep-2003	12.18°N	113.43°W	3	94.6 (3.99)	−16.0	14.3, 14.5 (0.23)
11[a]	6-Oct-2003	11.68°N	120.28°W	5	80.6 (7.21)	−16.2	13.3, 13.6 (0.26)
12	7-Oct-2003	11.18°N	119.18°W	6	69.0 (7.84)	−16.5	13.8
13[b]	7-Oct-2003	11.18°N	119.18°W	1	90.0	−16.2	13.1
14	8-Oct-2003	11.73°N	120.73°W	4	74.8 (2.95)	−16.2	13.9
15	8-Oct-2003	11.73°N	120.73°W	5	120.1 (21.11)	−16.0	13.9
16[a,b]	14-Oct-2003	11.78°N	114.68°W	6	85.3 (3.64)	−15.8	14.0, 14.4 (0.16)
17	14-Oct-2003	11.78°N	114.68°W	3	101.2 (16.7)	−15.8	15.5
18	21-Oct-2003	13.75°N	113.07°W	6	80.1 (14.77)	−16.0	14.7
19	21-Oct-2003	13.75°N	113.07°W	1	93.4	−15.9	14.7
20	24-Oct-2003	10.53°N	109.02°W	6	68.2 (8.00)	−16.1	13.5
21	27-Oct-2003	11.35°N	113.00°W	4	61.1 (6.54)	−16.3	13.7
22	27-Oct-2003	11.35°N	113.00°W	2	94.1 (4.74)	−15.7	14.1
23[a]	27-Oct-2003	21.65°N	110.68°W	3	82.6 (6.74)	−16.2	15.5, 15.5 (0.10)
24	27-Oct-2003	21.65°N	110.68°W	6	98.8 (2.32)	−16.3	15.3
25	14-Mar-2004	9.55°N	103.22°W	6	66.8 (11.70)	−16.2	13.2
26	14-Mar-2004	9.55°N	103.22°W	2	93.4 (0.14)	−15.8	13.8
27[a]	15-Mar-2004	13.40°N	101.77°W	6	65.2 (4.89)	−15.4	13.3, 13.4 (0.13)
28	16-Mar-2004	16.12°N	101.53°W	6	57.9 (10.40)	−15.7	13.1
29[a]	19-Mar-2004	14.63°N	97.73°W	6	68.7 (8.36)	−15.5	13.2, 13.8 (0.11)
30[a]	31-May-2004	14.73°N	121.12°W	6	72.4 (7.74)	−16.3	14.6, 14.6 (0.18)
31[b]	31-May-2004	14.73°N	121.12°W	2	99.7 (10.89)	−15.8	14.6
32	5-Jun-2004	15.40°N	109.87°W	6	73.3 (8.52)	−16.0	14.2
33[b]	5-Jun-2004	15.40°N	109.87°W	1	106.2	−15.7	14.8
34[a,b]	13-Jun-2004	16.25°N	106.27°W	6	70.5 (5.22)	−15.6	14.0, 14.1 (0.08)
35	23-Jun-2004	21.12°N	114.60°W	6	58.0 (13.25)	−16.4	14.2
36	2-Jul-2004	17.70°N	114.25°W	6	60.8 (5.52)	−16.2	14.4
37	13-Jul-2004	18.08°N	119.60°W	6	52.5 (3.62)	−16.3	14.7
38	14-Jul-2004	7.17°N	111.95°W	6	45.8 (4.57)	−16.3	13.7
39	21-Jul-2004	19.02°N	115.68°W	3	72.8 (13.27)	−16.1	14.5
40	21-Jul-2004	19.02°N	115.68°W	1	129.3	−16.4	15.3
41[a]	4-Aug-2004	11.65°N	107.35°W	6	81.9 (5.02)	−15.4	14.0, 14.1 (0.05)
42	4-Aug-2004	11.65°N	107.35°W	3	94.2 (7.19)	−15.4	14.4
43	6-Aug-2004	8.85°N	106.93°W	6	67.8 (15.85)	−15.9	14.0
44	6-Aug-2004	8.85°N	106.93°W	6	102.5 (16.46)	−15.4	13.7
45	14-Aug-2004	18.05°N	117.25°W	6	64.0 (2.42)	−16.5	14.7
46	16-Aug-2004	18.15°N	116.95°W	6	78.9 (11.14)	−16.2	14.4
47	16-Aug-2004	18.15°N	116.95°W	5	122.2 (14.34)	−16.0	14.9
48	12-Sep-2004	7.75°N	116.33°W	6	50.0 (5.97)	−16.1	15.4
49	25-Sep-2004	9.37°N	116.25°W	5	70.8 (12.88)	−15.9	13.9
50	25-Sep-2004	9.37°N	116.25°W	6	119.5 (23.12)	−15.6	13.4

[a] Samples analyzed for within-composite sample (within-school) variability.
[b] Samples analyzed for compound-specific isotope analysis (CSIA) of amino acids.

The $TP_{GLU\text{-}GLY}$ estimates are based on the assumption that glutamic acid is enriched in ^{15}N by 7‰ relative to glycine with each trophic transfer above phytoplankton (McClelland and Montoya, 2002; Popp et al., 2007).

3. Results

We report carbon and nitrogen isotope values (Tables 1 and 2), but did not analyze the $\delta^{13}C$ data here because they showed little spatial and intraspecific variability.

3.1. Omnivorous copepod $\delta^{15}N$

Copepod samples were taken from a wide range of locations in the ETP, and they showed large spatial variability in $\delta^{15}N$ values (Fig. 1, Table 1). Abundance-weighted-average $\delta^{15}N$ values of the omnivorous copepods ranged from 5.7‰ at 11.60°N–88.58°W to 11.9‰ at 24.22°N–111.73°W (Table 1). Of the samples that contained sufficient copepod biomass for stable-isotope analysis, *Subeucalanus subcrassus* and *Euchaeta indica* were the dominant species in occurrence, followed by *Subeucalanus subtenuis*, *Centropages furcatus*, *Pleuromamma abdominalis*, and *P. gracilis* (Table 1). Three other species occurred infrequently in the samples, five times or less.

The estimated latitude–longitude surface from the GAM fitted to the omnivorous copepod $\delta^{15}N$ values shows a strong gradient of $\delta^{15}N$ values increasing from south to north and a crest of higher values in a north–north-westerly plane angling from the southerly end of the distribution at about 102–103°W toward the tip of Baja California (Fig. 1). This two-dimensional surface was significant ($p < 0.01$ for a test of the null hypothesis of the surface equal to zero), and explained about 71% of the deviance.

