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Compound-specific isotopic analysis of amino acids reveals dietary changes in mesophotic coral-reef fish

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ABSTRACT: Shallow-water (<30 m) coral-reef fishes are under threat from multiple environmental and anthropogenic impacts. Typically spatially removed from such impacts, mesophotic coral ecosystems (MCEs) at intermediate depths (30–150 m) may serve as refugia for these fishes. In the main Hawaiian Islands, efforts to protect and manage coral-reef fisheries are underway. However, the inclusion of MCEs within management plans has been limited by a lack of information about the trophic structure of resident fish communities. Because of physical environment changes associated with increasing depth, we hypothesized that the diets of shallow and mesophotic fish would differ, with mesophotic fish relying less on benthic macroalgae and more on higher trophic-level prey. To address these possible ecological dietary changes, we examined the bulk tissue nitrogen and carbon isotopic compositions of 319 samples of reef fish from shallow and mesophotic depths, further analyzing the amino acid nitrogen isotopic composition of 81 samples. Significant differences were found in the bulk isotopic compositions of omnivores and benthic invertivores, whereas planktivores showed overlap between depths. Results of compound-specific isotopic analyses of amino acids indicated slightly but significantly higher trophic positions of mesophotic benthic invertivores compared to shallow-water members. Ecosystem models need to reflect the differences in food webs between shallow and mesophotic habitats, and results here place important constraints on outputs and assumptions for Hawaiian coral-reef habitats and potentially other reef tracts. Our results will allow better understanding of mesophotic fish ecology and stress the importance of MCE research in understanding trophic connectivity.

KEY WORDS: Mesophotic · Coral reef fishes · Amino acids · ^{15}N · Trophic ecology · Isotope analysis · Marine food webs

INTRODUCTION

Mesophotic coral ecosystems (MCEs) are reefs with light-dependent corals found in depths of approximately 30 to 150 m. At the upper bound, these depth limits reflect changes in coral species composition, while the lower bounds are set by the deepest records of hermatypic corals (Hinderstein et al. 2010). Interest in understanding MCEs has gained much attention in the last 2 decades (Glynn 1996, Pyle 1996, 2000, Riegl & Piller 2003, Lesser et al. 2009, Bongaerts et al. 2010, Hinderstein et al. 2010). The depth and often remote nature of MCEs may provide residents a buffer from anthropogenic and climatic influences (Glynn 1996), but may also create a separation of mesophotic and shallow populations through physical distance.

Despite increased interest in MCEs, there remains a dearth of scientific information on the ecology of these communities, especially when compared to

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studies of shallow reefs (Menza et al. 2008). This lack of knowledge is even greater for Hawaiian MCEs. In the main Hawaiian Islands where MCEs are present (Mauʻi, Lanaʻi, Oʻahu, and Kauaʻi), large, dense reefs of *Leptoseris* spp. corals are separated from one another and from shallow reef ecosystems by several kilometers of sandy patches and macroalgal fields (Rooney et al. 2010). Despite awareness of these expansive mesophotic reefs, the handful of studies that exist mainly focus on the lucrative black coral industry (Grigg et al. 2002, Boland & Parrish 2005, Grigg 2006, Kahng & Kelley 2007, Chan et al. 2009).

While recent focus is closing many of the critical gaps in our knowledge of MCEs, a clear picture of the trophic structure of resident fishes has remained elusive (Lesser et al. 2009). The few descriptions of ichthyofauna within MCEs show a tremendous overlap in species with shallow reef faunas (Boland & Parrish 2005, Feitoza et al. 2005, Brokovich et al. 2007). Despite this, there are distinct differences in community structure between shallow reefs and MCEs (Feitoza et al. 2005, Brokovich et al. 2010, Garcia-Sais 2010). Much effort has focused on observed decreases in herbivore abundance with depth, which has been attributed not to the obvious light limitation, but rather to undocumented differences in nutritional ecology of herbivorous fish in MCEs that may be caused by altered nutritional value and palatability of the deep-growing algae (Brokovich et al. 2010). A similar case can be made for invertebrate-feeding fish, whose prey items may also show different nutritional ecology in response to changing food sources. While changes in physical parameters with increasing depth are unlikely to impact planktivorous fish, documented increases in their abundance in mesophotic reefs (Feitoza et al. 2005) indicate that more information on trophic relationships of these fish is needed.

Current models of biomass flow in MCEs use descriptions of diet and feeding habits from shallow water (<30 m) representatives (Parrish et al. 2012), because similar fish species populate both *Leptoseris* reefs at mesophotic depths and *Montipora*- and other coral-dominated reefs at shallow depths. However, if nutritional ecology changes with depth, model assumptions of trophic similarity would be violated, potentially impacting accuracy of model results. Such trophic plasticity in fishes can have major impacts on biomass flows (Pinnegar & Polunin 1999, Hernández-León et al. 2010, Bollens et al. 2011). If shallow (<30 m) and mesophotic (>30 m) habitats are combined for the purposes of developing management tools in an effort to preserve and protect coral-reef ecosystems, there is a clear need for a better understanding of the trophic structure and nutrient flow in MCEs in order to determine if assumptions of management models are accurate.

Stable isotope analyses are one of several available tools used to trace nutrient flow through food webs or ecosystems. $\delta^{13}C$ values of metazoans can reflect the isotopic composition of primary producers from different habitats (Post 2002). Calculations of trophic position can be made from isotopic analysis of nitrogen in bulk tissue or whole organisms, but require knowledge of baseline isotopic values to correct for spatial and temporal variations in the isotopic composition of nutrients, such as NO_3, NO_2, and NH_4, which are propagated through the food web. Stomach content analysis (SCA) is useful in its ability to accurately identify and inventory prey items, but is limited by knowledge of the prey items' precise trophic positions as well as variable digestion of prey types (Hyslop 1980, Cortés 1997).

Results of compound-specific isotopic analysis (CSIA), in particular that of amino acids (AA-CSIA), can elucidate information about both nutrient sources and trophic interactions from a single organism (McClelland & Montoya 2002, Chikaraishi et al. 2007, 2009, Popp et al. 2007, Hannides et al. 2009, Lorrain et al. 2009). Nitrogen isotopic analyses reveal 2 groups of amino acids (AAs): 'source' AAs that appear to retain the $\delta^{15}N$ values of the primary source of nitrogen (McClelland & Montoya 2002), and 'trophic' AAs that fractionate consistently with each trophic step (McClelland & Montoya 2002, Chikaraishi et al. 2007, 2009, Popp et al. 2007, Hannides et al. 2009, Lorrain et al. 2009).

Results of AA-CSIA allow information about ecosystem interactions to be gathered from discrete samples of targeted species without the need for exhaustive isotopic analysis of primary producers or lower trophic position organisms typically associated with the use of bulk tissue isotopic analysis and SCA. Combined with information from $\delta^{13}C$ and $\delta^{15}N$ values of bulk muscle tissue, comparisons of nutrient flow and trophic interactions can be made between ecosystems. The goal of this study was to compare nutrient sources and trophic levels of fish feeding guilds occurring in both mesophotic and shallow coral reef habitats. Because of a changing physical environment due to increasing depth, we hypothesized that the diets of shallow and mesophotic fish would differ, with mesophotic fish relying less on benthic macroalgae and more on higher trophic-level prey items. We used bulk tissue isotopic analysis and AA-CSIA to determine the extent of changes,

from baseline to trophic level, within commonly used feeding-guild groupings of fish communities at mesophotic depths in the Au'au channel (60–140 m) near Mau'i, Hawai'i and off the southern shore of O'ahu, Hawai'i in comparison to shallow (<30 m) communities in both areas.

