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Evidence for benthic primary production support of an apex predator–dominated coral reef food web

Anna K. Hilting · Carolyn A. Currin ·
Randall K. Kosaki

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Abstract Five hundred and ninety-nine primary producers and consumers in the Papahānaumokuākea Marine National Monument (PMNM) (22°N–30°N, 160°W–180°W) were sampled for carbon and nitrogen stable isotope composition to elucidate trophic relationships in a relatively unimpacted, apex predator–dominated coral reef ecosystem. A one-isotope (δ^{13}C), two-source (phytoplankton and benthic primary production) mixing model provided evidence for an average minimum benthic primary production contribution of 65 % to consumer production. Primary producer δ^{15}N values ranged from −1.6 to 8.0 ‰ with an average (2.1 ‰) consistent with a prevalence of N_2 fixation. Consumer group δ^{15}N means ranged from 6.6 ‰ (herbivore) to 12.1 ‰ (*Galeocerdo cuvier*), and differences between consumer group δ^{15}N values suggest an average trophic enrichment factor of 1.8 ‰ Δ^{15}N. Based on relative δ^{15}N values, the larger *G. cuvier* may feed at a trophic position above other apex predators. The results provide baseline data for investigating the trophic ecology of healthy coral reef ecosystems.

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A. K. Hilting (✉) · C. A. Currin
NOAA NOS NCCOS CCFHR, 101 Pivers Island Road,
Beaufort, NC, USA
e-mail: anna.hilting@noaa.gov

R. K. Kosaki
NOAA NOS Papahānaumokuākea Marine National Monument,
6600 Kalanianaʻole Highway, Honolulu, HI, USA

Abbreviations

BMA	Benthic macroalgae
BMI	Benthic microalgae
C	Carbon
FL	Fork length
FFS	French Frigate Shoals
HCl	Hydrochloric acid
IRMS	Isotope ratio mass spectrometer
Lu	Ludox
PMNM	Papahānaumokuākea Marine National Monument
N	Nitrogen
SD	Standard deviation
SI	Stable isotope
TEF	Trophic enrichment factor
TL	Total length
TP	Trophic position
VM	Vertical migration

Introduction

Coral reefs worldwide are at risk from geochemical and physical changes in the ocean driven by geologically unprecedented rates of CO_2 release (Kiehl 2011; Hoenisch et al. 2012). Although the capacity of reefs to acclimate to rapid global change is uncertain, reef resiliency may be enhanced or protected by reducing local disturbance (Hughes et al. 2003; Hoegh-Guldberg et al. 2007). Understanding the trophic ecology of healthy, minimally impacted coral reef ecosystems improves the ability to evaluate resiliency and establish guidelines for protected areas (Hughes et al. 2003; Vroom and Braun 2010). Remote, minimally impacted coral reef ecosystems differ from disturbed coral reef ecosystems by their greater biomass and higher relative proportions of apex predators to herbivores (Friedlander and DeMartini 2002; Stevenson et al. 2007). The multiple species of herbivores, predators, and competitors of intact systems help prevent phase shifts in coral reef ecosystem trophic structure that lead to reductions in coral cover in favor of macroalgal cover and to reductions in reef resiliency to disease and bleaching (Sandin et al. 2008; Hixon 2011). Although often associated with reduced coral reef health in tropical systems (Hughes et al. 2010), high macroalgal cover is typical for subtropical systems (Harriott and Banks 2002). In relatively undisturbed subtropical systems, high macroalgal cover may provide critical habitat maintaining ecosystem health (Vroom and Braun 2010) or, in co-occurrence with low coral recruitment and low coral growth rates, limit potential resilience (Hoey et al. 2011).

The Northwestern Hawaiian Islands, designated as Papahānaumokuākea Marine National Monument (PMNM) in 2006, consists of atolls, reefs, islands, and submerged banks extending 2,000 km northwest of the main Hawaiian Islands (Friedlander et al. 2008). The PMNM is one of the few, relatively undisturbed, remote coral reef ecosystems serving as a reference point for restoration research (Knowlton and Jackson 2008). Like the unpopulated Kingman Reef and Palmyra Atoll in the Central Pacific Line Islands, the PMNM is dominated by apex predators (Friedlander and DeMartini 2002; Stevenson et al. 2007; Sandin et al. 2008). Unlike those tropical coral reef ecosystems, the subtropical PMNM has greater macroalgal cover than coral reef cover (Vroom et al. 2005). PMNM benthic communities are typified by dense, healthy patches of coral surrounded by hard-bottom habitat dominated by algae (Parrish and Boland 2004; Friedlander et al. 2008; Vroom and Braun 2010).

Understanding the trophic ecology of the PMNM includes determining the relative contribution of benthic primary production to the food web and the number of trophic positions between primary producers and apex predators. Stable isotope (SI) analyses have provided insight into energy pathways in coral reef ecosystem trophic ecology (Carassou et al. 2008; Greenwood et al. 2010), as has mass-balance ecosystem modeling. The ECOPATH mass-balance model was applied to the shallow region of French Frigate Shoals (FFS) in the PMNM to estimate mean annual biomass, production, and consumption for primary producers and consumers (Polovina 1984). Model input included assigning the contribution of benthic primary production to the atoll food web at 90 %, an assumption supported by comparisons of ECOPATH model results with metabolism studies (Atkinson and Grigg 1984). In contrast, an ECOPATH model coupling pelagic and benthic systems of lagoonal communities of an open atoll in New Caledonia predicted that benthic primary production was responsible for 50 % of total net primary production and 61 % of total consumption (Bozec et al. 2004). The FFS ECOPATH model predicted a food web of almost five trophic positions (TP) with piscivores, jacks, and sharks between positions four and five (Grigg et al. 2008). The New Caledonia model predicted just over four TPs with sharks and pelagic piscivores at the highest positions (Bozec et al. 2004).

Stable isotope analysis is commonly used to trace food sources and energy flow in food webs (Post 2002). Algal carbon (C) and nitrogen (N) SI composition is a function of ocean or sediment chemistry, photosynthesis, growth rates, and specific nitrogen uptake mechanisms. Typically, phytoplankton have lower δ^{13}C (~ -22 ‰) than benthic algae (~ -17 ‰) (France 1995), but similar δ^{15}N values (Owens 1987). Consumer SI values are higher than their food sources by a trophic enrichment factor (TEF) reported in mixing model literature as ranging from 0 to 1.4 ‰ Δ^{13}C and 2.2 to 3.5 ‰ Δ^{15}N (Galván et al. 2012). Because primary producer δ^{13}C signatures tend to be preserved in consumers at higher TPs, δ^{13}C values help distinguish the relative importance of different primary producers as sources of energy supporting the food web. Consumer δ^{15}N values provide useful information about TP because of the relatively large TEF (Δ^{15}N). Linear mixing models are based on mass-balance equations and the assumption that, after adjustment for TEF and TP, the consumer isotope ratios are identical to the isotopic base of the consumer's food web ($\delta^{15}N_{\text{food web base}}$ and/or $\delta^{13}C_{\text{food web base}}$), which is usually a mixture of potential primary producers. A range of feasible contributions by potential primary producer sources can be calculated and depend on the position of adjusted consumer isotope value(s) along a mixing line (one-isotope/two-source models) or within a polygon formed by values of three or more sources (primary producers) in a two-isotope mixing model. Mixing models and the relationships between TEF, TP, and consumer values were used to evaluate trophic relationships based on a large dataset of consumer and primary producer SI values collected, opportunistically, over the length of the PMNM in 3 years (2001, 2004, and 2005).

Methods

Sample collection and shipboard processing

Primary producer ($n = 50$) and consumer ($n = 549$) samples were collected for SI analysis between August 2001 and October 2005 on four research cruises at locations spanning the length of the PMNM (Fig. 1). Sampling was temporally and spatially opportunistic, and the majority of

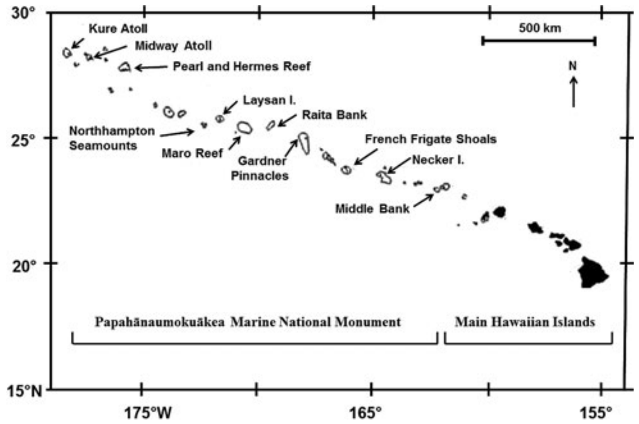

Fig. 1 Map of the Hawaiian Archipelago showing the locations sampled in the Papahānaumokuākea Marine National Monument. Map modified from Marine Mammal Commission (2001)

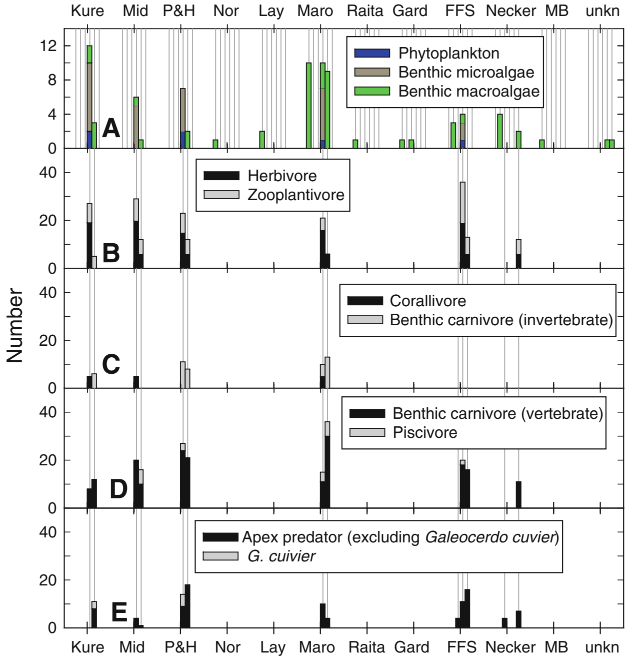

Fig. 2 Spatial and temporal sampling frequency of major primary producer (**a**) and consumer groups (**b–e**). Locations are ordered geographically from northwest to southeast. Location abbreviations are Kure (Kure Atoll), Mid (Midway Island), P&H (Pearl and Hermes Atoll), Nor (Northampton Seamounts), Lay (Laysan Island), Maro (Maro Reef), Raita (Raita Bank), Gard (Gardner Pinnacles), FFS (French Frigate Shoals), Necker (Necker Island), MB (Middle Bank), and unknown. In the first panel, each location is bordered by six *gray vertical lines* representing sampling months in chronological order. Lines representing August 2001, August 2004, and September 2004 precede each *tick line,* and lines representing May 2005, September 2005, and October 2005 follow each *tick line*. Lines are extended into panels (**b–e**) for months in which consumers were sampled. Note the change in the *vertical scale* between primary producer and consumer panels