3.2. Yellowfin tuna $\delta^{15}N$

The $\delta^{15}N$ values from 50 composite samples of up to six yellowfin tuna each (Table 2), totaling 231 fish, sampled in areas overlapping the copepod sample locations, are displayed as contours in Fig. 2a. The $\delta^{15}N_{YFT}$ values spanned a range of 3.2‰ over a latitude range of about 18° (2046 km). As with the copepods, yellowfin tuna showed a general south-to-north gradient in $\delta^{15}N$ values, with the highest values in the north. These similar spatial trends are consistent with limited movement rates of yellowfin tuna over the time scale of their muscle nitrogen turnover.

A plot of the measured $\delta^{15}N$ values of yellowfin tuna versus the GAM-estimated $\delta^{15}N$ values of omnivorous copepods at each sample location (Fig. 3) shows considerable variability around a positive overall relationship. Given that the composite yellowfin tuna samples comprised several individual fish, we examined intrasample (i.e. intraschool) variability by separately analyzing the isotopic composition of each fish in nine composite samples (Table 2). Analysis of variance showed that the within-composite mean square error (MSE, 0.12) was many times smaller than the among-composite MSE (1.69, $p < 0.01$). Therefore, intraschool isotopic variability does not have a confounding effect on the spatial variability represented in Fig. 2a).

Variability in the size of yellowfin tuna was suspected to have contributed to the observed isotopic spatial variability. The per-sample mean FL of yellowfin tuna that were analyzed isotopically, ranged between 46 and 129 cm (Table 2), different sized yellowfin were distributed fairly evenly across the study area (Fig. 2b), and sample sizes varied a small amount across the length range (Fig. 4). The multiple linear regression model used to test the effect of copepod $\delta^{15}N$ values and fish size on yellowfin tuna $\delta^{15}N$ values showed coefficients for copepod $\delta^{15}N$ values and yellowfin tuna FL of 0.52 ($p < 0.01$) and 0.0008 ($p = 0.07$), respectively. The adjusted R-square for this model was 0.37, and the overall model was significant ($p < 0.01$). The differences in the yellowfin tuna $\delta^{15}N$ values and those predicted from the multiple regression model show spatial structure, with the greatest residuals offshore and the smallest residuals inshore (Fig. 5a).

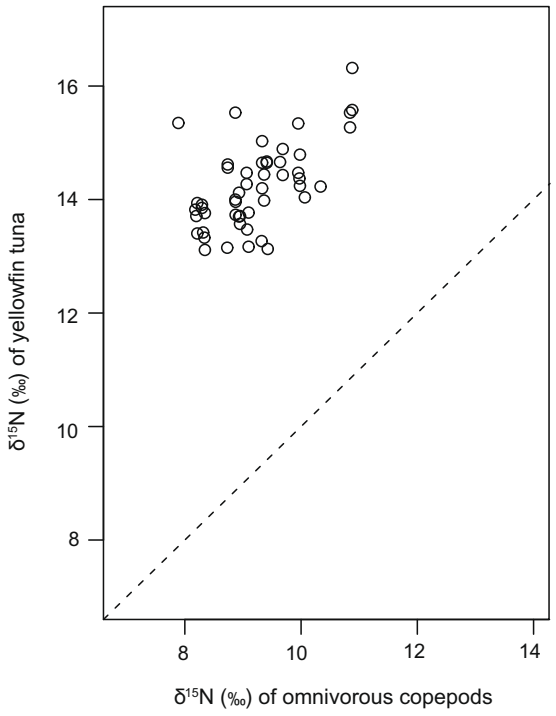

Fig. 3. Measured $\delta^{15}N$ values of yellowfin tuna versus the GAM-predicted $\delta^{15}N$ values of omnivorous copepods. The broken line is the 1:1 reference line.

The spatial distribution of the $\Delta_{YFT\text{-}COP}$ values (i.e., the difference between the measured $\delta^{15}N$ values of the yellowfin tuna and the GAM-estimated $\delta^{15}N$ values of copepods at each location) show an increasing gradient from onshore to offshore (Fig. 5b). The range of the $\Delta_{YFT\text{-}COP}$ values was substantial, 4.0–7.6‰. Comparing the spatial distribution of the residuals from the multiple regression model (Fig. 5a) to that of the $\Delta_{YFT\text{-}COP}$ values (Fig. 5b) shows only small differences when we removed the effect of yellowfin tuna size. Given this result, and that the effect of yellowfin tuna size was non-significant in the multiple regression model, we did not consider yellowfin tuna size in our subsequent analyses.

3.2.1. Trophic position – $\delta^{15}N$

Similar spatial patterns of nitrogen isotope ratios in yellowfin tuna and copepods offer justification for using $\delta^{15}N$ data to estimate yellowfin tuna TP. The $\Delta_{YFT\text{-}COP}$ values provided 50 estimates of the numerator of Eq. (1), representing 231 fish. Overall, the TP estimates based on $\delta^{15}N$ values (hereafter $TP_{ISOTOPES}$) averaged 4.7 ± 0.05 SE, and ranged from 4.1 to 5.7, spanning 1.6 trophic levels. The spatial distribution of the $TP_{ISOTOPES}$ estimates indicates lower values inshore and increasing to higher values offshore, in the same pattern as the $\Delta_{YFT\text{-}COP}$ values (Fig. 5b). This apparent spatial gradient in trophic status requires further examination of the underlying isotope values before food-web inferences can be justified.

3.2.2. Compound-specific isotope analysis (CSIA)

Sample selection for CSIA was based on (1) sample location along an onshore–offshore transect and (2) sample date, so that three samples were from yellowfin tuna caught during the same months in 2003 as those of the copepods, and three samples were from fish caught during the first half of 2004. The $\delta^{15}N$ values of glutamic acid (Table 3, Fig. 6), a trophic amino acid, varied little east to west, consistent with those of bulk white-muscle tissue (Fig. 2a). There were non-significant relationships between the $\delta^{15}N$ values of glutamic acid and longitude ($p = 0.50$) and between bulk white muscle $\delta^{15}N$ values and longitude ($p = 0.13$) for the six samples. The glycine $\delta^{15}N$ values, however, decreased significantly from east to west ($p = 0.02$, weighted least-squares regression, weights = 1/variance; Table 3, Fig. 6). Glycine $\delta^{15}N$ values in yellowfin tuna muscle tracked copepod $\delta^{15}N$ trends at the sample locations (Fig. 1). This result supports our contention that omnivorous copepods provide a reasonable proxy for the isotopic variation at the base of the food web in the ETP.

The $TP_{GLU\text{-}GLY}$ estimates (Eq. (3)) ranged from 4.8 to 5.7 (Table 3), and the values increased from east to west. A linear regression model to test the relationship between $TP_{GLU\text{-}GLY}$ and longitude showed a coefficient of −0.05‰ per degree longitude ($p = 0.055$) and an adjusted R-square of 0.55. The CSIA also provided insight about seasonal differences in basal isotope values in our study area (see Section 3.3).