MATERIALS AND METHODS

Fish collection

Over 750 fish specimens were opportunistically collected from sites along the coasts and offshore of Mau'i and O'ahu in the main Hawaiian Islands (Table 1; for a description of locations, see Costa et al. 2015). Fishes were collected from shallow depths (<30 m) using Hawaiian sling spears, monofilament nets, and spear guns by divers using closed-circuit rebreathers (CCR). At depths >50 m, manned submersibles used the non-selective ichthyocide, rotenone, to collect cryptic species, and CCR divers used spears to catch conspicuous species. The selection of depth definitions for shallow and mesophotic ecosystems was based upon previous definitions for MCE habitats (Lesser et al. 2009, Hinderstein et al. 2010, Rooney et al. 2010); however, sampling was influenced by coral reef distributions. The majority of the shallow fish collections, both on Mau'i and O'ahu, were made between 10 and 20 m as a result of the vast expanses of reef ecosystems in this depth range. MCE specimens were mainly collected in the Au'au channel in reefs of *Leptoseris hawaiiensis* at depths ranging from 90–120 m. However, fishes from O'ahu mesophotic depths were collected on *Leptoseris* reefs only from 50–70 m depth.

Table 1. Locations of fish collections (2009–2011)

Latitude (°N)	Longitude (°W)	Depth (m)
O'ahu		
21° 16.910	157° 40.081	20
21° 17.088	157° 52.099	22
21° 17.195	157° 51.934	22
21° 15.641	157° 50.246	25
21° 16.805	157° 51.579	31
21° 15.330	157° 50.040	33
21° 15.106	157° 49.796	55
21° 17.257	157° 59.717	55
21° 14.574	157° 46.114	66
21° 16.065	157° 50.918	66
Mau'i		
20° 47.162	156° 33.592	22
20° 47.093	156° 33.525	28
20° 52.515	156° 45.747	35
20° 52.722	156° 46.046	50
20° 53.170	156° 43.603	50
20° 52.610	156° 43.683	50
20° 52.947	156° 43.782	55
20° 46.575	156° 40.335	89
20° 45.462	156° 40.740	102
20° 45.479	156° 40.729	104
20° 46.414	156° 40.295	109
20° 45.509	156° 40.750	110
20° 46.622	156° 40.964	112
20° 45.611	156° 40.710	113
20° 45.611	156° 40.929	114
20° 45.600	156° 40.992	115
20° 45.513	156° 40.827	116

Once divers or submersibles returned to the surface, specimens were immediately packed in ice for transport to the lab. Each fish was identified to species, excluding undescribed species, which were designated 'nsp'. Species were grouped into appropriate feeding guilds using results of published SCA and observational studies (Randall 1967, 1985, 1998, Hobson 1974, Harmelin-Vivien 1979, Sano et al. 1984, Lieske & Myers 1994, Piché et al. 2010). All specimens were weighed and measured (total, fork, and standard lengths). Muscle tissue was biopsied from just below the second dorsal spine for isotopic analysis and frozen at –20°C.

Preparation of tissue for isotopic analysis

Frozen biopsied tissue was dried at 60°C for 24–48 h. Dried tissue was ground using a mortar and pestle, after which approximately 0.5 mg was used for bulk tissue isotopic analysis and ~10 mg for compound-specific isotopic analysis.

Muscle tissue was prepared for AA-CSIA using established methods (Popp et al. 2007, Hannides et al. 2009). Dried, ground tissue was weighed into acid-rinsed, pre-combusted vials and subsequently hydrolyzed (6 N HCl, 150°C for 70 min). These conditions convert glutamate and aspartate to glutamic and aspartic acid, and destroy tryptophan and cystine. After drying, the hydrolysate was redissolved (0.01 N HCl), filtered (0.45 µm hydrophilic filter), and AAs separated from sugars and organic acids using cation exchange chromatography (Dowex 50WX8-400). An AA fraction was eluted using 4 ml of 2 N NH_4OH and after being evaporated to dryness, was esterified (4:1 isopropanol:acetyl chloride, 110°C for 60 min) and finally acylated (3:1 methylene chloride: trifluoracetic anhydride, TFAA, 100°C for 15 min). Samples were evaporated to dryness and then further purified by solvent extraction (Ueda et al. 1989). A final acylation step was repeated and samples

were stored at −20°C in 3:1 methylene chloride: TFAA for up to 6 mo before isotope analysis.

Isotope analysis

Bulk tissue stable isotope analyses were performed using an on-line carbon-nitrogen analyzer coupled with an isotope ratio mass spectrometer. Isotope ratios are reported as δ-values relative to the international standards VPDB or AIR by:

$$\delta Z = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1\right) \times 1000 \quad (1)$$

where δZ is either $\delta^{13}C$ or $\delta^{15}N$, R_{sample} is the ratio of rare to common isotope within the sample, and R_{standard} is the isotope standard. Isotope values are reported in per mille (‰). Accuracy was checked by well-characterized reference samples of glycine and ground-tuna, which were analyzed after every ~10 samples. Additionally, sample duplicates were analyzed on 1–2 samples per species (~15% duplicates). The standard deviation of duplicates analyzed was ≤0.1‰ for carbon and nitrogen isotope analysis.

The $\delta^{15}N$ values of derivatized AA samples were determined using a Delta V 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. Samples were analyzed in triplicate and normalized to the known $\delta^{15}N$ values of internal references norleucine and aminoadipic acid. Samples were injected (split-splitless injector, either splitless or using 10:1 split ratio) onto a *forte* BPx5 capillary column (either 30 m × 0.32 mm × 1.0 µm or 60 m × 0.32 mm × 1.0 µm film thickness) at an injector temperature of 180°C with a constant helium flow rate of 1.4 ml min^{-1}. The column was initially held at 50°C for 2 min and then increased to 190°C at a rate of 8°C min^{-1}. Once at 190°C, the temperature was increased at a rate of 10°C min^{-1} to 300°C where it was held for 7.5 min. The average standard deviation of measured $\delta^{15}N$ values of AAs based on multiple (at least triplicate) analyses was 0.57‰ and ranged from 0.03 to 2.1‰.

This method yields values for 16 AAs: alanine (Ala), glycine (Gly), threonine (Thr), serine (Ser), valine (Val), leucine (Leu), isoleucine (Ile), proline (Pro), aspartic acid (Asp), methionine (Met), glutamic acid (Glu), phenylalanine (Phe), tyrosine (Tyr), lysine (Lys), arginine (Arg), and histidine (His). Of those, 7 AAs were chosen for use in further analyses. Three trophic AAs (Glu, Ala, and Leu) and 3 source AAs (Phe, Gly, and Lys) were chosen due to their prevalence in previous literature as reliable representatives from each group (see Bradley et al. 2015). Thr was also chosen as it has shown unique ^{15}N depletion relative to all other AAs (Hare et al. 1991, Sherwood et al. 2011) across trophic levels, but does not conform to the definition of trophic or source AA.

Calculation of trophic position

Trophic position (TP) was calculated from AA-CSIA using a method modified after Chikaraishi et al. (2009):

$$\text{TP}_{\text{trp–src}} = \left(\frac{\delta^{15}\text{N}_{\text{trp}} - \delta^{15}\text{N}_{\text{src}} - \beta}{\text{TEF}}\right) + 1 \quad (2)$$

where $\delta^{15}N_{trp}$ and $\delta^{15}N_{src}$ are the nitrogen isotopic compositions of chosen trophic and source AAs, respectively (Chikaraishi et al. 2007, 2009, 2010, Popp et al. 2007, Hannides et al. 2009, Lorrain et al. 2009, Dale et al. 2011, Choy et al. 2012). The difference in $\delta^{15}N$ values between the trophic and source AAs at the primary-producer trophic level is represented by β. TEF, the trophic enrichment factor, is the average ^{15}N enrichment per trophic level between trophic and source AAs.