samples were obtained from six locations: Kure Atoll, Midway Island, Pearl and Hermes Atoll, Maro Reef, FFS, and Necker Island (Fig. 2). Targeted species include those classified in trophic groups as herbivore and apex predator by Friedlander and DeMartini (2002); as herbivore, zooplanktivore, benthic carnivore, and piscivore by Parrish and Boland (2004); and the most common macroalgae *Microdictyon* and *Halimeda* (Parrish and Boland 2004). Primary producers included three main groups: phytoplankton, benthic microalgae (BMI), and benthic macroalgae (BMA) (Table 1). In 2005, we collected 314 samples on NOAA ship Hiʻialakai cruises HI-05-04 (May) and 253 samples during the HI-05-10 Northwestern Hawaiian Islands Reef Assessment and Monitoring Program (NWHIRAMP) (September–October). An additional 32 samples from cruises in August 2001 and August 2004 were obtained to double the number of macroalgae SI measurements and provide samples from otherwise unsampled locations (Fig. 2). With the exception of eight *Caranx* samples collected in September 2004 from FFS and Necker Island, all consumers were sampled in 2005 (Fig. 2; Table S1). Benthic macroalgae and shark sampling mostly occurred at depths >30 m; invertebrates were collected in depths <20 m; and fish and BMI were sampled in both shallow and deeper waters (3–60 m). Seawater (6–10 L) was collected from four sites with Niskin bottles from 1 to 35 m depths. Water samples were pre-filtered through 200-µm mesh to remove zooplankton and then filtered on pre-combusted glass fiber filters (GF/F) to obtain phytoplankton samples. Surface (top 0.5–1 cm) sediment samples were collected by divers or by Ponar grab, and BMI were separated using two methods. Eighteen samples from five sites were concentrated on pre-combusted glass fiber filters (GF/F) following vertical migration (VM) through a 63-µm Nitex mesh and silica layer (Wainright et al. 2000). In addition, six samples from two locations were collected on pre-combusted glass fiber filters (AH) following rinsing and density centrifugation with Ludox (Lu; colloidal Si) (Moseman et al. 2004). Both techniques were utilized in an attempt to sample the entire BMI community. All filtered phytoplankton and BMI samples were frozen immediately. In May 2006, they were thawed, fumed with concentrated HCl to remove carbonates, and shipped for SI analysis.

Fifty samples of 13 BMA genera (Table 1) (~100 mg wet weight, above-ground portion) were collected by divers. Benthic macroalgae samples were rinsed in freshwater and frozen immediately. All BMA samples were thawed in January 2008, rinsed with distilled water and cleaned of epiphytes, photographed under a dissecting microscope to aid identification, dried at 60 °C, ground with a mortar and pestle or Wig-L-Bug® grinding mill, acidified with 1 N HCl to remove carbonates, rinsed, and redried in preparation for SI analysis. Some samples were also acidified prior to grinding.

Table 1 Stable isotope values of the primary producer groups, combined groups, subgroups, and species

Primary producers		(*n*)	δ^{13}C (‰ VPDB)			δ^{15}N (‰ Air)		
Groups and combined groups	Subgroups and species		Mean ± SD	Min	Max	Mean ± SD	Min	Max
Phytoplankton		6	−23.4 ± 0.8	−24.4	−22.3	1.4 ± 1.8	−1.0	4.5
BMI		26	−9.5 ± 4.7	−18.5	−1.6	3.0 ± 1.9	0.5	7.9
	High N BMI	6	−15.7 ± 2.3	−18.5	−12.5	5.8 ± 1.2	4.7	7.9
	Low N BMI	20	−7.7 ± 3.5	−14.9	−1.6	2.2 ± 1.2	0.5	4.2
	BMI (Lu)	8	−8.6 ± 3.2	−14.9	−5.2	1.4 ± 0.9	0.5	3.1
	BMI (VM)	18	−9.9 ± 5.3	−18.5	−1.6	3.7 ± 1.8	0.5	7.9
BMA		50	−18.7 ± 4.0	−31.5	−12.8	1.5 ± 2.1	−1.6	8.0
	High N BMA	4	−15.6 ± 2.6	−19.1	−12.8	6.7 ± 1.3	5.3	8.0
	Low N BMA	46	−19.0 ± 4.0	−31.5	−13.8	1.1 ± 1.5	−1.6	3.5
	Low C Low N BMA	5	−29.2 ± 2.0	−31.5	−27.6	2.2 ± 1.0	0.9	3.3
	High C Low N BMA	41	−17.8 ± 1.8	−21.8	−13.8	0.9 ± 1.5	−1.6	3.5
	Caulerpa racemosa (Chl)	1	−14.8			7.6		
	Codium cf. arabienn (Chl)	1	−16.1			2.7		
	Dictyota friabilis (Pha)	1	−15.6			8.0		
	Galaxaura cf. rugosa (Rho)	1	−16.3			3.6		
	Halimeda cf. discoidea (Chl)	2	−19.6 ± 3.0	−21.8	−17.5	1.3 ± 0.4	1.0	1.6
	Halimeda sp. (Chl)	7	−20.1 ± 1.0	−20.9	−18.1	1.1 ± 1.0	−0.3	2.4
	Halimeda velasquezii (Chl)	5	−18.9 ± 1.1	−20.8	−17.8	2.8 ± 1.5	1.4	5.3
	Laurencia sp. (Rho)	5	−16.3 ± 1.2	−17.7	−15.2	2.0 ± 0.6	1.2	2.8
	Liagora sp. (Rho)	3	−17.8 ± 1.8	−19.0	−15.8	2.8 ± 0.1	2.7	3.0
	Microdictyon setchellianum (Chl)	11	−17 ± 3.4	−18.0	−14.0	−0.5 ± 2.7	−1.4	−0.1
	Microdictyon sp. (Chl)	4	−17.7 ± 0.3	−18.0	−17.2	−0.5 ± 0.7	−1.1	0.4
	Padina sp. (Pha)	3	−14.2 ± 1.7	−16.1	−12.8	1.9 ± 3.6	−0.7	6.0
	Portieria hornemanni (Rho)	1	−27.6			2.6		
	Sporochnus dotyi (Pha)	2	−27.7 ± 0.1	−27.8	−27.7	2.1 ± 1.1	1.3	2.9
	Ulva cf. flexuosa (Chl)	1	−18.6			3.0		
	Unidentified red algae (Rho)	2	−31.4 ± 0.2	−31.6	−31.2	2.1 ± 1.7	0.9	3.3
Combined BMI and BMA		76	−15.6 ± 6.1	−31.5	−1.6	2.0 ± 2.2	−1.6	8.0
	High N BMI and BMA	10	−15.6 ± 2.3	−19.1	−12.5	6.2 ± 1.3	4.7	8.0
Combined primary producers		82	−16.2 ± 6.2	−31.5	−1.6	2.1 ± 2.2	−1.6	8.0

Sampling frequency and distribution for major groups are shown in Fig. 2

The abbreviation following benthic macroalgae species names indicates its phyletic group: Chlorophyta (Chl), Phaeophyta (Pha), or Rhodophyta (Rho)

BMI benthic microalgae, *BMA* benthic macroalgae. *BMI* are grouped by nitrogen values and by method: *Lu* for Ludox and *VM* vertical migration, *VPDB* Vienna Pee Dee Belemnite, *SD* standard deviation

Invertebrates were captured by divers, and fish and sharks were captured by divers or by hook and line. Larger specimens, including sharks and jacks, were released live after white muscle plugs were obtained. White muscle tissue from smaller specimens was obtained by dissection. Samples were frozen immediately after collection and stored until processing (2006–2008). In the laboratory, muscle tissue was thawed, rinsed with distilled water, dried at 60 °C, and ground with a mortar and pestle or Wig-L-Bug$^{\circledR}$ grinding mill prior to SI analysis. We did not correct δ^{13}C values for lipid content, as all consumer groups had C:N ratios of 3.4 or less (Post et al. 2007).

Stable isotope analysis

Dried, ground samples were weighed, placed into tin capsules, and sent to the University of California-Davis (UC-D) SI Laboratory ($n = 588$) or the University of Washington (UW) SI Core Laboratory ($n = 11$) for analysis. Replicate samples were run on a regular basis to check for consistency, and a few of the reported results represent the average of two replicates. Stable isotope analyses were conducted using a Sercon Ltd. PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (IRMS)

(UC-D) or a continuous flow Thermo Finnigan Delta Plus XP IRMS and Costech Analytical ECS 4010 elemental analyzer (UW). Carbon and nitrogen isotope ratios are reported in units per mil (‰) relative to the standards Vienna Peedee Belemnite (VPDB) for carbon and atmospheric nitrogen (air) for nitrogen:

$$\delta^{13}\mathrm{C} = \left[\left(\frac{Ratio_{sample}}{Ratio_{VPDB}}\right) - 1\right] \times 1{,}000 \qquad (1)$$

$$\delta^{15}\mathrm{N} = \left[\left(\frac{Ratio_{sample}}{Ratio_{Air}}\right) - 1\right] \times 1{,}000 \qquad (2)$$

Trophic group assignment

Consumers were classified into trophic groups. For species not classified by Parrish and Boland (2004) or Friedlander and DeMartini (2002), we referred to Hiatt and Strasburg (1960), Hobson (1974), Allen et al. (1998), DeFelice and Parrish (2003), and Piché et al. (2010) for trophic information (Table 2). We classified six species as herbivores ($n = 119$), four species as zooplanktivores ($n = 77$), and the butterflyfish *Chaetodon lunulatus* ($n = 15$) was the sole corallivore. Piché et al. (2010) included invertebrates and vertebrates in their benthic carnivore group, but we created two classes of benthic carnivores. The benthic carnivore (invertebrate) group included a single *Octopus cyanea* sample and 42 lobsters (*Panulirus marginatus*). Seven fish species were classified as benthic carnivore (vertebrate) ($n = 138$) including the introduced snapper *Lutjanus kasmira*. *L. kasmira* was classified as an omnivore by Piché et al. (2010) based on diet information in FishBase (Froese and Pauly 2012), whereas we based our classification on the DeFelice and Parrish (2003) report. The piscivore group ($n = 21$) consists of two species that occupy different habitats: the benthic bigeye (*Priacanthus meeki*) and the pelagic tuna (*Euthynnus affinis*). An alternate classification for *P. meeki* is zooplanktivore (Piché et al. 2010). Seven species (a snapper, two jacks, a grouper, and three sharks) were classified as apex predators ($n = 104$). Trophic position assignments (Table 2) were based on estimates of the ECOPATH model of FFS [modified from Polovina (1984) in Grigg et al. (2008)] with a few exceptions. The corallivore group (unrepresented in the ECOPATH model) was assigned the FishBase TP of 3.3 (Froese and Pauly 2012). ECOPATH estimated cephalopods at TP 3.9, but we included our single specimen with lobsters in the benthic carnivore (invertebrate) group (TP 3.5). ECOPATH estimates for reef sharks (TP 4.7) were higher than their estimates for *G. cuvier* (TP 4.5) and jacks (TP 4.1), but we placed all apex predators excluding *G. cuvier* at TP 4.0, consistent with most FishBase estimates (Froese and Pauly 2012).

