# Intraspecific variation and energy channel coupling within a Chilean kelp forest

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*Abstract.* The widespread importance of variable types of primary production, or energy channels, to consumer communities has become increasingly apparent. However, the mechanisms underlying this "multichannel" feeding remain poorly understood, especially for aquatic ecosystems that pose unique logistical constraints given the diversity of potential energy channels. Here, we use bulk tissue isotopic analysis along with carbon isotope ($\delta^{13}$C) analysis of individual amino acids to characterize the relative contribution of pelagic and benthic energy sources to a kelp forest consumer community in northern Chile. We measured bulk tissue $\delta^{13}$C and $\delta^{15}$N for >120 samples; of these we analyzed $\delta^{13}$C values of six essential amino acids (EAA) from nine primary producer groups ($n = 41$) and 11 representative nearshore consumer taxa ($n = 56$). Using EAA $\delta^{13}$C data, we employed linear discriminant analysis (LDA) to assess how distinct EAA $\delta^{13}$C values were between local pelagic (phytoplankton/particulate organic matter), and benthic (kelps, red algae, and green algae) endmembers. With this model, we were able to correctly classify nearly 90% of producer samples to their original groupings, a significant improvement on traditional bulk isotopic analysis. With this EAA isotopic library, we then generated probability distributions for the most important sources of production for each individual consumer and species using a bootstrap-resampling LDA approach. We found evidence for multichannel feeding within the community at the species level. Invertebrates tended to focus on *either* pelagic or benthic energy, deriving 13–67% of their EAA from pelagic sources. In contrast, mobile (fish) taxa at higher trophic levels used more equal proportions of each channel, ranging from 19% to 47% pelagically derived energy. Within a taxon, multichannel feeding was a result of specialization among individuals in energy channel usage, with 37 of 56 individual consumers estimated to derive >80% of their EAA from a single channel. Our study reveals how a cutting-edge isotopic technique can characterize the dynamics of energy flow in coastal food webs, a topic that has historically been difficult to address. More broadly, our work provides a mechanism as to how multichannel feeding may occur in nearshore communities, and we suggest this pattern be investigated in additional ecosystems.

*Key words:* $^{13}$C; compound-specific stable isotope analysis; essential amino acids; marine ecology; multichanneling; multiple energetic pathways; stable isotopes; upwelling.

## Introduction

The use of multiple sources of primary production by consumer communities is now thought to be common across terrestrial, freshwater, and marine food webs (Wolkovich et al. 2014). In particular, the use of asymmetric channels, or fast and slow sources of production, has received much attention in recent years (Rooney et al. 2006, Rooney and McCann 2012, Wolkovich et al. 2014, Ward et al. 2015). Termed "multichanneling" at

the ecosystem scale, or "multichannel feeding" in reference to particular consumers, much of the research on this topic has documented its prevalence across different ecosystems (Wolkovich et al. 2014), examined temporal variation in multichannel feeding (García et al. 2017), explored the implications for food web stability (Rooney et al. 2006, Rooney and McCann 2012), and examined how this phenomenon alters our understanding of trophic cascades (Ward et al. 2015). However, one aspect of multichannel feeding that remains understudied is the mechanism underlying this consumer generalism (Perkins et al. 2018). More simply, is energy channel coupling achieved through populations of individuals that switch from fast to slow energy channels as they become

available? Or, within a population that uses multiple sources of energy, do individual consumers specialize on a particular energy channel?

Kelp forests are highly productive nearshore marine ecosystems that provide an excellent framework for exploring the processes governing energy channel coupling and multichannel feeding. These systems are characterized by expansive swaths of macroalgae, particularly order Laminariales (kelps), and occur globally in nutrient-rich waters at temperate latitudes (Steneck et al. 2002). Consequently, the highly diverse consumer communities within kelp forests are potentially able to utilize a combination of fast (pelagic phytoplankton) and slow (benthic macroalgae) energy channels. The degree to which members of these communities rely on kelp-derived energy is a highly debated issue (e.g., Duggins et al. 1989, Docmac et al. 2017) and likely depends locally on the invertebrate herbivore community (Yorke et al. 2019), concentration of secondary metabolites in kelp tissues (Steinberg et al. 1995), and extent and consistency of adjacent phytoplankton production. For example, in kelp forests at high latitudes, the oceanographic conditions that promote phytoplankton production are highly seasonal (Westberry et al. 2016), and local consumers can only occasionally utilize this ephemeral energy channel during their annual life cycle. Thus, kelp-derived production is an important energy source for consumer communities in these habitats (Duggins et al. 1989, Elliott Smith et al. 2018). In contrast, in low latitude regions with high amounts of pelagic production nearly year-round, this energy channel provides a more consistent basal resource for consumers, and kelp-derived energy may be less important (Vargas et al. 2007, Docmac et al. 2017). Kelp forests act as key habitat for many species and provide important goods and services to human populations throughout their distribution, including fisheries (Vásquez et al. 2014). As kelp forests experience escalating anthropogenic impacts, quantifying which energy channels are important to local consumers will thus be vital in setting management targets.

Traditional methods of studying energy flow in food webs have been challenging or entirely intractable in kelp forests. Because direct observations are time and cost intensive in marine ecosystems, researchers have increasingly relied on stable isotope analysis as a method for characterizing the transfer of nutrients across trophic linkages (e.g., Layman et al. 2012). In particular, measurements of stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes in whole (bulk) tissues can be used as biomarkers, with $\delta^{13}C$ values typically varying among marine producer groups, and $\delta^{15}N$ values increasing systematically as a function of trophic level (Fogel and Cifuentes 1993, Hobson et al. 1994). However, the inferences that can be drawn from bulk tissue isotope analysis are limited by substantial spatiotemporal isotopic variation in producer tissues (e.g., Larsen et al. 2009, Whiteman et al. 2019). In particular, bulk tissue analysis often fails to adequately resolve pelagic and benthic endmembers in highly productive coastal areas characterized by high levels of upwelling (Fredriksen 2003, Page et al. 2008). In addition, trophic discrimination factors (e.g., $\Delta^{13}C$ and $\Delta^{15}N$) between consumers and food sources can vary widely depending on consumer tissue type, life history, diet, and physiological state (Ben-David and Flaherty 2012). Together, these factors make it difficult to "match" the isotopic composition of basal producers to those of local consumers, and thus to estimate the importance of different energy channels to various food web compartments.