Values for both β (2.7 ± 2.2‰) and TEF (7.6 ± 1.3‰) can be derived from the literature for the combination of Glu and Phe from feeding studies and wild samples (Chikaraishi et al. 2009, McCarthy et al. 2013). However, increasingly, unrealistic trophic level estimates have been derived (Dale et al. 2011, Choy et al. 2012, Bradley et al. 2015), suggesting values of β and TEF might be more variable than previously believed and that the use of multiple AAs may provide a more accurate estimate of trophic position (McCarthy et al. 2007, Sherwood et al. 2011, Décima et al. 2013). The difference between weighted mean isotopic values of trophic and source AAs eliminates the uncertainty in using TEF and β to calculate trophic position and gives a relative measure of trophic enrichment as a proxy to trophic level. Weighted mean ($\delta^{15}N_{\overline{x_w}}$) values for groups of AAs were calculated:

$$\delta^{15}N_{\overline{x_w}} = \frac{\sum \frac{\delta^{15}N_x}{\sqrt{\sigma_x^2}}}{\sum \frac{1}{\sqrt{\sigma_x^2}}} \quad (3)$$

where $\delta^{15}N_x$ is the nitrogen isotopic composition of a specified AA within the grouping, and σ_x is the standard deviation of the specific AA. Using 3 trophic AAs (Ala, Leu, Glu) and 3 source AAs (Gly, Lys, Phe), the difference in $\delta^{15}N$ values (Δ_{TS}) is calculated:

$$\Delta_{TS} = \delta^{15}N_{\overline{trp}} - \delta^{15}N_{\overline{src}} \quad (4)$$

where $\delta^{15}N_{\overline{trp}}$ and $\delta^{15}N_{\overline{src}}$ are the weighted mean values of trophic and source AA groups, respectively.

Given the uncertainty in TEF, all trophic position calculations including those using Glu and Phe and the difference between weighted means of trophic and source AAs are considered relative, but allow comparison of the trophic ecology of fish communities. The uncertainty in trophic position was estimated using propagation of measured analytical uncertainty and the variations in published estimates of TEF and β (Dale et al. 2011, Blum et al. 2013, Bradley et al. 2015).

Statistical analyses

Due to the opportunistic nature of sampling at mesophotic depths, low sample sizes during certain time periods and at some locations did not allow for a full comparative analysis of all factors contributing to isotopic variation. However, carbon and nitrogen isotopic compositions grouped by feeding guild and depth provide insight into the trophic ecology of resident fish communities. To better understand some of the factors affecting the trophic ecology of feeding guilds within the 2 depth ranges studied, we performed regression analyses on bulk nitrogen and carbon isotopic values as a function of fish length. ANCOVA tests were performed to compare the isotopic compositions of feeding guilds from mesophotic and shallow depths (independent variables) with standard length as a covariate, testing similarities of intercepts using a post hoc multiple comparison test. Where no significant differences between regression equations were found, groups were compared using a *t*-test (for normally distributed samples) or a Mann-Whitney *U*-test. Isotopic data that were not found to vary significantly between islands were pooled at the given depth range. Likewise, data that varied between islands, but not between depths, were pooled by island.

Principal component analyses (PCA) were used to compare differences in $\delta^{13}C$ and $\delta^{15}N$ values across species, feeding guilds, and depth locations. PCA were conducted on isotope values, all of which were normalized by their standard deviation:

$$N\delta z = \frac{\delta z}{\sigma} \quad (5)$$

$N\delta z$ is the normalized $\delta^{13}C$ or $\delta^{15}N$ value, δz is the measured isotope value, and σ is the standard deviation. This transformation was chosen to minimize the effect of large differences in $\delta^{15}N$ values of trophic AAs (up to 28‰) compared to bulk muscle tissue ($\delta^{13}C$: 9‰, $\delta^{15}N$: 6‰) and source AAs ($\delta^{15}N$: <15‰). All statistical analyses were performed using Matlab version R2010b or R2012b.

RESULTS

Collections

A total of 756 reef fish representing 45 species, 30 genera, and 18 families were collected off the coasts of Mauʻi and Oʻahu from 2009 to 2011. Of these, 22 species from 7 families were selected for further comparison (Table 2). These families were present and collected in both mesophotic and shallow reef communities. This resulted in 319 samples for bulk stable isotope analysis, 81 of which were chosen for AA-CSIA. Of the selected fishes, 21 species belonged to 2 feeding guilds (planktivores and invertivores) of approximately similar trophic position. The remaining species, *Centropyge potteri*, was included for not only its abundance in both depth ranges, but also as a representative omnivore, with distinct ties to the benthos (Piché et al. 2010).

Bulk tissue isotopic analysis

Carbon isotopic values of mesophotic and shallow fishes had similar ranges (−13.5 to −20.9‰ and −12.5 to −19.6‰, for mesophotic and shallow, respectively), but had different means (Table 3; *t*-test, $p < 0.05$ for both Oʻahu and Mauʻi). Bulk tissue $\delta^{15}N$ values ranged from 6.2 to 10.8‰ in mesophotic fish and from 6.8 to 10.6‰ in shallow reef fish (Table 3). Ranges of $\delta^{13}C$ and $\delta^{15}N$ values in planktivorous fish were smaller than those of benthic invertivores in both shallow and deeper communities (Table 3). *C. potteri* showed a greater range in carbon isotopic composition at shallow depths, and in $\delta^{15}N$ values at mesophotic depths.

The $\delta^{13}C$ values of the solitary omnivore in this study, *C. potteri*, were different at mesophotic and shallow depths (Fig. 1a). Results of a *t*-test revealed significantly lower $\delta^{13}C$ values for mesophotic individuals compared to shallow individuals ($p = 0.0007$; Table 3). Although there were a number of mesophotic *C. potteri* with high $\delta^{15}N$ values, the overall mean values were slightly but significantly higher in the shallow conspecifics (*t*-test, $p < 0.002$).

Table 2. Numbers of fish species (Mauʻi/Oʻahu), mean standard length (SL), and weight (wt), collected at 2 depth ranges in Auʻau Channel, Mauʻi, and south shore of Oʻahu, Hawaiʻi. I = invertivore, PK = planktivore, O = omnivore, na = no weight available

	Feeding guild	Mesophotic			Shallow		
		Sample size (Mauʻi/Oʻahu)	Mean SL (mm)	Mean wt (g)	Sample size (Mauʻi/Oʻahu)	Mean SL (mm)	Mean wt (g)
Apogonidae		**17/1**			**3/0**		
Apogon deetsie	I	3/0	68.3	10.0			
Apogon sp.	I				3/0	90.0	18.8
Pristiapogon kallopterus	I	6/0	106.3	42.6			
Ostorhinchus maculiferus	I	8/1	105.1	39.4			
Chaetodontidae		**5/23**			**12/37**		
Chaetodon miliaris	PK	2/15	114.6	67.7	6/25	108.7	62.1
Forcipiger flavissimus	I	0/7	132.3	43.6	4/12	120.9	25.8
F. longirostris	I	3/1	136.7	37.9	2/0	156.5	54.1
Holocentridae		**22/19**			**12/24**		
Myripristis berndti	PK	0/14	179.2	202.6	4/3	152.0	90.2
M. chryseres	PK	11/2	137.7	121.8			
M. kuntee	PK				0/5	121.8	71.1
Sargocentron diadema	I				3/3	113.2	na
S. ensifer	I	6/0	161.1	139.6			
S. xantherythrum	I	5/3	107.8	38.5	5/13	98.1	25.6
Labridae		**4/0**			**9/5**		
Pseudocheilinus evanidus	I	4/0	41.8	1.8	7/5	60.3	4.7
P. octotaenia	I				2/0	85.0	13.5
Mullidae		**12/1**			**7/7**		
Parupeneus multifasciatus	I	12/1	154.4	57.0	4/7	156.0	92.5
P. porphyreus	I				3/0	193.0	na
Pomacanthidae		**16/3**			**5/10**		
Centropyge potteri	O		82.2	31.9		67.7	18.7
Pomacentridae		**13/12**			**20/15**		
Chromis hanui	PK				2/0	55.5	9.0
C. lecura	PK	1/1	54.0	9.1			
C. verater	PK	6/11	133.6	106.8	4/7	126.3	110.4
Dascyllus albisella	PK	6/0	80.2	32.4	14/8	86.6	32.9