Statistics and modeling

Ontogenetic shifts in shark diet are associated with species-specific size classes (Lowe et al. 1996; Wetherbee et al. 1996, 1997). We examined the relationship between total length and δ^{15}N for six *Carcharhinus amblyrhynchos* (medium and large size classes), 16 *Carcharhinus galapagensis* (small, medium, and large size classes), and eight *Galeocerdo cuvier* (large size class) that were sampled in May and September 2005 at four locations. Fork length (FL), recorded instead of total length (TL) for two *C. amblyrhynchos* and one *C. galapagensis* specimens, was converted to total length using ratios (1.2 TL:FL) measured during this study.

Although sampling frequency, distribution, and replication were inadequate for spatial, temporal, or comparative analysis of the major groups, subgroups, and species (Fig. 2), SI values averaged by subgroup, species, location, and date (Table S1) provide clues to potential sources of the observed variability. We used mixing models to analyze trophic relationships between consumer and primary producer groups. Mixing model solutions depend on the isotopic values of primary producer source groups and values of TEF and TP used to adjust consumer values to the base of their food web. The relationship between TEF, TP, consumer isotopic values, and the isotopic value of the base of the food web is defined by Eq. (3).

$$\mathrm{X}_{food\,web\,base} = \mathrm{X}_{consumer} - [(TP - 1) \times TEF] \qquad (3)$$

where X is either δ^{13}C or δ^{15}N.

We first estimated the percent contribution of phytoplankton and benthic (combined BMI and BMA) primary production to consumer production using the one-isotope, two-source mixing model, IsoError (Phillips et al. 2005). Model input included the δ^{13}C mean ± standard deviation (SD) of both primary producers and each consumer group (after adjustment to the base of the consumer's food web). $\delta^{13}\mathrm{C}_{\mathrm{food\ web\ base}}$ values were calculated using Eq. 3, an assumed TEF of 0.5 Δ^{13}C (McCutchan et al. 2003), and the assigned TP (Table 2). Results are reported as mean and 95 % confidence intervals (CI) of percent contribution to consumer production.

We also attempted to estimate the separate contribution by BMI and BMA and utilize δ^{15}N data with a three-source, two-isotope mixing model. A requirement of three-source mixing models is that adjusted consumer values lie within the polygon formed by the SI values of sources (primary producer groups). We found that the low and similar δ^{15}N means of the three main groups (phytoplankton, BMI, and BMA) resulted in a source polygon too small to solve the model unless consumers were adjusted

Table 2 Stable isotope values for consumer groups and species

Trophic group (TP)	Genus species (trophic group reference)	(*n*)	δ^{13}C (‰ VPDB)			δ^{15}N (‰ Air)		
			Mean ± SD	Min	Max	Mean ± SD	Min	Max
Herbivore (2.2)	**Herbivore**	119	**−16.2 ± 2.1**	−22.8	−11.5	**6.6 ± 0.9**	4.5	9.2
	Acanthurus nigroris (2)	23	−16.3 ± 1.2	−18.4	−14.1	7.0 ± 0.8	5.7	8.8
	Acanthurus olivaceus (2)	30	−16.2 ± 1.5	−20.0	−13.3	6.6 ± 0.9	5.2	9.2
	Acanthurus triostegus (2)	35	−14.6 ± 0.8	−15.9	−12.4	6.5 ± 0.4	5.8	7.8
	Ctenochaetus cf. strigosus (1)	4	−13.3 ± 2.0	−15.8	−11.5	6.9 ± 0.6	6.3	7.7
	Stegastes fasciolatus (3)	5	−17.1 ± 0.9	−17.9	−16.1	7.4 ± 0.3	6.9	7.7
	Zebrasoma flavescens (2)	22	−19.2 ± 1.5	−22.8	−15.7	6.0 ± 0.9	4.5	8.0
Zooplanktivore (3.0)	**Zooplanktivore**	77	**−16.9 ± 1.5**	−18.8	−12.0	**8.3 ± 0.7**	6.8	9.7
	Chaetodon miliaris (2)	53	−16.7 ± 1.7	−18.6	−12.0	8.4 ± 0.7	7.0	9.7
	Dascyllus albisella (2)	18	−17.3 ± 0.9	−18.8	−16.3	8.3 ± 0.8	6.8	9.3
	Myripristis amaena (5)	3	−17.9 ± 0.8	−18.4	−17.0	7.5 ± 0.2	7.3	7.8
	Myripristis berndti (5)	3	−17.5 ± 0.1	−17.6	−17.4	7.2 ± 0.2	6.9	7.3
Corallivore (3.3)	***Chaetodon lunulatus*** (7)	15	**−13.2 ± 0.5**	−13.9	−12.3	**8.3 ± 0.4**	7.8	9.1
Benthic carnivore (invertebrate) (3.5)	**Benthic Carnivore (invertebrate)**	43	**−15 ± 1.1**	−16.6	−12.1	**8.4 ± 0.6**	7.3	9.7
	Octopus cyanea (3)	1	−15.3			8.0		
	Panulirus marginatus (3)	42	−15.0 ± 1.0	−16.6	−12.1	8.4 ± 0.6	7.3	9.7
Benthic carnivore (vertebrate) (3.7)	**Benthic carnivore (invertebrate)**	138	**−15.7 ± 1.4**	−18.2	−10.7	**9.2 ± 1.1**	7.1	13.1
	Bodianus bilunulatus (2)	15	−15.1 ± 1.6	−18.1	−11.7	9.0 ± 0.5	8.4	9.9
	Chaetodon fremblii (2)	30	−15.6 ± 1.1	−17.2	−13.0	8.7 ± 0.5	7.5	9.6
	Lutjanus kasmira (6)	11	−15.8 ± 0.8	−17.3	−14.9	8.8 ± 0.4	8.2	9.3
	Parupeneus cyclostomus (4, 5)	2	−16.8 ± 0.4	−17.1	−16.5	8.4 ± 0.1	8.3	8.4
	Parupeneus multifasciatus (2)	37	−16.3 ± 1.5	−18.2	−12.8	8.8 ± 1	7.1	12.4
	Parupeneus porphyreus (3)	14	−14.6 ± 2.0	−16.9	−10.7	9.7 ± 0.6	8.6	10.9
	Thalassoma ballieui (3)	29	−15.6 ± 1.2	−17.9	−14.0	10.2 ± 1.2	9.2	13.1
Piscivore (4.0)	**Piscivore**	21	**−17.8 ± 1.3**	−21.7	−15.8	**9.3 ± 0.5**	8.5	10.2
	Euthynnus affinis (4)	5	−18.6 ± 2.5	−21.7	−15.8	9.8 ± 0.3	9.4	10.2
	Priacanthus meeki (3)	16	−17.5 ± 0.5	−18.1	−16.0	9.1 ± 0.4	8.5	9.7
Apex predator (excluding *G. cuvier*) (4.0)	**Apex predator (excluding *G. cuvier*)**	96	**−14.6 ± 1.4**	−17.6	−10.7	**10.2 ± 0.7**	8.8	11.7
	Aprion virescens (1)	6	−15.4 ± 1.5	−17.6	−13.1	9.4 ± 0.2	9.0	9.6
	Caranx ignobilis (1)	35	−14.5 ± 1.5	−17.2	−10.7	10.5 ± 0.7	8.8	11.7
	Caranx melampygus (1)	19	−15 ± 1.3	−16.9	−11.9	9.9 ± 0.7	8.8	11.0
	Carcharhinus amblyrhynchos (1)	6	−14.2 ± 1.1	−15.6	−13.0	10.0 ± 0.7	9.4	11.1
	Carcharhinus galapagensis (1)	28	−14.2 ± 1	−15.7	−12.3	10.3 ± 0.5	8.9	11.3
	Hyporthodus quernus (1)	2	−16.7 ± 0.7	−17.2	−16.2	10.5 ± 0.4	10.2	10.8
G. cuvier (4.5)	***Galeocerdo cuvier*** (1)	8	**−14.5 ± 1.2**	−16.6	−13.2	**12.1 ± 0.7**	11.2	13.7

Trophic positions (TP) are based on FFS ECOPATH model results (Grigg et al. 2008) and FishBase reports (Froese and Pauly 2012)

Trophic group classification references are in parentheses following taxonomic names: 1. Friedlander and DeMartini (2002); 2. Parrish and Boland (2004); 3. Piché et al. (2010); 4. Hiatt and Strasburg (1960); 5. Hobson (1974); 6. DeFelice and Parrish (2003); and 7. Allen et al. (1998)

Bold font indicates values that were used in the mixing models

VPDB Vienna Pee Dee Belemnite, *SD* standard deviation

using Δ^{15}N (TEF) or TP values that were higher than literature values. Therefore, we used Eq. 3 with a range of literature values for Δ^{15}N TEF and the TPs assigned in Table 2 to explore feasible values for $\delta^{15}\mathrm{N}_{\mathrm{food\ web\ base}}$. Two alternate (solvable) mixing model source polygons that included sources with higher δ^{15}N values were created by (1) increasing the δ^{15}N value of the phytoplankton group above the measured mean to a value consistent with literature values (Owens 1987) and (2) replacing the BMI and BMA endmember groups with four subgroups of benthic algal values (High N BMI and BMA, Low N BMI, and Low N BMA).