3.3. Temporal effects

We considered seasonality over 6-month periods in the ETP (Kessler, 2006). The copepod samples were taken during the second half of 2003 (Table 1), whereas the yellowfin tuna were captured during the opposite season, during March, May, and June 2004, as well as during the second half of the year, August–October 2003 (same as the copepods) and July–September 2004. For the most part, the $\Delta_{YFT\text{-}COP}$ values of fish caught during the first half of the year (Fig. 5b, points surrounded by boxes) were lower than those of fish caught during the second half of 2003 and 2004, and those fish were collected primarily inshore. If the $\delta^{15}N$ values of the copepods in the first half of the year were higher than those in the

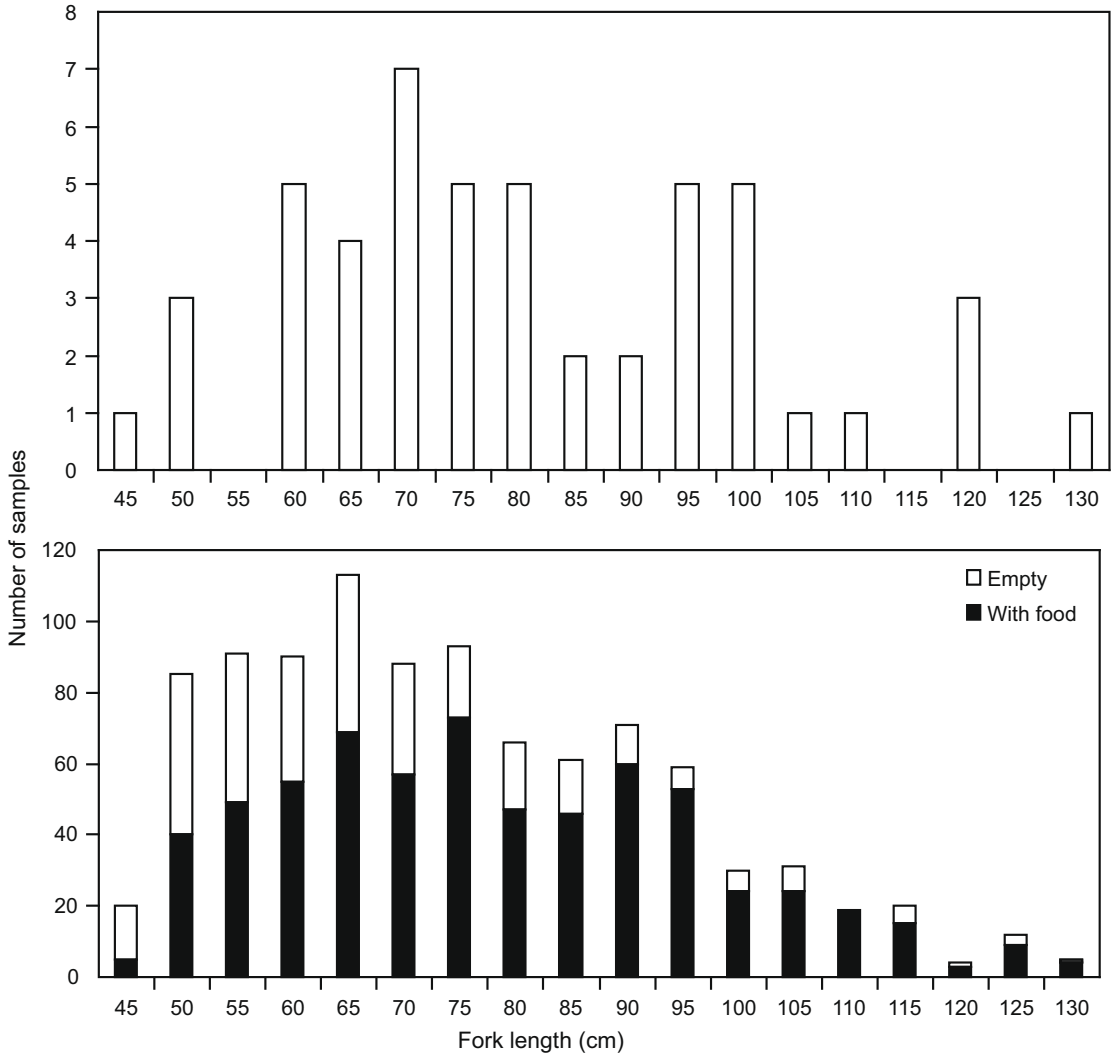

Fig. 4. Size-frequency of yellowfin tuna sampled for stable-isotope analysis (upper panel), and for stomach-contents analysis (lower panel).

second half, then this could explain or contribute to the onshore–offshore gradient of $\Delta_{YFT\text{-}COP}$ values. The isotopic variability at the base of the food web has not been investigated in our study area during the entire year, and we cannot test the yellowfin $\delta^{15}N$ data for seasonal effects because the samples were not taken in the same locations during both seasons. The ENSO conditions, as indicated by the Southern Oscillation Index, were similar during both years of the isotope samples and all 3 years of the stomach samples. However, the results from the CSIA of amino acids (Section 3.2.2) allowed us to draw inferences about seasonal differences in baseline isotope values.

There was no significant difference ($p > 0.05$) between the glycine amino acid $\delta^{15}N$ values for samples taken during September–October 2003 and May–June 2004. Although the sample size was small, these CSIA results support a null hypothesis of no seasonal differences in the N isotopic values at the base of the food web. It is unlikely that seasonal differences influenced the onshore–offshore pattern of $\Delta_{YFT\text{-}COP}$ values.

3.4. Diet composition

We analyzed the stomach-contents data from yellowfin tuna sampled during 2003–2005 in the areas overlapping the copepod sample distribution (Fig. 1). Stomach samples were taken from 1000 yellowfin tuna caught in 77 purse-seine sets; of these 34% of the stomachs were empty or contained only residual hard parts (FL distribution in Fig. 4). We stratified the stomach-contents data into 21 5-deg areas, and present the diet composition as mean proportions by weight (Eq. (2)) for two taxonomic groups (crustaceans, cephalopods) and two guilds (small fishes, medium fishes; Fig. 7). We present a low level of taxonomic detail for ease of display, while still revealing diet diversity. Sample sizes within the area strata were insufficient to allow additional stratification by yellowfin tuna size.

The dominant species in the crustacean diet group was the galatheid crab *Pleuroncodes planipes*, while the most common cephalopod taxa were *Argonauta* spp., *Dosidicus gigas*, and *Sthenoteuthis oualaniensis*. The flyingfish *Exocoetus volitans*, the half-beak *Oxyporhamphus micropterus*, *Lactoria diaphanum* (Ostraciidae), and *Vinciguerria lucetia* (Phosichthyidae) were the most common small fishes. The medium-sized fishes were primarily *Auxis* spp. (Scombridae).

