Dietary connections of marine species to kelp and eelgrass

Paul Chittaro (paul.chittaro@noaa.gov)
NOAA NWFSC: NOAA Fisheries Northwest Fisheries Science Center https://orcid.org/0000-0001-9310-7314

Kelly Andrews
NOAA NWFSC: NOAA Fisheries Northwest Fisheries Science Center

Nick Tolimieri
NOAA NWFSC: NOAA Fisheries Northwest Fisheries Science Center

Jonelle Gates
NOAA NWFSC: NOAA Fisheries Northwest Fisheries Science Center

Emily Buckner
Puget Sound Restoration Fund

Gina Ylitalo
NOAA NWFSC: NOAA Fisheries Northwest Fisheries Science Center

Dan Tonnes
NOAA Fisheries Western Regional Center: NOAA Fisheries Oregon and Washington Coastal Office

Research Article

Keywords: trophic relationships, mixing model, stable isotope

Posted Date: June 15th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2809764/v1

Abstract

Ecosystem-based fisheries management requires an understanding of the interactions between managed and unmanaged species and the connections they have with their habitats. Although aquatic plants are known to provide important shelter for a variety of species, an often-overlooked component is the extent to which primary producers contribute to their diet. In this study, we reconstructed the dietary connections between 18 consumers, including vulnerable rockfish species, and several primary producers. Two of these primary producers, *Zostera marina* (eelgrass) and *Nereocystis luetkeana* (bull kelp), have experienced spatial variation in abundance and substantial declines, respectively, within Puget Sound, Washington, USA. Using stable isotope ratio data, we estimated that both bull kelp and *Smithora naiadum* (an epiphytic algae) were the most important sources of carbon in consumer diets, followed by particulate organic matter and eelgrass. Our results indicated strong dietary connections for certain consumers, such that epiphytic algae was found to comprise greater than 40% of the diets of copper rockfish (*Sebastes caurinus*), while bull kelp contributed most to the diets of Pacific staghorn sculpin (*Leptocottus armatus*) and quillback rockfish (*S. maliger*). For several consumers, we observed habitat-mediated prey choice because the relative importance of bull kelp or epiphytic algae in their diets increased when these consumers were collected from sites dominated by kelp or eelgrass, respectively. Understanding the strength of these trophic relationships is an important step for predicting ecosystem consequences following perturbations within these habitats, and such information is vital to managers making decisions related to the conservation of valuable populations.

Introduction

Biogenic habitats, such as kelp forests and seagrass beds, support important ecosystem functions such as predation refugia, movement corridors, and carbon sequestration (Duggins et al. 1989, Beck et al. 2001, Graham 2004, Olson et al. 2019) and ecosystem services like tourism, commercial and recreational harvest, and coastline protection (Arkema et al. 2013, Krumhansl et al. 2016, Unsworth et al. 2019). Many of these functions and services are at risk because coastal habitats are declining in density and extent (Steneck et al. 2002, Lotze et al. 2006, Krumhansl et al. 2016, Berry et al. 2021) due to a variety of stressors including climate change, pollution, coastal development, and species invasions (Connell et al. 2008, Filbee-Dexter et al. 2016, Filbee-Dexter and Wernberg 2018, Caro et al. 2022). Seagrasses, for example, have declined worldwide by 29% since their first recording in 1879 (Waycott et al. 2009); yet, they are believed to provide important nursery habitat to over 20% of the worlds' largest fisheries (Unsworth et al. 2019). Overall, even though biogenic habitats are important to food webs and ecosystem functions, little is understood about how changes in abundance, quality, and/or distribution of biogenic habitats impact the status and trends of managed species (Scheffer et al. 2001, Kritzer et al. 2016, Rogers-Bennett and Catton 2019). Importantly, because biogenic habitats are often primary producers, changes in their presence and abundance have the potential to impact food webs by altering the quantity and source of energy available to higher level consumers.

In the U.S. Pacific Northwest, *Nereocystis luetkeana* (bull kelp) and *Zostera marina* (eelgrass) are highly productive habitat-forming species that create large forests/beds in shallow subtidal rocky and sandy areas, respectively (Rehr et al. 2014, Calloway et al. 2020). The study of bull kelp and eelgrass is particularly interesting in Puget Sound (Washington, USA). Long-term declines in bull kelp have been reported (i.e., a 63% reduction in extent when compared to the earliest baseline in1878) with some historical kelp forests being completely lost (Berry et al. 2020). Currently, bull kelp is being petitioned for listing under the U.S. Endangered Species Act (ESA) (Kelkar and Carden 2022). In contrast, eelgrass beds in Puget Sound appear to be relatively resilient, although highly variable at small spatial scales (Shelton et al. 2017). Furthermore, both bull kelp and eelgrass are reported to provide shelter, because of their three-dimensional structure, for a variety of valued species in Puget Sound. These include species listed as threatened or endangered under the ESA, such as *Sebastes ruberrimus* (yelloweye rockfish), *S. paucispinis* (bocaccio) (NMFS 2017), and several Pacific salmon (*Oncorhynchus* spp.) stocks (NMFS 1999). However, less is understood about the extent to which bull kelp forests and eelgrass beds are important as primary producers in the food webs that support aquatic and terrestrial organisms in Puget Sound. These biogenic habitats are worthwhile studying to better understand their food web linkages with valued and ESA-listed species.

To investigate dietary linkages to kelp and seagrass, methods traditionally used, such as stomach content analysis, are limited because these species can be rapidly digested, leading to their underestimation in a consumer's diet (Pethybridge et al. 2018). Moreover, higher-level consumers like rockfishes or salmon species, do not consume these primary producers directly but do rely on food chains grounded in some basal energy source like eelgrass, kelp or particulate organic matter (POM). A more robust and effective alternative method is stable isotope analysis, which can help quantify the proportional contribution of primary producer-based food chains to consumer diets.

For more than 30 years, stable isotope analyses of carbon and nitrogen have proven to be a powerful means to examine trophic ecology (Fry 2006). Stable isotope ratios in a consumer's tissues reflect those in their diet in a predictable manner. Carbon stable isotope ratios (i.e., $^{13}C/^{12}C$), for example, change very little between diet and consumer, which makes this ratio a convenient tracer of habitats the consumer moved through indicating the source of primary production (or energy in the case of POM). In contrast, nitrogen stable isotope ratios (i.e., $^{15}N/^{14}N$) become more enriched at each trophic level making it suitable for estimating a consumer's trophic position (Post 2002).

Here, we used stable isotopes as a tool to identify trophic relationships between primary producers and several herbivorous and carnivorous species, including species of concern quillback and copper rockfish (*S. maliger* and *S. caurinus*) and Chinook salmon (*O. tshawytscha*). Our specific objective was to determine the extent to which four primary producers (bull kelp, eelgrass, epiphytic algae (*Smithora naiadum*), and POM) were the basal dietary resources for marine invertebrates, fishes, and a seabird in Puget Sound. Understanding the trophic connections between these consumers and biogenic habitats is critical for making informed management decisions related to the recovery, conservation and sustainability of these marine species and habitats.

Materials & Methods

Study area & sampling

This study was conducted within Puget Sound, the southern portion of the Salish Sea within coastal waters of Washington state, U.S.A., from June through October 2018 and July through August 2019. We collected primary producers and primary, secondary, tertiary, and quaternary consumers from 31 sites (Fig. 1). At 14 of these sites, we sampled primary producers of bull kelp, eelgrass, epiphytic red algae, and/or POM. Many of the 14 sites sampled had either bull kelp or eelgrass as the dominant biogenic habitat, and at each of these sites, we sampled bull kelp or eelgrass by removing approximately 20 cm or 10 cm, respectively, from the growing end of at least five blades. Epiphytic algae were also sampled along with bull kelp and eelgrass since it is an epiphyte that grows on their surfaces. We collected POM by sampling 1–4 L of seawater at the surface using clean 1 L Nalgene bottles that were pre-rinsed in seawater. Collected primary producers were placed in Ziploc® bags and put on ice until we returned to the Northwest Fisheries Science Center (NWFSC, Seattle, Washington) where they were stored at -20°C. In summary, we collected bull kelp, eelgrass, and seawater from 6, 7, and 9 sites, respectively (Fig. 1; Supplementary Table 1).

To collect invertebrate and vertebrate consumers, we used a variety of techniques and gear: modified crab traps (61 cm x 61 cm x 25 cm), minnow traps (30 cm x 25 cm x 15 cm), standard monitoring units for the recruitment of fish (SMURF; Ammann 2004), a beach seine (10 m wide, 2 m high, 2 m x 2 m x 2 m bag in the center; 3.175 mm nylon mesh), a lampara net (37 m and 32 m in length on the float and lead line, respectively and a maximum of 4.6 m in depth; mesh size ranges from 6 to 48 mm), and SCUBA divers with hand nets. We deployed crab traps, minnow traps, and SMURFs for 7–14-day intervals at each kelp- and eelgrass-dominated site (Fig. 1). Crab and minnow traps were placed a minimum of 10 m apart and at a depth of 4–6 m, while SMURFs were positioned 1–2 m above the substrate on anchored lines with surface floats. Egg shells of the seabird *Cepphus columbus* (pigeon guillemot) were collected opportunistically from nesting burrows around Whidbey Island (Fig. 1) between June 12, 2019 and September 1, 2019 during other research efforts (Buckner et al. 2022). Egg shells from different burrows were stored separately. Collected fish, invertebrates, and egg shells were placed on ice and transported to the NWFSC where they were stored at -20°C.

Sample processing & stable isotope analysis:

We filtered water samples for POM through pre-combusted (400°C) 47-mm Whatman GF/C filter papers using an oilless vacuum pump. The filter containing POM was then transferred to a 20-ml scintillation vial and placed, uncapped, in a laboratory drying oven and dried overnight at 65°C. Next, we removed the particulate inorganic carbon from our sampled POM by placing the vial, uncapped, in a desiccator containing concentrated, high grade (Sigma-Aldrich 37%) hydrochloric acid overnight, followed by a second overnight, uncapped, in a drying oven at 65°C. Last, we used a scalpel to scrape POM from the filter paper and placed this material into a clean, 20-ml scintillation vial and stored the vials in a

desiccator until further analysis. We pooled some POM samples from adjacent sites because we had an insufficient amount of POM material (< 1.5mg) for stable isotope analysis (Supplementary Table 1).

We scraped epiphytic red algae from blades of eelgrass and bull kelp using a scalpel and this material was then transferred into separate clean 20ml scintillation vials and immediately stored at -20°C. Next, bull kelp and eelgrass were rinsed in deionized water and placed into individual 200ml pre-combusted oyster jars and stored at -20°C. We sterilized the jars prior to use by placing them in a muffle furnace overnight at 400°C. We had an insufficient amount of epiphytic algae from sampled bull kelp to permit stable isotope analysis, thus all references to epiphytic algae are those removed from eelgrass.

Fish and invertebrates were identified to species and their sizes were measured: total length (mm) for fish, maximum carapace width (mm) for crab, test diameter for urchin, and carapace length (mm) for shrimp and *Idotea*. Sampled Chinook salmon are presumed to be wild because they lacked an artificial tag and had an intact adipose fin. However, not all hatchery-produced fish are tagged and/or clipped. Some rockfish were only classified to genus because they were too young to identify using meristic and morphometric traits. These fish are referred to as young-of-year (YOY). For fishes and crabs, we extracted dorsal muscle tissue and claw muscle tissue, respectively, from each individual. For urchins we extracted ampullae, while shrimp and *Idotea* muscle tissue was taken after we removed the carapace, pleopods, abdominal segments, and uropod. Muscle tissue from each individual was placed in a clean 20ml scintillation vial and immediately stored at -20°C.