Table 3. Range and mean (±SD) values of ^{13}C and ^{15}N in bulk tissue isotopic analyses of fish from shallow and mesophotic depths from the Oʻahu south shore and Auʻau Channel, Mauʻi, Hawaiʻi. Values sharing the same superscript are significantly different pairs ($p < 0.05$): a: *t*-test, $p = 0.0007$; b: *t*-test, $p < 0.002$; c: ANCOVA, df = 1, $F = 4.33$, $p = 0.04$; d: multiple comparison, $p < 0.05$; e,f: multiple comparison, $p < 0.05$

	$\delta^{13}C$ (‰)					$\delta^{15}N$ (‰)				
	Mean for Mauʻi	Mean for Oʻahu	All islands	Min.	Max.	Mean for Mauʻi	Mean for Oʻahu	All islands	Min.	Max.
Shallow										
Centropyge potteri	−18.1 ± 1.5	−17.6 ± 0.6	−17.8 ± 1.0[a]	−19.6	−16.1	7.8 ± 0.6	7.8 ± 0.2	7.8 ± 0.4[b]	6.9	8.2
Planktivores	−17.1 ± 0.5	−17.6 ± 0.3	−17.4 ± 0.5[c]	−18.3	−15.9	8.0 ± 0.3	8.3 ± 0.5		7.5	8.6
Benthic invertivores	−15.1 ± 1.6	−15.6 ± 0.8	−15.4 ± 1.2[d]	−18.2	−12.5	8.7 ± 0.9	9.1 ± 0.7		6.8	10.6
Mesophotic										
C. potteri	−18.8 ± 0.8	−18.8 ± 0.7	−18.8 ± 0.7[a]	−20.9	−18.0	7.2 ± 0.8	7.5 ± 0.3	7.3 ± 0.8[b]	6.2	9.2
Planktivores	−18.1 ± 0.7	−17.3 ± 0.4	−17.7 ± 0.7[c]	−19.3	−16.8	8.0 ± 0.7	8.2 ± 0.5		7.0	9.6
Benthic invertivores	−17.0 ± 0.9	−16.5 ± 0.6	−16.9 ± 0.8[d]	−18.2	−13.5	8.9 ± 1.0	9.4 ± 0.4		7.2	10.8
All depths										
Planktivores						8.2 ± 0.5[e]	8.0 ± 0.6[e]			
Benthic invertivores						9.2 ± 0.7[f]	8.8 ± 0.9[f]			

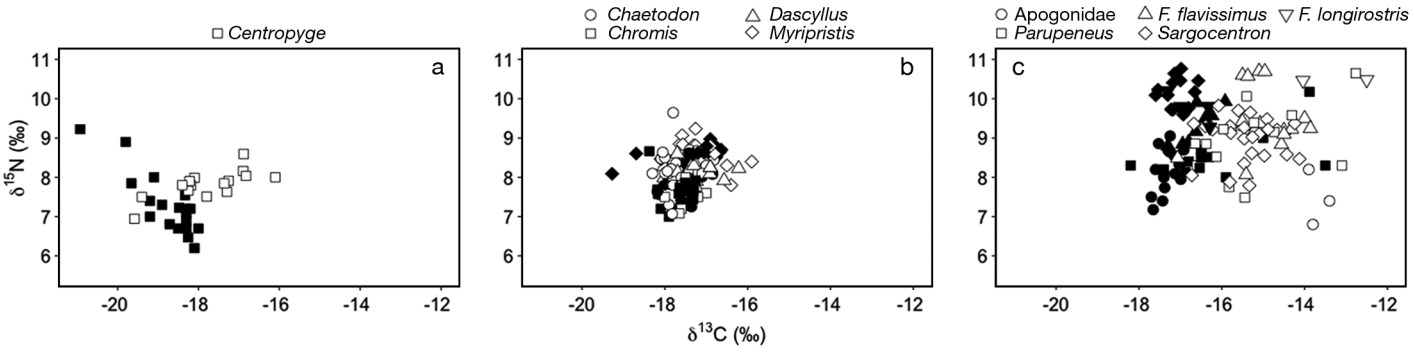

Fig. 1. Bulk muscle tissue carbon-nitrogen biplot of different feeding guilds at 2 depths. Carbon versus nitrogen isotopic compositions in (a) omnivores, (b) planktivores, and (c) benthic invertivores from shallow (unfilled symbols) and mesophotic (filled symbols) depths. See Table 2 for full species names

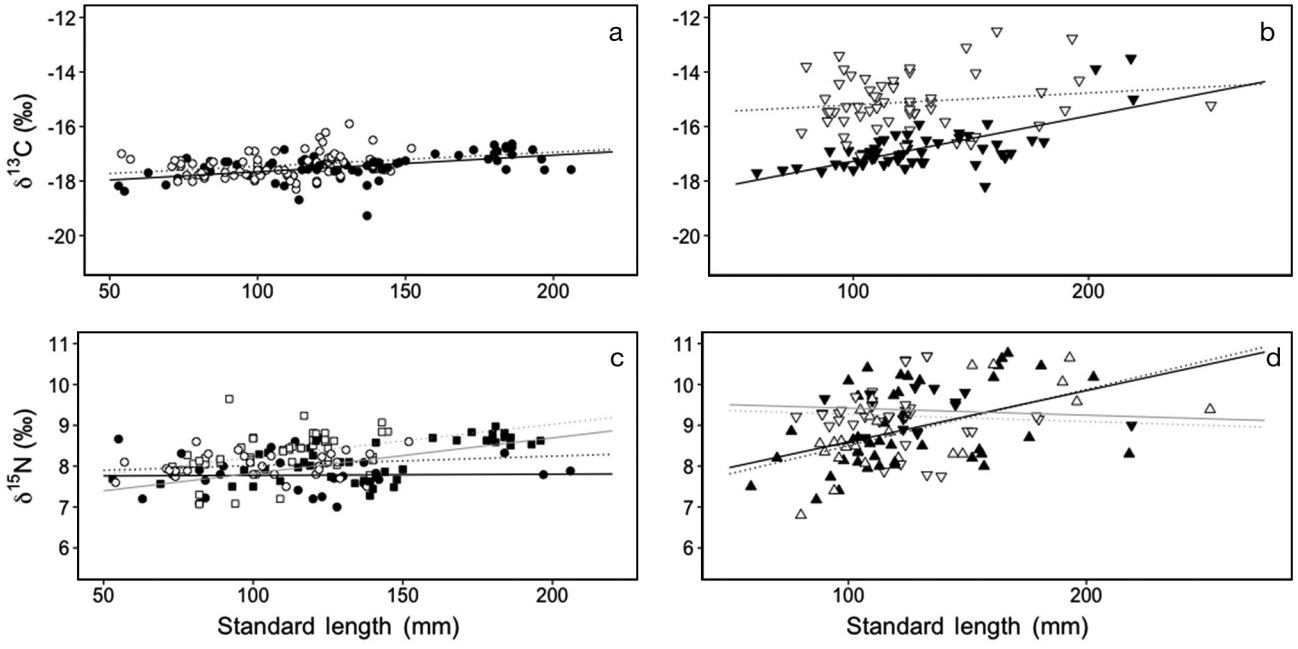

Fig. 2. Carbon and nitrogen isotopic values in fishes at 2 depth ranges versus standard length. $\delta^{13}C$ and $\delta^{15}N$ values of (a,c) planktivores and (b,d) benthic invertivores from shallow (unfilled symbols) and mesophotic (filled symbols) depths. Significant regressions ($p < 0.05$) are shown where present for mesophotic (solid lines) and shallow (dotted lines) fish. Islands were pooled for $\delta^{13}C$ values, as no significant differences were observed. Differences were seen in trends of $\delta^{15}N$ values between islands in (c) planktivores (Oʻahu: squares, Mauʻi: circles) and in (d) benthic invertivores (Oʻahu: inverted triangles, Mauʻi: triangles), and are shown in regression lines (Oʻahu: grey, Mauʻi: black)

Carbon and nitrogen isotopic compositions of mesophotic and shallow planktivores overlapped (Fig. 1b). Interestingly, diurnal feeding planktivores were depleted in ^{13}C and ^{15}N relative to nocturnal planktivores at both depths (*t*-test, $p < 0.05$); this did not drive differences overall in nitrogen (ANOVA, $p = 0.78$, $F = 0.082$) or carbon among species (ANOVA, $p = 0.98$, $F = 0.01$). $\delta^{13}C$ values of mesophotic and shallow benthic invertivores were clearly different (Fig. 1c); however, there were no significant differences between nocturnal and diurnal benthic invertivores ($p > 0.05$). Of the benthic invertivores, apogonids had the lowest $\delta^{15}N$ values, while *Forcipiger* species tended to be more enriched in ^{15}N relative to all other benthic invertivores.