3.4.1. Trophic position–stomach contents

We applied TP estimates of each prey taxon, based on a trophic mass-balance model for the ETP (Olson and Watters, 2003), to compute weighted-average trophic positions of the diet of yellowfin tuna caught in each 5-deg area. The mean TP ± SD of the stom-

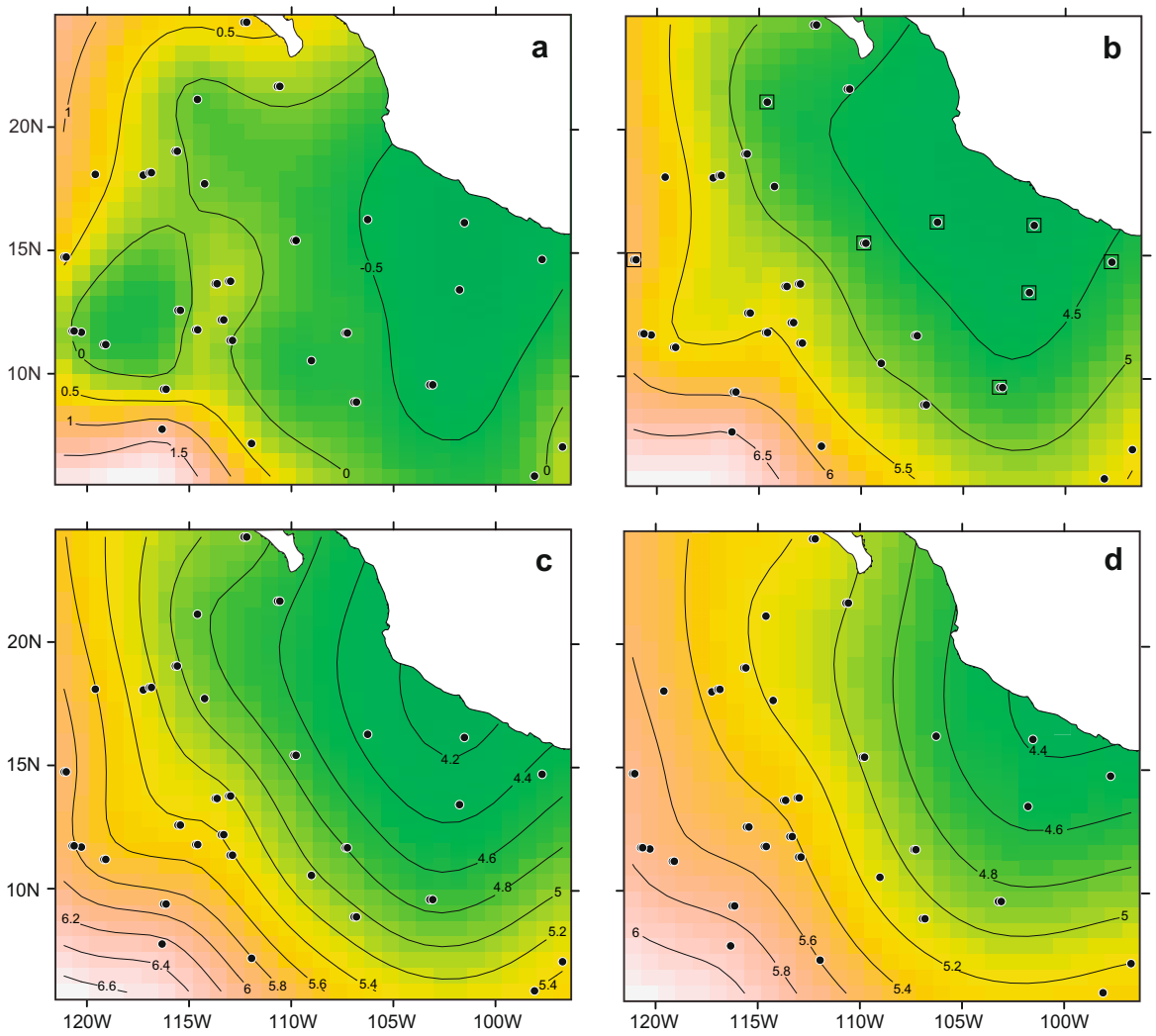

Fig. 5. Contour plots of (a) residuals of a multiple linear regression of the effect of copepod $\delta^{15}N$ and yellowfin tuna size on the $\delta^{15}N$ values of yellowfin tuna, (b) $\Delta_{YFT\text{-}COP}$ values, difference between the measured $\delta^{15}N$ values of yellowfin tuna and the $\delta^{15}N$ values of omnivorous copepods predicted from the GAM. The black dots bordered by black boxes are locations where yellowfin tuna were sampled in the first semester (March, May, June) of 2004, (c) $\Delta_{YFT\text{-}COP}$ values adjusted for yellowfin tuna movement behavior, and (d) $\Delta_{YFT\text{-}COP}$ values adjusted for bigeye tuna movement behavior. The black dots in all panels are the locations where the yellowfin tuna were caught in purse-seine sets during 2003–2004, and the dots are jittered slightly when two samples are taken from the same location.

Table 3
Stable N isotope values of bulk white-muscle tissue and two amino acids from composite samples of yellowfin tuna. $TP_{GLU\text{-}GLY}$ values are trophic-position estimates for yellowfin tuna in each sample, based on Eq. (3). Sample information corresponding to each sample number is listed in Table 2.

Sample number	Bulk white muscle $\delta^{15}N$ (‰)	Glutamic acid mean $\delta^{15}N$ (SE) (‰)	Glycine mean $\delta^{15}N$ (SE) (‰)	$TP_{GLU\text{-}GLY}$ mean (SE)
10	14.3	27.0 (0.01)	−0.1 (0.01)	4.9 (0.02)
13	13.1	27.0 (0.28)	−2.1 (0.00)	5.2 (0.08)
16	14.0	25.0 (0.04)	−5.4 (0.13)	5.3 (0.06)
31	14.6	28.7 (0.03)	−3.9 (0.34)	5.7 (0.09)
33	14.8	26.7 (0.05)	−1.5 (0.14)	5.0 (0.06)
34	14.0	27.1 (0.83)	0.4 (0.99)	4.8 (0.19)

ach contents (hereafter TP_{DIET}) for all 21 areas was 3.6 ± 0.31 (range 3.2–4.4, spanning 1.2 trophic levels). The mean TP_{DIET} + 1.0 (i.e. the estimated mean TP of the yellowfin tuna sampled for stomach contents; hereafter "diet-based TP_{YFT}"), 4.6 ± 0.07 SE was not significantly different ($p > 0.05$) than the mean $TP_{ISOTOPES}$ (4.7 ± 0.05 SE). The inter-quartile ranges of the diet-based TP_{YFT} and $TP_{ISOTOPES}$ estimates were 0.43 and 0.35, and the coefficients of variation were 0.07 and 0.06, respectively.