Pigeon guillemot egg shells were prepared for stable isotope analysis using standard procedures described in Buckner et al. (2020). In summary, the egg shell membrane was separated from the shell and the former was cleaned with deionized water and gently wiped/scraped with a laboratory grade cotton swab. Cleaned membrane tissue was placed in clean 20-ml scintillation vials and dried in an oven at 60°C for 24 hours, after which they were stored at room temperature until further analysis.

We used clean instruments (i.e., scalpels, scissors, and forceps) when removing tissues from each individual and each egg shell. Tissues were finely blended using scissors or a Baymix blender, freeze-dried for 24 hours, and then ground into a homogenous powder using a SPEX Sample Prep 5100 Mixer Mill for 3 minutes. All samples were then weighed into specified amounts (6–8 mg for POM, 0.5–0.7 mg for epiphytic algae, eelgrass, and bull kelp, 0.2–0.3 mg for fish and invertebrates, and 0.25–0.35 mg for egg shell membranes), placed into 6 mm x 4 mm tin capsules, and analyzed for carbon ratios ($^{13}C/^{12}C$) and nitrogen ratios ($^{15}N/^{14}N$) via a Thermo Scientific Flash 2000 Organic Elemental Analyzer Flash HT Plus coupled with a Thermo Scientific Delta V Advantage IRMS continuous flow stable isotope ratio mass spectrometer at the NWFSC. Isotope values are expressed in the δ notation:

$$\delta\left(‰\right) = \left[\frac{(Rsample - Rstandard)}{Rstandard}\right]$$

where *R* is the ratio of heavy to light isotope (e.g., $^{13}C/^{12}C$) in both a sample and a standard. The standard for nitrogen was atmospheric nitrogen, and the standard for carbon was Vienna-Pee Dee

Belemnite (V-PDB). Stable isotope ratios were reported as per mil (‰) using standard delta notation ($\delta^{13}C$ and $\delta^{15}N$). Overall, we analyzed 8, 9, 11, and 10 samples of POM, epiphytic algae, eelgrass, and bull kelp, respectively, for carbon and nitrogen stable isotopes along with 142 fish and 119 invertebrate muscle samples, and 17 seabird egg shell membranes (Table 1).

Table 1

Mean (standard deviation) of body size (mm) and stable isotopes (δ13C and δ15N; ‰) for each of our sampled species associated with one of four trophic groupings (primary producer, and primary, secondary, tertiary, and quaternary consumers). Sample size (n) per species is indicated.

Trophic grouping	Species		Body size	δ 13C	δ 15N	n
	Common name	Scientific name				
1° producer	Particulate organic matter	-	- (-)	-22.7 (1.6)	5.1 (1.4)	8
	Bull kelp	*Nereocystis luetkeana*	- (-)	-16.4 (1.7)	6.1 (1.1)	10
	Epiphytic algae	*Smithora naiadum*	- (-)	-13.2 (1.7)	6.9 (0.9)	9
	Eelgrass	*Zostera marina*	- (-)	-9.7 (0.9)	4.8 (1.5)	11
1° consumer	-	*Idotea* sp.	23.9 (6.6)	-12.4 (1.3)	9.2 (0.5)	15
	Green urchin	*Strongylocentrotus droebachiensis*	62.4 (18.0)	-18.1 (1.0)	7.1 (0.4)	12
2° consumer	California green shrimp	*Hippolyte californiensis*	41.8 (12.9)	-14.4 (1.2)	10.9 (0.4)	11
	Dock shrimp	*Pandalus danae*	24.7 (8.4)	-14.3 (1.1)	10.6 (0.6)	10
	Humpback shrimp	*Pandalus hypsinotus*	35.3 (10.7)	-14.6 (1.3)	11.4 (0.7)	20
	Red rock crab	*Cancer productus*	91.8 (26.4)	-15.0 (1.0)	11.9 (0.4)	13
	Sharp-nosed crab	*Scyra acutifrons*	23.5 (5.9)	-15.6 (0.4)	11.5 (0.5)	5
	Graceful kelp crab	*Pugettia gracilis*	38.4 (17.6)	-13.7 (1.8)	10.1 (0.5)	13
	Northern kelp crab	*Pugettia producta*	39.8 (7.9)	-14.1 (1.4)	10.5 (1.1)	10
	Helmet crab	*Telmessus cheiragonus*	38.7 (16.3)	-12.6 (1.8)	11.1 (1.2)	10
3° consumer	Penpoint gunnel [a]	*Apodichthys flavidus*	167.9 (88.7)	-13.5 (1.0)	13.0 (0.4)	6

[a] Species of gunnel were aggregated for mixing model analysis.

Trophic grouping	Species		Body size	δ 13C	δ 15N	n
	Common name	Scientific name				
	Crescent gunnel [a]	*Pholis laeta*	100.9 (17.8)	-13.9 (0.8)	12.9 (0.1)	3
	Saddleback gunnel [a]	*Pholis ornata*	105.5 (29.9)	-13.6 (1.9)	13.1 (0.6)	12
	Shiner perch	*Cymatogaster aggregata*	85.4 (21.5)	-16.1 (1.0)	12.9 (0.3)	27
	Pacific staghorn sculpin	*Leptocottus armatus*	124.0 (64.8)	-14.8 (1.4)	13.5 (1.1)	25
	Chinook salmon	*Oncorhynchus tshawytscha*	119.3 (19.6)	-18.4 (1.1)	12.2 (1.1)	9
	Young-of-year rockfish	*Sebastes* sp.	54.5 (10.1)	-14.1 (1.6)	12.8 (0.3)	16
	Copper rockfish	*Sebastes caurinus*	121.9 (42.4)	-13.6 (1.1)	13.9 (0.6)	32
	Quillback rockfish	*Sebastes maliger*	119.7 (31.5)	-14.6 (0.9)	14.0 (0.5)	12
4° consumer	Pigeon guillemot	*Cepphus columba*	-	-13.7 (0.7)	16.1 (0.6)	17

[a] Species of gunnel were aggregated for mixing model analysis.

$\delta^{13}C$ values are known to vary with respect to the lipid content of protein, the latter of which is the common molecule in animal tissue. We did not lipid extract prior to analysis, but instead evaluated the lipid content of each of our sampled species by calculating their carbon to nitrogen ratios (C:N) from percentage element weight. C:N values greater than 3.5 are considered to have a major amount of lipids and are often lipid-corrected mathematically (Post et al. 2007). The majority of our 20 sampled consumers had C:N values less than 3.5 except *Cymatogaster aggregata* (shiner perch, C:N values ranged from 3.1–3.7), gunnels (*Pholis ornate*, P. *laeta* & *Apodichthys flavidus*, 3.2–3.7), crabs (*Pugettia gracilis*, 3.4–4.7; *P. producta*, 3.2–4.3), *Idotea* sp. (isopod, 4.3–5.3), and *Strongylocentrotus droebachiensis* (purple urchin, 5.7–7.1). Because of the high C:N values for all individuals, *Idotea* sp. and purple urchin were excluded from our mixing models. Shiner perch, gunnels, and crabs were included in our mixing models despite some of their individuals showing C:N values > 3.5. We did not lipid-correct these values because this would violate the assumption of mass balance in our mixing models (Arostegui et al. 2019).

To monitor the analytical accuracy of our instruments, a minimum of three blanks, five National Institute of Standards and Technology (NIST) Standard Reference Material (lipid-extracted lake trout muscle SRM 1946) and 20 calibration standards (aspartic acid and histidine28-17b) were processed with every set of tissues containing up to 20 tissue samples. All quality assurance measures associated with isotope

analyses met our established quality criteria (Sloan et al. 2019), including the within-run standard deviation of $\delta^{13}C$ and $\delta^{15}N$ reference materials being ≤ 0.2‰, which were calibrated to internationally accepted V-PDB and atmospheric N2, respectively. Duplicate or triplicate samples, representing 25% of all analyses, were run for quality assurance and had standard deviations that ranged from 0.01–0.46‰ and 0.01–0.12‰ for δ15N and δ13C, respectively. When more than two $\delta^{13}C$ and $\delta^{15}N$ measurements were available for an individual, we randomly selected one of the replicates for inclusion in our analyses.

Statistical analyses:

We estimated the relative percent contribution of primary producers to the diets of each consumer (Table 1) using MixSIAR (Stock and Semmens 2016), a package in R statistical software (RStudio version 1.2.5033 and R version 4.0.3)(R Core Team 2021). MixSIAR is a Bayesian mixing model that incorporates $\delta^{13}C$ and $\delta^{15}N$ variability of primary producers to estimate probability distributions describing the percent contribution of each primary producer to a consumer's diet (Stock and Semmens 2016, Stock et al. 2018a). Because mixing models are sensitive to isotopic separation of primary producers, we evaluated, a priori, whether primary producers should be aggregated based on overlapping $\delta^{13}C$ and $\delta^{15}N$ values (Phillips et al. 2014). To evaluate isotopic similarity among primary producers, we used a multivariate analysis of variance (MANOVA) with $\delta^{13}C$ and $\delta^{15}N$ values as dependent variables and primary producers as the independent variable. If significant differences in isotopic ratios were found among primary producers, separate ANOVA analyses were performed for $\delta^{13}C$ and $\delta^{15}N$, followed by Tukey's post-hoc pairwise comparisons to determine which species differed significantly. Primary producers were pooled for MixSIAR analysis if their $\delta^{13}C$ and $\delta^{15}N$ values did not differ significantly.

Similarly, we also used the approach outlined above (i.e., MANOVA and Tukey's post-hoc test) to evaluate whether $\delta^{13}C$ and $\delta^{15}N$ values differed among consumer species. We were specifically interested in whether there were similarities (or significant differences) in isotopic ratios among closely related species of consumers that would support their aggregation (or separation, respectively): a) shrimp (*Hippolyte californiensis*, *Pandalus danae*, and *P. hypsinotus*), b) crabs (*Cancer productus*, *Scyra acutifrons*, *Pugettia gracilis*, *P. producta*, and *Telmessus cheiragonus*), c) gunnels (*Apodichthys flavidus*, *Pholis laeta*, and *P. ornata*), and d) rockfishes (*Sebastes caurinus*, *S. maliger*, and YOY *Sebastes* spp.) (Table 1). The results of these analyses determined whether we aggregated consumers for use in the mixing models. For example, if isotope ratios differed significantly among our sampled species of crab, then a separate mixing model would be run for each species of crab, otherwise crab species would be aggregated and run in a single mixing model.