Results

Primary producers

Primary producer SI values averaged −16.2 ‰ δ^{13}C and 2.1 ‰ δ^{15}N (Table 1). Mean δ^{15}N values of the three main groups were 1.4 (phytoplankton), 1.5 (BMA), and 3.0 ‰ (BMI) (Table 1). Within groups, the range of δ^{15}N values was ≥5.5 ‰ (Table 1; Fig. 3). Relative to BMI and BMA, phytoplankton had a narrow range of δ^{13}C values and a low δ^{13}C mean. The eight BMI (Lu) measurements had a smaller range in SI values than 18 BMI (VM) measurements (Table 1; Fig. 3). Six of the BMI (VM) samples (from three locations) had δ^{15}N values >4.5 ‰ and relatively low values of δ^{13}C (<−12.4 ‰) (Fig. 3). The δ^{13}C and δ^{15}N values of these six measurements (the subgroup High N BMI) averaged 8.0 ‰ lower and 3.6 ‰ higher, respectively, than the average of the remaining 20 measurements (Low N BMI) (Table 1). Benthic macroalgae also exhibited a wide range of SI values (Table 1; Fig. 3). Five samples (two unidentified red algae, two *Sporochnus dotyi,* and one *Portieria hornemanni*) from three locations had δ^{13}C values <−27 ‰ (Fig. 3) and formed a Low C Low N BMA subgroup (Table 1). With the exception of these five specimens, BMA δ^{13}C values were ≥−21.8 ‰ (High C Low N BMA subgroup; Table 1). Four δ^{15}N values between 5.3 and 8.0 ‰ were obtained in September 2005 at Kure Atoll (*Dictoyta friabilis* and *Padina sp.)* and in October 2005 at Necker Island (*Halimeda velasquezii* and *Caulaerpa racemosa*) (Table S1). The High N BMA subgroup formed by these four specimens had δ^{13}C and δ^{15}N means similar to those of the High N BMI subgroup, and the δ^{15}N values of the remaining BMA (Low N BMA subgroup) ranged from −1.6 to 3.5 ‰ (Table 1). Although the Low C Low N BMA and High N BMA subgroups had δ^{13}C and δ^{15}N values that were 11.4 ‰ lower and 5.8 ‰ higher (respectively) than the remaining 41 measurements (the High C Low N BMA subgroup), they had minimal influence on the overall BMA mean (Table 1). Finally, the averages of all (combined) BMI and BMA δ^{13}C and δ^{15}N values were slightly higher than BMA means (Table 1).

Consumers

Consumer group δ^{13}C means were centered near the combined BMI and BMA mean (−15.6 ‰) although zooplanktivore (−16.9 ‰) and piscivore (−17.8 ‰) means were slightly lower and the corallivore mean (−13.2 ‰) was slightly higher (Table 2). Herbivores had a wider range of individual δ^{13}C values than all other consumer groups (Table 2; Fig. 4) and a δ^{15}N mean (6.6 ‰) that was 4.5 ‰> the mean of all sampled primary producers (Table 1). The zooplanktivore, corallivore, and the benthic carnivore (invertebrate) groups had similar δ^{15}N mean values (∼8.3 ‰) as did the vertebrate benthic carnivore and piscivore groups (∼9.3 ‰) (Table 2). Apex predator δ^{15}N mean values were higher than piscivore and benthic carnivore (vertebrate) groups by ∼1 ‰ (excluding *Galeocerdo cuvier*) to ∼3 ‰ (*G. cuvier*) (Table 2).

Within consumer groups, several species exhibit distinctive isotopic signatures, including some with apparent spatial variability. Relative to all other species, the herbivore *Zebrasoma flavescens* stands out as having the lowest δ^{13}C and δ^{15}N means and largest range of δ^{13}C values

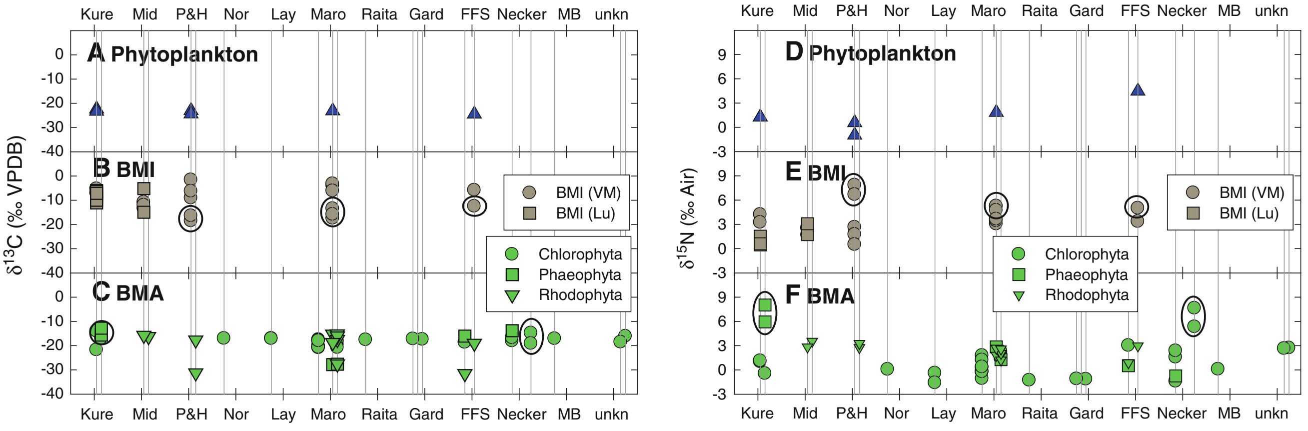

Fig. 3 δ^{13}C (**a–c**) and δ^{15}N (**d–e**) values of individual phytoplankton, benthic microalgae (BMI), and benthic macroalgae (BMA) samples are presented in spatial and temporal context. The relative position of vertical *gray lines* represents months sampled at each location in chronological order. Lines representing August 2001, August 2004, and September 2004 precede *tick lines,* and lines representing May 2005, September 2005, and October 2005 follow *tick lines*. Only months sampled for primary producers are shown; refer to Fig. 2a for relative *spacing of lines* representing all 6 months. Benthic microalgae are separated by methodology: *VM* vertical migration and *Lu* Ludox. Benthic macroalgae values are shown by phyletic groups. Samples with δ^{15}N values >4.5 ‰ are indicated by the larger circles. See Fig. 2 for location abbreviations and Table S1 for average values per location and month. *VPDB* Vienna Pee Dee Belemnite

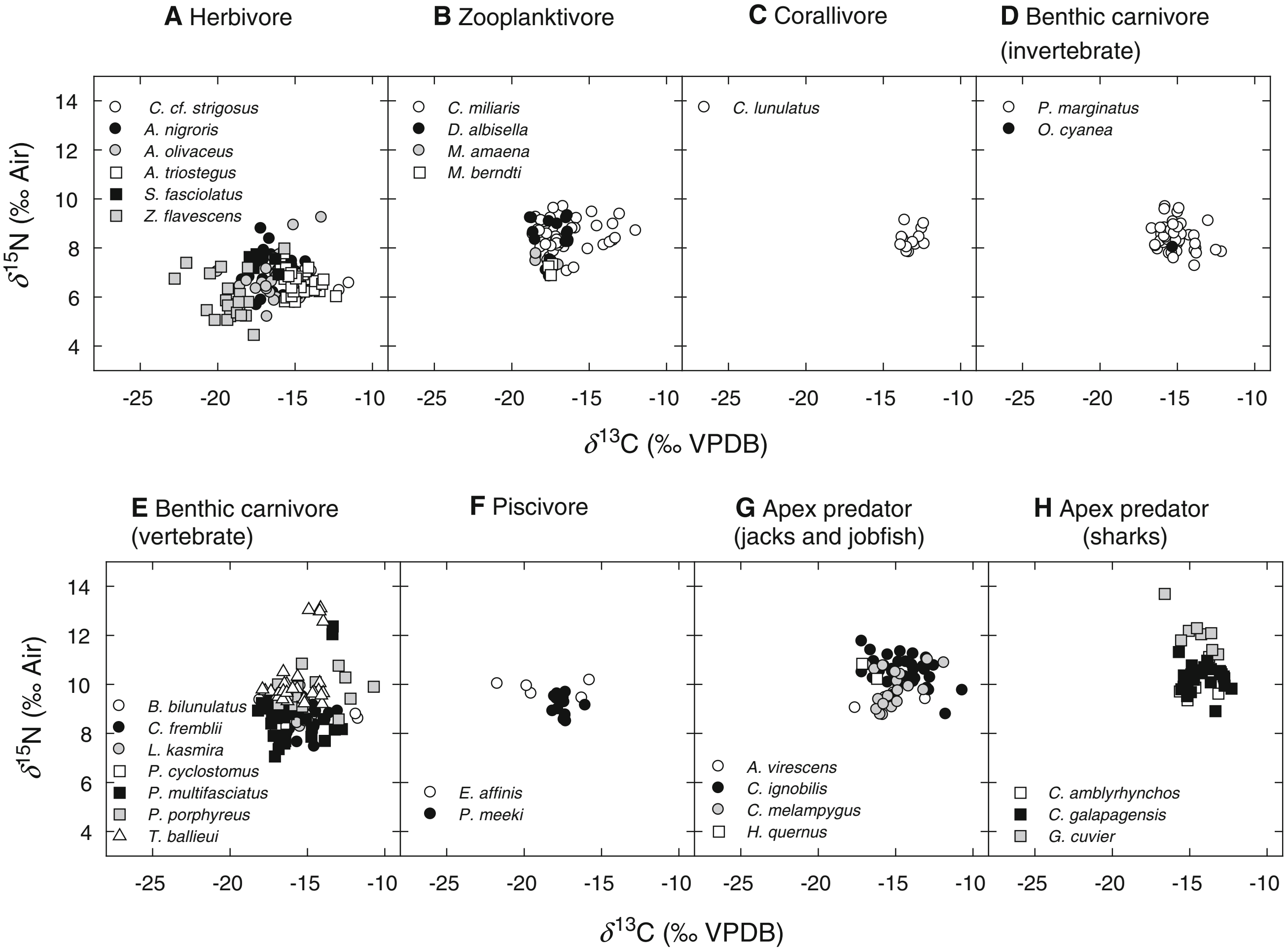

Fig. 4 The individual carbon and nitrogen stable isotope values of each consumer species plotted by trophic group. Apex predators are shown in two panels, G (jacks and jobfish) and H (sharks). The full names and basic statistics for each species are provided in Table 2. *VPDB* Vienna Pee Dee Belemnite

(Table 2; Fig. 4). The herbivore *Ctenochaetus cf. strigosus* ($n = 4$) and the sole corallivore *Chaetodon lunulatus* had similar δ^{13}C means (~ -13.3 ‰) that were at least 0.9 ‰ higher than means of all other consumer species (Table 2). The few individual herbivore δ^{15}N values >7.5 ‰ were found only at Kure Atoll (three species) and Necker Island (one species) (Table S2). The wide range in zooplanktivore δ^{13}C values was driven by *Chaetodon miliaris*, the only zooplanktivore with δ^{13}C values > -16.3 ‰. The eight highest *C. miliaris* δ^{13}C values (> -14.5 ‰) occurred at Kure Atoll (Table S2), and relatively high δ^{13}C values were also found at Kure Atoll for the lobster *Panulirus marginatus* (Table S2). Individual zooplanktivore δ^{15}N were, generally, ~ 1 ‰ lower at FFS and Necker Island than at other locations (Table S1).