There was a significant positive relationship between $\delta^{13}C$ values and standard length for both benthic invertivores and planktivores (Fig. 2a,b; linear regression, $p < 0.05$). In the absence of significant effects of species (multiple comparison test, $p > 0.05$), benthic invertivores were grouped and the slopes of these regressions were significantly different between depths (df = 1, $F = 4.38$, $p = 0.039$). Smaller mesophotic invertivores were depleted by >1‰ in ^{13}C

Table 4. $\delta^{15}N$ values (±SD) of select amino acids for 3 feeding guilds of mesophotic (M) and shallow (S) fish from O'ahu and Mau'i, Hawai'i. Significant differences ($p < 0.05$) between depths or islands are in **bold**; superscripts indicate paired comparisons. Sample sizes given (n) are from both Mau'i and O'ahu islands. Mean trophic (trp) and mean source (src) values are calculated as a weighted mean (±SD) (see 'Materials and methods')

Island	Depth	n	Trophic amino acids (‰) Ala	Glu	Leu	$\delta^{15}N_{\overline{trp}}$ (‰)	Source amino acids (‰) Gly	Phe	Lys	$\delta^{15}N_{\overline{src}}$ (‰)	Thr (‰)
Centropyge potteri											
Mau'i	S	7	16.4 ± 1.4	15.6 ± 0.6	**15.4 ± 0.6**[a]	15.4 ± 0.6	4.3 ± 2.1	2.7 ± 0.6	2.9 ± 0.9	2.9 ± 0.6	**−3.7 ± 1.6**[c]
	M	9	17.3 ± 1.4	15.2 ± 1.6	**14.3 ± 0.9**[a]	15.2 ± 1.3	3.1 ± 2.2	**1.9 ± 0.9**[b]	3.0 ± 1.1	2.4 ± 0.8	**−8.5 ± 1.8**[c]
O'ahu	S	3	15.4 ± 0.5	14.3 ± 0.5	13.3 ± 0.7	14.5 ± 0.3	4.2 ± 0.8	1.6 ± 0.1	3.8 ± 0.9	3.0 ± 0.9	**−5.7 ± 1.8**[d]
	M	3	16.9 ± 0.8	15.4 ± 0.7	14.3 ± 0.5	15.5 ± 0.5	2.6 ± 1.9	**3.0 ± 0.4**[b]	3.1 ± 0.7	3.0 ± 0.7	**−8.4 ± 2.8**[d]
Planktivores											
Mau'i	S	5	**20.1 ± 2.2**[e]	17.7 ± 1.8	17.4 ± 1.8	18.1 ± 1.5	1.4 ± 2.5	1.8 ± 1.9	0.7 ± 0.7	1.2 ± 1.7	**−18.9 ± 3.6**[g]
	M	5	20.3 ± 2.5	17.6 ± 1.6	17.4 ± 2.2	18.0 ± 1.9	0.3 ± 2.5	**0.6 ± 0.9**[f]	0.9 ± 0.8	0.4 ± 0.9	**−19.9 ± 5.1**[h]
O'ahu	S	3	**23.3 ± 2.5**[e]	19.2 ± 1.4	19.1 ± 1.2	19.3 ± 1.8	3.0 ± 2.1	2.9 ± 1.2	1.3 ± 0.8	2.7 ± 1.3	**−15.5 ± 3.3**[g]
	M	3	24.3 ± 3.3	19.7 ± 2.6	19.6 ± 1.9	20.0 ± 2.3	3.2 ± 2.0	**4.6 ± 1.3**[f]	0.7 ± 0.3	2.5 ± 0.1	**−20.3 ± 5.3**[h]
Benthic invertivores											
Mau'i	S	4	21.8 ± 2.6	18.4 ± 1.8	18.7 ± 2.4	19.0 ± 2.1	2.5 ± 2.7	2.4 ± 1.4	**2.3 ± 1.2**[i]	2.6 ± 1.4	**−17.1 ± 3.4**[j]
	M	2	22.0 ± 2.5	19.0 ± 2.1	18.9 ± 2.5	19.4 ± 2.4	1.2 ± 3.3	1.6 ± 1.4	**1.4 ± 1.3**[i]	1.4 ± 1.5	**−22.6 ± 4.9**[j,k]
O'ahu	S	7	21.9 ± 3.5	17.7 ± 2.0	17.6 ± 2.1	18.1 ± 1.9	3.0 ± 1.9	2.6 ± 1.5	2.4 ± 0.7	2.6 ± 0.7	**−15.5 ± 1.8**[k]
	M	3	23.5 ± 3.3	19.0 ± 1.2	18.7 ± 2.3	19.4 ± 2.0	3.5 ± 2.4	3.3 ± 1.6	2.5 ± 1.0	3.3 ± 1.1	**−18.0 ± 2.2**[k]

compared to shallow congeners. In contrast, shallow and deep planktivores had similar increases in $\delta^{13}C$ values with size ($df = 1$, $F = 0.13$, $p = 0.72$), with the exception of *Myripristis berndti*, which had decreasing isotopic values with increasing size. Overall, $\delta^{13}C$ values of planktivores were not significantly different in $\delta^{13}C$ (multiple comparison test, $p > 0.05$) between shallow and deep guild members.

Nitrogen isotopic compositions of benthic invertivores and planktivores were not significantly different between depths (Fig. 2c,d). When data were pooled for each feeding guild across depths, significant differences were found between islands (multiple comparison test, $p < 0.05$ for both). $\delta^{15}N$ values of Mau'i planktivores increased with increasing size (simple regression, $p < 0.01$), while those from O'ahu did not (simple regression, $p = 0.86$). The opposite was true for invertivores; fish from O'ahu became enriched in ^{15}N with increasing size and Mau'i fish showed no significant trend (simple regression, $p = 0.00$ and 0.66, respectively). In both feeding guilds, mean $\delta^{15}N$ values of fish from Mau'i were greater than those from O'ahu (Table 3).

AA-CSIA

AA-CSIA data was obtained for 81 samples, 42 from shallow and 39 from mesophotic depths. Samples included 14 species from 7 families. AA $\delta^{15}N$ values ranged from 9.8‰ (Val) to 27.3‰ (Ala) for trophic AAs (Ala, Val, Leu, Ile, Pro, Asp, Met, and Glu) and −3.9‰ (Gly) to 8.9‰ (Tyr) for source AAs (Gly, Ser, Phe, Lys, Tyr, and His). Average individual AA nitrogen isotope values for each species group are listed for mesophotic and shallow depths in Table S1 in the Supplement at www.int-res.com/articles/suppl/m558p065_supp.pdf.

