Trophic Ecology of Deep-Sea Megafauna in the Ultra-Oligotrophic Southeastern Mediterranean Sea

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The trophic ecology of fourteen species of demersal fishes and six species of demersal decapod crustaceans from the continental slope and rise of the Southeastern Mediterranean Sea (SEMS) was examined using stable isotope analysis. Mean δ13C values among fish species varied by ca. 4.0‰, from -20.85‰ (Macroramphosus scolopax) to -16.57‰ and -16.89‰ (Conger conger and Centrophorus granulosus), showing an enrichment in 13C as a function of depth (200 – 1400 m). Mean δ13C values of the crustaceans showed smaller variation, between -18.54‰ (Aristeus antennatus) and -16.38‰ (Polycheles typhlops). This suggests a shift from pelagic to regenerated benthic carbon sources with depth. Benthic carbon regeneration is further supported by the low benthic-pelagic POM-δ13C values, averaging -24.7 ± 1.2‰, and the mixing model results, presenting relatively low contribution of epipelagic POM to the deep-sea fauna. Mean δ15N values of fish and crustacean species ranged 7.91 ± 0.36‰ to 11.36 ± 0.39‰ and 5.96 ± 0.24‰ to 7.73 ± 0.46‰, respectively, resulting in trophic position estimates, occupying the third and the fourth trophic levels. Thus, despite the proximity to the more productive areas of the shelf, low number of trophic levels (TL ~1.0) and narrow isotopic niche breadths (SEA C<1) were observed for demersal crustaceans (TL = 2.94 ± 0.18) and fishes (TL = 3.62 ± 0.31) in the study area – probably due to the ultra-oligotrophic state of the SEMS resulting in limited carbon sources. Our results, which provide the first trophic description of deep-sea megafauna in the SEMS, offer insight into the carbon sources and food web structure of deep-sea ecosystems in oligotrophic marginal seas, and can be further used in ecological modeling and support the sustainable management of marine resources in the deep Levantine Sea.

Keywords: stable isotope analysis, trophic level, vertical vs. lateral transport, Bayesian mixing models, benthic carbon regeneration, carbon limitation, continental slope and rise

INTRODUCTION

Deep-sea ecosystems cover much of the oceans seafloor and play a major role in large-scale biogeochemical cycles (Walsh, 1991; Drazen and Sutton, 2017). They provide ecosystem services that are important to humans, including carbon sequestration, nutrient recycling and burial, waste accumulation and fisheries production (Danovaro et al., 2008; Mengerink et al., 2014; Thurber et al., 2014). Recent studies have shown that an increasing number of stressors, including climate change (warming), deoxygenation, ocean acidification, as well as, overfishing, and natural resource extraction...
(e.g., Stramma et al., 2008; Yashuhara et al., 2008; Stramma et al., 2010; Helm et al., 2011; Tecchio et al., 2015) are expanding into deep environments, thus threatening the diversity and stability of deep-sea ecosystems. Consequently, studying the status of deep-sea communities and describing deep-sea ecosystem structures are currently gaining more and more attention.

Continental slopes account for ~11% of the total ocean floor (Ramirez-Llodra et al., 2010), connecting the shallow shelf productive areas with the abyssal plains along steep seabed gradients. Covering large bathymetric ranges (~200 – 2000 m), these dynamic habitats exhibit strong spatial differences in temperature, salinity, nutrient concentrations, sedimentological features, like organic carbon content, and consequently, in habitat suitability (Koslow, 1993; Gordon et al., 1995; Neat et al., 2008; Bergstad, 2013; Pajuelo et al., 2016). Bathyal habitats also support diverse deep-sea fauna (Gordon and Swan, 1997; Kelly et al., 1998; Menezes et al., 2006; Neat et al., 2008), even in ultra-oligotrophic basins, such as the easternmost Mediterranean Sea (Goren et al., 2008). In deep-sea benthic ecosystems, fish can play key ecological and biogeochemical roles (Drazen and Sutton, 2017) by regulating nutrient limitation and zooplankton populations (Hopkins and Gartner, 1992; Pakhomov et al., 1996).

Deep-sea benthic ecosystems largely rely on particulate organic matter (POM) that passively sinks from the surface waters or by lateral transport as a primary source of nutrients (Tecchio et al., 2013). Marine organisms that carry out vertical diel migrations through the water column (Trueman et al., 2014) and occasional sink of large animal carcasses is another important food source to deep-sea ecosystems (Smith and Baco, 2003). Each of these primary food sources may carry a distinct isotopic signature that reflects its origin, resulting from different chemo-physical processes. Thus, by knowing the isotopic composition of the food sources that fuels a specific food web, it is possible to reconstruct the trophic structure and dynamics of specific habitats (Post, 2002).