Our inputs into MixSIAR included $\delta^{15}N$ and $\delta^{13}C$ values for each of our four primary producers and 18 consumers, as well as trophic discrimination factors to correct isotope enrichment in consumer tissues. The trophic discrimination factors account for the process by which the heavy isotope (^{15}N of $^{15}N/^{14}N$ and ^{13}C of $^{13}C/^{12}C$) from the primary producers are preferentially incorporated into consumer tissue resulting in a bias in consumer isotopic signatures (Zanden and Rasmussen 2001). For our consumers,

we adopted three different trophic discrimination factors based on those reported by Polito et al. (2009) and Olson et al. (2019) (Table 2). Estimates of the contributions of each primary producer to consumer diets are sensitive to trophic discrimination factors (Phillips et al. 2014). To investigate this sensitivity, we varied our trophic discrimination factors by ± 0.5‰ for $\delta^{15}N$ and $\delta^{13}C$ following procedures reported by (Resano-Mayor et al. 2014) while the reported standard deviation associated with each trophic discrimination factor remained unchanged. For example, for tertiary consumers, we varied the trophic discrimination factors from 6.4‰ to 7.4‰ for $\Delta\delta^{15}N$ and from 0.7‰ to 1.7‰ for $\Delta\delta^{13}C$.

Table 2
Trophic discrimination factors (Δ) applied to consumers to correct for isotope enrichment. Standard deviation (SD) is also indicated.

Trophic grouping	Species	Δδ15N (± SD)	Δδ13C (± SD)	Reference
2˚ consumer	Omnivorous invertebrate: shrimp and crab	4.6 ± 0.25‰	0.8 ± 0.16‰	Olson et al. 2019
3˚ consumer	carnivorous vertebrate: fish	6.9 ± 0.34‰	1.2 ± 0.22‰	Olson et al. 2019
4˚ consumer	carnivorous vertebrate: seabird	11.1 ± 0.34‰	4.1 ± 0.22‰	Polito et al. 2009; Olson et al. 2019

Several consumer species were collected in both bull kelp- and eelgrass-dominated sites therefore permitting habitat specific estimates of the contribution of primary producers to their diets. To obtain these habitat specific estimates, habitat was included as a fixed effect in those mixing models. We arbitrarily required a minimum of five individuals per habitat so as to provide an estimate of habitat specific variability in terms of $\delta^{15}N$ and $\delta^{13}C$ values. Having habitat as a fixed effect meant that dietary contributions were estimated separately for kelp- and eelgrass-dominated sites.

For all mixing models, we used an uninformative prior to allow an equal starting probability of consumption among basal sources. To retrieve the posterior density estimates of basal source contribution, each mixing model was run with a 1,000,000-chain length, 500,000 burn-in, 3 chains, and a residual-error structure. Gelman-Rubin and Geweke diagnostics were used to evaluate whether model run length was within acceptable ranges (Stock et al. 2018b). Our discussion of the MixSIAR results focuses on the primary producer that represents the greatest proportion of a consumers' diet followed by any remaining primary producer(s) that substantially contribute to a consumer's diet; the latter of which we arbitrarily define as having a dietary contribution that is at least 0.1 greater than the next ranked primary producer.

An assumption of the mixing model is that the consumer isotope values are within the isotope space (i.e., polygon) defined by the isotope values of the primary producers (Stock and Semmens 2016). To evaluate if this assumption was violated, we ran a Monte Carlo simulation method using 1500 iterations, prior to running the mixing models, and calculated the probability that each individual consumer could be

explained by the primary producer isotope data (Smith et al. 2013). An individual consumer was excluded from the subsequent mixing model if it had a low probability (i.e., < 0.05) of being within isotope space of the primary producers. It is important to note that we did not account for all possible basal energy sources, including the microbial contributions (Sosik and Simenstad 2013, Dethier et al. 2014), so individuals that fell outside of the isotope space of the primary producers analyzed here, may have been feeding on food chains based on unsampled producers.

Results

Ratios of stable isotopes varied across primary producers and consumers, with the former group of species having the lowest $\delta^{15}N$ values (mean = 5.76 ‰; range = 2.43 to 8.31 ‰) and a wide range of $\delta^{13}C$ values (mean = -15.05 ‰; range = -25.84 to -7.74‰; Fig. 2, Table 1, Supplementary Table 1) relative to the consumers. Primary and secondary consumers (i.e., invertebrates) had $\delta^{15}N$ values that were intermediate between those of primary producers and tertiary and quaternary consumers (i.e., vertebrates). In comparison, $\delta^{13}C$ values of most consumers ranged between − 16 ‰ and − 10 ‰, with the exception of Chinook salmon and green urchin both of which had the most depleted $\delta^{13}C$ values. Finally, pigeon guillemot had the highest $\delta^{15}N$ values (mean = 16.18 ‰; range = 15.47 to 17.92 ‰).

Aggregation of primary producers or consumers:

Significant differences in $\delta^{13}C$ and $\delta^{15}N$ values were observed when comparing primary producers (MANOVA $F_{3,34}$= 17.9; $p < 0.001$). ANOVA and Tukeys post-hoc test revealed significant differences among each primary producer with respect to $\delta^{13}C$ values, while $\delta^{15}N$ values differed only between epiphytic algae and eelgrass or POM. Due to these differences in $\delta^{13}C$ and $\delta^{15}N$ values, primary producers were not aggregated for the purpose of the mixing model analyses. With regards to consumers, statistical analyses revealed that three species of gunnel (penpoint, crescent, and saddleback gunnels) had overlapping $\delta^{13}C$ and $\delta^{15}N$ values, and therefore we aggregated these species for the purpose of the mixing model analyses (Table 1). We ran a separate mixing model for each of the 16 consumers (including the aggregated gunnels). Of these 16 consumers, 7 had a sufficient number of individuals collected from both kelp- and eelgrass-dominated sites to allow us to include habitat as a fixed effect in their mixing model analyses. The Gelman-Rubin and Geweke diagnostics indicated that each model was within acceptable ranges.

Overall, epiphytic algae or bull kelp ranked as the most important primary producer in a consumer's diet. We found that 83% of the mixing models had either of these species as the highest proportional contribution to a consumers' diet. In contrast, eelgrass had the lowest contribution to a consumers' diet in 57% of the models (Fig. 3).

We present habitat-based results for those consumers with sufficient sample sizes to include habitat in the model. In other cases, we present the aggregate results with habitat excluded from the model. Lastly, we present median values of the MixSIAR results to summarize the relative importance of each primary

producer to a consumers' diet and note that mean, standard deviation, and quantiles are reported in Supplementary Table 2.

Secondary consumers:

MixSIAR results indicated that epiphytic algae contributed the most to the diet of secondary consumer California green shrimp based on the median (38%), while epiphytic algae and bull kelp were equally important (31% and 32%, respectively) to dock shrimp (Fig. 3, 4a and 4b; Supplementary Table 2). For Humpback shrimp, model results indicated that epiphytic algae contributed most (45%) to the diets of individuals collected from eelgrass-dominated sites, while bull kelp was important (51%) to the diets of individuals collected from kelp-dominated sites (Figs. 3 and 4c; Supplementary Table 2).

For the omnivorous red rock crab, model results indicated that the importance of epiphytic algae and bull kelp varied by 5–9% depending on what habitat individuals were sampled. Specifically, epiphytic algae contributed the most (44%) to the diets of individuals collected from eelgrass-dominated sites, while bull kelp contributed the most (45%) to the diets of individuals collected from kelp-dominated sites (Figs. 3 and 4d; Supplementary Table 2). For sharp-nosed crab, both bull kelp and epiphytic algae contributed equally (32%) to its diet, followed by POM (Figs. 3 and 4e; Supplementary Table 2). Model results for graceful kelp crab revealed that eelgrass was most important (41%) to the diets of individuals collected from eelgrass-dominated sites, while bull kelp, POM, and eelgrass all contributed about the same (24–27%) to diets in kelp-dominated sites (Figs. 3 and 4f; Supplementary Table 2). Among all the evaluated consumers, the diets of northern kelp crab were relatively similar (20% – 29%) across the four primary producers (Fig. 3, 4g; Supplementary Table 2). Lastly, MixSIAR results for helmet crab indicated that epiphytic algae contributed the most (45%) to their diets (Fig. 3, 4h; Supplementary Table 2).

Tertiary and quaternary consumers:

The three aggregated species of gunnels (penpoint, crescent, and saddleback gunnels) fed on food chains based equally (median of 35%) on epiphytic algae and bull kelp production at eelgrass-dominated sites. In kelp-dominated sites, bull kelp was more important (43%) to gunnels than epiphytic algae (33%), while the contribution of eelgrass was lower and comparable to POM (10% and 8%, respectively) (Figs. 3 and 5a; and Supplementary Table 2).

The food chain for shiner perch indicated that POM and bull kelp were the most important basal energy sources, (36% and 31%, respectively) (Figs. 3 and 5b; and Supplementary Table 2). Estimates of diet contribution for Pacific staghorn sculpin indicated that bull kelp (43%-53%), followed by epiphytic algae (23%-28%), were the most important primary producers regardless of whether individuals were collected from eelgrass- or kelp-dominated sites (Figs. 3 and 5c; and Supplementary Table 2). For Chinook salmon, a POM-based food chain was the primary source of carbon (62%) with contribution from bull kelp, epiphytic algae, and POM ranging from 8%-16% (Figs. 3 and 5d; Supplementary Table 2).

Epiphytic algae contributed the most to the diet of copper rockfish regardless of habitat type (60% and 63%), followed by bull kelp (23%) (Figs. 3 and 5e; Supplementary Table 2). For quillback rockfish, epiphytic algae and bull kelp contributed equally (37%-38%) to the diets of individuals in eelgrass-dominated sites, while bull kelp was more important (50%) than epiphytic algae (31%) at kelp-dominated sites (Figs. 3 and 5f; Supplementary Table 2). The diets of YOY rockfish were based primarily on bull kelp derived carbon (32%), followed by epiphytic algae (26%), and both POM and eelgrass contributing approximately equivalent amounts (19%) (Figs. 3 and 5g; Supplementary Table 2). Lastly, results for pigeon guillemot, a quaternary consumer, indicated that the primary producer contributing to egg membranes was based largely on POM (48%), with the remaining primary producers each contributing 11–18% to their diets (Figs. 3 and 5h; Supplementary Table 2).

Sensitivity of trophic discrimination factors:

Analyses investigating the sensitivity of consumption estimates to inaccuracies in trophic discrimination factors indicated that, on average, consumption estimates varied less than 9% for the median contributions to the diet among all consumers. Therefore, we are confident with our results since changes of ± 0.5‰ in the trophic discrimination factors did not substantially influence our median consumption estimates from MixSIAR.

Discussion

High-quality habitat is a basic requirement for the recovery and persistence of fish populations (Levin and Stunz 2005). Although aquatic plants provide important shelter for higher trophic level species (Iglesias et al. 2007, Valinoti et al. 2011, Grutters et al. 2015), a component often overlooked is the extent to which these biogenic habitats contribute to the assimilated energy of consumers. Understanding these trophic relationships is an important consideration for defining critical and essential fish habitat (NMFS 2006, 2014) and for ecosystem-based fisheries management (Francis et al. 2007). This is especially important because of global declines in biogenic habitats like kelp forests, mangroves, seagrass meadows and coral reefs (Waycott et al. 2009, Polidoro et al. 2010, Krumhansl et al. 2016, Hughes et al. 2017, Filbee-Dexter and Wernberg 2018). Quantifying the extent of these connections and how they vary among species could prove invaluable in calculating tradeoffs and designing effective management strategies (Levin and Stunz 2005).