Our analysis of stomach contents does not show an inshore–offshore gradient in TP_{DIET} (Fig. 7), which is not consistent with the inshore–offshore gradient in yellowfin tuna-copepod ^{15}N enrichment (Fig. 5b). The diet diversity over the region is considerable, with the highest TP_{DIET} estimates (about 5.4) for areas where yellowfin tuna had the greatest proportions of cephalopods and medium fishes (e.g. *Auxis* spp.) in their diet, while the crustaceans and small fishes dominated the diets in areas with the lowest TP estimates (about 4.2).

4. Discussion

The broad-scale pattern of nitrogen isotope values in omnivorous copepods (Fig. 1) is remarkably consistent over a large region of the ETP, comprising some 3 million km^2. The south–north gradient of increasing $\delta^{15}N$ values of this important component of the food web (Hays et al., 2005) is thought to be induced by spatial trends of $\delta^{15}N$ values in the dissolved nitrate pool, which supports growth at the base of the food web. The spatial variability in $\delta^{15}NO_3^-$ values, as well as particulate and sedimentary nitrogen, have been described for the ETP (Farrell et al., 1995; Voss et al.,

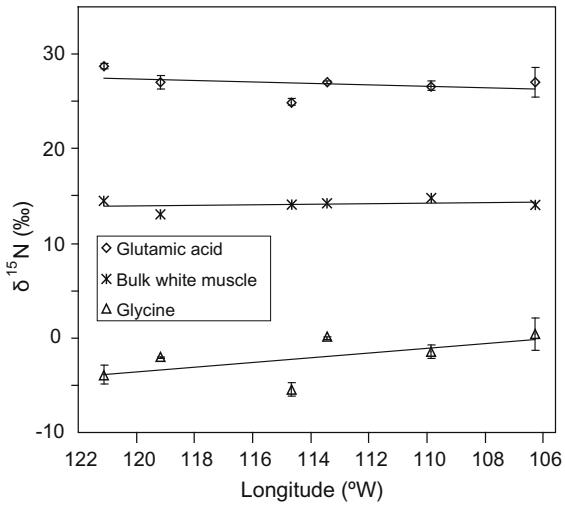

Fig. 6. Stable N isotope values of glutamic acid, a "trophic" amino acid, glycine, a "source" amino acid, and bulk white-muscle tissue from composite samples of yellowfin tuna in the ETP. Error bars represent SD.

2001; Sigman et al., 2005), and has been related to spatial patterns of phytoplankton drawdown of upwelled NO_3^- at the equator, and to the north, the effects of denitrification that occur in the oxygen minimum layer. The ETP oxygen minimum layer is remarkable for its size and degree of hypoxia (Fiedler and Talley, 2006).

The isotopic data for yellowfin tuna also display a general south–north trend in the $\delta^{15}N$ values of their white muscle (Fig. 2a). This broad-scale consistency (Fig. 3) among components of the food web that are separated by several trophic levels suggests that (1) spatially-explicit $\delta^{15}NO_3^-$ trends are conserved from the base of the food web into the upper-trophic levels and (2) yellowfin tuna, a highly-active fish (Brill, 1996), appear to move only a limited amount in the region (Hunter et al., 1986; Deriso et al., 1991; Popp et al., 2007; Schaefer et al., 2007). To be reflected in the tissues of upper-level predators, residency of predators and prey is required for spatial trends in $\delta^{15}N$ values to propagate up the food web (Section 4.3).

We focus, however, not on the consistencies but on the differences between yellowfin tuna and copepod $\delta^{15}N$ patterns. A GAM describing the spatial structure of omnivorous copepod $\delta^{15}N$ data afforded us a means to examine the departures of yellowfin tuna $\delta^{15}N$ values from the basal isotopic variability, under our working hypothesis that the $\delta^{15}N$ spatial distribution of omnivorous copepods provides a proxy for the isotope variability at the base of the food web. The mathematical difference between the measured $\delta^{15}N$ value of each yellowfin tuna sample and the GAM-estimated $\delta^{15}N$ value of omnivorous copepods at the yellowfin sampling locations ($\Delta_{YFT\text{-}COP}$) showed an increasing onshore–offshore, east–west gradient. We explored four plausible mechanisms that might explain this east to west gradient: spatial differences in yellowfin tuna size, seasonal isotopic variability at the base of the food web, yellowfin tuna movement patterns, and spatial differences in diet composition. The $\Delta_{YFT\text{-}COP}$ spatial pattern is unlikely to be due to errors in the copepod GAM surface because there was no significant correlation between the yellowfin $\delta^{15}N$ residuals from the multiple linear regression model (Section 3.2) and the standard errors of the GAM-predicted copepod $\delta^{15}N$ values (Spearman rank correlation coefficient = 0.06, $p = 0.66$). The $\Delta_{YFT\text{-}COP}$ spatial pattern is also unlikely due to different species-specific spatial distributions of copepod $\delta^{15}N$ values. López-Ibarra (2008) presented maps of the $\delta^{15}N$ values for the six most abundant copepod species in our samples, and none showed an east–west gradient.

4.1. Yellowfin tuna size

The yellowfin tuna sampled for this study spanned a considerable size range (46–129 cm FL). Larger consumers often have higher $\delta^{15}N$ values than smaller individuals because they can eat larger prey with higher trophic status (Jennings et al., 2002; Estrada et al., 2003; Sarà and Sarà, 2007). Our data, however, showed that yellowfin tuna length was not as useful a predictor of the $\delta^{15}N$ values