Three pairs of benthic carnivore (vertebrate) species had similar SI signatures or patterns in variability (within pairs). *Chaetodon frembii* and *Lutjanus kasmira* had similar δ^{13}C and δ^{15}N means. *Bodianus bilunulatus* and *Parupeneus porphyreus* had similar δ^{13}C ranges and means, and, for both species, the highest individual δ^{13}C values (> -12 ‰) occurred at Midway Island in May 2005 (Table S2). Although *Parupeneus multifasciatus* mean SI values were lower than those of *Thalassoma ballieu* (Table 2), both species exhibited similar spatial variability with lower δ^{13}C means and ranges at Maro Reef and higher δ^{15}N means and ranges at Necker Island (Table S1). In addition, the *T. ballieu* δ^{15}N mean (10.2 ‰) was highest of all benthic carnivores and equal to the mean of apex predators (excluding *Galeocerdo cuvier*) (Table 2).

Euthynnus affinis had the second lowest δ^{13}C mean of all consumer species and differed from the only other piscivore *Priacanthus meeki* by a wider δ^{13}C range and higher δ^{15}N mean (Table 2). Except for *Hyporthodus quernus* ($n = 2$) and *Aprion virescens* ($n = 6$), all apex predator species exhibited ranges in δ^{13}C values ≥ 2.6 ‰ (Table 2), and *Caranx ignobilis* had the widest range in δ^{13}C values (6.5 ‰). Average δ^{15}N values of *A. virescens* were similar to piscivore and benthic carnivore (vertebrate) means (~9.3 ‰). *G. cuvier* specimens were at least 90 cm longer than other apex predators and had average δ^{15}N values 1.6–2.7 ‰> the means of other apex predator species. There was a positive but insignificant relationship between δ^{15}N and TL for *G. cuvier* ($n = 8$, from two locations) and *Carcharhinus amblyrhnochos* ($n = 6$, all from FFS) (Fig. 5). δ^{15}N values of large, medium, and small *Carcharhinus galapagensis* ($n = 16$, from four locations) overlapped with those of medium and large *C. amblyrhynchos*.

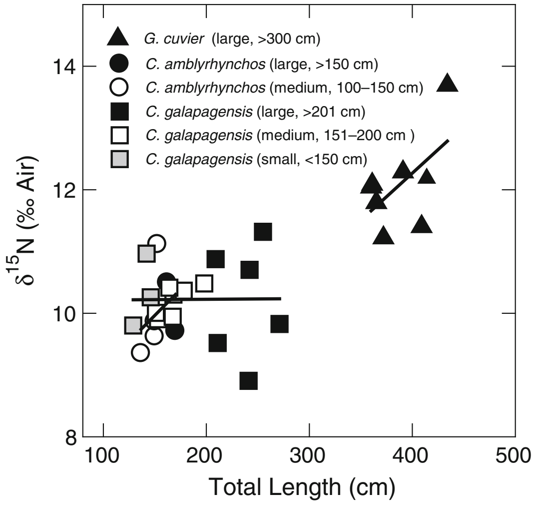

Fig. 5 The relationship between total length and δ^{15}N values for three shark species. Individuals are plotted by species-specific size classes (Lowe et al. 1996; Wetherbee et al. 1996, 1997) and regression lines are shown for each species

Mixing models

Results of the one-isotope (δ^{13}C), two-source mixing model, IsoError (Phillips et al. 2005) indicated that benthic primary production contributed, on average, a minimum of 65 % to consumer production with a lower minimum contribution (40 and 56 %, respectively) to piscivore and zooplanktivore groups than to other groups (≥66 %) (Table 3). Except for the corallivore group, mean benthic primary production contributions ranged from 53 (piscivore) to >90 % (both apex predator groups). The adjusted corallivore δ^{13}C value was higher than the means of both primary producer sources, which resulted in a negative phytoplankton contribution and a benthic primary producer contribution >100 %. The selection of TEF affected model solutions. Increasing Δ^{13}C had the effect of lowering adjusted consumer values ($\delta^{13}C_{\text{food web base}}$) closer to the phytoplankton mean and away from the benthic primary producer mean. For each incremental 0.5 ‰ increase in TEF, the average relative contribution by benthic primary production decreased by 7–22 % with largest changes for consumers at higher trophic levels (not shown). Decreasing Δ^{13}C had the opposite effect.

Although consumer and primary producer individual δ^{15}N values had similar ranges (9.2 and 9.6 ‰, respectively), the range in the δ^{15}N means of three main primary producer groups was compressed (1.6 ‰) relative to the range in consumer group means (5.5 ‰) (Tables 1, 2; Fig. 6). This disparity imposed tight modeling constraints on the selection of TEF and TP values used to adjust consumer means to the base of the food web. Assuming the ECOPATH TP of 2.2 (Grigg et al. 2008) and applying the full range of literature values for Δ^{13}C and Δ^{15}N TEFs (Galván et al. 2012), all of the calculated herbivore

Table 3 Results of the two-source [phytoplankton and combined benthic microalgae (BMI) and benthic macroalgae (BMA)], one-isotope (δ^{13}C) mixing model, IsoError

Consumer group	Percent source contribution to consumer production [mean ± SD (95 % CI)]	
	Phytoplankton	Combined BMI and BMA
Herbivore	16 ± 8 (0–32)	84 ± 8 (68–100)
Zooplanktivore	30 ± 7 (17–44)	70 ± 7 (56–83)
Corallivore	<0	>100
Benthic carnivore (invertebrate)	8 ± 8 (0–25)	92 ± 8 (75–100)
Benthic carnivore (vertebrate)	19 ± 7 (4–34)	81 ± 7 (66–96)
Piscivore	47 ± 6 (34–60)	53 ± 6 (40–66)
Apex predator (excluding *Galeocerdo cuvier*)	7 ± 9 (0–24)	93 ± 9 (76–100)
G. cuvier	9 ± 10 (0–29)	91 ± 10 (71–100)

Assigned TPs (Table 2) and a TEF of 0.5 ‰ Δ^{13}C were used to adjust consumer values to the base of each consumer's food web ($\delta^{13}C_{\text{food web base}}$)

The adjusted corallivore value was outside of the mixing model line formed by the two sources, which resulted in a contribution by combined benthic microalgae and benthic macroalgae of >100 %

SD standard deviation, *CI* confidence interval

$\delta^{15}N_{\text{food web base}}$ values were outside of the mixing model polygon formed by the three major primary producer groups (Fig 7a). This suggests that either TP or $\Delta^{15}N$ was too low or that an important source of primary production with higher $\delta^{15}N$ values was underrepresented. There is no evidence that the presumed herbivores have a higher TP than the ECOPATH estimate of 2.2. Although the 4.5 ‰ difference between the $\delta^{15}N$ means of the herbivore (Table 2) and combined primary producers (Table 1) suggests a TEF of 3.8 ‰ $\Delta^{15}N$ (calculated as using Eq. 3 and the TP of 2.2), this is 0.3–1.6 ‰ higher than all published estimates (Galván et al. 2012). We note also that this estimate of TEF is based on the assumption that the sampled primary producers represent the base of the herbivore food web. Another estimate of TEF is the difference between consumers and their presumptive prey, which averages 1.8 ‰ $\Delta^{15}N$ for the sampled consumer groups. With no compelling support for an herbivore TP >2.2 or that PMNM consumers have a TEF > literature values, it is likely that primary producers with $\delta^{15}N$ values higher than the means of the sampled primary producers were important to the PMNM food web. This conclusion is supported by a number of published primary producer $\delta^{15}N$ values [e.g., Owens (1987) and (MacArthur et al. 2011)].

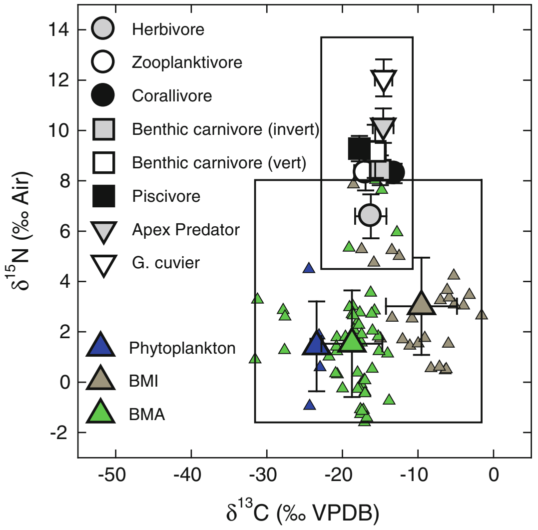

Fig. 6 Primary producer and consumer group $\delta^{13}C$ and $\delta^{15}N$ means (± SD; *large symbols*) plotted in relationship to individual primary producer values (*small symbols*). The ranges of consumer and primary producer values are represented by boxes. *BMI* benthic microalgae, *BMA* benthic macroalgae, *G. cuvier Galeocerdo cuvier*, *VPDB* Vienna Pee Dee Belemnite

We used Eq. 3 and a range of TEFs (2.0–3.5 ‰ $\Delta^{15}N$) (Galván et al. 2012) and $\delta^{15}N_{\text{food web base}}$ values (2.0, 3.0, and 4.0 ‰ $\delta^{15}N$) to calculate potential TPs for herbivore and *Galeocerdo cuvier* groups. The calculated TPs were compared with ECOPATH TPs to evaluate feasible TEF and $\delta^{15}N_{\text{food web base}}$ values (Fig. 8). Calculated TP varied inversely with TEF and $\delta^{15}N_{\text{food web base}}$, and the sensitivity of TP increased with higher values of either variable. For $\delta^{15}N_{\text{food web base}}$ values of 2.0, 3.0, and 4.0 ‰, the ECOPATH TP for *G. cuvier* could be calculated using TEFs ranging of 2.9, 2.6, and 2.3 ‰ $\Delta^{15}N$, respectively. The herbivore ECOPATH TP could be calculated for $\delta^{15}N_{\text{food web base}}$ values of 3.0 and 4.0 using TEFs of 3.0 and 2.2 ‰ $\Delta^{15}N$, respectively. However, the herbivore ECOPATH TP could not be calculated for a $\delta^{15}N_{\text{food web base}}$ of 2.0 ‰