Recent developments in the isotopic analysis of individual compounds provide an improved method of characterizing food web structure and energy flow (Whiteman et al. 2019). The analysis of individual essential amino acids (EAA) is particularly useful for community ecology, as these molecules are a major conduit of energy flow between trophic levels (Larsen et al. 2009, Ruess and Müller-Navarra 2019). Because only autotrophs and microbes can synthesize EAA, higher order consumers must acquire them via direct consumption (Fantle et al. 1999, Howland et al. 2003) or possibly from their gut microbiome if dietary protein content is insufficient (Newsome et al. 2011, 2020). Consequently, EAA are minimally altered by consumers during assimilation and the isotopic composition of these molecules remains consistent across trophic linkages (Fantle et al. 1999, Howland et al. 2003). Furthermore, recent work reveals that co-occurring producer taxa within coastal ecosystems have distinct patterns of EAA $\delta^{13}C$ values, or "fingerprints," depending on the metabolic pathways they use to synthesize these compounds de novo (Vokhshoori et al. 2014, Elliott Smith et al. 2018). These fingerprints do not appear to change across growth or nutrient gradients (Larsen et al. 2013, 2015), and can thus be identified in consumers and used to quantify the importance of distinct energy pathways to any food web compartment.

Here, we examine the processes governing the coupling of pelagic (phytoplankton and particulate organic matter) and benthic (kelps and other macroalgae) energy channels in a highly productive Chilean kelp forest. We present bulk tissue isotopic analysis, as well as a library of essential amino acid $\delta^{13}C$ values for pelagic organic material, and three benthic macroalgae groups. We use EAA $\delta^{13}C$ data to quantify the relative importance of pelagic/benthic production to representative invertebrate ($n = 6$) and fish ($n = 5$) taxa spanning a range of trophic and functional groups. At both the individual and species level, we were able to characterize the proportional contribution of pelagic and benthic production to consumers, and from there the overall degree of "multichannel feeding" in this system. Our results reveal a high degree of individual variation in energy channel utilization by consumers, and we suggest that intraspecific diet specialization may play an important role in mediating multichannel feeding.

METHODS

*Sites and sample collection*

We collected the majority of samples from two localities near Antofagasta in northern Chile (Appendix S1: Fig. S1): Isla Santa María (ISM, 23°27' S, 70°36' W) and Coloso (COL, 23°46' S, 70°29' W). Both sites are shallow, characterized by rocky substrates and strong, consistent upwelling (Paredes et al. 2018), although upwelling influence is likely less intense at COL (Reddin et al. 2015). Sampling at these sites was conducted on a bimonthly basis from January 2016 to December 2017; most of the samples analyzed here are from the austral winter and summer of 2016. Information on the collection season for every sample can be found in Data S1. Because of the close geographic proximity and similar abiotic environments of ISM and COL, data from these sites were grouped by species or functional group (e.g., "kelps"); there were no differences in the bulk tissue isotope values among sites (Appendix S4: Table S1).

At each site, we collected four groups of marine producers representing the dominant local algae/functional groups (Table 1; Fariña et al. 2008). These included kelps (*Lessonia* sp., *Macrocystis pyrifera*), red algae (*Chondrus canaliculatus*, *Plocamium cartilagineum*, *Rhodymenia corallina*, and one unidentified sample), green algae (*Ulva* sp.), and phytoplankton/particulate organic matter (POM) samples. In addition, we collected one sample of epilithic biofilm from each site. Kelps and red algae were collected via SCUBA at 5 and 10 m depth. *Ulva* and biofilm were collected from the intertidal. Phytoplankton and particulate organic matter were sampled close to (100–200 m) and further (1,000–1,500 m) offshore at several stations (Appendix S1: Fig. S1). For phytoplankton samples, verticals tows were made using a $40 \times 100$ cm standard phytoplankton net with 20 µm mesh (Hydro-Bios GmbH, Altenholz, Germany); for POM samples, a 5-L Niskin water sampler (Hydro-Bios) was deployed. In the laboratory, POM and phytoplankton samples were filtered at 200 µm to screen out larger detritus and zooplankton, then passed through pre-combusted (450°C, 4 h) 0.7 µm GF/F membranes. These filters were checked visually with a binocular microscope, and large particles of obvious non-phytoplankton origin were removed using forceps.

TABLE 1. Humboldt Current producer and consumer samples from near Antofagasta, Chile.

| Functional group | Species | ISM: bulk tissue (EAA) | COL: bulk tissue (EAA) | Regional totals: bulk tissue (EAA) | Habitats and collection notes |
|---|---|---|---|---|---|
| **Fish** | | | | | |
| Carnivore (large inverts, fish) | *Paralabrax humeralis* | 4 (3) | 4 (3) | 8 (6) | subtidal fyke nets |
| Carnivore (small inverts) | *Cheilodactylus variegatus* | 4 (4) | 4 (2) | 8 (6) | subtidal fyke nets |
| Omnivore (nearshore) | *Isacia conceptionis* | 4 (4) | 3 (3) | 7 (7) | subtidal fyke nets |
| Herbivore | *Aplodactylus punctatus* | 4 (4) | 4 (4) | 8 (8) | subtidal fyke nets |
| Planktivore (offshore) | *Engraulis ringens* | – | – | 4 (4) | stranded alive onshore |
| **Invertebrates** | | | | | |
| Carnivore | *Concholepas concholepas* | – | 2 (2) | 2 (2) | intertidal/upper subtidal |
| Herbivore | *Taliepus* sp. | – | – | 6 (6) | subtidal fyke nets |
| Herbivore/grazer | *Tegula atra* | 4 (4) | 4 (3) | 8 (7) | intertidal/upper subtidal |
| Herbivore/grazer | *Tetrapygus niger* | 3 (2) | 2 (2) | 5 (4) | intertidal/upper subtidal |
| Filter feeder | *Perumytilus purpuratus* | 2 (2) | 3 (3) | 5 (5) | intertidal |
| Planktivore | zooplankton | 1 (1) | 3 (0) | 4 (1) | pelagic tows |
| **Producers** | | | | | |
| Epilithic film | biofilm | 1 (1) | 1 (1) | 2 (2) | intertidal |
| Red algae | *Chondrus canaliculatus*, *Plocamium cartilagineum*, *Rhodymenia corallina*, unidentified red | 4 (3) | 4 (4) | 8 (7) | subtidal |
| Green algae | *Ulva* sp. | 3 (3) | 4 (4) | 7 (7) | intertidal |
| Kelp | *Lessonia* sp., *Macrocystis pyrifera* | 15 (5) | 9 (7) | 24 (12) | subtidal at different depths |
| Phytoplankton/POM | unknown | 6 (6) | 9 (7) | 15 (13) | pelagic tows/upper subtidal subsurface water samples |

*Notes:* Sample sizes, mode and location of collections, and functional groups of each species sampled in the present study. Dashes indicate no samples were collected for these species/taxa at the indicated locality. ISM, Isla Santa María; COL, Coloso. Samples of *Engraulis ringens* and *Taliepus* sp. were collected near but not directly at these sites (see Appendix S1: Fig. S1). The subset of samples with essential amino acid (EAA) $\delta^{13}$C data are presented in parentheses. Note that for two phytoplankton/particulate organic matter (POM) samples and one kelp we have only EAA $\delta^{13}$C data. See Metadata S1 and Data S1 for details.