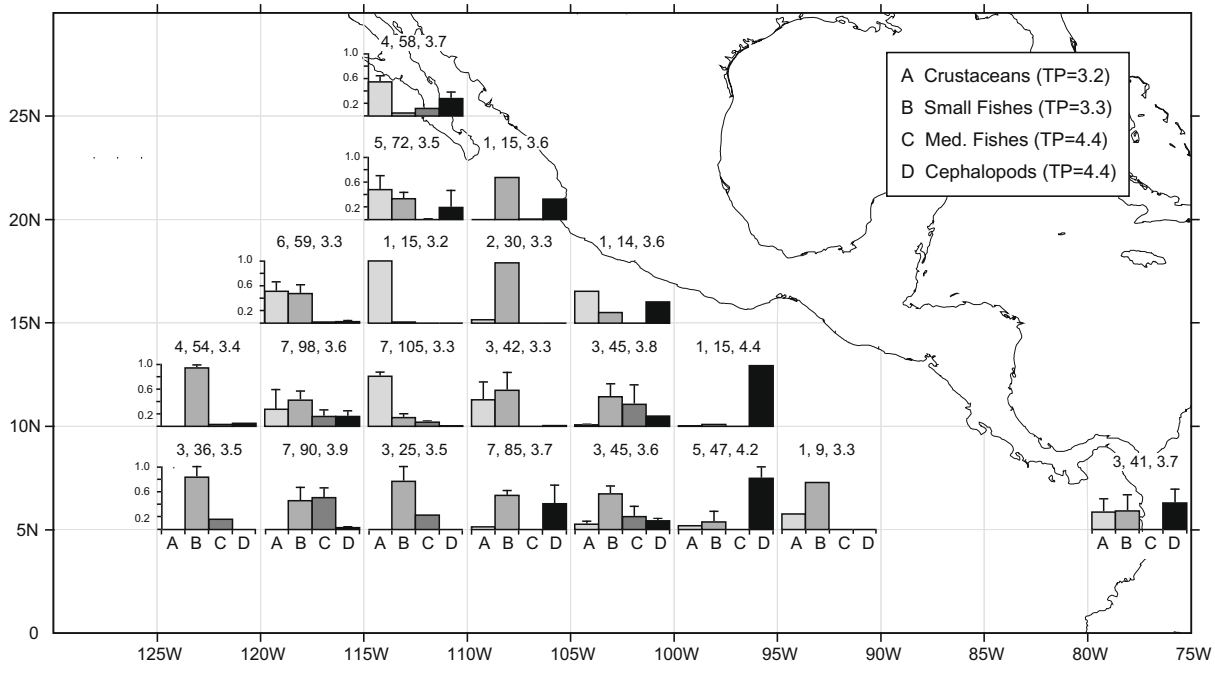

Fig. 7. Proportional composition by weight of yellowfin tuna stomach contents pooled by 5-deg areas in the ETP. The nominal average trophic position (TP) for four prey groups from Olson and Watters (2003) are in the legend. The numbers in each 5-deg area are number of purse-seine sets, number of stomach samples, and weighted-average TP estimate of the stomach contents. Error bars represent SE.

($p = 0.07$) as were the copepod $\delta^{15}N$ values ($p < 0.01$). Furthermore, the large fish were not caught further offshore than the smaller fish in our study (Fig. 2b). It is clear that the spatial distribution of small and large yellowfin in our samples did not explain the onshore–offshore gradient in $\Delta_{YFT\text{-}COP}$ values.

Ménard et al. (2007) also found low variability in the $\delta^{15}N$ values of yellowfin tuna over a FL range of 39–164 cm in the western Indian Ocean. Sarà and Sarà (2007), however, found increasingly higher $\delta^{15}N$ values with age in Atlantic bluefin tuna (*Thunnus thynnus*), up to 13-year-old fish weighing 225 kg. Atlantic bluefin live longer and reach larger maximum sizes than do yellowfin tuna. Graham et al. (2007) described trophic ontogeny in small, 40-cm, yellowfin tuna in nearshore waters around Oahu, Hawaii, based on $\delta^{15}N$ values and stomach contents. Marked trophic ontogeny is not characteristic of yellowfin tuna in general (King and Ikehara, 1956; Alverson, 1963; Olson and Boggs, 1986; Buckley and Miller, 1994; Ménard et al., 2006), except for very small individuals (Maldeniya, 1996). The diet data analyzed in this study did not contain evidence of trophic ontogeny, but our smallest sample was 46 cm in fork length.

4.2. Temporal effects

The primary producers that support marine food webs typically have $\delta^{15}N$ values that change not only spatially, as indicated by our copepod isotopic data, but also seasonally (Dugdale and Goering, 1967; Cline and Kaplan, 1975; Altabet, 2001; reviewed by Popp et al. (2007)). The stable-isotopic composition of organisms depends on the time scales over which the isotope tracers are integrated into the organisms, and temporal integration is an especially important consideration when comparing $\delta^{15}N$ values of organisms with different trophic status (O'Reilly et al., 2002). Seasonal and inter-annual physical variability affect different components of the food web at different time scales.

Omnivorous copepods in our study were sampled during a 4-month interval in the second half of 2003 (Table 1) while the yellowfin tuna chosen for isotopic analysis were sampled over a 13-month period in 2003–2004 (Table 2), and most of the $\Delta_{YFT\text{-}COP}$ values for yellowfin tuna sampled during the first half of 2004 were slightly lower than those during the second half of 2003 and 2004. Our amino-acid isotopic data indicated that seasonal differences in phytoplankton were not important. The possibility remains, however, of seasonally specific feeding patterns of copepods, with a greater proportion of their diet acquired by predation than by grazing during the first half of the year. Seasonal diet switching has not been demonstrated for the tropical Pacific. It is noteworthy that the $TP_{ISOTOPES}$ estimates for fish sampled in the first half (4.5) and the second half of the year (4.7) converge by increasing TP_{COP} in Eq. (1) from 2.5 to 2.8 for only the fish sampled in semester 1.

Vargas et al. (2006) provided evidence for seasonal changes in copepod feeding behavior in a temperate region in the southern hemisphere (36°S). Copepods collected in a bay off central Chile alternated between diets of mostly dinoflagellates and ciliates in autumn and winter and diatoms during the spring and summer upwelling period. Seasonal diet switching would be expected to change the $\delta^{15}N$ values of copepods, but this has not been demonstrated for the tropical Pacific. We encourage future research to tease apart the complex effects of seasonal variability in the baseline $\delta^{15}N$ values of the pelagic food web from the trophic effects on the $\delta^{15}N$ values of key ecosystem components.

4.3. Movement effects

The foraging histories of mobile animals and their $\delta^{15}N$ values can be partly influenced by recent movements. Whether recent movements are pertinent to the stable-isotopic composition of animals is determined by the temporal scale of their tissue turnover due to metabolism and growth. If their movements over a pertinent period of time includes areas that have markedly different $\delta^{15}N$ values at the base of the food web compared to the area where they were sampled, then the $\delta^{15}N$ values of their body tissues will reflect some integrated value of the different areas. Teasing apart the relative influence of movements versus trophic dynamics on stable-isotope values of a mobile animal is a challenge for making ecological inferences from stable isotope data (Graham, 2008).

As previously noted, the yellowfin tuna $\delta^{15}N$ values followed the spatial trends of $\delta^{15}N$ values near the base of the food web, which in general, implies a level of residency. In theory, if yellowfin tuna mixed completely and foraged over the entire study area (Fig. 1), within a time period determined by tissue turnover rates, they would all experience the same isotopic baseline, which is approximated as the mean $\delta^{15}N$ value over the area. Yellowfin tuna clearly move much less in the ETP (Hunter et al., 1986; Deriso et al., 1991; Schaefer et al., 2007). The effect of recent movements on $\delta^{15}N$ values of a fish is greater and more variable in areas where the baseline is spatially heterogeneous compared to regions where it changes smoothly and gradually (e.g. Fig. 1). By means of the following calculations, we address whether published "home range" estimates for yellowfin tuna are large enough in relation to the isotopic baseline variability to have had a role in determining the $\Delta_{YFT\text{-}COP}$ pattern (Fig. 5b).