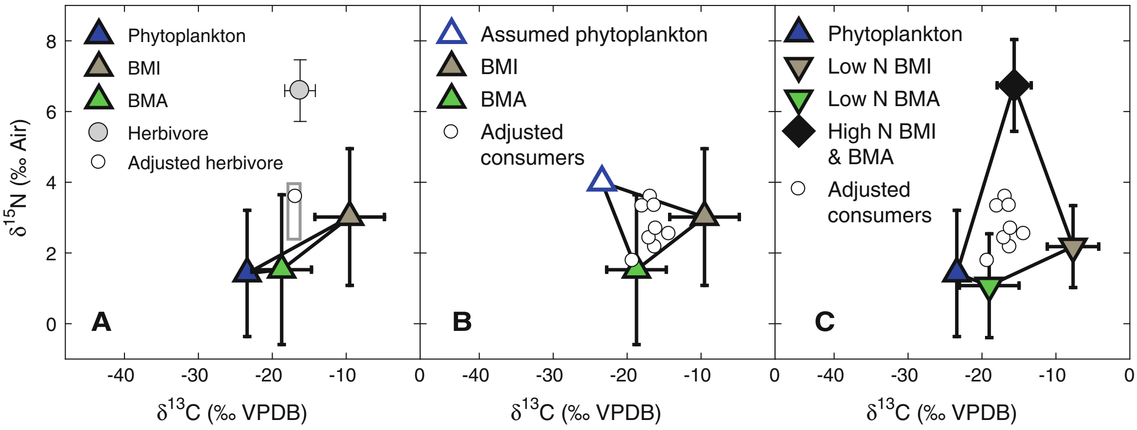

Fig. 7 Three mixing model source polygons are shown relative to $\delta^{15}N_{\text{food web base}}$ and $\delta^{13}C_{\text{food web base}}$ values (*open circles*) of the herbivores **a** and all consumer groups **b** and **c**. Isotopic signatures of the base of food web were calculated using Eq. 3, assumed TPs (Table 2), and TEFs of 0.5 ‰ $\Delta^{13}C$ and 2.5 ‰ $\Delta^{15}N$. The *gray box* **a** represents the full range of potential herbivore food web base values calculated using a TP of 2.2 and literature ranges of TEFs (0–1.4 ‰ $\Delta^{13}C$ and 2.2–3.5 ‰ $\Delta^{15}N$). The polygon formed by the average values of three main primary producer groups is shown in **a**. Alternate polygons were formed by increasing the measured phytoplankton average $\delta^{15}N$ value (1.4 ‰) to 4.0 ‰ **b** and by forming subgroups of benthic algae (Table 2) **c**. Benthic microalgae and benthic macroalgae are abbreviated as BMI and BMA, respectively. *Error bars* represent standard deviation. *VPDB* Vienna Pee Dee Belemnite

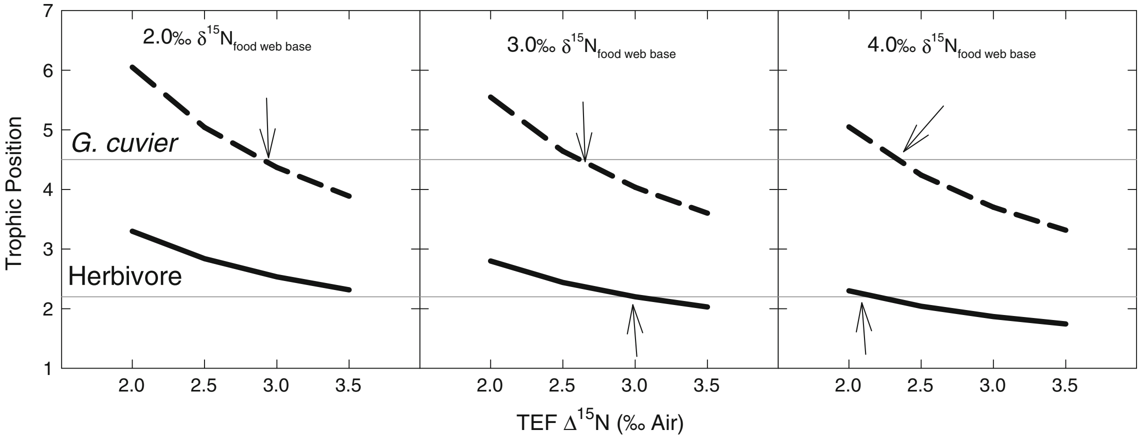

Fig. 8 Calculated potential trophic positions (TP) for the herbivore group (*solid black line*) and *Galeocerdo cuvier* (*dashed black line*) are shown relative to ECOPATH TP estimates (*horizontal lines*) for herbivores (2.2) and *G. cuvier* (4.5). Potential TP was calculated using Eq. 3 and a range of TEFs (2.0, 2.5, 3.0, and 3.5 ‰ Δ^{15}N) for three different $\delta^{15}N_{\text{food web base}}$ values (2.0, 3.0, and 4.0 ‰). *Arrows* indicate the TEF value required to calculate ECOPATH TPs for each consumer using the different $\delta^{15}N_{\text{food web base}}$ values

unless a TEF > literature values was applied. This exercise demonstrates that a TEF near 2.5 ‰ Δ^{15}N is feasible and that a primary food source with an average δ^{15}N value of 3 ‰ or greater was needed to solve a two-isotope, multi-source mixing model for the herbivore group using the ECOPATH TP, as suggested by Fig. 7a.

To explore the isotopic *signature* of potentially important missing or undersampled sources of primary production, we created two alternate source polygons. The first was formed by increasing the average phytoplankton δ^{15}N value from 1.4 to 4.0 ‰ (Owens 1987) (Fig. 7b). For the second, we created a subgroup of the six BMI and four BMA samples with δ^{15}N values >4.5 ‰ and with similar ranges of δ^{13}C values (High N BMI and BMA) (Table 2; Fig. 7c). The remaining BMI and BMA samples formed the Low N BMI and Low N BMA groups. Both polygons were sufficient to solve mixing models for all consumer groups which were adjusted to their food web base values using TPs listed in Table 2 and TEFs of 0.5 ‰Δ^{13}C and 2.5 ‰Δ^{15}N. Within each polygon, adjusted consumer δ^{15}N values formed two clusters, with values of zooplanktivore, herbivore, and *G. cuvier* somewhat higher than other consumers (Fig. 7b, c).

Discussion

Primary producers

We observed large ranges in δ^{13}C and δ^{15}N values of primary producers with no strong evidence of phyletic, temporal, spatial, or methodological trends (Fig. 3). Much of the variability in BMI and BMA was driven by a few samples (BMA δ^{13}C values ≤ -27.6 ‰ and BMI and BMA δ^{15}N values >4.5 ‰) that had little effect on the mean values of the two groups (Table 2). The δ^{13}C results were typical for marine phytoplankton and BMA (France 1995; MacArthur et al. 2011); however, the BMI δ^{13}C results were ~8 ‰ lower than the average reported in France (1995) and may be indicative of cyanobacteria communities or a higher ratio of photosynthetic HCO_3^-:CO_2 utilization (Yamamuro et al. 1995; Kolasinski et al. 2011). Coral, unsampled in this study, is also a source of benthic primary production, but it is unlikely that its inclusion would have altered our results, as PMNM benthic algae δ^{13}C values encompass the range of reported coral flesh and zoozanthellae δ^{13}C values from around the world (−15.1 to −10.4 ‰) (Heikoop et al. 2000).

Based on the mean δ^{15}N values (1.4–3.0 ‰) of the three main groups of primary producers, N_2 fixation appears to be an important source of N to both planktonic and benthic algal communities. However, an undersampled primary producer source group with δ^{15}N values >3 ‰ was required to solve a two-isotope, multi-source mixing model. Consumers may have preferentially utilized the High N BMI and BMA subgroup or phytoplankton with higher δ^{15}N values. However, we note that only one individual (*Halimeda velasquezii*) in the High N BMA group was a member of the genera representing the majority of PMNM primary production biomass. In addition, several other *Halimeda* specimens had lower δ^{15}N values (Table S2). Alternatively, phytoplankton and/or benthic primary producers with higher δ^{15}N values may have been more prevalent on an annual basis than in our May–October sampling window.

Seasonality, or a temporal lag between primary producer and consumer values, is often the source of putative variability in TEF (Hannides et al. 2009; Wyatt et al. 2010).

Integration of the isotopic signature of food sources in consumer muscle tissue can take several months in some species (Logan and Lutcavage 2010; Madigan et al. 2012), and variability in primary producer δ^{15}N values can be driven by seasonality in the N sources for new production in the water column (Dore et al. 2002; Hannides et al. 2009) and the isotopic signature of N in advected or upwelled waters (O'Reilly et al. 2002).

Primary producers with lower δ^{15}N values may also be important to the PMNM food web, as indicated by the position of the adjusted values of several consumer groups within the alternate polygons (Fig. 7a, b). There is little fractionation associated with N_2 fixation (Fogel and Cifuentes 1993), and low δ^{15}N values (~2.0 ‰) are consistent with a primary producer community utilizing N originating with N_2 fixers (Dore et al. 2002). Particularly low near-reef macroalgae δ^{15}N values (~0.3 ‰) have been suggested to originate from cyanophytes (France et al. 1998), and even lower values are reported for *Trichodesmium* in cultures and field studies (McClelland et al. 2003). The importance of benthic cyanobacteria in food webs has been demonstrated in a diet study in Mariana Islands coral reefs (Cruz-Rivera and Paul 2006) and an SI study of a California salt marsh (Currin et al. 2011), while *Trichodesmium* values were the source of low zooplankton δ^{15}N values found in the tropical North Atlantic (McClelland et al. 2003).

Consumers

IsoError mixing model results indicated that benthic primary production (potentially including unsampled coral) provided the majority of support for the PMNM consumer groups (Table 3). However, consumer groups encompass species with a range of habitats and feeding habits, so it is worth noting instances where the SI values of species or individuals differ from their assigned trophic group. For example, similar δ^{13}C values of the corallivore *Chaetodon lunulatus;* the herbivore *Ctenochaetus cf. strigosus*; and some individuals of the zooplanktivore *Chaetodon miliaris* (Fig. 4) suggest mutual dependence on sources with high δ^{13}C values, which could include BMI or unsampled coral. The herbivore *Zebrasoma flavescens* had a δ^{13}C mean similar to that of the piscivore group (the midwater tuna *Euthynnus affinis* and the benthic bigeye *Priacanthus meeki*). The wide (similar) ranges in *Z. flavescens* and *E. affinis* δ^{13}C values (~−22 to −16 ‰) (Table 2) suggest these are opportunistic or generalist feeders (Bearhop et al. 2004) with some individuals perhaps more dependent on a phytoplankton-based food web (or, alternatively, a food web based on BMA species with relatively lower δ^{13}C values).

Similar δ^{13}C values of *P. meeki* and the *Dascyllus* and soldierfish zooplanktivores suggest a common reliance on primary producers with lower δ^{13}C values. The relatively higher δ^{15}N values of *P. meeki* support its inclusion in the piscivore group (Table 2). Also based on relative δ^{15}N values, the benthic carnivore *Thalassoma ballieu* may have been feeding at a higher TP than other benthic carnivores, and the apex predator *Aprion virescens* may have been feeding at a TP similar to that of the piscivores and benthic carnivore (vertebrate) groups (Table 2). The remarkable similarity of *Chaetodon fremblii* and *Lutjanus kasmira* δ^{13}C and δ^{15}N values suggests that these species occupy similar trophic niches; both were classified as benthic carnivores, but an alternate classification is omnivore (Piché et al. 2010; Froese and Pauly 2012). The wide range in *Caranx ignobilis* δ^{13}C and δ^{15}N values may be indicative of an opportunistic diet (Bearhop et al. 2004) or individual dietary specialization (Matich et al. 2011).