For analysis, phytoplankton/POM samples were incorporated into a single "pelagic" category.

We also sampled local macroinvertebrates and fish from different trophic levels and feeding modes (Table 1). Among the invertebrate community, we collected filter-feeding mussels (*Perumytilus purpuratus;* Santelices and Martínez 1988), offshore pelagic zooplankton, and three herbivore species including a grazing gastropod (*Tegula atra;* Camus et al. 2013), grazing sea urchin (*Tetrapygus niger;* Camus et al. 2013), and a predominantly macroalgae herbivore *Taliepus* sp. (kelp crab; Jofré Madariaga et al. 2013). We also opportunistically collected the carnivorous gastropod *Concholepas concholepas* (Stotz et al. 2003). Invertebrates, except *Taliepus* and zooplankton, were collected at each site by hand from the lower intertidal/upper subtidal zone. Zooplankton were sampled from the phytoplankton tows during laboratory filtration, and *Taliepus* were collected from fyke nets from a locality ~7 km south of COL. In addition, we collected individuals from five fish species belonging to different foraging guilds (Table 1, Data S1): (1) *Aplodactylus punctatus*, an herbivore (Pérez-Matus et al. 2012), (2) *Paralabrax humeralis*, an invertivore/piscivore (Docmac et al. 2017), (3) *Cheilodactylus variegatus*, an invertivore (Docmac et al. 2017), (4) *Isacia conceptionis*, a nearshore omnivore (Pérez-Matus et al. 2012), and (5) the anchoveta, *Engraulis ringens*, an offshore planktivore (Pizarro et al. 2019). With the exception of anchoveta, all fish samples were collected at each site via spearfishing, or double fyke-net fished overnight (Doppelreuse Typ 3, Engel-Netze GmbH & Co. Kg, KG, Bremerhaven, Germany). Anchoveta were found stranded alive on the coast by local fisherman; we assume these individuals were feeding locally, as previous research suggests there is limited immigration of non-local anchoveta into the northern Chile stock (Garcés et al. 2019). All producers and consumer samples were kept on ice for 2–8 h immediately post collection, and then either processed and lyophilized, or stored frozen at −20°C until analysis.

*Sample preparation and isotopic analysis*

In preparation for isotopic analysis, samples were rinsed with deionized water and then lyophilized (producers), and lipid extracted (consumers). We did not acidify phytoplankton/POM filters as this procedure has minimal effects on bulk tissue isotopic values (Barrios-Guzmán et al. 2019), and our amino acid purification and data processing procedures ensure only amino acid carbon is measured. Prior to lipid extraction, invertebrate consumers were shucked, and where possible muscle was preferentially excised; a few smaller individuals were roughly homogenized and subsampled. For fish specimens, a piece of the dorsal muscle was removed for analysis. For all consumer subsamples, lipids were removed via four 24 h soaks in petroleum ether, followed by thorough rinsing in deionized water and

lyophilization. These treatments ensured that we conducted isotopic analysis on only muscle for all consumers except zooplankton ([C]:[N] ratios < 4, Data S1). Samples were subsequently analyzed for bulk tissue $\delta^{13}C$ and $\delta^{15}N$.

Bulk tissue $\delta^{13}C$ and $\delta^{15}N$ values of all samples were measured using a Costech 4010 elemental analyzer (Valencia, California, USA) interfaced with a Thermo Scientific Delta V Plus isotope ratio mass spectrometer (Bremen, Germany) at the University of New Mexico Center for Stable Isotopes (UNM-CSI, Albuquerque, New Mexico, USA). The within-run standard deviation of organic reference materials was ≤0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$ values. For both bulk tissue and amino acid analyses, we report all isotopic results as $\delta$ values. $\delta^{13}C$ or $\delta^{15}N = 1,000 \times ([R_{sample}/R_{standard}] - 1)$, where $R_{sample}$ and $R_{standard}$ are the $^{13}C$:$^{12}C$ or $^{15}N$:$^{14}N$ ratios of the sample and standard, respectively. The internationally accepted standards for $\delta^{13}C$ and $\delta^{15}N$ analysis are Vienna-Pee Dee Belemnite limestone (V-PDB) and atmospheric $N_2$. The units are expressed as parts per thousand, or per mil (‰).

For EAA $\delta^{13}C$ analysis, we hydrolyzed 2–5 mg of lipid-extracted consumer tissues, and 4–6 mg of macroalgae, in 1–1.5 mL of 6mol/L hydrochloric acid at 110°C for 20 h. Hydrolysis tubes were flushed with $N_2$ gas before sealing. For pelagic (phytoplankton/POM) samples, approximately one-half of each filter was added to 6N HCl and treated as above. Producer hydrolysates were then passed through a cation exchange resin column (Dowex 50 W X8 100-200 mesh, Alfa Aesar, Tewksbury, MA, USA) to isolate AAs from other contaminants/metabolites (Amelung and Zhang 2001). Purified amino acids were derivatized to N-trifluoroacetic acid isopropyl esters following established protocols (Silfer et al. 1991, Newsome et al. 2011). Samples were derivatized in batches alongside an in-house reference material containing all AAs measured for $\delta^{13}C$ (Appendix S2).

Derivatized samples were injected into a 60 m BPX5 gas chromatograph column for AA separation (0.32 ID, 1.0 μm film thickness, SGE Analytical Science, Victoria, Australia) in a Thermo Scientific Trace 1300. Samples were then combusted into $CO_2$ with a GC Isolink II interfaced to a Thermo Scientific Delta V Plus isotope ratio mass spectrometer at UNM-CSI. Samples were injected in duplicate or triplicate (for two samples, HUM-POM-COL2-B2 and HUM-ISM-ULA3-B4, we were only able to get a single injection) and bracketed with the in-house AA reference material. The within-run standard deviations of $\delta^{13}C$ values of the reference material ranged from 0.1‰ (isoleucine) to 1.7‰ (threonine). We reliably measured $\delta^{13}C$ values of 12 AAs including six considered non-essential (alanine [Ala], aspartic acid [Asp], glutamic acid [Glu], glycine [Gly], proline [Pro], serine [Ser]) and six considered essential (isoleucine [Ile], leucine [Leu], lysine [Lys], phenylalanine [Phe], threonine [Thr], and valine [Val]). We here present and interpret *only* the essential amino acid $\delta^{13}C$ data. The reagents

used during derivatization add carbon to the side chains of amino acids, and the $\delta^{13}C$ measured via GC-C-IRMS is thus a combination of original amino acid and reagent carbon. See Appendix S2 for the correction equations used to calculate the intrinsic EAA $\delta^{13}C$.