For the majority of source and trophic AAs, $\delta^{15}N$ values did not vary greatly between depths for the 3 feeding guilds (Table 4). Thr $\delta^{15}N$ values were the most variable between depths and were always significantly lower in mesophotic fish compared to shallow fish (Mann-Whitney U, $p < 0.05$). Weighted-mean source AA isotopic values, $\delta^{15}N_{\overline{src}}$, ranged only 3‰ across feeding guilds studied, but some distinctions were made between depths. Similar to bulk nitrogen data, $\delta^{15}N_{\overline{src}}$ values of the omnivore *C. potteri* did not vary significantly across islands or depths ($p > 0.05$ for both depths and islands; Fig. 3a). No depth differences were found in planktivores $\delta^{15}N_{\overline{src}}$ values ($p = 0.08$ and 0.40, for Mau'i and O'ahu, respectively), although fish from Mau'i were significantly depleted in ^{15}N compared to those from O'ahu ($p = 0.001$). Benthic invertivores were more complex; shallow and mesophotic invertivores from O'ahu were indistinguishable from one another ($p = 0.20$). Invertivores from mesophotic depths on Mau'i had significantly lower $\delta^{15}N_{\overline{src}}$ values than those from O'ahu, either shallow or mesophotic ($p = 0.009$). Shallow Mau'i benthic invertivores had source AAs significantly enriched in ^{15}N compared to mesophotic

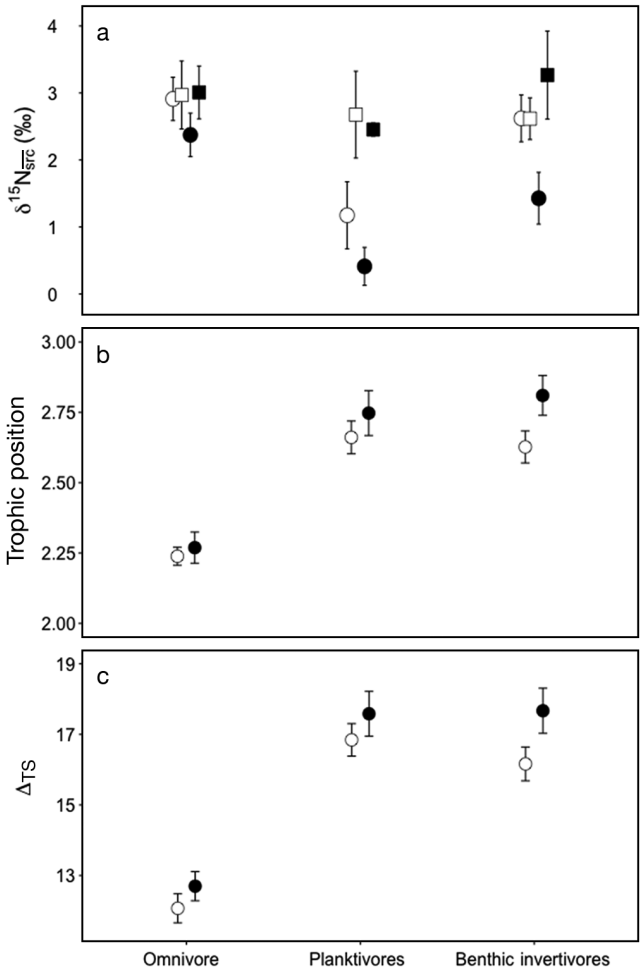

Fig. 3. Source amino acid $\delta^{15}N$ values and trophic positions of different feeding guilds from mesophotic and shallow reef fishes. Compound-specific isotopic analysis of amino acids (AA-CSIA) of mesophotic (filled) and shallow (unfilled) reef fish. (a) Weighted mean $\delta^{15}N$ value of source amino acids of fish from O'ahu (squares) and Mau'i (circles). (b) AA-CSIA derived trophic position calculations using Glu and Phe, using values pooled across islands. (c) Difference between weighted mean values for trophic and source amino acids (Δ_{TS}) as a measure of trophic fractionation. Error bars: SD of the mean

counterparts ($p = 0.01$), but were not significantly different from O'ahu fish ($p = 0.22$).

The lack of variation in isotopic compositions of source AAs was matched by a corresponding lack of significant differences found in trophic positions estimated from AA-CSIA for both planktivores and *C. potteri* (Fig. 3b). Table S2 in the Supplement lists average measurements of standard length, trophic position, and Δ_{TS} for each species within feeding guilds. Trophic positions and source AAs of diurnal and nocturnal predators from the planktonic and benthic invertivore feeding guilds were not significantly different from each other and were subsequently grouped as one ($p > 0.05$, ANOVA). Source differences between islands in benthic invertivores were not reflected in trophic position estimates ($p = 0.95$ and 0.053 for Mau'i and O'ahu, respectively). Grouped across islands, the trophic position estimated for shallow benthic invertivores was slightly, but significantly, lower than that of mesophotic invertivores ($p = 0.04$). As there have been recent indications of variability in the trophic discrimination factor (TDF_{AA}), previously TEF, used in estimation of TP from Glu and Phe (Eq. 2), Δ_{TS} gives a measure of isotopic fractionation from source to consumer, without assuming constant fractionation with trophic step. Δ_{TS} values also indicated little difference in trophic position of different feeding guilds from shallow and mesophotic depths, with the exception of benthic invertivores (Fig. 3c). In the benthic invertivore group, mesophotic fish had slightly but significantly higher trophic proxy values compared to shallow invertivores ($p = 0.04$).

Results of PCA using $N\delta^{13}C$, $N\delta^{15}N_{\overline{src}}$, $N\delta^{15}N_{Thr}$, and NTP_{GluPhe} (TP estimated using normalized values of Glu and Phe) values yielded 3 significant principal components which explained 93.0% of the variance in the data. A weak separation of mesophotic and shallow fish could be seen in the biplot of PC1, mainly driven by trophic position and $\delta^{15}N_{\overline{src}}$, versus PC2 (Fig. 4). A somewhat clearer separation of benthic feeding guilds by depth was produced by the interaction of PC2 and PC3 (Fig. 4b). PC2 was mainly influenced by values of $\delta^{13}C$ and $\delta^{15}N_{Thr}$; PC3 was driven by values of $\delta^{15}N_{Thr}$ and $\delta^{15}N_{\overline{src}}$.

DISCUSSION

The mesophotic reef-fish communities of the main Hawaiian Islands have similarities to those of the neighboring shallow reefs, in both resident species (Boland & Parrish 2005) and, as found in this study, trophic ecology. Bulk tissue carbon and nitrogen isotopic compositions of planktivorous fish showed considerable overlap between depths (Fig. 2c), suggesting similar sources of carbon, nitrogen, and trophic positions of both mesophotic and shallow species. In contrast, $\delta^{13}C$ values of mesophotic benthic invertivores were considerably lower and trophic positions slightly but significantly higher than those from euphotic depths (Figs. 2 & 3). Together, these observations suggest differential utilization of nutrient resources by invertebrate-feeding fish in mesophotic coral-reef ecosystems compared to resident benthic fish communities from shallow counterparts, with potential effects on trophic ecology.