Schaefer et al. (2007) presented estimates of 95% utilization distributions for 12 yellowfin tuna that had shown non-random movement histories during more than 154 days at liberty. The utilization distribution (UD) is based on a bivariate probability density function that estimates the probability of finding an animal at a particular location on a plane (Anderson, 1982). The 95% UD estimated for yellowfin tuna is the area encompassed by the 95% probability contour, derived from tagging data, over which the animal likely moved during the time at liberty. We estimated the movement history (hereafter, the "ambit") of yellowfin tuna (FL 67–130 cm) that were at liberty for the maximum period of time during which isotope values of the diet eaten throughout the entire 95% UD would be represented in the $\delta^{15}N$ values of the white muscle. Graham (2008) derived estimates of the nitrogen isotope half-life of white-muscle tissue of juvenile, fast-growing yellowfin tuna. The best estimate of the half-life of yellowfin white muscle is 37 days, which is equivalent to 94% turnover in 148 days (~5 months). We estimated a UD of 129,650 km^2 by fitting a linear model to these UD data versus days at liberty (DAL; UD = 1722 DAL −135,539, $R^2 = 0.44$). We used DAL = 154 days because Schaefer et al. (2007) did not estimate UD for fish at liberty less than 154 days. We then recalculated the $\Delta_{YFT\text{-}COP}$ values as: measured $\delta^{15}N$ value of each yellowfin tuna at the capture location minus the mean $\delta^{15}N$ value of omnivorous copepods over twice the yellowfin tuna ambit around the capture location, estimated from the copepod GAM surface. We doubled the yellowfin tuna ambit estimate because a fish sampled for stable-isotope analysis could have been at the edge of its ambit when captured.

In essence, we have adjusted the isotopic increment represented in yellowfin tuna muscle to account for the background variability at the base of the food web encountered during their likely ambit over the past 154 days. After making this adjustment, a similar substantial spatial pattern in the $\Delta_{YFT\text{-}COP}$ values remains (Fig. 5c). In Schaefer et al.'s (2007) study, however, the data were from yellowfin tuna in coastal areas, and fish further offshore may disperse more. We explored the effect of a larger ambit (UD = 763,280 km^2) using archival tag data for 17 bigeye tuna (*Thunnus obesus*) at liberty for 154 days (Schaefer and Fuller, 2002), based on our same calculations for yellowfin tuna, and a

substantial spatial gradient in $\Delta_{YFT\text{-}COP}$ values remained (Fig. 5d). This suggests that the movements of yellowfin tuna that we sampled would have been larger than typical movement rates of yellowfin and bigeye tunas recorded in the ETP, in order to have influenced the onshore–offshore spatial gradient in $\Delta_{YFT\text{-}COP}$ values. While this simple analysis does not preclude the possibility that some of the yellowfin tuna we sampled had made extensive migrations prior to being captured, it seems highly unlikely that the consistently higher $\Delta_{YFT\text{-}COP}$ values in yellowfin tuna offshore were due to long-range movements from another region with higher $\delta^{15}N$ values. Long-range movements by yellowfin tuna are thought to be rare events (Hunter et al., 1986; Deriso et al., 1991; Sibert and Hampton, 2003).

Our assertion that yellowfin tuna showed considerable residency in the ETP, within the time scale of muscle turnover, is corroborated by a study in the western Indian Ocean. Ménard et al. (2007) found a significant effect of latitude on the $\delta^{15}N$ values of yellowfin tuna, and concluded that yellowfin tuna is a relatively resident species at the time scale of tissue isotopic turnover. Yellowfin tuna are not considered highly migratory, whereas other tunas [e.g. bluefin tunas (*Thunnus thynnus*, *T. orientalis*, and *T. maccoyii*), and albacore (*T. alalunga*)] do exhibit migratory behavior. Estrada et al. (2005) found evidence of isotopic variability due to migrations in Atlantic bluefin tuna. Similarly, Das et al. (2000) explained isotopic differences in co-occurring albacore tuna, striped dolphins (*Stenella coeruleoalba*), and common dolphins (*Delphinus delphis*) by the strong migration cycle of albacore tuna.

4.4. Trophic structure

The results of our study point to small but consistent zonal differences in the trophic position of yellowfin tuna in the ETP, increasing in an offshore direction proportional to the gradient of $\Delta_{YFT\text{-}COP}$ (Fig. 5b) and indicative of increasing food chain length (defined here as maximum number of trophic levels). Having considered several factors that can influence the stable-isotope values of yellowfin tuna and omnivorous copepods, variability in trophic relations is the most parsimonious and robust interpretation of these stable isotope data. The CSIA of trophic and source amino acids corroborated this gradient in TP. Similarly, resident groups of killer whales (*Orcinus orca*) showed a west (central Aleutian Islands) to east (Gulf of Alaska) gradient in stable-isotope values, due to differences in prey taxa (Krahn et al., 2007).

Variability in food chain length can result from processes both at the base of the food web and by differences in foraging by predators. In oligotrophic waters, the dominant phytoplankton taxa typically comprise very small forms, more trophic steps are required between primary producers and predatory fishes, and longer food chains (i.e. higher TP of apex predators) can result (Seki and Polovina, 2001). Large diatoms dominate in nutrient-rich areas, however, and are directly fed upon by large zooplankton or planktivorous fishes, creating shorter food chains (Seki and Polovina, 2001). Inshore upwelling areas in the ETP are more productive than offshore areas (Fiedler and Talley, 2006; Pennington et al., 2006), and east–west differences in food chain length could explain a higher TP for yellowfin tuna offshore. Our results are consistent with this mechanism because, although the $\delta^{15}N$ values of copepods do not increase offshore, the glycine $\delta^{15}N$ values decreased from east to west, indicating lower bulk $\delta^{15}N$ values of primary producers offshore and therefore implying higher copepod TP. Longer food chains in the offshore ETP can also be related to trophic differences above the macrozooplankton level.

Stomach-contents data in our study were spatially variable and did not show a spatial gradient in TP. This result is not surprising because stomach contents represent only a limited depiction of the diet due to the sample comprising only the most-recent, several hours of feeding (for gastric evacuation rates see Olson and Boggs (1986)) on a diverse prey base, while the stable-isotope values of a wide-ranging, opportunistic predator can provide a record of the assimilated diet and movement history during the previous 4–5 months (Graham, 2008). Furthermore, tuna stomach samples are collected only during the daytime and do not represent feeding at night. Despite the different time scales of feeding recorded by isotopes and stomach contents, the mean TP estimates of yellowfin tuna based on bulk stable-isotope values were not significantly different than those based on diet data, and the variability of the estimates derived from both methods was similar (see Section 3.4.1). The mean TP estimates are comparable to those estimated by other methods. Popp et al. (2007) were the first to perform CSIA of amino acids on tunas. They computed the weighted mean difference between the $\delta^{15}N$ of glutamic acid and glycine, using the same trophic-enrichment assumption as our Eq. (3). The TP estimates from CSIA averaged 4.5 ± 0.1 SD (Popp et al., 2007) and 5.1 ± 0.05 SE (this study). The higher amino-acid TP estimates from this study are not surprising because the samples we analyzed included those with the highest bulk $TP_{ISOTOPES}$ offshore (Fig. 2a), while those analyzed by Popp et al. (2007) were inshore and oriented north–south. TP 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). All the above TP estimates for the ETP are higher than those based on Sibert et al.'s (2006) model relating TP to length of yellowfin tuna in the Western and Central Pacific Ocean (WCPO); approximately 4.1–4.3, for fish of the size in our study. This is likely due to the WCPO ecosystem model omitting the microbial loop, resulting in TP estimates about 0.5 trophic levels lower than estimates of the ETP model that contains two producer groups (see Hinke et al. (2004) for comparisons of both models).