Of the total 123 samples analyzed for bulk tissue isotopic analyses, we obtained EAA $\delta^{13}C$ data for 97 (Table 1, Data S1). All samples except HUM-ISM-Biofilm-Junio had standard deviations across injections of <1.1‰ for all EAA. For two phytoplankton/POM samples (HUM-ISM-PEL3-FITO and HUM-ISM4-FITO), and one kelp sample (HUM-ISM12-MIN3-B3), initial bulk tissue analysis failed and we did not have enough remaining material to rerun the sample after EAA $\delta^{13}C$ analysis. Thus, we only have amino acid isotopic data for these samples (Data S1).

### Statistical analyses

We present bulk tissue $\delta^{13}C$ and $\delta^{15}N$ data here largely for comparative purposes with other studies; the majority of our analyses relied on EAA $\delta^{13}C$ values. For all isotopic data, we assessed the assumptions of normality and equal variances within and among groups using the Cramér-von Mises test with quantile-quantile plots, and Bartlett's test, respectively. Following this, for bulk tissue $\delta^{13}C$ and $\delta^{15}N$ values we used the nonparametric Wilcoxon-signed rank test with a Benjamini and Hochberg correction (1995) to determine whether isotopic data for each species/producer group differed between ISM and COL. We also used these analyses to test if we could distinguish among our two primary energy channels, kelp and phytoplankton/POM, using bulk tissue isotopic values. EAA $\delta^{13}C$ values for producers and consumers passed normality checks and we thus used MANOVA to compare EAA $\delta^{13}C$ values among producer and consumer groups. Individual pairwise contrasts for each EAA between groups were subsequently conducted using ANOVA and Tukey's HSD. We ran statistical analyses in R (v 3.3.1; R Core Team 2013) with RStudio interface (v 0.99.903).

To characterize the proportional contribution of each producer group to our sampled consumers, we applied a bootstrapping resampling approach (e.g., Fox et al. 2019) to linear discriminant analysis (LDA). We first examined the classification error rate for the four producer groups (phytoplankton/POM, green algae, red algae, kelps) using a leave-one-out, cross-validation procedure to establish if our sources were statistically distinct. We then used this training data set to predict group membership for each consumer sample. We ran 10,000 iterations of the LDA model, using a random sample removal with replacement from our producer training data set. For each iteration, LDA output included the posterior probabilities of individual consumer classification with each producer group. For individuals and species, we calculated a mean posterior probability of classification with each producer, and

associated error, across all bootstrapped iterations. These posterior probabilities of classification represent the likelihood of unknown samples having derived from the distribution of the sources included in the training data set. These probabilities are calculated across all linear discriminant axes and sum to 1 (Tabachnick and Fidell 2013). With sufficient discriminatory power between sources in LDA space, the posterior probabilities of classification may be predictably related to the distance between an unknown (consumer) sample and each producer centroid, as is the case for our system (Appendix S3: Figs. S1, S2). We thus assume that for each consumer, the calculated posterior probabilities of classification represent an estimate for the percent reliance on each producer group; a detailed discussion on this topic is included in Appendix S3.

Following LDA bootstrapping, we calculated a posteriori percent reliance on pelagic (phytoplankton/POM) vs. benthic (macroalgae) endmembers. We considered an individual to be an energy channel "specialist" if they exhibited ≥80% posterior probability of classification with a single producer group across all bootstrap iterations. This threshold is toward the upper, but not extreme, range of these previously employed in studies of energy channel coupling (e.g., Wolkovitch et al. 2014, Ward et al. 2015). We were unable to include epilithic biofilm as a possible source for consumers due to LDA sample size requirements ($n_{samples} = n_{amino\ acids} + 1$; Tabachnick and Fidell 2013). Instead, we treated biofilm as a consumer group and classified it with the producer groups for comparison. We chose not to use isotopic mixing models (Phillips et al. 2014) to estimate contribution of pelagic vs. benthic endmembers to our consumers (see Appendix S3).

### RESULTS

#### Bulk tissue $\delta^{13}C$ and $\delta^{15}N$ values

Kelp forest consumers showed substantial variation in bulk tissue $\delta^{13}C$ and $\delta^{15}N$ values (Fig. 1, Data S1). However, Wilcoxon Signed-Ranks tests showed that isotope values of each species/producer group did not differ between our sample sites (Appendix S4: Table S1). Consumer groups varied widely in bulk tissue $\delta^{15}N$ values: mean ± SD $\delta^{15}N$ values ranged from 10.0‰ ± 3.2‰ (zooplankton) to 18.2‰ ± 1.1‰ (Concholepas) for invertebrates, and 15.4‰ ± 1.2‰ (Engraulis) to 22.0‰ ± 0.7‰ (Cheilodactylus) for fish (Fig. 1). Among primary producers, we found minimal variation in mean $\delta^{15}N$ values (10.8‰ to 12.0‰), with the exception of two offshore phytoplankton samples with notably $^{15}N$ depleted values of 4.2‰. In contrast, we found a large range in producer bulk tissue $\delta^{13}C$ values. The red algae Plocamium and Rhodymenia had the lowest mean ± SD $\delta^{13}C$ values of −33.0‰ ± 1.1‰ and −31.9‰ ± 1.6‰ respectively, whereas green algae (Ulva) and biofilm had the highest (−13.3‰ ± 2.3‰ and −9.6‰ ± 2.0‰, respectively).
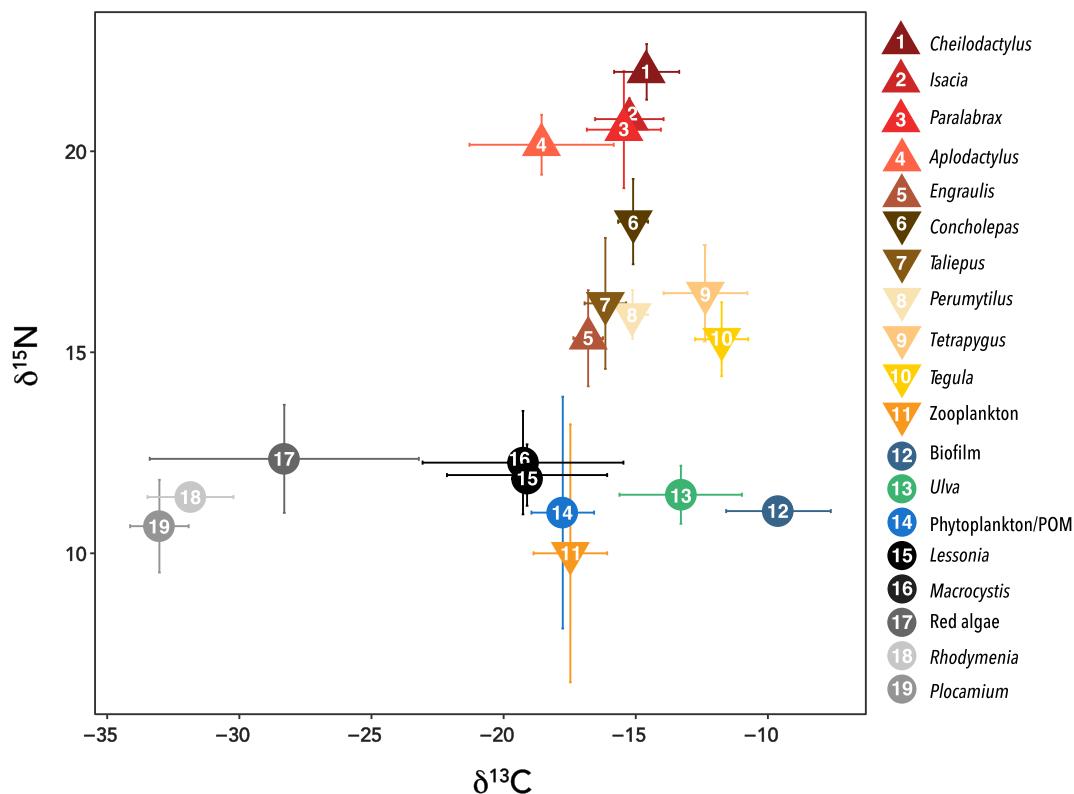
Fig. 1. Variation in bulk tissue $\delta^{13}C$ and $\delta^{15}N$ values for Humboldt Current samples. Circles represent primary producer taxa, inverted triangles represent invertebrate consumers, triangles represent fish. The group "Red algae" represents one unidentified red and one *Chondrus canaliculatus* sample. Note the overlap between phytoplankton/particulate organic matter (14) and the two sampled kelp species (15 and 16), a common issue with bulk tissue isotopic analysis in productive coastal systems.