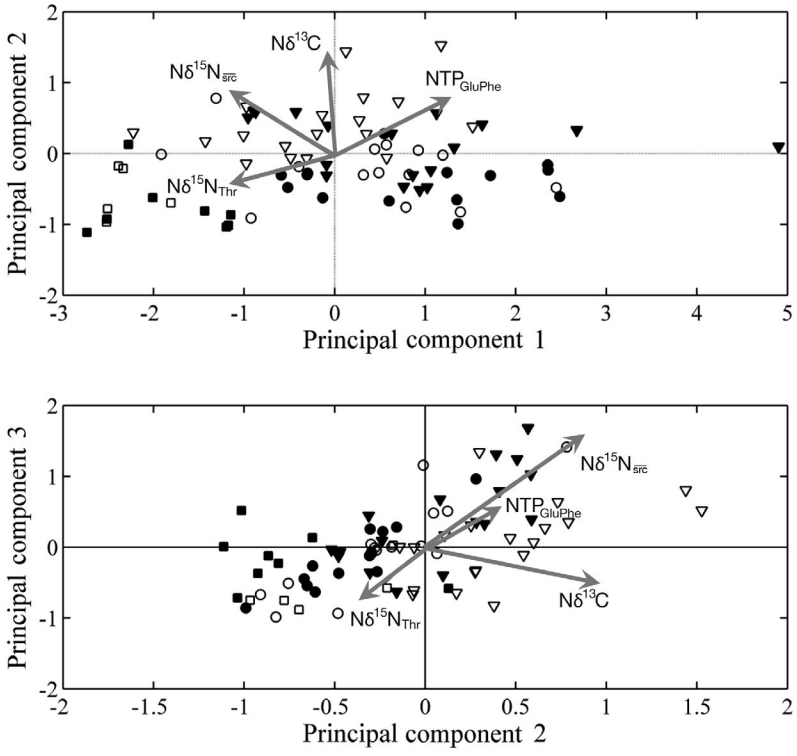

Fig. 4. PCA of mesophotic and shallow reef fish normalized (N) $\delta^{13}C$, $\delta^{15}N_{\overline{src}}$, $\delta^{15}N_{Thr}$ values and trophic position (TP). Biplots of (a) first 2 PCA axes and (B) PCA axes 2 and 3 showing scores for shallow (○) and mesophotic (●) planktivores, shallow (□) and mesophotic (■) omnivores, and shallow (▽) and mesophotic (▼) benthic invertivores

Baseline nutrient shifts

Differences in baseline nutrients can be detected using carbon isotopic composition of bulk muscle tissue and the $\delta^{15}N$ values of source AAs because both change little through food webs (Peterson & Fry 1987, McClelland & Montoya 2002, Post 2002, Popp et al. 2007, Chikaraishi et al. 2009, 2010). Results of this study revealed the separation of mesophotic- and shallow-dwelling fishes belonging to the benthic invertivore and omnivore feeding guilds, by both bulk tissue $\delta^{13}C$ values (Fig. 1) and source AA $\delta^{15}N$ values (Fig. 3). Decreases in the carbon isotopic composition with increasing depth in sessile photoautotrophs has been shown and attributed to increased isotopic fractionation from decreased photosynthesis in low light conditions (Muscatine et al. 1989). It is possible that differences seen in carbon isotopic composition of benthic feeding fishes reflect this change in the $\delta^{13}C$ values of sessile photoautotrophs (Fig. 1), rather than dietary differences between depths.

Similarly to carbon, low light conditions may result in a larger nitrogen isotope fractionation in primary producers that utilize nitrate and that are growing under light limitation (Needoba & Harrison 2004). However, this larger net isotope fractionation will occur only if nitrate concentrations are in excess of that utilized. That is, if all available nitrate in the environment is utilized, isotope mass balance dictates that there will be no net fractionation. Although the open waters surrounding the Hawaiian Island archipelago are classified as oligotrophic, the MCEs sampled in this study are located at the approximate depth range of seasonal thermoclines in the main Hawaiian Islands, between 60 and 120 m (Grigg 2006, Kahng & Kelley 2007). Localized upwelling around the thermocline could increase nitrate concentrations in these low light environments, and if so, increased nitrogen fractionation in MCE photoautotrophs would lead to changes in source AA $\delta^{15}N$ compared to shallow environments. Conversely, shallow coral reefs are known to be nutrient-limited and light conditions are not expected to limit photosynthesis and increase nitrogen isotopic fractionation in shallow sessile photoautotrophs. The observed significant enrichment of ^{15}N in source AAs in shallow invertivores relative to mesophotic invertivores is not likely driven by photosynthesis-associated changes in isotopic fractionation of sessile photoautotrophs.

We suspect that upwelling of deep-water nitrate enriched in ^{15}N provides a potential source of nitrogen to mesophotic reefs. Although nutrient sources in shallow-water reefs are commonly autochthonous (Kleypas et al. 1999), the presence of internal tides propagating along the thermocline can transport additional allochthonous nutrient inputs through transient upwelling events (Leichter et al. 1998, Leichter & Genovese 2006, Lesser et al. 2009). Additionally, the $\delta^{15}N$ value of deep-water nitrate increases by up to 6‰ over the depth range of ~175 to 600 m in the pelagic ocean north of O'ahu (Casciotti et al. 2008), so it is possible that upwelling events may result in the delivery of ^{15}N-enriched nitrate to the base of the mesophotic food web. While internal tides have not been directly measured in the Au'au Channel, current and temperature measurements in the MCE indicate their presence (C. Bradley unpubl. data). Time series temperature data in the Au'au channel has revealed episodic pulses of cold water to mesophotic depths (C. Bradley unpubl. data)

similar to those observed on reef slopes and in the pelagic ocean that have been attributed to deep-water upwelling (Leichter & Genovese 2006, Johnson et al. 2010, Sevadjian et al. 2012).

As no significant differences were found between depths in the $\delta^{15}N$ values of source AAs in the omnivorous *Centropyge potteri* (Fig. 3a), 3 likely conclusions are possible. First, upwelled nitrate could have been completely used by photoautotrophs, resulting in a lack of isotopic fractionation. Alternatively, input of a lighter source of nitrogen at depth, in addition to ^{15}N-enriched upwelled nitrate, may have resulted in a similar isotopic composition between depths or deep-water derived ^{15}N enriched nitrate could have been upwelled to shallow depths. Upwelled nitrogen would be expected to increase the $\delta^{15}N$ values of benthic-associated taxa compared to those feeding higher in the water column, which is observed in omnivores compared to planktivores (mean $\delta^{15}N_{src}$: 2.7 ± 0.7‰ and 1.2 ± 1.5‰, respectively). Because of this difference, it is not likely that complete assimilation is responsible for observed trends. The observed isotopic changes between fish from different depths strongly suggests the use of separate suites of resources, derived from either benthic or planktonic sources. The ^{15}N enrichment of the benthic food web was not apparent in mesophotic benthic invertivores (1.7 ± 1.6‰), which had statistically similar $\delta^{15}N$ values to planktivores. Instead, benthic invertivores from shallow depths were enriched in ^{15}N (2.6 ± 1.2‰) relative to mesophotic invertivores and not significantly different from omnivores from both shallow and mesophotic depths. This suggests that the source of nitrogen found in mesophotic waters may not be distinct from shallower waters, but that a nutrient source from the planktonic food web is being utilized by the benthic food web at mesophotic depths. It is possible that either reduced benthic primary production or lack of palatable algae is creating a coupling of the planktonic and benthic food webs in mesophotic environments. However, isotope fractionation can be variable across taxa (Muscatine et al. 1989, Needoba & Harrison 2004) and could account for isotopic differences seen in some benthic-feeding fish and not others.

Conversely, the lack of differences observed in $\delta^{13}C$ values of mesophotic and shallow planktivores indicates utilization of similar carbon resources and suggests feeding on the same or similar food resources. For planktonic animals feeding in the water column, the impact of horizontal and vertical separation between shallow and mesophotic ecosystems is probably smaller than for benthic animals. Diurnal horizontal migrations of zooplankton occur along the island shelf in the Hawaiian Islands (Benoit-Bird et al. 2008). Additionally, the fluctuation and breaking of internal tides is observed to be a large transporter of plankton across depths (Leichter et al. 1998). It is reasonable that a combination of both zooplankton migration and advection has resulted in a planktonic food source with relatively uniform $\delta^{13}C$ values across mesophotic and shallow depths off Hawai'i.

Shifts in trophic position

Trophic levels did not vary between shallow and mesophotic depths for omnivores and planktivores (Fig. 3b), indicating that any potential changes in baseline nutrients have not affected their position within a food web. A small, statistically significant difference in trophic position exists between benthic invertivore feeding guilds from mesophotic and shallow depths, which could be driven by a number of factors. Reduced consumption of macroalgae by grazers, both vertebrate and invertebrate, as has been documented in other MCEs (Brokovich et al. 2010, Garcia-Sais 2010), could impact benthic-associated food webs, driving diet changes to higher trophic-level prey items and subsequently impacting trophic levels of consumers. The difference of 0.2 trophic levels in benthic invertivores can be traced to a change in Δ_{TS} of 1.2 ± 3.5‰ between shallow and mesophotic depths (Fig. 3c). Feeding guilds contained the same representation of families at each depth, with few differences at the generic level. Therefore, differences in the $\delta^{15}N$ value of trophic and source AAs may not be from physiological differences, but from differences in diet, either due to prey items from differing trophic levels, or from different food webs.