In the current study, we reconstructed the dietary connections among 16 Puget Sound consumers, including species of concern, such as Chinook salmon, quillback and copper rockfish, and four primary producers, one of which, bull kelp, has experienced substantial local declines (Berry et al. 2021) and is being considered for listing under the U.S. Federal Endangered Species Act. Our results indicated that bull kelp and an epiphytic algae, *S. naiadum*, were the largest sources of carbon for most diets, although consumers exhibited some variability in the strength of their trophic connection to these primary producers (Fig. 3). Eelgrass was not prevalent as the basal energy source for most species in this study. However, our results demonstrate that eelgrass likely plays an important role in the Puget Sound

ecosystem as substratum for the epiphytic algae, which colonizes its blades (O'Clair and Lindstrom 2021), and as a significant source of POM in estuarine systems (Yoon et al. 2011, Conway-Cranos et al. 2015). Our results highlight the importance of both kelp and eelgrass habitats to the functioning of the Puget Sound ecosystem, albeit in potentially different ways. Below we describe the findings of our study in terms of the relative importance of different primary producers to the foraging of consumer species, how that importance varies with respect to the habitat in which a consumer resides, and lastly, cross-habitat trophic subsidies.

Trophic connections to primary producers

Because of their three-dimensional structure and, consequently, reduced visibility and accessibility, kelp forests and eelgrass beds have been reported to provide predation refugia for a variety of species (Beck et al. 2001, O'Brien et al. 2018, Shaffer et al. 2020). Further, with their large volume of primary production, kelp forests and eelgrass beds function as an important food source that supports neighboring food webs (Duggins et al. 1989, Harvey et al. 2012, Olson et al. 2019, Cartraud et al. 2021), including those with connections to economically valuable species such as ESA-listed salmon and rockfish (NMFS 2017). Most of the species we examined (13/16) relied on kelp or epiphytic algae as the primary source of carbon in their diets including a range of secondary (e.g., shrimp and crabs) to tertiary consumers (e.g., rockfish and sculpins). The median proportional contribution of kelp to the diets of many of our species varied from 15–52%, which was comparable to that of other studies. For example, Koenigs et al. (2015) reported that giant kelp (*Macrocystis pyrifera*) represented approximately 20–40% of the carbon in several resident fishes (with the remaining percentages attributed to phytoplankton), and that contribution increased with fish trophic level. Further, von Biela et al. (2016) reported that kelp carbon contributions ranged from 32–89% in the diets of kelp greenling (*Hexagrammos decagrammus*) and black rockfish (*Sebastes melanops*).

The relative importance of both bull kelp and epiphytic algae, as seen in the diet estimates of our sampled tertiary consumers of rockfish, sculpin, and gunnels, was also observed for several of the secondary consumers (California green shrimp, dock shrimp, humpback shrimp, red rock crab, and sharp-nosed crab; Fig. 3), some of which could be prey for these aforementioned fishes. Previous stomach content studies support this possibility by indicating that shrimp, crab, and other arthropods represent substantial components in the diets of juvenile copper rockfish and Pacific staghorn sculpin. For example, the diet of copper rockfish in Puget Sound consisted most frequently of arthropods (75%, including 29% from dock shrimp; Patten 1973, Washington et al. 1978), while crustaceans, including dock shrimp, red rock crab, and graceful kelp crab represented approximately 86% of their diets in Saanich Inlet, British Columbia, Canada (Murie 1991). Studies on copper rockfish conducted in Humboldt Bay, California, showed that the largest portion of their diet consisted of amphipods (55% at age-0 and 27% at age-1; Prince 1972) and copepods (59% and 24% of harpacticoid and calanoid, respectively, at age-0; Studebaker and Mulligan 2009). Stomach contents of Pacific staghorn sculpin also showed the importance of shrimp and crab, which together represented 85% of their total diet (Armstrong et al. 1996). Although estimates of the proportional contributions of secondary consumers to the diets of tertiary

consumers was beyond the scope of our study, the overlap in the importance of kelp and epiphytic algae among these consumers, together with stomach content studies, corroborate the existence of these trophic linkages.

Among our sampled consumers, eelgrass was often ranked as the least important primary producer. The average median proportional contribution of eelgrass across consumers was 12% (range of 2–41%), with graceful kelp crab, northern kelp crab, helmet crab, YOY rockfish, and pigeon guillemot showing the highest dietary contributions. Although eelgrass as a basal energy source was not as important as kelp and/or epiphytic algae, the fact that epiphytic algae grow on eelgrass highlights the significance of eelgrass within the Puget Sound food web. Similarly, few of our sampled species relied upon a POM-based food web. The exceptions being shiner perch, Chinook salmon, and pigeon guillemot. Their dependence on POM is not entirely surprising as they are not necessarily long-term residents of either demersal habitat and may feed more directly on a pelagic food chain supported by POM (Malick et al. 2015, Batten et al. 2018). The importance of POM across these consumers might also indicate that these fishes are preyed upon by pigeon guillemot. Pigeon guillemot are known to prey on a wide range of fish species within several kilometers of their nest sites (Litzow et al. 2000), but are also known to specialize in their selection of prey, potentially in response to prey abundance (Golet et al. 2000). According to a companion study, adult pigeon guillemot were observed delivering gunnels with greater frequency to burrows across Whidbey Island in Puget Sound than Pacific staghorn sculpin or other taxa (Buckner et al. 2022), despite gunnels having a lower abundance (Buckner 2020). In that same study, isotope analysis indicated that gunnels, Pacific staghorn sculpin, and rockfish represented 74% of the diet of adult female pigeon guillemot, followed by shiner perch (13%) and Chinook salmon (6%).

Interspecific and habitat-specific differences in energy sources

The relative importance of each primary producer, as observed in the diet estimates of our sampled consumers, varied depending on the habitat in which the consumer resided. Specifically, in four of seven consumers (i.e., graceful kelp crab, red rock crab, humpback shrimp, and copper rockfish), in which individuals were collected from both eelgrass- and kelp-dominated habitats, we found that the rank order of primary producers contributing to the consumers' diet differed between eelgrass and kelp habitats. Some of these species showed increases in relative importance of kelp when individuals were sampled from kelp-dominated sites and increased importance of epiphytic algae and/or eelgrass when sampled from eelgrass-dominated sites. Of greatest interest, is a comparison between copper and quillback rockfish, which could have important implications for conservation and management. Copper rockfish assimilated carbon from an epiphyte-based food web at more than twice the rate than that from a kelp-based food web regardless of whether individuals were collected from kelp or eelgrass sites. In contrast, quillback rockfish relied more on a kelp-based food web when they were found in kelp habitats and relied equally on epiphytic algae and kelp as their basal source of energy when they were found in eelgrass habitats. The causal mechanism(s) for these differences in diet between habitats are likely related to differential prey selectivity that could be due to differences in prey assemblages and total prey

abundance between habitats (Siddon et al. 2008, Shaffer et al. 2020), but needs further study in Puget Sound.

Inter- and intra-specific differences in the primary source of energy, particularly between these two rockfish species that are generally managed as a single complex, creates an interesting set of questions related to whether eelgrass or kelp habitats are “essential” and/or “critical” (i.e., provide significant benefits to the sustainability of a species; Levin and Stunz 2005) to these species or these specific life stages. The epiphytic algae, and thus eelgrass habitat, appears to be particularly central to the diet of juvenile copper rockfish and the protection of this habitat could benefit this rockfish. It is important to note that this epiphytic algae is also present on kelp and so extending any conservation measures to kelp habitat might also be beneficial. In Alaska, age-1 copper rockfish in eelgrass habitats had a higher energy content but lower relative growth rates compared to individuals from kelp habitats, even though similar prey items, shrimp and amphipods, were dominant in their diets in both habitats (Byerly 2001). Conversely, quillback rockfish appear to be more of a generalist in its overall assimilation of energy from both kelp- and epiphyte-based food webs; although to a greater extent for quillback in kelp habitats, suggesting that kelp may be particularly vital to the overall population. It’s important to consider the proportion of the population found in each of these habitats as to the overall importance of each habitat and the number of subtidal areas consisting of kelp and eelgrass habitats (Beck et al. 2001, Dahlgren et al. 2006). If the majority of juvenile quillback reside in kelp forest habitat, then kelp as a basal source of energy is quite meaningful; however, if the majority of juvenile quillback reside in eelgrass habitat, then kelp and eelgrass are equally relevant. Understanding these subtleties will provide managers with useful information to make decisions related to how resources are used to protect, conserve or restore eelgrass and kelp habitats in Puget Sound (e.g., (Calloway et al. 2020).

Contributions and connections among food webs

Trophic subsidies represent an important mechanism that connects habitats, communities and ecosystems across the marine environment (Zuercher and Galloway 2019). The import and export of primary production from different primary producers across habitat types creates a web of interactions and consequences that have hindered our abilities to adequately define the importance of habitats to species and to fully operationalize ecosystem-based management (Duggins et al. 1989, Conway-Cranos et al. 2015, Cartraud et al. 2021).

In Puget Sound, across nearly every species investigated, we found multiple primary producers contributing a relatively large (> 20%) amount to consumers’ diets. This pattern was consistent for all species in which we were able to compare across and within habitat types, with Chinook salmon being the only exception. For example, the proportion of diet based on epiphytic algae was greater for four species (i.e., humpback shrimp, red rock crab, gunnels, and quillback rockfish) sampled from eelgrass-dominated sites, but the proportion of diet based on kelp was still relatively high (> 30%); and this pattern was exactly the opposite when examining these same species in kelp habitats (i.e., kelp was nominally the greatest contributor to diet, but epiphytic algae contributed > 30%). Subsidies of bull kelp to other

habitats has also been observed in British Columbia, Canada estuaries in which 41%-51% of the diet of YOY rockfish was attributed to kelp regardless of whether fish were collected from eelgrass meadows, or the edge of kelp beds or sand habitats (Olson et al. 2019). Evidence of trophic subsidies from eelgrass to kelp habitats was also noted by Olson and colleagues (2019), but there appears to be very limited research on this specific pathway, with the vast majority of research dedicated to the importance of kelp subsidies to eelgrass habitats (e.g., Doropoulos et al. 2009, Krumhansl and Scheibling 2012, Cartraud et al. 2021).

Our understanding of which habitats represent “essential” or “critical” habitat to protected or socially-valuable species will need to account for the types of cross-habitat trophic subsidies identified here. In Puget Sound, there are numerous efforts to protect, conserve, and restore nearshore eelgrass and kelp habitats (e.g., Calloway et al. 2020, PSP 2022). With limited resources to address the needs of these and other efforts across the globe, it will be important to advance our understanding of the relative importance of multiple habitats to the goals of management efforts. More broadly, these results highlight the importance of trophic connectivity among nearshore habitats and the need to employ ecosystem-based management efforts capable of accounting for these cross-habitat subsidies and trophic interactions.