Phytoplankton/POM and our sampled kelps (*Lessonia, Macrocystis*) had overlapping mean $\delta^{13}C$ (phytoplankton/POM, –17.8‰ ± 1.2‰; kelps, –19.3‰ ± 3.8‰) and $\delta^{15}N$ (phytoplankton/POM, 11.0‰ ± 2.9‰; kelps, 12.1‰ ± 1.0‰) values. Wilcoxon Signed-Ranks tests confirmed phytoplankton/POM and kelps were statistically indistinguishable in bulk tissue $\delta^{13}C$ ($W = 233$, $P = 0.26$) and $\delta^{15}N$ ($W = 156$, $P = 0.33$), limiting our ability to distinguish between these major energy channels using bulk tissue stable isotope analysis (Fig. 1).

### Amino acid $\delta^{13}C$ values

In contrast to bulk tissue isotope data, we found significant differences in EAA $\delta^{13}C$ values among producers (MANOVA, $F_{3,35} = 12.07$, $P < 0.01$) and consumers ($F_{10,45} = 2.58$, $P < 0.01$; Appendix S4: Table S2). All producers were statistically distinct from one another in $\delta^{13}C$ values for at least one EAA, however, we noted variation in which EAA differed among the four producer groups. For example, phytoplankton/POM and kelps only differed in mean isoleucine $\delta^{13}C$ values, whereas red algae showed strikingly different $\delta^{13}C$ values for all six essential amino acids (Appendix S4: Table S2). A number of consumer taxa stood apart in

their EAA $\delta^{13}C$ values. Among fish, the herbivorous *Aplodactylus*, offshore planktivore *Engraulis*, and invertivore/piscivore *Paralabrax* exhibited significantly different $\delta^{13}C$ values for multiple EAAs in comparison to other fish species (Appendix S4: Table S2). For the invertebrate community, *Tegula* showed the greatest number of EAA $\delta^{13}C$ values that were significantly different with those of other species (Appendix S4: Table S2). We note that the consumer EAA $\delta^{13}C$ data set passed normality tests but exhibited mild kurtosis.

### Linear discriminant analysis: energy channel usage of consumers

Results from multivariate analysis of EAA $\delta^{13}C$ values showed strong separation among producer groups (Fig. 2, Appendix S4: Tables S3, S4). For the entire producer EAA $\delta^{13}C$ data set, the first two linear discriminant axes explained a cumulative 95.1% of the variation, driven mostly by differences in isoleucine, lysine, and valine $\delta^{13}C$ values. Importantly, cross-validation of the LDA found a high overall success rate (89.7%) in distinguishing among our four primary producer groups (Fig. 2, Appendix S4: Table S4). Within groups, the results varied: no red algae samples were incorrectly classified, green algae and
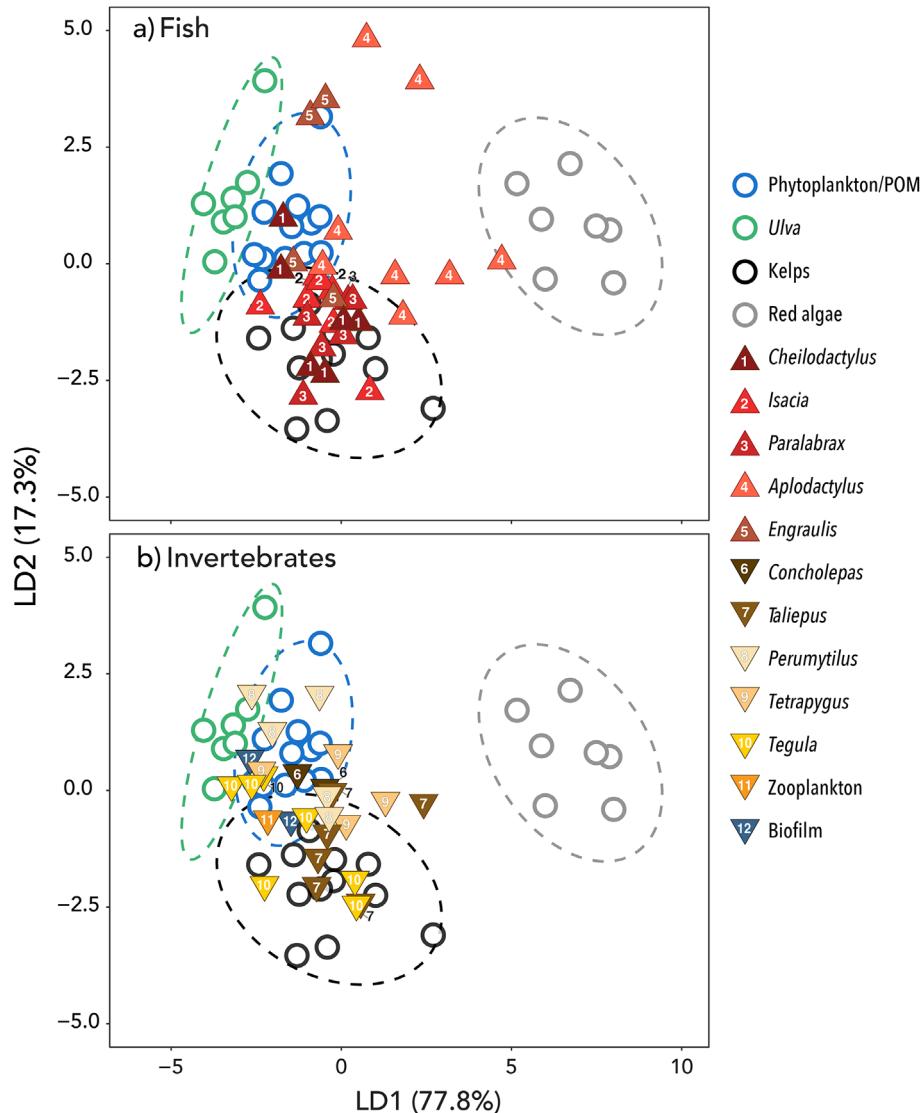
FIG. 2. Fingerprinting of Humboldt Current producers and consumers. Results from a single iteration of linear discriminant analysis (LDA) using essential amino acid δ13C data of producers and consumers. Circles represent linear discriminant loading of individual producer samples; ellipses represent 90% confidence intervals. Triangles (panel a), and inverted triangles (panel b) represent linear discriminant loadings for fish and invertebrates respectively. The first two axes accounted for 95% of the variation and clearly separated producer functional groups. Where a consumer plots in this space is a thus reflection of the predominant source of production from which their essential amino acids are sourced.