While results of isotope analyses can be used to evaluate differences in trophic position or chemical baselines, differences in prey composition may occur, and these are best investigated using SCA. Published SCA of the fishes collected in this study are limited, especially for targeted mesophotic representatives (Randall 1967, 1985, 1998, Hobson 1974, Harmelin-Vivien 1979, Sano et al. 1984, Masuda & Allen 1993, Lieske & Myers 1994). We examined the stomach content of the specimens collected, but most were everted or damaged during collection. The lack of comprehensive information on feeding habits of mesophotic reef fishes in the Hawaiian Islands prevents us from identifying the cause of these small but nonetheless significant trophic position differences in benthic invertivores between depth ranges studied.

Implications for coral-reef management

In fisheries management, there is an increasing awareness of the need to take an ecosystem approach (Polovina 1984, Pikitch et al. 2004, Gascuel & Pauly 2009). Movement of biomass through an ecosystem is modeled 'flowing' through trophic levels and lost to either natural mortality or removal from the system (Gascuel & Pauly 2009). This form of modeling has become widespread to predict factors influencing changes to ecosystems and fisheries. In the case of Hawaiian coral-reef habitats, models need to reflect the differences in food webs between the mesophotic and shallow depths, and results here place important constraints on model output and assumptions (Fig. 5). Our results indicate that the differences in the trophic positions of fishes at mesophotic and shallow depths are small to nonexistent, so models that predict the trophic levels of fishes as an output can be guided by our findings. Changes at the base of the food web from one including both algal and phytoplankton primary production in shallow waters, to one primarily driven by the planktonic sources, could have far-reaching implications. Macroalgae production and grazing are major components of shallow water coral-reef ecosystems, and the stability of these ecosystems has been linked to the stability of grazer populations (Jackson et al. 2001). Mesophotic fish populations clearly lack herbivores (Brokovich et al. 2010), and unfortunately, the present study was not able to compare any herbivore conspecifics between depths. Therefore it is difficult to determine the nature of the effects of the mesophotic shift to planktonic food sources, but it is clear that there are functional differences between depths. The impact of a 0.2 trophic level shift in mesophotic benthic invertivores, through lengthening of the food web, could potentially result in a less productive ecosystem. However, without information on the specific sizes of nutrient pools in the 2 environments, it is impossible to determine the extent of that change in productivity with much certainty.

Despite differences, mesophotic and shallow coral-reef ecosystems in the main Hawaiian Islands are not completely separate from one another in terms of their ecology. A better understanding of the movement of large, mobile predatory fishes between the 2 depths is also required to fully determine this link, but there are common energy pathways and trophic relationships between fish populations in both ecosystems (Fig. 5). For management of Hawaiian reef fisheries, this link must be reflected while still acknowledging that parts of the system are separate. The benthic invertivore functional group may need to be split into 2 groups for mesophotic and shallow habitats, reflecting the slight difference in trophic

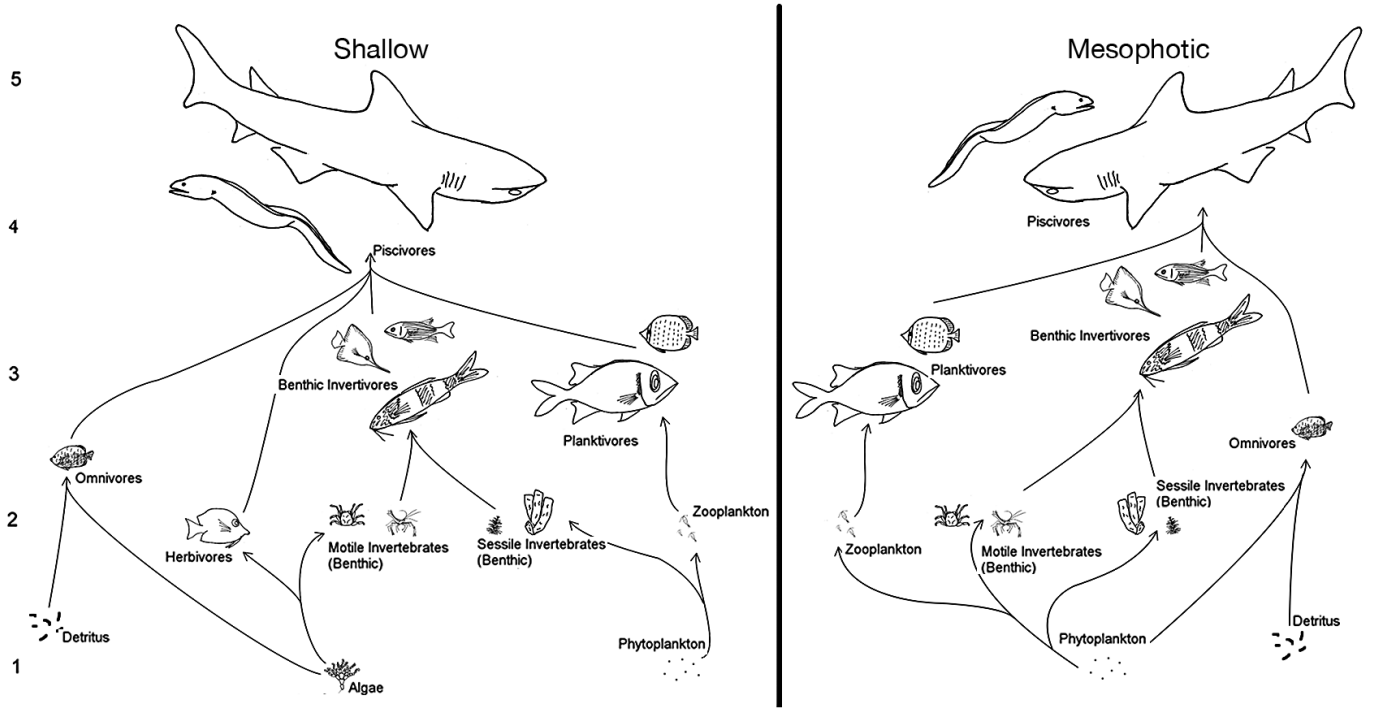

Fig. 5. Coral-reef food web including mesophotic and shallow depths for Hawaiian Islands of Mauʻi and Oʻahu. Functional groups are shown by trophic position (left). Energy flow (arrows) is depicted for each depth grouping

level, and the potential for different nutrient inputs into the 2 ecosystems warrants further investigation to determine the impact of this change.

Understanding the ecology of MCEs and the connectivity to shallow waters is critical to their assessment and evaluation as refugia (Glynn 1996, Riegl & Piller 2003, Lesser et al. 2009, Bongaerts et al. 2010, Hinderstein et al. 2010). Typically, connectivity between habitats in juvenile and adult fish is determined by movement into and out of an area and modeled as a diffusion across a boundary (Botsford et al. 2009). However, in the case of the MCEs on O'ahu and Mau'i, connectivity between shallow and mesophotic fish populations appears to be the result of a physical environment connecting food sources rather than actual movement of fish. Still, in both types of connectivity, resources are shared and sustainability can be affected. While random movement into and out of a protected area can have negative effects on sustainability and yield, the effects of established movements within a home range have not been studied (Botsford et al. 2009). Managing Hawaiian coral-reef fisheries will require a better understanding of how shared resources between spatially distinct habitats can impact the ecosystem. Additionally, further studies are needed, in particular SCA, to fully understand the complexities of the nutritional ecology of mesophotic fish.

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