Conclusion

Identifying and prioritizing habitats used by specific life stages is critical as fisheries managers shift to ecosystem-based approaches to management (Levin and Stunz 2005). Although aquatic plants are known to provide important shelter for a variety of species, an often-overlooked component is the extent to which biogenic habitats contribute to their diet. Understanding these trophic relationships is especially crucial since biogenic habitats, like seagrass and kelp, are experiencing severe declines globally. Our specific objective was to determine the extent to which primary producers of bull kelp, eelgrass, epiphytic algae, and particulate organic matter are dietary resources for marine invertebrates, fishes, and a seabird in Puget Sound, an understanding of which is critical to inform ongoing recovery planning. We determined that bull kelp and epiphytic algae had the greatest contribution to the diets of most of the sampled 16 consumers, yet each primary producer was utilized to some degree across consumers. Overall, this work highlighted the complex and interdependent nature of feeding relationships within Puget Sound. Understanding the strength of these trophic relationships is an important step for predicting ecosystem consequences following natural and anthropogenic perturbations within these habitats, and such information is vital to managers making decisions related to the conservation of valuable populations. This study adds to growing research aimed at improving our understanding of the role primary producers such as kelp play in the Puget Sound food web and the essential ecosystem services they provide. This knowledge will enhance our ability to advocate for the conservation of biogenic habitats as a means to better the ecosystem health and sustainability.

Declarations

Acknowledgements:

Special thanks to B Filipiak, R Doung, D Robichaux, W Platt, K Frick, G Williams, G Sullaway, J Pohl, and M Delgado who provided field and lab support. Additional thanks to H Garfield, E Sosik, and S Naman for productive discussions and to J Gosselin and J Smith for their constructive comments on an earlier version of this manuscript. Funding provided by the Protected Resources Division, West Coast Regional Office National Marine Fisheries Service, National Oceanic and Atmospheric Administration.

Funding: Funding provided by the Protected Resources Division, West Coast Regional Office National Marine Fisheries Service, National Oceanic and Atmospheric Administration.

Conflicts of interest/Competing interests: The authors declare that they have no conflict of interest.

Ethics approval: All applicable institutional and/or national guidelines for the care and use of animals were followed. Collections of biological materials were conducted under applicable federal and state permits. Specifically, sampling permits provided by Washington Department of Fish and Wildlife (#18-140 and 19-142) and NOAA Fisheries (ESA section 10 permit #17062-6R and 1583-4R).

Consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and material: Data is available upon request.

Code availability: Not applicable

Author Contributions: PC, NT, KA, DT, and GY conceived and designed the study; PC, NT, KA, and EB conducted fieldwork; PC, EB, and JG conducted stable isotope analysis; PC analyzed the data; PC, NT, and KA wrote the manuscript; JG, EB, GY, and DT provided editorial advice.

References

1. Ammann, A. J. 2004. SMURFs: standard monitoring units for the recruitment of temperate reef fishes. J Exp Mar Biol Ecol 299:135-154.
2. Arkema, K., G. Guannel, G. Verutes, S. Wood, A. Guerry, M. Ruckelshaus, P. Kareiva, M. Lacayo-Emery, and J. Silver. 2013. Coastal habitats shield people and property from sea-level rise and storms. Nature Climate Change 3:913-918.
3. Armstrong, J. L., D. A. Armstrong, and S. B. Mathews. 1996. Food habits of estuarine staghorn sculpin, *Leptocottus armatus*, with focus on consumption of juvenile Dungeness crab, *Cancer magister*. Oceanogr Lit Rev 43:595.
4. Arostegui, M. C., D. E. Schindler, and G. W. Holtgrieve. 2019. Does lipid-correction introduce biases into isotopic mixing models? Implications for diet reconstruction studies. Oecologia 191:745-755.

5. Batten, S. D., G. T. Ruggerone, and I. Ortiz. 2018. Pink Salmon induce a trophic cascade in plankton populations in the southern Bering Sea and around the Aleutian Islands. Fish Oceanogr 27:548-559.
6. Beck, M. W., K. L. Heck, K. W. Able, D. L. Childers, D. B. Efleston, B. M. Gillanders, B. Halpern, C. G. Hays, K. Hoshino, T. J. Minello, R. J. Orth, P. F. Sheridan, and M. P. Weinstein. 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. BioScience. 51:633-641.
7. Berry, H. D., T. F. Mumford, B. Christiaen, P. Dowty, M. Calloway, L. Ferrier, E. E. Grossman, and N. R. VanArendonk. 2020. Long-term changes in kelp forests in an inner basin of the Salish Sea. bioRxiv:2020.2002.2013.947309.
8. Berry, H. D., T. F. Mumford, B. Christiaen, P. Dowty, M. Calloway, L. Ferrier, E. E. Grossman, and N. R. VanArendonk. 2021. Long-term changes in kelp forests in an inner basin of the Salish Sea. PLoS ONE 16:e0229703.
9. Buckner, E., P. Chittaro, F. Wood, and T. Klinger. 2022. Identifying dietary preferences in breeding pigeon guillemot (*Cepphus columba*) using different methods. Northwestern Naturalist 103:42-50.
10. Buckner, E. 2020. Spatial foraging patterns in Puget Sound pigeon guillemot (*Cepphus columba*): an investigation using stable isotopes and community science. M.Sc thesis University of Washington, Marine and Environmental Affairs.
11. Byerly, M. M. 2001. The ecology of age-1 copper rockfish (*Sebastes caurinus*) in vegetated habitats of Sitka Sound, Alaska.
12. Calloway, M., D. Oster, H. Berry, T. Mumford, N. Naar, B. Peabody, L. Hart, D. Tonnes, S. Copps, J. Selleck, B. Allen, and J. Toft. 2020. Puget Sound kelp conservation and recovery plan. Prepared for NOAA-NMFS, Seattle, WA. 52 pages plus appendices. Available at: https://nwstraits.org/our-work/kelp/.
13. Caro, T., Z. Rowe, J. Berger, P. Wholey, and A. Dobson. 2022. An inconvenient misconception: Climate change is not the principal driver of biodiversity loss. Conserv Lett e12868. https://doi.org/10.1111/conl.12868
14. Cartraud, A. E., P. S. Lavery, C. M. Rae, and G. A. Hyndes. 2021. Pathways to spatial subsidies by kelp in seagrass meadows. Estuaries and Coasts 44:468-480.
15. Connell, S., B. Russell, D. Turner, S. Shepherd, T. Kildea, D. Miller, L. Airoldi, and A. Cheshire. 2008. Recovering a lost baseline: Missing kelp forests from a metropolitan coast. Mar Ecol Prog Ser 360:63-72.
16. Conway-Cranos, L., P. Kiffney, N. Banas, M. Plummer, S. Naman, P. Maccready, J. Bucci, and M. Ruckelshaus. 2015. Stable isotopes and oceanographic modeling reveal spatial and trophic connectivity among terrestrial, estuarine and marine environments. Mar Ecol Prog Ser 533.
17. Dahlgren, C. P., G. T. Kellison, A. J. Adams, B. M. Gillanders, M. S. Kendall, C. A. Layman, J. A. Ley, I. Nagelkerken, and J. E. Serafy. 2006. Marine nurseries and effective juvenile habitats: concepts and applications. Mar Ecol Prog Ser 312:291-295.

18. Dethier, M. N., A. S. Brown, S. Burgess, M. E. Eisenlord, A. W. E. Galloway, J. Kimber, A. T. Lowe, C. M. O'Neil, W. W. Raymond, E. A. Sosik, and D. O. Duggins. 2014. Degrading detritus: Changes in food quality of aging kelp tissue varies with species. J Exp Mar Biol Ecol 460:72-79.
19. Doropoulos, C., G. A. Hyndes, P. S. Lavery, and F. Tuya. 2009. Dietary preferences of two seagrass inhabiting gastropods: Allochthonous vs autochthonous resources. Estuar Coast Shelf Sci 83:13-18.
20. Duggins, D., C. Simenstad, and J. A. Estes. 1989. Magnification of secondary production by kelp detritus in coastal marine ecosystems. Science (New York, N.Y.) 245:170-173.
21. Filbee-Dexter, K., C. Feehan, and R. Scheibling. 2016. Large-scale degradation of a kelp ecosystem in an ocean warming hotspot. Mar Ecol Prog Ser 543.
22. Filbee-Dexter, K., and T. Wernberg. 2018. Rise of Turfs: A new battlefront for globally declining kelp forests. Bioscience 68:64-76.
23. Francis, R. C., M. A. Hixon, M. E. Clarke, S. A. Murawski, and S. Ralston. 2007. Ten commandments for ecosystem-based fisheries scientists. Fisheries 32:217-233.
24. Fry, B. 2006. Stable isotope ecology. Springer, New York, New York, USA. Pp. 308.
25. Golet, G. H., K. J. Kuletz, D. D. Roby, and D. B. Irons. 2000. Adult prey choice affects chick growth and reproductive success in Pigeon Guillemots. The Auk 117:82-91.
26. Graham, M. H. 2004. Effects of local deforestation on the diversity and structure of Southern California giant kelp forest food webs. Ecosystems 7:341-357.
27. Grutters, B. M. C., B. J. A. Pollux, W. C. E. P. Verberk, and E. S. Bakker. 2015. Native and non-Native plants provide similar refuge to invertebrate prey, but less than artificial plants. PLoS ONE 10:e0124455.
28. Harvey, C. J., G. D. Williams, and P. S. Levin. 2012. Food web structure and trophic control in central Puget Sound. Estuaries and Coasts 35:821-838.
29. Hughes, T. P., M. L. Barnes, D. R. Bellwood, J. E. Cinner, G. S. Cumming, J. B. C. Jackson, J. Kleypas, I. A. van de Leemput, J. M. Lough, T. H. Morrison, S. R. Palumbi, E. H. van Nes, and M. Scheffer. 2017. Coral reefs in the Anthropocene. Nature 546:82-90.
30. Iglesias, C., G. Goyenola, N. Mazzeo, M. Meerhoff, E. Rodó, and E. Jeppesen. 2007. Horizontal dynamics of zooplankton in subtropical Lake Blanca (Uruguay) hosting multiple zooplankton predators and aquatic plant refuges. Hydrobiologia 584:179-189.
31. Kelkar, M., and K. Carden. 2022. Petition to list bull kelp under the U.S. Endangered Species Act.
32. Koenigs, C., R. J. Miller, and H. M. Page. 2015. Top predators rely on carbon derived from giant kelp *Macrocystis pyrifera*. Mar Ecol Prog Ser 537:1-8.
33. Kritzer, J. P., M.-B. DeLucia, E. Greene, C. Shumway, M. F. Topolski, J. Thomas-Blate, L. A. Chiarella, K. B. Davy, and K. Smith. 2016. The Importance of benthic habitats for coastal fisheries. Bioscience 66:274-284.
34. Krumhansl, K., and R. Scheibling. 2012. Production and fate of kelp detritus. Mar Ecol Prog Ser 467:281-302.