The lack of significant correlation between length and δ^{15}N values for PMNM shark species and the overlapping δ^{15}N values of species-specific size classes provided no SI evidence for ontogenetic changes in diet. However, overlapping δ^{15}N values of large *Carcharhinus amblyrhynchos* and small *Carcharhinus galapagensis* are consistent with the high dietary overlap observed in a previous study (Papastamatiou et al. 2006). With a δ^{15}N mean value ~2 ‰ > other apex predators, *Galeocerdo cuvier,* at least 90 cm longer than other apex predators, appears to have been feeding at a higher TP. There was no isotopic support for the FFS ECOPATH model estimates placing the reef shark *C. amblyrhynchos* at a higher TP than *G. cuvier.*

There were indications of apparent spatial variability in some primary producer and consumers. Higher δ^{13}C values of *C. miliaris* and *P. marginatus* occurred at Kure Atoll, and, for some benthic carnivore species, lower δ^{13}C values were observed at Midway Island and Maro Reef. Within trophic groups, most exceptionally high δ^{15}N values were from Kure Atoll and Necker Island (Table S2). These include BMA values >5 ‰ δ^{15}N ($n = 4$), herbivore values >8 ‰ δ^{15}N ($n = 4$), benthic carnivore values >10.8 ‰ δ^{15}N ($n = 7$), and the *G. cuvier* value of 13.7 ‰ δ^{15}N ($n = 1$) (Fig. 3; Table S2). Although the trend was not observed in all trophic groups (e.g., zooplanktivore δ^{15}N values were low at Necker Island and FFS relative to other locations), some of the locational differences are consistent with previous findings. Significant differences in δ^{15}N values of the spiny lobster *Panulirus marginatus* at two PMNM locations were traced to relatively higher δ^{15}N values at the base of the Necker Island food web (O'Malley et al. 2012).

The data highlight the need for identifying sources of apparent spatial isotopic variability in consumers and for investigating the temporal scale of external and internal nitrogen supply processes and phytoplankton dynamics that influence primary producer isotopic variability. Observed spatial variation in SI values of New Caledonia sources and consumers have been ascribed to heterogenetic water column productivity (Carassou et al. 2008), and higher δ^{13}C and lower δ^{15}N values are associated with *Trichodesmium* (Carpenter et al. 1997). In addition, diel, tidal, lunar, and seasonal changes in connectivity among PMNM apex predator species (Dale et al. 2011) can complicate interpretations of spatial variability between sites. Variability in the observed consumer SI values may also be driven by ontogenetic variations, omnivory, or tissue turnover time (Sweeting et al. 2005).

Mixing models

Mixing model evidence for a major contribution of benthic primary production to the PMNM food web is consistent with ecosystem mass-balance model results. The average contribution by benthic primary production to the piscivore group was slightly lower than the 61 % benthic algal contribution for the entire food web in the ECOPATH model of a New Caledonia lagoon (Bozec et al. 2004). For other consumer groups, the average benthic primary producer contribution was 70 % or greater (Table 3), but, generally, lower than the 90 % assigned for the FFS food web in the FFS ECOPATH model (Polovina 1984). Mixing model performance is highly sensitive to TEF (Bond and Diamond 2011), and qualitative differences in model solutions can be examined using a range of TEF values (Galván et al. 2012). If the isotopic signatures of utilized primary producers were adequately represented in mixing models, the SI analysis would provide a test of TP estimates and TEF assumptions. However, this study provided qualitative evidence that the base of the PMNM food web included a source with a higher δ^{15}N value than the averages of the sampled primary producer groups. For alternate mixing model polygons (including a high δ^{15}N source), model solutions required a TEF in the lower range of literature values (2.5 ‰ Δ^{15}N) (Fig. 7b, c). A low TEF (1.8 ‰ Δ^{15}N) was also supported by differences between consumer δ^{15}N values. A TEF of 3.4 ‰ Δ^{15}N (DeNiro and Epstein 1981; Vander Zanden and Rasmussen 2001; Post 2002) is frequently cited, but evidence for lower TEF values (McCutchan et al. 2003; Vanderklift and Ponsard 2003; Caut et al. 2009) is increasing. In a paired tissue and gut sample analysis of 152 coral reef fish from Western Australia, Wyatt et al. (2010) found an average TEF of 2.4 ‰ Δ^{15}N. In addition, a TEF of $\sim$2 ‰ Δ^{15}N was required to match the North Pacific subtropical gyre zooplankton TP calculated using compound-specific isotope analysis (Hannides et al. 2009), and a TEF of 2.5 was required to match diet-based estimations of eastern Pacific Ocean yellow fin tuna TP (Olson et al. 2010).

Conclusion

The comprehensive SI analysis (599 measurements) provides a baseline for systematic investigations of PMNM trophic relationships and drivers of temporal and spatial variability in source isotopic signatures. Mixing model analysis provides support for mass-balance model estimates of the trophic importance of benthic primary production, demonstrating that BMA and BMI are vital components of a coral reef ecosystem with documented high macroalgal cover and high concentration of apex predator biomass (Friedlander and DeMartini 2002; Friedlander et al. 2008; Vroom and Braun 2010). The results suggest that the larger *G. cuvier* may be feeding at a higher TP above other apex predators and that TEF Δ^{15}N may be relatively low in the PMNM. In addition, we report significant variability in primary producer SI values, the prevalence of a N_2 fixation isotopic signature in all primary producers and qualitative evidence that undersampled non-N_2 fixing primary producers are important contributors to the PMNM food web. The results improve our understanding of the trophic ecology of healthy coral reef ecosystems.

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References

Allen GR, Steene R, Allen M (1998) A guide to angelfishes and butterflyfishes. Odyssey Publishing/Tropical Reef Research, Perth

Atkinson MJ, Grigg RW (1984) Model of a coral-reef ecosystem. 2. Gross and net benthic primary production at French Frigate Shoals, Hawaii. Coral Reefs 3:13–22. doi:10.1007/bf00306136

Bearhop S, Adams CE, Waldron S, Fuller RA, Macleod H (2004) Determining trophic niche width: a novel approach using stable isotope analysis. J Anim Ecol 73:1007–1012. doi: 10.1111/j.0021-8790.2004.00861.x

Bond AL, Diamond AW (2011) Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. Ecol Appl 21:1017–1023. doi:10.1890/09-2409.1

Bozec YM, Gascuel D, Kulbicki M (2004) Trophic model of lagoonal communities in a large open atoll (Ouvea, Loyalty islands, New Caledonia). Aquat Living Resour 17:151–162. doi:10.1051/alr:2004024

Carassou L, Kulbicki M, Nicola TJR, Polunin NVC (2008) Assessment of fish trophic status and relationships by stable isotope data in the coral reef lagoon of New Caledonia, southwest Pacific. Aquat Living Resour 21:1–12. doi:10.1051/alr:2008017

Carpenter EJ, Harvey HR, Fry B, Capone DG (1997) Biogeochemical tracers of the marine cyanobacterium Trichodesmium. Deep-Sea Res, Part I 44:27–38. doi:10.1016/s0967-0637(96)00091-x

Caut S, Angulo E, Courchamp F (2009) Variation in discrimination factors $\delta^{15}N$ and $\delta^{13}C$: the effect of diet isotopic values and applications for diet reconstruction. J Appl Ecol 46:443–453. doi:10.1111/j.1365-2664.2009.01620.x

Cruz-Rivera E, Paul VJ (2006) Feeding by coral reef mesograzers: algae or cyanobacteria? Coral Reefs 25:617–627. doi:10.1007/s00338-006-0134-5

Currin CA, Levin LA, Talley TS, Michener R, Talley D (2011) The role of cyanobacteria in Southern California salt marsh food webs. Mar Ecol 32:346–363. doi:10.1111/j.1439-0485.2011.00476.x

Dale JJ, Meyer CG, Clark CE (2011) The ecology of coral reef top predators in the Papahānaumokuākea Marine National Monument. J Mar Biol 2011:1–14. doi:10.1155/2011/725602

DeFelice RD, Parrish JD (2003) Importance of benthic prey for fishes in coral reef-associated sediments. Pac Sci 57:359–384

DeNiro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochim Cosmochim 45:341–351

Dore JE, Brum JR, Tupas LM, Karl DM (2002) Seasonal and interannual variability in sources of nitrogen supporting export in the oligotrophic subtropical North Pacific Ocean. Limnol Oceanogr 47:1595–1607

Fogel ML, Cifuentes LA (1993) Isotope fractionation during primary production. In: Engel MH, Macko SA (eds) Organic Geochemistry: Principles and applications. Plenum Publ, Corp, New York, pp 73–98

France RL (1995) Carbon-13 enrichment in benthic compared to planktonic algae: food web implications. Mar Ecol Prog Ser 124:307–312

France R, Holmquist J, Chandler M, Cattaneo A (1998) $\delta^{15}N$ evidence for nitrogen fixation associated with macroalgae from a seagrass-mangrove coral reef system. Mar Ecol Prog Ser 167:297–299. doi:10.3354/meps167297

Friedlander AM, DeMartini EE (2002) Contrasts in density, size, and biomass of reef fishes between the northwestern and the main Hawaiian islands: the effects of fishing down apex predators. Mar Ecol Prog Ser 230:253–264

Friedlander A, Aeby G, Balwani S, Bowan B, Brainard R, Clark A, Kenyon J, Maragos J, Meyer C, Vroom PS, Zamzow J (2008) The state of coral reef ecosystems of the Northwestern Hawaiian islands. In: Wadell JE, Clarke AM (eds) The state of coral reef ecosystems of the United States and Pacific freely associated states NOAA Technical Memorandum NOS NCCOS 73. NOAA/NCCOS Center for Coastal Monitoring and Assessment's Biogeography Team, Silver Spring, MD, pp 270–311

Friedlander A, Kobayashi D, Bowen B, Meyers C, Papastamatiou Y, DeMartini E, Parrish F, Treml E, Currin C, Hilting A, Weiss J, Kelley C, O'Conner R, Parke M, Clark RG, Toonen RJ, Wedding L (2009) Connectivity and integrated ecosystem studies. NCCOS's Biogeography Branch in cooperation with the Office of National Marine Sanctuaries Papahānaumokuākea Marine National Monument, Silver Spring, MD

Froese R, Pauly D (2012) FishBase World Wide Web electronic publication. www.fishbase.org, version (02/2012)

Galván DE, Sweeting CJ, Polunin NVC (2012) Methodological uncertainty in resource mixing models for generalist fishes. Oecologia 169:1083–1093. doi:10.1007/s00442-012-2273-4

Greenwood NDW, Sweeting CJ, Polunin NVC (2010) Elucidating the trophodynamics of four coral reef fishes of the Solomon Islands using $\delta^{15}N$ and $\delta^{13}C$. Coral Reefs 29:785–792. doi:10.1007/s00338-010-0626-1