phytoplankton/POM each had a single misclassified sample, and kelp had two misclassified samples (Appendix S4: Table S4). Given the differences in sample sizes for these latter three groups this resulted in successful group-specific reclassification rates ranging from 92% (phytoplankton/POM) to 83% (kelps).

The bootstrapped LDA analysis showed strong individual-, species-, and functional-level partitioning in the energy channels utilized by consumers. Phytoplankton/POM and kelps were the dominant producer groups supporting local consumers (Table 2, Fig. 2). However, species varied widely in which energy pathway they were

most reliant on. Invertebrates showed a greater degree of variation in energy channel usage, ranging from an average of 13% (*Taliepus*) to 98% (singleton zooplankton sample) pelagically derived EAA (Table 2, Figs. 2, 3). Fish taxa utilized a more mixed proportion of pelagic and benthic sources, from an average of 19% (*Paralabrax*) to 47% (*Isacia*) pelagically derived EAA (Table 2, Figs. 2, 3). Within each consumer species, the large variation in energy channel used was predominantly driven by variation among, rather than within individuals (Fig. 3, Appendix S4: Table S5). Of the 56 individual consumers analyzed, 37 had ≥80% posterior probability

TABLE 2. Percentage of EAA from each producer group routed to consumers.

| Species | N | Phytoplankton/POM (%) | Kelps (%) | Green (%) | Reds (%) | Energy channel specialists (%) |
|---|---|---|---|---|---|---|
| **Fish** | | | | | | |
| *Cheilodactylus variegatus* | 6 | 27 ± 38 | 71 ± 41 | 3 ± 4 | 0 ± 0 | 83 |
| *Isacia conceptionis* | 7 | 47 ± 36 | 53 ± 36 | 0.2 ± 0.4 | 0 ± 0 | 57 |
| *Paralabrax humeralis* | 6 | 19 ± 20 | 81 ± 20 | 0 ± 0 | 0 ± 0 | 50 |
| *Aplodactylus punctatus* | 8 | 25 ± 38 | 32 ± 42 | 21 ± 40 | 22 ± 38 | 63 |
| *Engraulis ringens* | 4 | 43 ± 45 | 24 ± 46 | 34 ± 45 | 0 ± 0 | 75 |
| **Invertebrates** | | | | | | |
| *Concholepas concholepas* | 2 | 89 ± 15 | 11 ± 15 | 0 ± 0 | 0 ± 0 | 50 |
| *Taliepus* sp. | 6 | 13 ± 25 | 86 ± 25 | 0.1 ± 0.3 | 0.3 ± 0.7 | 83 |
| *Perumytilus purpuratus* | 5 | 67 ± 31 | 20 ± 34 | 13 ± 22 | 0 ± 0 | 40 |
| *Tetrapygus niger* | 4 | 40 ± 38 | 54 ± 46 | 6 ± 8 | 0 ± 0 | 75 |
| *Tegula atra* | 7 | 32 ± 38 | 52 ± 48 | 16 ± 33 | 0 ± 0 | 71 |
| Zooplankton | 1 | 98 | 2 | 0 | 0 | 100 |

*Notes::* Results represent the species-level average posterior probability of 10,000 bootstrap iterations of linear discriminant analysis using EAA $\delta^{13}C$ values. POM, particulate organic matter. The percentage of energy channel specialists was calculated using the number of individuals within each species that had ≥80% mean posterior probability of classification with a single producer group across bootstrap iterations (see Appendix S4: Table S5).

of classification with a single producer group across randomized bootstrap iterations (Fig. 3, Appendix S4: Table S5).

## DISCUSSION

Characterizing the structure of biological communities has been a challenging task in marine ecosystems, where the very habitats that food webs exist in preclude the use of traditional observational methods. Through essential amino acid $\delta^{13}C$ analysis (EAA $\delta^{13}C$), we were able to quantify energy channel usage and ecological specialization by 11 species of marine consumers in a highly productive nearshore kelp forest. We found evidence for the coupling of pelagic (fast) and benthic (slow) energy channels by consumers, or "multichanneling," that is clearly an important component of food web structure in this ecosystem. However, in contrast to theoretical considerations (e.g., Post et al. 2000), we found that multichannel feeding was predominantly a species-level phenomenon, as the majority of our sampled individuals tended to utilize either the pelagic or benthic channel rather than a mixture of the two. Our results provide a possible mechanism for multichannel feeding in coastal food webs based on ecological variation among individuals.

Our findings demonstrate widespread use of both benthic and pelagic energy channels by nearshore consumers in northern Chile. This result differs from that of previous work in the Humboldt Current System (Docmac et al. 2017), which used bulk tissue isotope analysis to show that pelagic-derived materials overwhelmingly fueled marine fish assemblages. It is important to note that bulk tissue and EAA isotope analysis may not always agree because the former is a reflection of the entire mixture of macromolecules (proteins, carbohydrates, lipids) utilized by consumers, while the latter only traces essential nutrients (Ruess and Muller-Navarra 2019). The interpretation of bulk tissue isotopic data can be complicated by numerous physiological and environmental factors, which appear to be less problematic for the interpretation of EAA $\delta^{13}C$ patterns (Larsen et al. 2013, Whiteman et al. 2019). Furthermore, the previous isotope-based work in the Humboldt Current (Docmac et al. 2017) relied on consumer "indicator species" (sensu Post 2002) rather than direct sampling of primary producers to characterize energy channels. While this produced clean separation at a broad geographic scale (hundreds of kilometers), the bulk tissue isotope data we report here (Fig. 1) show substantial overlap between the two dominant producers in the system (phytoplankton and kelp), which suggests that reliance on a bulk tissue isotope approach is of limited utility at our study sites.