35. Krumhansl, K. A., D. K. Okamoto, A. Rassweiler, M. Novak, J. J. Bolton, K. C. Cavanaugh, S. D. Connell, C. R. Johnson, B. Konar, S. D. Ling, F. Micheli, K. M. Norderhaug, A. Perez-Matus, I. Sousa-Pintol, D. C. Reed, A. K. Salomon, N. T. Shears, T. Wernberg, R. J. Anderson, N. S. Barrett, A. H. Buschmanns, M. H. Carr, J. E. Caselle, S. Derrien-Courtel, G. J. Edgar, M. Edwards, J. A. Estes, C. Goodwin, M. C. Kenner, D. J. Kushner, F. E. Moy, J. Nunn, R. S. Stenecka, J. Vsquezb, J. Watsonc, J. D. Witmand, and J. E. K. Byrnese. 2016. Global patterns of kelp forest change over the past half-century. PNAS 113:13785-13790.
36. Levin, P. S., and G. W. Stunz. 2005. Habitat triage for exploited fishes: Can we identify essential "Essential Fish Habitat?": Connectivity in the life cycles of fishes and invertebrates that use estuaries. Estuar Coast Shelf Sci 64:70-78.
37. Litzow, M. A., J. F. Piatt, A. A. Abookire, A. K. Prichard, and M. D. Robards. 2000. Monitoring temporal and spatial variability in sandeel (*Ammodytes hexapterus*) abundance with pigeon guillemot (*Cepphus columba*) diets. ICES J Mar Sci 57:976-986.
38. Lotze, H. K., H. S. Lenihan, B. J. Bourque, R. H. Bradbury, R. G. Cooke, M. C. Kay, S. M. Kidwell, M. X. Kirby, C. H. Peterson, and J. B. C. Jackson. 2006. Depletion, degradation, and recovery potential of estuaries and coastal seas. Science 312:1806-1809.
39. Malick, M. J., S. P. Cox, F. J. Mueter, and R. M. Peterman. 2015. Linking phytoplankton phenology to salmon productivity along a north–south gradient in the Northeast Pacific Ocean. Can J Fish Aquat Sci 72:697-708.
40. Murie, D. J. 1991. Comparative ecology and interspecific competition between the sympatric congeners *Sebastes caurinus* (copper rockfish) and *S. maliger* (quillback rockfish) Department of Biology, University of Victoria. Pp. 310.
41. NMFS. 1999. Endangered and threatened species: threatened status for three chinook salmon evolutionarily significant units (ESUs) in Washington and Oregon, and endangered status for one chinook salmon ESU in Washington. Federal Register 64: 14308-14328.
42. NMFS. 2006. Magnuson-Stevens Act Provisions; Fisheries off West Coast States; Pacific Coast Groundfish Fishery. National Marine Fisheries Service, Federal Register 71 FR 27408.
43. NMFS. 2014. Endangered and threatened species; designation of critical habitat for the Puget Sound/Georgia Basin distinct population segments of yelloweye rockfish, canary rockfish and bocaccio; final rule. National Marine Fisheries Service, Federal Register 79 FR 68042.
44. NMFS. 2017. Rockfish Recovery Plan: Puget Sound / Georgia Basin yelloweye rockfish (*Sebastes ruberrimus*) and bocaccio (*Sebastes paucispinis*). National Marine Fisheries Service. Seattle, WA.
45. O'Brien, B. S., K. Mello, A. Litterer, and J. A. Dijkstra. 2018. Seaweed structure shapes trophic interactions: A case study using a mid-trophic level fish species. J Exp Mar Biol Ecol 506:1-8.
46. O'Clair, R., and S. Lindstrom. 2021. *Smithora naiadum* (Anderson) Hollenberg. In: Klinkenberg, Brian. (Editor) 2021. E-Flora BC: Electronic Atlas of the Flora of British Columbia [eflora.bc.ca]. Lab for Advanced Spatial Analysis, Department of Geography, University of British Columbia, Vancouver. https://ibis.geog.ubc.ca/biodiversity/eflora/.

47. Olson, A. M., M. Hessing-Lewis, D. Haggarty, and F. Juanes. 2019. Nearshore seascape connectivity enhances seagrass meadow nursery function. Ecol Appl 29:e01897.
48. Patten, B. G. 1973. Biological Information on copper rockfish in Puget Sound, Washington. Trans. Am. Fish 102:412-416.
49. Pethybridge, H. R., C. A. Choy, J. J. Polovina, and E. A. Fulton. 2018. Improving marine ecosystem models with biochemical tracers. Ann Rev Mar Sci 10:199-228.
50. Phillips, D. L., R. Inger, S. Bearhop, A. L. Jackson, J. W. Moore, A. C. Parnell, B. X. Semmens, and E. J. Ward. 2014. Best practices for use of stable isotope mixing models in food-web studies. Can J Zool 92:823-835.
51. Polidoro, B. A., K. E. Carpenter, L. Collins, N. C. Duke, A. M. Ellison, J. C. Ellison, E. J. Farnsworth, E. S. Fernando, K. Kathiresan, N. E. Koedam, S. R. Livingstone, T. Miyagi, G. E. Moore, V. Ngoc Nam, J. E. Ong, J. H. Primavera, S. G. Salmo, III, J. C. Sanciangco, S. Sukardjo, Y. Wang, and J. W. H. Yong. 2010. The Loss of species: mangrove extinction risk and geographic areas of global concern. PLoS ONE 5:e10095.
52. Polito, M., S. Fisher, C. Tobias, and S. Emslie. 2009. Tissue-specific isotopic discrimination factors in gentoo penguin (*Pygoscelis papua*) egg components: Implications for dietary reconstruction using stable isotopes. J Exp Mar Biol Ecol 372:106-112.
53. Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83:703-718.
54. Post, D. M., C. A. Layman, D. A. Arrington, G. Takimoto, J. Quattrochi, and C. G. Montaña. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152:179-189.
55. Prince, E. D. 1972. The food and behavior of the copper rockfish, *Sebastes caurinus* richardson, associated with an artificial reef in south Humboldt Bay, California. Master of Science thesis, California State University, Humboldt.
56. Puget Sound Partnership 2022. 2022-2026 Action Agenda for Puget Sound. Puget Sound Partnership. Accessed November 23, 2022: https://www.psp.wa.gov/2022AAupdate.php.
57. R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
58. Rehr, A., G. Williams, N. Tolimieri, and P. Levin. 2014. Impacts of terrestrial and shoreline stressors on eelgrass in puget sound: an expert elicitation. Coast Manage 42. doi: 10.1080/08920753.2014.904195
59. Resano-Mayor, J., A. Hernández-Matías, J. Real, M. Moleón, F. Parés, R. Inger, and S. Bearhop. 2014. Multi-scale effects of nestling diet on breeding performance in a terrestrial top predator inferred from stable isotope analysis. PLoS ONE 9:e95320.
60. Rogers-Bennett, L., and C. A. Catton. 2019. Marine heat wave and multiple stressors tip bull kelp forest to sea urchin barrens. Sci Rep 9:15050.

61. Scheffer, M., S. Carpenter, J. A. Foley, C. Folke, and B. Walker. 2001. Catastrophic shifts in ecosystems. Nature 413:591-596.
62. Shaffer, J. A., S. H. Munsch, and J. R. Cordell. 2020. Kelp forest zooplankton, forage fishes, and juvenile salmonids of the northeast Pacific nearshore. Mar Coast Fish 12:4-20.
63. Shelton, A. O., T. B. Francis, B. E. Feist, G. D. Williams, A. Lindquist, and P. S. Levin. 2017. Forty years of seagrass population stability and resilience in an urbanizing estuary. J Ecol 105:458-470.
64. Siddon, E. C., C. E. Siddon, and M. S. Stekoll. 2008. Community level effects of Nereocystis luetkeana in southeastern Alaska. J Exp Mar Biol Ecol 361:8-15.
65. Sloan, C. A., B. F. Anulacion, K. A. Baugh, J. L. Bolton, D. Boyd, P. M. Chittaro, D. A. M. da Silva, J. B. Gates, B. L. Sanderson, K. Veggerby, and G. M. Ylitalo. 2019. Quality assurance plan for analyses of environmental samples for polycyclic aromatic hydrocarbons, persistent organic pollutants, dioctyl sulfosuccinate, estrogenic compounds, steroids, hydroxylated polycyclic aromatic hydrocarbons, stable isotope ratios, and lipid classes. U.S. Department of Commerce, NOAA Technical Memorandum NMFSNWFSC-147. https://doi.org/10.25923/kf28-n618.
66. Smith, J. A., D. Mazumder, I. M. Suthers, and M. D. Taylor. 2013. To fit or not to fit: evaluating stable isotope mixing models using simulated mixing polygons. Methods Ecol Evol 4:612-618.
67. Sosik, E., and C. Simenstad. 2013. Isotopic evidence and consequences of the role of microbes in macroalgae detritus-based food webs. Mar Ecol Prog Ser 494:107-119.
68. Steneck, R. S., M. H. Graham, B. J. Bourque, D. Corbett, J. M. Erlandson, J. A. Estes, and M. J. Tegner. 2002. Kelp forest ecosystems: biodiversity, stability, resilience and future. Environ Conserv 29:436-459.
69. Stock, B. C., and B. X. Semmens. 2016. MixSIAR GUI User Manual. Version 3.1. https://github.com/brianstock/MixSIAR/. doi:10.5281/zenodo.47719.
70. Stock, B. C., A. L. Jackson, E. J. Ward, A. C. Parnell, D. L. Phillips, and B. X. Semmens. 2018a. Analyzing mixing systems using a new generation of Bayesian tracer mixing models. PeerJ 6:e5096.
71. Stock, B. C., A. L. Jackson, E. J. Ward, A. C. Parnell, D. L. Phillips, and B. X. Semmens. 2018b. Analyzing mixing systems using a new generation of Bayesian tracer mixing models. PeerJ Preprints 6:e26884v26881.
72. Studebaker, R. S., and T. J. Mulligan. 2009. Feeding Habits of Young-of-the-Year Black and Copper Rockfish in Eelgrass Habitats of Humboldt Bay, California. Northwestern Naturalist 90:17-23.
73. Unsworth, R. K. F., L. M. Nordlund, and L. C. Cullen-Unsworth. 2019. Seagrass meadows support global fisheries production. Conserv Lett 12:e12566.
74. Valinoti, C. E., C.-K. Ho, and A. R. Armitage. 2011. Native and exotic submerged aquatic vegetation provide different nutritional and refuge values for macroinvertebrates. J Exp Mar Biol Ecol 409:42-47.
75. von Biela, V. R., S. D. Newsome, J. L. Bodkin, G. H. Kruse, and C. E. Zimmerman. 2016. Widespread kelp-derived carbon in pelagic and benthic nearshore fishes suggested by stable isotope analysis. Estuar Coast Shelf Sci 181:364-374.

76. Waycott, M., C. M. Duarte, T. J. B. Carruthers, R. J. Orth, W. C. Dennison, S. Olyarnik, A. Calladine, J. W. Fourqurean, K. L. Heck, A. R. Hughes, G. A. Kendrick, W. J. Kenworthy, F. T. Short, and S. L. Williams. 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. PNAS 106:12377.
77. Washington, P. M., R. Gowan, and D. H. Ito. 1978. A biological report on eight species of rockfish (*Sebastes* spp.) from Puget Sound, Washington. Northwest and Alaska Fisheries Center Processed Report. Pg. 63.
78. Yoon, S., Y. Sakanishi, and M. J. Kishi. 2011. Estimation of particulate organic carbon flux produced from eelgrass, Zostera marina L., in a subarctic estuary of Hokkaido, Japan. Curr Dev Ocean 3:1-32.
79. Zanden, M. J. V., and J. B. Rasmussen. 2001. Variation in δ15N and δ13C trophic fractionation: Implications for aquatic food web studies. Limn Ocean 46:2061-2066.
80. Zuercher, R., and A. W. E. Galloway. 2019. Coastal marine ecosystem connectivity: pelagic ocean to kelp forest subsidies. Ecosphere 10:e02602.