Caveats apply to each of the different methods of estimating TP that we have considered. The $TP_{ISOTOPES}$ estimates are influenced by the TP_{COP} and *TEF* estimates (Eq. (1)). TP_{COP} is a function of the relative amounts of grazing on primary producers and predation on micrograzers by omnivorous copepods, which is variable in the ETP (Fernández-Álamo and Färber-Lorda, 2006). Nitrogen utilization estimates, based on Chai et al.'s (2002) nutrient–phytoplankton–zooplankton–detritus model for the eastern equatorial Pacific, were used by Olson and Watters (2003) to derive diet proportions for mesozooplankton of 0.7 microzooplankton and 0.3 diatoms, (i.e. a TP of 2.7). Dam et al. (1995) reported up to 80% predation rates by mesozooplankton in the equatorial central Pacific (Fernández-Álamo and Färber-Lorda, 2006). Mesozooplankton assemblages, however, contain highly carnivorous taxa (e.g. chaetognaths) as well as omnivores, justifying our lower TP_{COP} estimate of 2.5 for omnivorous copepods.

The *TEF* value applied in Eq. (1) is based on a mean *TEF* of 2.4 for marine fishes. Vanderklift and Ponsard (2003) reviewed an extensive body of literature that reported consumer-diet ^{15}N enrichment in a variety of consumers that eat a variety of taxa and found patterns related to the biochemical form of nitrogen excretion, nutritional status, and marine versus terrestrial and freshwater habitats. In general, *TEF*s for fishes ranged between 1.5 and 3.2, and ammonotelic, carnivorous, marine fishes had low *TEF*s. Caut et al.'s (2009) literature review also found low nitrogen *TEF* values for muscle tissue in fishes, and the value we used is within confidence limits of their findings. Given that trophic pathways from copepods to yellowfin tuna pass through small fishes, cephalopods, and crustaceans, further support for a low *TEF* is provided by Vanderklift and Ponsard's (2003) mean estimates for invertebrate carnivores (approximately 2.0) and crustaceans (2.0). It is worth noting that convergence of the diet-based TP_{YFT} and $TP_{ISOTOPES}$ estimates in our data requires a *TEF* of only 2.5, well within the results of Vanderklift and Ponsard (2003) and Caut et al. (2009).

Similar uncertainty exists in the *TEF* used for the amino acid CSIA approach to estimate trophic position. Only one experimental determination of the isotopic fractionation between glutamic acid and glycine exists (McClelland and Montoya, 2002). On the other hand, a recent compilation of TPs based on results of amino acid CSIA, assuming a 7‰ difference between source and trophic amino acids, showed remarkable consistency with expectations regarding the pelagic marine ecosystem structure (Hannides et al., 2009). Further evaluation of this CSIA technique with more field and laboratory predator and prey tests will be necessary, however, before we can fully evaluate the utility of this method.

In computing the weighted-average TPs of the prey in the stomach contents, we categorized prey taxa by previous estimates of their TPs using diet data for a variety of predators sampled during 1992–1994 in the pelagic ETP (Olson and Watters, 2003). The 1992–1994 TP estimates could be different in 2003–2004 due to ecosystem and climate changes that might have taken place in the intervening decade.

Species diversity of forage taxa is generally greater in areas influenced by land masses than in open-ocean areas devoid of terrestrial discontinuities. Inshore areas in the ETP are more productive than offshore areas (Pennington et al., 2006), and ichthyoplankton assemblages indicate a more diverse prey base at more eastern longitudes (Vilchis et al., 2009). The thermocline and mixed layer are shallower inshore than offshore (Fiedler and Talley, 2006), and the volume of epipelagic habitat is proportional to the depth of the thermocline. Yellowfin tuna depend primarily on epipelagic and vertically migrating mesopelagic forage (Alverson, 1963; Olson and Boggs, 1986; Bertrand et al., 2002; Ménard and Marchal, 2003; Potier et al., 2007). Thus, a reduced habitat volume inshore concentrates a more diverse forage assemblage and lower-TP prey may be more available to epipelagic predators than offshore, where *Auxis* spp. dominate the diet (Galván-Magaña, 1999). Vilchis et al.'s (2009) analysis of ichthyoplankton assemblages in the eastern Pacific warm pool revealed a longitudinal gradient in abundance, species diversity, and species richness, increasing toward the east.

Whereas the stomach contents of a wide-ranging, opportunistic predator may not mirror persistent trophic interactions in a given region, stomach-contents analysis based on large sample sizes over a large spatial range is an important tool for trophic ecology studies. Our diet data summarized the food habits of 1000 yellowfin tuna sampled over more than a 2-year period. These data provide the taxonomic information required to define trophic links in ecosystem models (e.g. Cox et al., 2002; Olson and Watters, 2003), while isotope data may provide better estimates of biomass flow. Diet proportions estimated by isotopic mixing models (Phillips, 2001) are ideal, but obtaining isotope data for prey taxa is expensive and labor intensive. As an alternative, the diet proportions from stomach-contents analysis can be adjusted to match the $TP_{ISOTOPES}$ estimates, providing a more comprehensive view of trophic interactions than from stomach contents alone. We recommend an increased research emphasis on prey populations at middle trophic levels, focusing on both stable-isotope analysis and direct sampling (e.g. Vilchis et al., 2009), as well as sampling immediately following strong El Niño events.

In conclusion, we reiterate Murawski's (2000) counsel for additional ecosystem monitoring and research, with increased emphasis on species interactions and ecosystem diversity and variability at a variety of temporal and spatial scales. Appreciating ecosystem diversity, impacts on forage and ecologically dependent species, and indicators of trophic position (Gislason et al., 2000) requires an understanding of the trophodynamics of food webs (Marasco et al., 2007). Our study illustrates the utility of concurrent stable-isotope and stomach-contents analyses on key ecosystem components (Jennings and Kaiser, 1998), and may provide a standard approach for further analyses of the ecological effects of climate variability, climate change, and fisheries on trophic pathways.

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