Multichannel feeding by Humboldt Current consumers, defined here as utilization of both pelagic (phytoplankton/POM) and benthic (macroalgae) energy channels, was widespread in both the fish and invertebrate communities. This phenomenon was particularly evident within our sampled fish taxa, with species averaging 19–47% pelagically derived EAA. (Table 2, Fig. 3). Invertebrates showed less multichannel feeding overall, ranging from an average of 13–67% pelagically derived EAA, when considering only taxa with sample sizes >3 (e.g., excluding zooplankton and *Concholepas*, Table 2, Fig. 3). These patterns compliment previous empirical and theoretical research demonstrating that the coupling of slower (benthic) and faster (pelagic) energy channels is an important component of marine food web dynamics, and further, that this coupling is typically achieved by mobile top consumers like fish (Post et al. 2000, Wolkovitch et al. 2014, Ward et al. 2015).
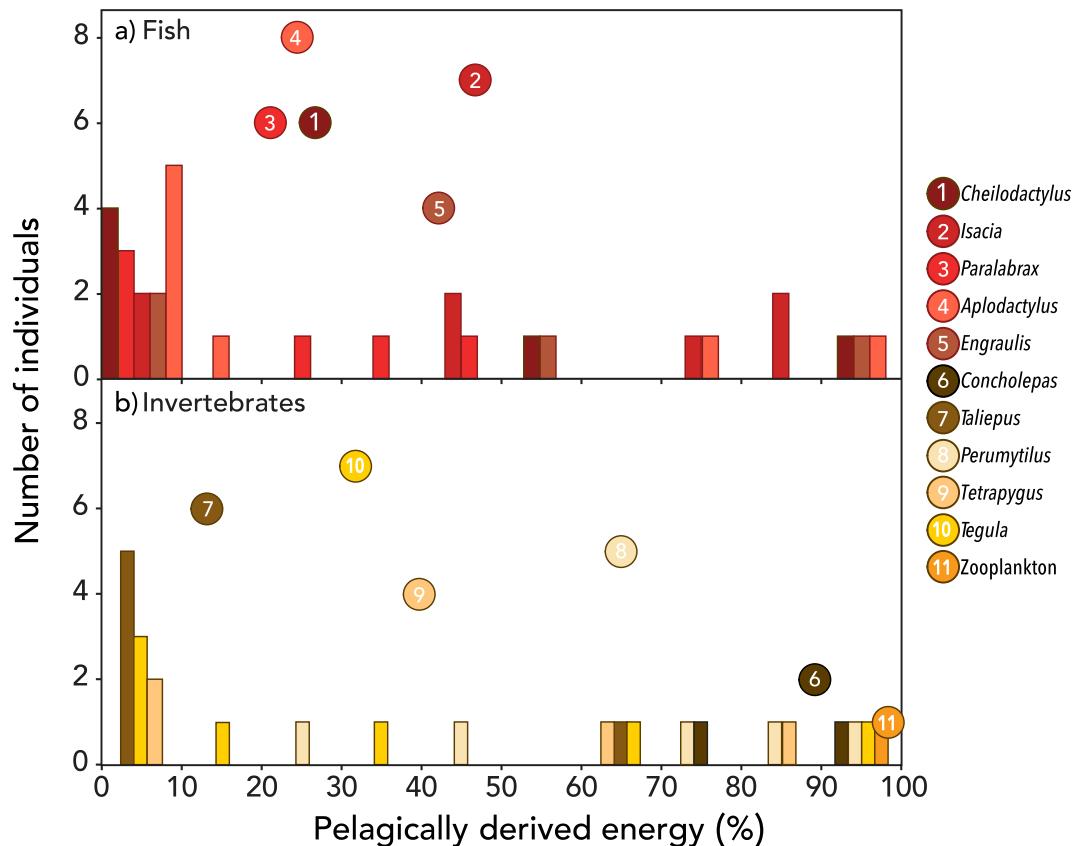
Fig. 3. Energy channel usage by individual fish (panel a) and invertebrates (panel b). Pelagically derived energy is here defined as essential amino acids (EAA) within consumer tissues that were ultimately sourced from phytoplankton; a value of 0 on the x-axis thus means the individual(s) derived their EAA from benthic macroalgae (kelps, red algae, or green algae). These percentages are calculated from the average posterior probabilities of the bootstrapped linear discriminant analysis using EAA $\delta^{13}C$ data. Bars are in intervals of 10%, circles represent species-level average (Table 2). We note that the small samples sizes for zooplankton and the carnivorous gastropod *Concholepas concholepas* (see Table 1) preclude robust statistical analysis, and the data for these taxa are thus presented here for comparative purposes.

Two species, the Peruvian anchoveta (*Engraulis ringens*) and jerguilla (*Aplodactylus punctatus*) showed contribution from unexpected sources given established understanding of their ecology. For *Engraulis*, we found two out of four individuals were reliant on benthic energy (one kelp, one *Ulva*), a stark contrast to expectations that this species is a pelagic planktivore. However, the diet of this taxa has been poorly studied (Pizarro et al. 2019), and the individuals sampled here were found stranded close to shore. Thus, contribution of kelp-derived energy in the form of detrital material consumed by the planktonic prey of anchoveta seems feasible. That said, we recognize that uncertainty of the exact origins of these stranded individuals measured here means this result should be verified with additional studies. In contrast, for the benthic herbivore *Aplodactylus*, the substantial contribution of pelagic material for two out of eight individuals is surprising. It is possible that phytoplankton-derived detritus either settled on the surface of macroalgae, or was utilized by epiphytes that were subsequently consumed by *Aplodactylus*.