Figures

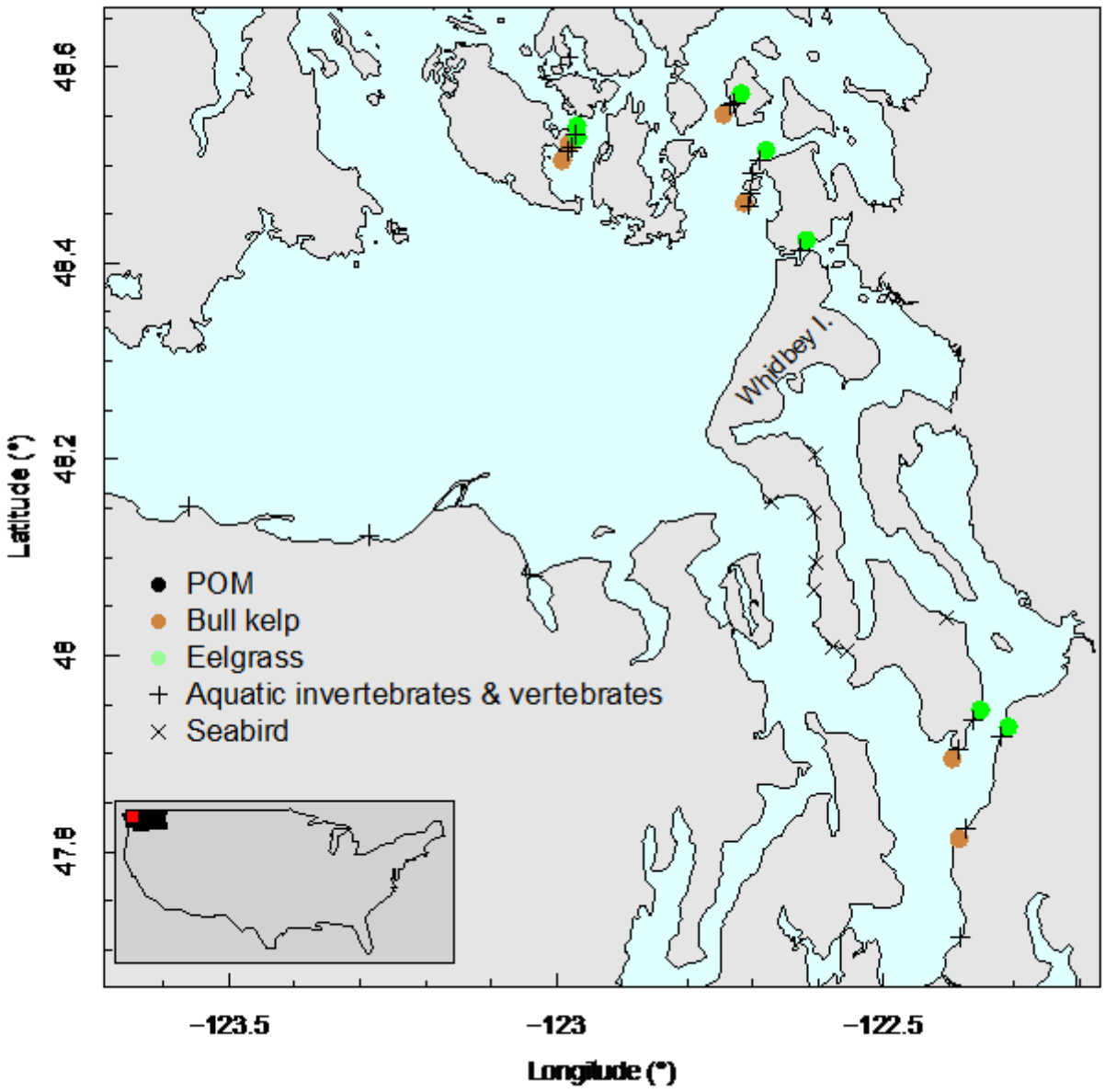

Figure 1

Sites (n=31) from which primary producers, and primary, secondary, tertiary, and quaternary consumers were collected in Puget Sound during the summers of 2018 and 2019. Brown and green circles represent sites where *Nereocystis luetkeana*(bull kelp) and *Zostera marina*(eelgrass), respectively, were the dominant biogenic habitats. *Cepphus columbus* (pigeon guillemot) egg shells were collected from nests (represented as 'x' symbol) exclusively around Whidbey Island.

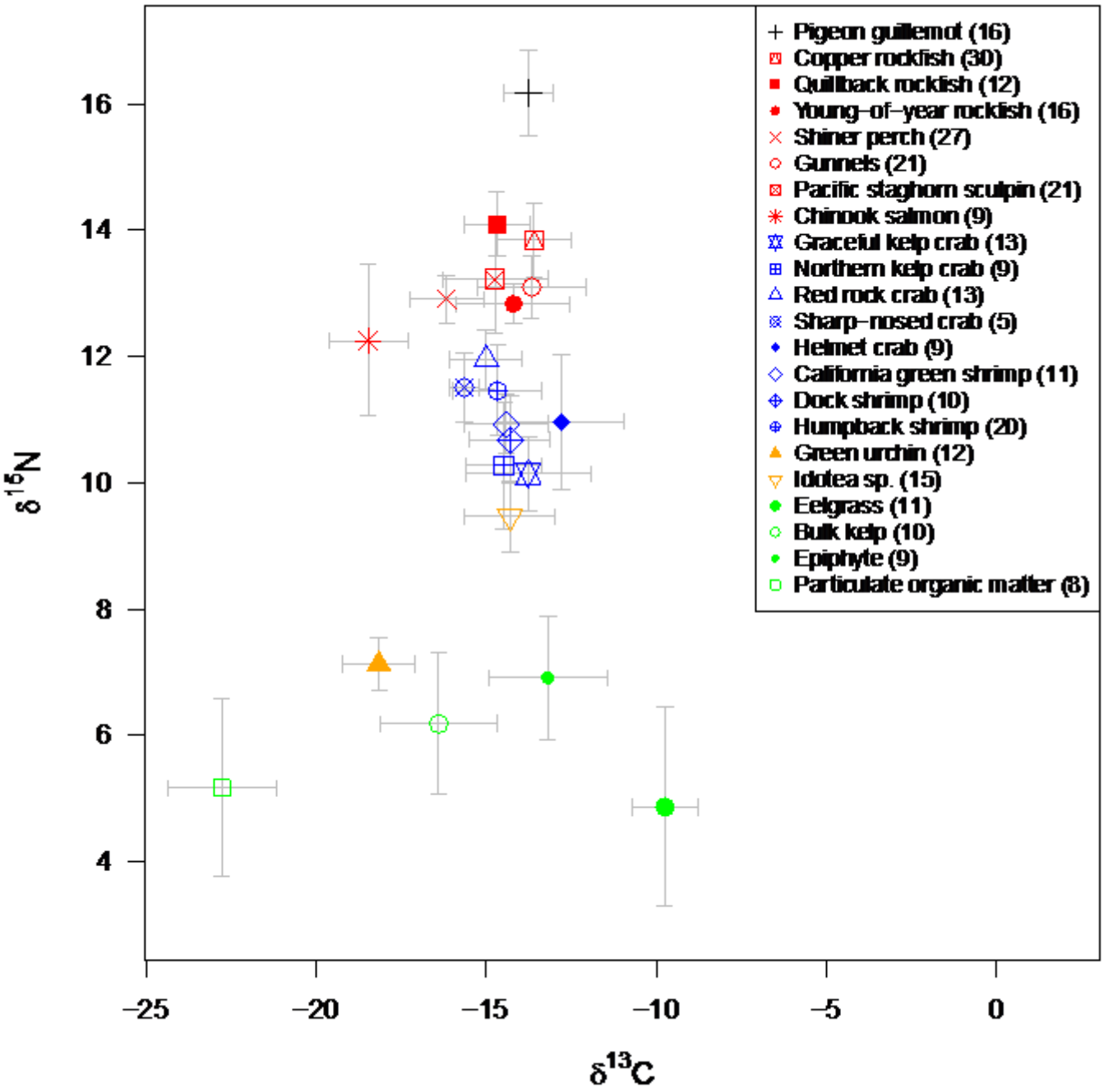

Figure 2

Plot of stable isotopic ratios of nitrogen ($\delta^{15}N$) to carbon ($\delta^{13}C$) from sampled taxa. Means (symbol) and standard deviation (whisker) are plotted for each primary producer (green), and for primary (orange), secondary (blue), tertiary (red) and quaternary consumers (black). Sample sizes are provided in parentheses.

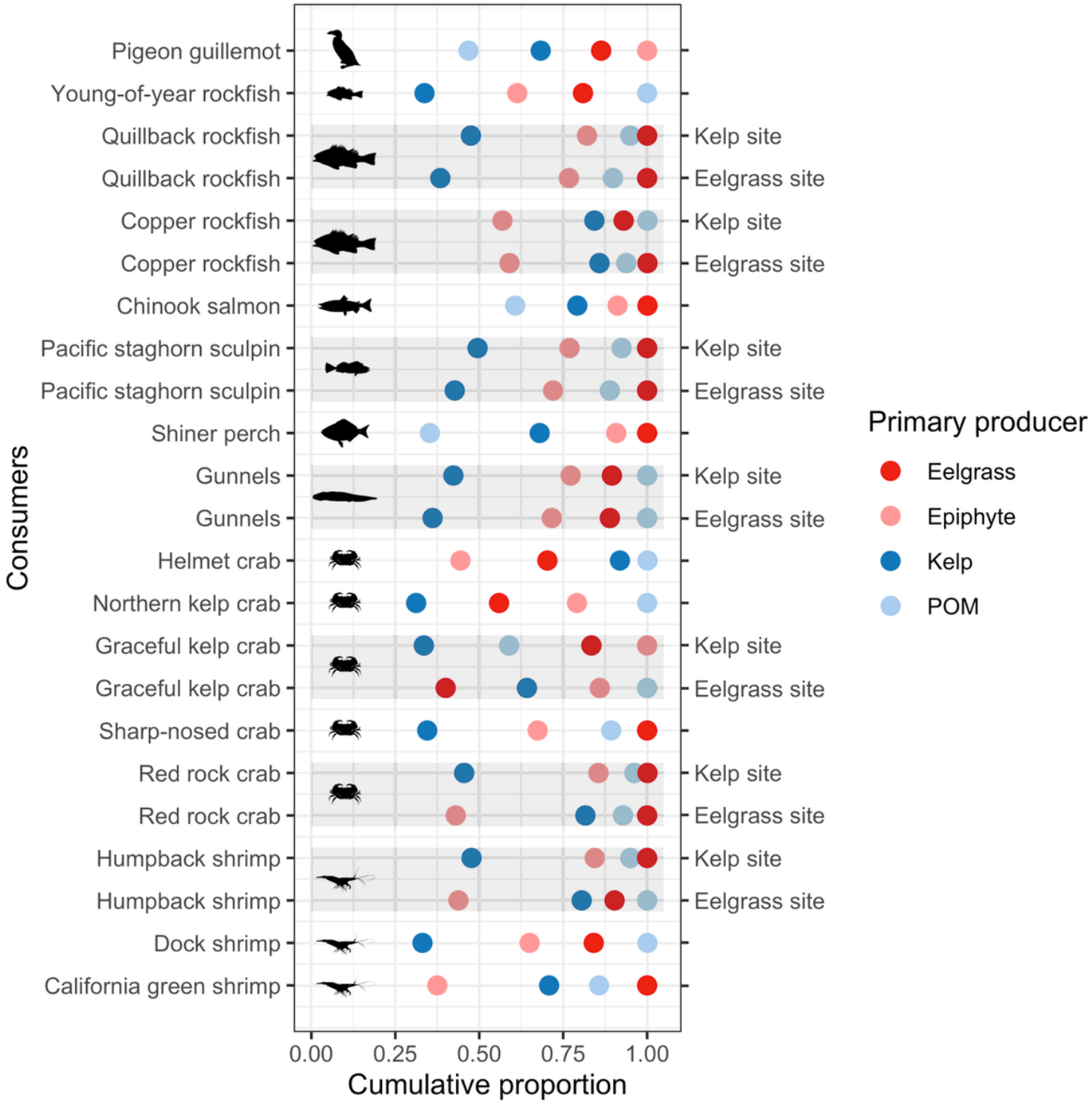

Figure 3

Cumulative proportion of the mean contribution of four primary producers to the diets of each of 18 consumers. A sufficient number of individuals were collected from both eelgrass- and kelp-dominated sites (grey shading) to permit estimates of diet composition within these habitats for the following species: Humpback shrimp, red rock crab, graceful kelp crab, gunnels (i.e., penpoint, crescent, and saddleback gunnels), Pacific staghorn sculpin, copper rockfish, and quillback rockfish.