Grigg RW, Polovina JJ, Friedlander AM, Rohmann SO (2008) Biology of Coral Reefs in the Northwestern Hawaiian Islands. In: Riegel BM, Dodge RE (eds) Coral Reefs of the USA. Springer, New York, pp 573–594

Hannides CCS, Popp BN, Landry MR, Graham BS (2009) Quantification of zooplankton trophic position in the North Pacific Subtropical Gyre using stable nitrogen isotopes. Limnol Oceanogr 54:50–61. doi:10.4319/lo.2009.54.1.0050

Harriott VJ, Banks SA (2002) Latitudinal variation in coral communities in eastern Australia: a qualitative biophysical model of factors regulating coral reefs. Coral Reefs 21:83–94. doi:10.1007/s00338-001-0201-x

Heikoop JM, Dunn JJ, Risk MJ, Tomascik T, Schwarcz HP, Sandeman IM, Sammarco PW (2000) $\delta^{15}N$ and $\delta^{13}C$ of coral tissue show significant inter-reef variation. Coral Reefs 19:189–193

Hiatt RW, Strasburg DW (1960) Ecological relationships of the fish fauna on coral reefs of the Marshall Islands. Ecol Monogr 30:65–127. doi:10.2307/1942181

Hixon MA (2011) 60 years of coral reef fish ecology: past, present, future. Bull Mar Sci 87:727–765. doi:10.5343/bms.2010.1055

Hobson ES (1974) Feeding relationships of teleostean fishes on coral reefs in Kona Hawaii. Fish Bull 72:915–1031

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R, Muthiga N, Bradbury RH, Dubi A, Hatziolos ME (2007) Coral reefs under rapid climate change and ocean acidification. Science 318:1737–1742. doi:10.1126/science.1152509

Hoenisch B, Ridgwell A, Schmidt DN, Thomas E, Gibbs SJ, Sluijs A, Zeebe R, Kump L, Martindale RC, Greene SE, Kiessling W, Ries J, Zachos JC, Royer DL, Barker S, Marchitto TM Jr, Moyer R, Pelejero C, Ziveri P, Foster GL, Williams B (2012) The geological record of ocean acidification. Science 335:1058–1063. doi:10.1126/science.1208277

Hoey AS, Pratchett MS, Cvitanovic C (2011) High macroalgal cover and low coral recruitment undermines the potential resilience of the world's southernmost coral reef assemblages. PLoS One 6(10):e25824. doi:10.1371/journal.pone.0025824

Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR, Folke C, Grosberg R, Hoegh-Guldberg O, Jackson JBC, Kleypas J, Lough JM, Marshall P, Nystrom M, Palumbi SR, Pandolfi JM, Rosen B, Roughgarden J (2003) Climate change, human impacts, and the resilience of coral reefs. Science 301:929–933. doi:10.1126/science.1085046

Hughes TP, Graham NAJ, Jackson JBC, Mumby PJ, Steneck RS (2010) Rising to the challenge of sustaining coral reef resilience. Trends Ecol Evol 25:633–642. doi:10.1016/j.tree.2010.07.011

Kiehl J (2011) Lessons from Earth's past. Science 331:158–159. doi:10.1126/science.1199380

Knowlton N, Jackson JBC (2008) Shifting baselines, local impacts, and global change on coral reefs. PLoS Biol 6:e54. doi:10.1371/journal.pbio.0060054

Kolasinski J, Rogers K, Cuet P, Barry B, Frouin P (2011) Sources of particulate organic matter at the ecosystem scale: a stable isotope and trace element study in a tropical coral reef. Mar Ecol Prog Ser 443:77–93. doi:10.3354/meps09416

Logan JM, Lutcavage ME (2010) Stable isotope dynamics in elasmobranch fishes. Hydrobiologia 644:231–244. doi:10.1007/s10750-010-0120-3

Lowe CG, Wetherbee BM, Crow GL, Tester AL (1996) Ontogenetic dietary shifts and feeding behavior of the tiger shark, *Galeocerdo cuvier*, in Hawaiian waters. Environ Biol Fishes 47:203–211. doi:10.1007/bf00005044

MacArthur LD, Phillips DL, Hyndes GA, Hanson CE, Vanderklift MA (2011) Habitat surrounding patch reefs influences the diet and nutrition of the western rock lobster. Mar Ecol Prog Ser 436:191–205. doi:10.3354/meps09256

Madigan D, Litvin S, Popp B, Carlisle AB, Farwell C, Block B (2012) Tissue turnover rates and isotopic trophic discrimination factors in the endothermic teleost, Pacific Bluefin Tuna (Thunnus orientalis). PLoS One 7:e49220. doi:10.1371/journal.pone.0049220

Marine Mammal Commission (2001) Annual Report to Congress 2000, Bethesda, Maryland, pp 1–253

Matich P, Heithaus MR, Layman CA (2011) Contrasting patterns of individual specialization and trophic coupling in two marine apex predators. J Anim Ecol 80:294–305. doi:10.1111/j.1365-2656.2010.01753.x

McClelland JW, Holl CM, Montoya JP (2003) Relating low δ^{15}N values of zooplankton to N_2-fixation in the tropical North Atlantic: insights provided by stable isotope ratios of amino acids. Deep-Sea Res, Part I 50:849–861. doi:10.1016/s0967-0637(03)00073-6

McCutchan JH, Lewis WM, Kendall C, McGrath CC (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen and sulfur. Oikos 102:378–390

Moseman SM, Levin LA, Currin C, Forder C (2004) Colonization, succession, and nutrition of macrobenthic assemblages in a restored wetland at Tijuana Estuary, California. Estuar Coast Shelf Sci 60:755–770

Olson RJ, Popp BN, Graham BS, Lopez-Ibarra GA, Galvan-Magana F, Lennert-Cody CE, Bocanegra-Castillo N, Wallsgrove NJ, Gier E, Alatorre-Ramirez V, Ballance LT, Fry B (2010) Food-web inferences of stable isotope spatial patterns in copepods and yellowfin tuna in the pelagic eastern Pacific Ocean. Prog Oceanogr 86:124–138. doi:10.1016/j.pocean.2010.04.026

O'Malley JM, Drazen JC, Popp BN, Gier E, Toonen RJ (2012) Spatial variability in the growth and prey availability of lobsters in the northwestern Hawaiian Islands. Mar Ecol Prog Ser 449:211–220. doi:10.3354/meps09533

O'Reilly CM, Hecky RE, Cohen AS, Plisnier PD (2002) Interpreting stable isotopes in food webs: recognizing the role of time averaging at different trophic levels. Limnol Oceanogr 47:306–309

Owens NJP (1987) Natural variations in ^{15}N in the marine environment. Adv Mar Biol 24:389–451

Papastamatiou YP, Wetherbee BM, Lowe CG, Crow GL (2006) Distribution and diet of four species of carcharhinid shark in the Hawaiian Islands: evidence for resource partitioning and competitive exclusion. Mar Ecol Prog Ser 320:239–251. doi:10.3354/meps320239

Parrish FA, Boland RC (2004) Habitat and reef-fish assemblages of banks in the Northwestern Hawaiian Islands. Mar Biol 144:1065–1073. doi:10.1007/s00227-003-1288-0

Phillips DL, Newsome SD, Gregg JW (2005) Combining sources in stable isotope mixing models: alternative methods. Oecologia 144:520–527. doi:10.1007/s00442-004-1816-8

Piché J, Iverson SJ, Parrish FA, Dollar R (2010) Characterization of forage fish and invertebrates in the Northwestern Hawaiian Islands using fatty acid signatures: species and ecological groups. Mar Ecol Prog Ser 418:1–15. doi:10.3354/meps08814

Polovina JJ (1984) Model of a coral reef ecosystem. I. The ECOPATH model and its application to French Frigate Shoals. Coral Reefs 3:1–11

Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703–718

Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montana CG (2007) Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152:179–189. doi:10.1007/s00442-006-0630-x

Sandin SA, Smith JE, DeMartini EE, Dinsdale EA, Donner SD, Friedlander AM, Konotchick T, Malay M, Maragos JE, Obura D, Pantos O, Paulay G, Richie M, Rohwer F, Schroeder RE, Walsh S, Jackson JB, Knowlton N, Sala E (2008) Baselines and degradation of coral reefs in the Northern Line Islands. PLoS One 3:e1548. doi:10.1371/journal.pone.0001548

Stevenson C, Katz LS, Micheli F, Block B, Heiman KW, Perle C, Weng K, Dunbar R, Witting J (2007) High apex predator biomass on remote Pacific islands. Coral Reefs 26:47–51

Sweeting CJ, Jennings S, Polunin NVC (2005) Variance in isotopic signatures as a descriptor of tissue turnover and degree of omnivory. Funct Ecol 19:777–784. doi:10.1111/j.1365-2435.2005.01019.x

Vander Zanden MJ, Rasmussen JB (2001) Variation in δ^{15}N and δ^{13}C trophic fractionation: implications for aquatic food web studies. Limnol Oceanogr 46:2061–2066

Vanderklift MA, Ponsard S (2003) Sources of variation in consumer-diet δ^{15}N enrichment: a meta-analysis. Oecologia 136:169–182. doi:10.1007/s00442-003-1270-z

Vroom PS, Braun CL (2010) Benthic composition of a healthy subtropical reef: baseline species-level cover, with an emphasis on algae, in the Northwestern Hawaiian Islands. PLoS One 5:e9733. doi:10.1371/journal.pone.0009733

Vroom PS, Page KN, Peyton KA, Kukea-Shultz JK (2005) Spatial heterogeneity of benthic community assemblages with an emphasis on reef algae at French Frigate Shoals, Northwestern Hawaiian Islands. Coral Reefs 24:574–581. doi:10.1007/s00338-005-0028-y

Wainright SC, Weinstein MW, Able KW, Currin CA (2000) Relative importance of benthic microalgae, phytoplankton and the detritus of smooth cordgrass *Spartina alterniflora* and the common reed *Phragmites australis* to brackish-marsh food webs. Mar Ecol Prog Ser 200:77–91

Wetherbee BM, Crow GL, Lowe CG (1996) Biology of the Galapagos shark, *Carcharhinus galapagensis*, in Hawai'i. Environ Biol Fishes 45:299–310. doi:10.1007/bf00003099

Wetherbee BM, Crow GL, Lowe CG (1997) Distribution, reproduction and diet of the gray reef shark *Carcharhinus amblyrhynchos* in Hawaii. Mar Ecol Prog Ser 151:181–189. doi:10.3354/meps151181

Wyatt ASJ, Waite AM, Humphries S (2010) Variability in isotope discrimination factors in coral reef fishes: implications for diet and food web reconstruction. PLoS One 5:e13682. doi:10.1371/journal.pone.0013682

Yamamuro M, Kayanne H, Minagawa M (1995) Carbon and nitrogen stable isotopes of primary producers in coral-reef ecosystems. Limnol Oceanogr 40:617–621