Alternatively, it may be a result of our undersampling of benthic microalgae, which is currently poorly characterized in terms of EAA $\delta^{13}C$ (Phillips et al. 2020). The suggested reliance of individuals of both *Aplodactylus* and *Engraulis* on *Ulva* presents more of a challenge to explain, as *Ulva* is not a dominant macroalgae at our sites in terms of biomass, although *Aplodactylus* are known to consume *Ulva* in large volumes when available (Caceres et al. 1994). Although we found good separation here between *Ulva* and phytoplankton/POM, our previous work in Alaska (Elliott Smith et al. 2018) has noted difficulties in distinguishing among these primary producer groups in EAA $\delta^{13}C$ multivariate space, and the importance of *Ulva* to subtidal fish taxa should thus be verified with additional studies. Finally, it should be noted that we cannot account for the potential synthesis of EAA by microbial communities, either within the gut (e.g., Newsome et al. 2011, 2020), or during the decomposition of particulate organic matter. Although we currently know very little about the contribution of these microbial communities to the EAA

budget of wild consumers, it is possible that this accounts for some of our signal here. Previous studies have assessed this possibility by looking for consumer samples that fall outside of the expected linear discriminant "mixing space" (Fox et al. 2019). This is arguably the case for one of the *Aplodactylus* samples discussed above (HUM-ISM-JER9-B2), and we suggest that assessing the importance of microbial amino acid synthesis to higher order consumers will be a fruitful avenue for future research.

The coupling of pelagic and benthic energy channels we found by kelp forest consumers was facilitated by intraspecific variation. While many species as a whole utilized a combination of energy channels, our data indicate that the majority of *individuals* tended to use either pelagic or benthic production, rather than a mixture of the two (Figs. 2, 3, Appendix S4: Table S5). We quantified such "energy channel specialization" as any individual with ≥80% posterior probability of classification with a single producer group across 10,000 LDA iterations. In LDA, posterior probability of classification represents the likelihood of an unknown sample having derived from the distribution of the sources included in the training data set. These probabilities are calculated across all linear discriminant axes and sum to 1 (Tabachnick and Fidell 2013). With sufficient discriminatory power between sources in LDA space, the posterior probabilities of classification may be predictably related to the distance between an unknown (consumer) sample and each producer centroid, as is the case for our system (see Appendix S3: Figs. S1, S2). Given this, these probabilities can provide estimates for the contribution of each marine producer group to our sampled consumers, and our bootstrapping approach provides associated error around these estimates. If nearshore consumers in northern Chile were energy channel generalists, the majority of individuals should have exhibited classification probabilities of ~20–80% for each energy channel. In contrast, we found that nearly two-thirds of individuals (37/56) exhibited extremely high (>80–90%) probabilities of classification with a single producer group (Fig. 3, Appendix S4: Table S5). This result provides strong evidence that the majority of our consumers were "specializing" on a particular energy channel (Fig. 2). As an example, in the fish species *Isacia* that averaged nearly 50% pelagically derived EAA at the species level, the majority of individuals (4/7) were considered to be specialists on either pelagic or benthic sources (Table 2, Fig. 3). This pattern held across nearly all species examined (Table 2, Fig. 3). The notable exception was the bivalve *Perumytilus*, where only two out of five individuals had greater than 80% probability of a single source classification (Table 2, Fig. 3), a sensible result for a putatively non-discriminant filter feeder. Individual specialization within populations along the pelagic-benthic niche axis has been noted for a number of aquatic species such as the three-spine stickleback (*Gasterosteus aculeatus*; Matthews et al. 2010, Ravinet et al. 2013),

European whitefish (*Coregonus lavaretus*; Harrod et al. 2010), and Midas cichlid (*Amphilophus tolteca*; Kusche et al. 2014). However, to our knowledge we are the first to document this ecological specialization across an entire consumer community.

Importantly, what we measured here was individual specialization in the use of a single *energy channel*, rather than consumption of a particular prey type. The latter is the traditional metric of individual dietary specialization, which is now recognized as a widespread and ecologically significant phenomenon, particularly in aquatic communities (e.g., Bolnick et al. 2003, Araújo et al. 2011). We were unable to empirically determine individual-level prey specialization as we did not collect multiple tissues from individuals required to generate a longitudinal record of dietary variation, nor did we exhaustively collect potential prey items. Despite this limitation, we suggest that specialization on particular functional groups of prey may well be linked to specialization on a given energy channel. Favored prey items may often be associated with particular habitats that are fueled by a single energy channel. In sea otters, for example, individual specialization on infaunal bivalves requires a particular set of foraging skills (e.g., digging and shucking) that are uniquely suited to soft-sediment habitats fueled primarily by phytoplankton production (Tinker et al. 2008). In contrast, sea otter individuals that specialize on urchins, which, in turn, are predominantly dependent on macroalgae energy, utilize a very different set of foraging behaviors, and would manifest as a "preference" for the kelp-derived energy channel with our metrics (Tinker et al. 2008). Thus, individual dietary specialization on a suite of ecologically similar species that belong to particular functional groups may inadvertently lead to specialization on a particular energy channel. However, we recognize that our results here are for a single coastal ecosystem, and we suggest that future research explore this pattern in additional habitats and localities.

The connection between individual specialization in energy channel usage and multichannel feeding is overlooked in the literature. The study of energy channel coupling by mobile consumers is extensive (e.g., Rooney et al. 2006, Wolkovitch et al. 2014, Ward et al. 2015). However, most of this work has focused on how energy channel coupling affects food web stability (Rooney and McCann 2012), trophic cascades (Ward et al. 2015), or how widespread multichanneling is across diverse ecosystems (Wolkovitch et al. 2014). Little work has been done on the mechanisms by which energy channel coupling is achieved within consumer species or functional guilds (but see Wimp et al. 2013, García et al. 2017, Perkins et al. 2018). Similarly, there is a large body of literature on individual diet specialization (Bolnick et al. 2003, Araújo et al. 2011), specifically how phenotypic variation (Mathews et al. 2010), behavior (Werner and Sherry 1987), and population dynamics (Tinker et al. 2008) mediate intraspecific dietary differences, and

how this in turn impacts food web stability (Kondoh 2003). Although previous work has reported specialization in energy channel usage within sympatric populations (e.g., Harrod et al. 2010, Matthews et al. 2010, Kusche et al. 2014), this research focused on the mechanisms behind adaptive radiations and thus on individual species. Our study is among the first to empirically show a link between individual specialization and multichannel feeding across a diverse community containing multiple functional groups and species.

By using a cutting-edge isotopic technique, we were able to characterize kelp forest food web structure in northern Chile with a high degree of precision; this would have been impossible with bulk analysis where basal production sources exhibited overlapping isotopic values (Fig. 1). This allowed us to confidently characterize the importance of benthic vs pelagic production to a suite of local consumers, a question of long-standing interest to marine ecologists (e.g., Duggins et al. 1989). Our results from this diverse community suggest that the processes of individual specialization and energy channel coupling are linked, with multichannel feeding in fish and invertebrate species facilitated by intraspecific dietary differences. With this new tool in hand, future research should assess (1) the relative importance of pelagic and benthic energy to nearshore communities in other dynamic coastal ecosystems, (2) differences among functional/trophic groups in energy channel specialization, and (3) the impact of consumer energy specialization on food web stability. An understanding of the latter will be particularly important for vulnerable marine ecosystems in this era of increasing anthropogenic change.

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