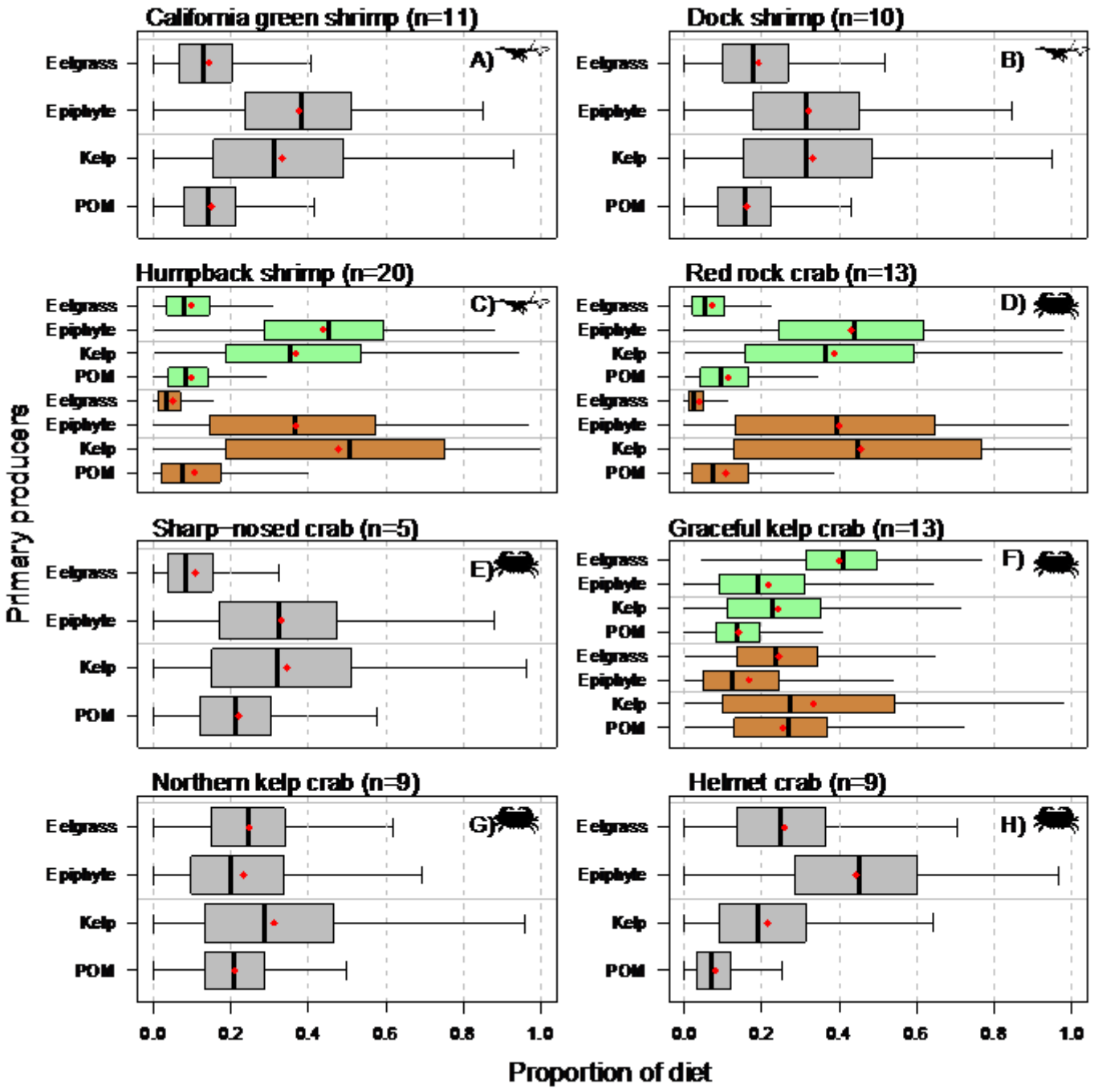

Figure 4

Relative proportional contribution of each of four primary producers to the diet of ten invertebrate primary and secondary consumers. Primary producers are *Zostera marina* (“Eelgrass”), *Smithora naiadum*(“Epiphyte”), *Nereocystis luetkeana*(“Kelp”), and particulate organic matter (“POM”). Results of MixSIAR analysis are shown for A) California green shrimp, B) Dock shrimp, C) Humpback shrimp, D) Red rock crab, E) Sharp-nosed crab, F) Graceful kelp crab, G) Northern kelp crab, and H) Helmet crab. A sufficient number of individuals were collected from both eelgrass- and kelp-dominated sites to permit estimates of diet composition within these habitats (green and brown, respectively) for the following species: humpback shrimp, red rock crab, and graceful kelp crab. Grey shading corresponds to species

whose individuals were collected from a mix of sites that were kelp- and eelgrass-dominated. Black vertical line, box, and whiskers represent median, first and third quartile (i.e., 25th and 75th percentiles), and minimum and maximum values, respectively, and red dot corresponds to mean.

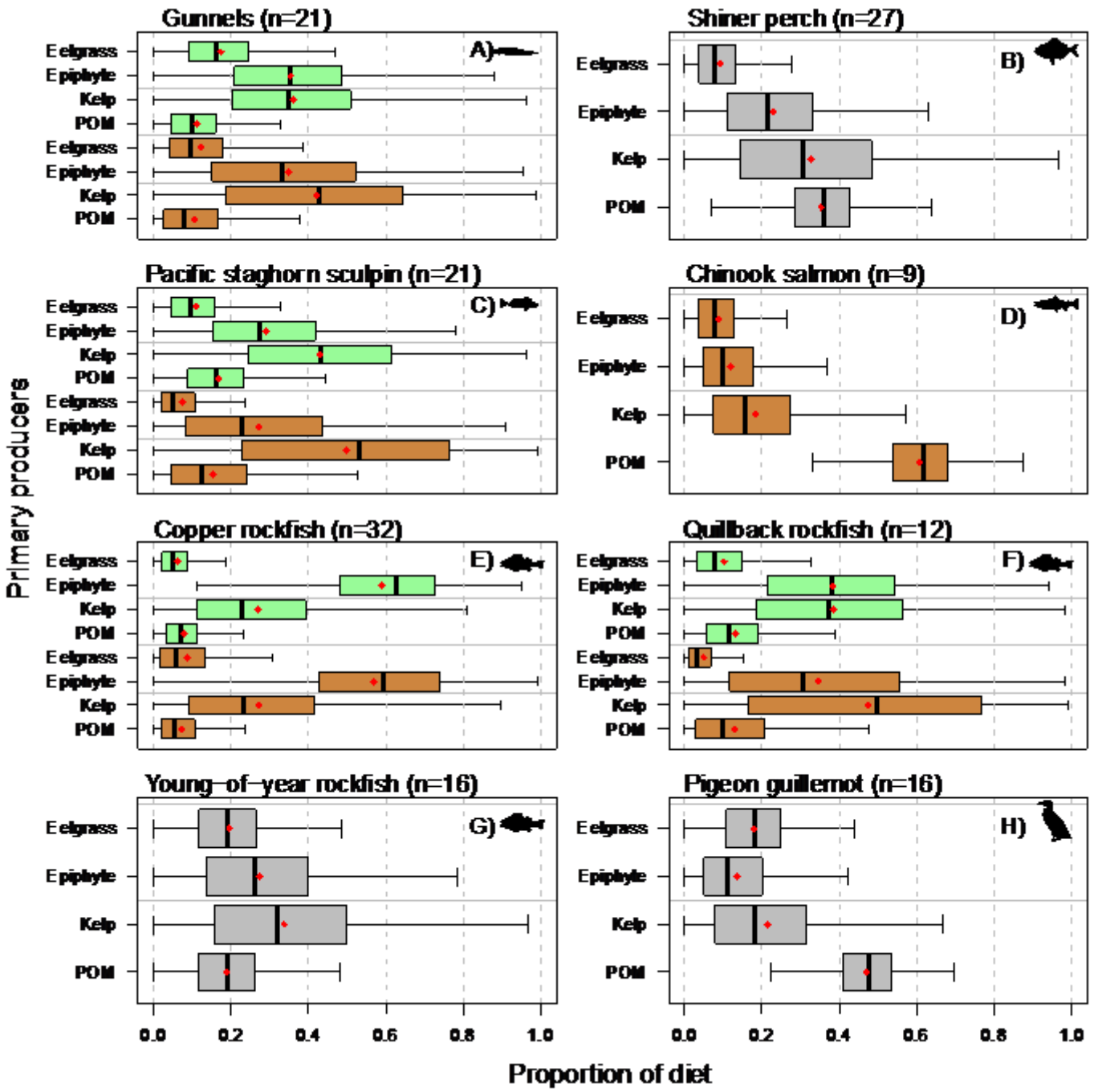

Figure 5

Relative proportional contribution of each of four primary producers to the diet of eight vertebrate tertiary and quaternary consumers. Primary producers are *Zostera marina* (“Eelgrass”), *Smithora naiadum*(“Epiphyte”), *Nereocystis luetkeana*(“Kelp”), and particulate organic matter (“POM”). Results of the MixSIAR analysis are shown for A) Gunnels (i.e., penpoint, crescent, and saddleback gunnels), B)

Shiner perch, C) Pacific staghorn sculpin, D) Chinook salmon, E) Copper rockfish, F) Quillback rockfish, G) Young-of-year rockfish, H) Pigeon guillemot. A sufficient number of individuals were collected from both eelgrass- and kelp-dominated sites to permit estimates of diet composition within these habitats (green and brown, respectively) for the following species: Gunnels, Pacific staghorn sculpin, and copper and quillback rockfish. Grey shading corresponds to species whose individuals were collected from a mix of sites that were kelp- and eelgrass-dominated. Chinook salmon were collected from kelp-dominated sites. Black vertical line, box, and whiskers represent median, first and third quartile (i.e., 25th and 75th percentiles), and minimum and maximum values, respectively, and red dot corresponds to mean.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTables.pdf