Stable isotope analysis (SIA) has been used successfully to study trophic level, important prey types, and trophic niche breadth in deep-sea ecosystems (e.g., Boyle et al., 2012; Shipley et al., 2017a). Nitrogen stable-isotope composition (δ15N) is used to determine the trophic position of an animal, as it preferentially fractionates as a function of its diet, where the heavy isotopes are retained in the consumers in respect to their prey by 2 – 4‰ (Post, 2002). Carbon stable isotopes (δ13C) fractionate much less with each trophic step (<1‰), but can be effectively used to infer basal sources of carbon. Moreover, SIA provides an integrated view of an organism’s diet over timescales relevant to tissue turnover rates rather than digestion rates (Peterson and Fry, 1987; Post, 2002), thereby providing estimates of the trophic position of an organism within a specific food web.

Knowledge of food web structure and dynamics is key to our understanding of ecological communities and their functioning (Polis and Strong, 1996; Winemiller and Polis, 1996). This fundamental information is, however, lacking in many oceanographic regions, including the Southeastern Mediterranean Sea (hereafter, SEMS) (Parzanini et al., 2019) — one of the most oligotrophic, nutrient-impoveryed marginal basin, worldwide (Kress et al., 2014). This basin exhibits extremely low open water primary production (~60 g C m−2 yr−1) merely half of that measured in other oligotrophic areas of the ocean (Hazan et al., 2018), resulting in limited energy sources to its deep-sea habitats (Rahav et al., 2019). The greatest fraction of particulate flux to the SEMS deep-seafloor is transported laterally from the continental shelf at intermediate depths (Katz et al., 2020) and is considered to be highly refractory (Rubin-Blum et al., 2022). The SEMS, therefore, provides a miniature model of processes occurring in vast oligotrophic marginal seas, an ideal location to study food web structure and functioning under severe nutrient limitation. Furthermore, the SEMS is one of the regions where sea surface temperatures are rising at the fastest rates under recent climate changes (Sisma-Ventura et al., 2014; Ozer et al., 2017) and is one of most vulnerable marine regions to species invasions (Rilov and Galil, 2009), which have been also reported from deep-sea habitats (Galil et al., 2019). Understanding deep-sea community structure and functioning is of prime importance for developing better predictions regarding the ecological effects of future climate change. The main objective of this study was to elucidate the energy sources that sustain the deep-sea food-webs of the SEMS.

To date, much of the research describing the trophic ecology of the Eastern Mediterranean Sea has focused on zooplankton groups (Koppelmann et al., 2003; Koppelmann et al., 2009; Hannides et al., 2015; Protopapa et al., 2019), shallow rocky reefs (Fanelli et al., 2015), and on anthropogenically-influenced coastal environments (Grossowicz et al., 2019), while less attention has been paid to deep-sea fishes and crustaceans that occupy higher trophic levels. Here we used bulk carbon and nitrogen stable isotopes (δ13C and δ15N) of demersal and bathybenthic fishes and crustaceans from the southeast Mediterranean continental slope and rise. We explored potential factors that may explain the variability in stable isotope values across species. These data offer insights into the carbon sources and trophic complexity of deep-sea ecosystems in oligotrophic marginal seas.

MATERIALS AND METHODS

Study Sites and Sampling Design

Sampling campaigns were conducted in the course of three oceanographic cruises during 2017 – 2019, as part of the national deep-water monitoring program of the Israeli Mediterranean Sea performed by Israel Oceanographic and Limnological Research (IOLR). Sampling sites were divided to three major benthic habitats: (1) the end of the continental shelf, with an average depth of 200 m; (2) the continental slope with depth range of 500 – 600 m; and (3) the deep bathyal plateau (continental rise) with depth range of 1000 – 1400 m (Figure 1). Specimens were collected onboard the R/V Bat-Galim, using a semi-ballooned trawl net with an opening of eight meters and mesh size of 10 mm. Once the trawls were retrieved, specimens were sorted, enumerated, weighted and visually identified to species level. The
FIGURE 1 | Map of sampling sites in the SEMS. The locations of POM samples are presented in black circles, and locations of fishes and crustaceans by trawl net are presented in black lines.

| Sample type/Species(abbreviation) | n | Depth (m) | Total length (cm) | Total weight (g) | δ¹³C (%o) | δ¹⁵N (%o) | C/N |
|-----------------------------------|---|-----------|--------------------|------------------|-----------|-----------|-----|
| Fish                              |   |           |                    |                  |           |           |     |
| Bathytroctes mediterraneus (Bm)   | 3 |           | 9.73 ± 1.63        | 5.00 ± 2.08      | -17.6 ± 0.21 | 9.62 ± 0.01 | 2.94 ± 0.02 |
| Centrophorus granulosus (Cg)      | 1 | 600       | n/a                | n/a              | -16.89    | 9.55      | 2.39 |
| Coelorinchus caelarhincus (Cc)    | 3 | 200       | 18.47 ± 4.00       | 26.33 ± 18.88    | -17.88 ± 0.32 | 10.88 ± 0.56 | 3.02 ± 0.02 |
| Coelorinchus caelarhincus (Cc)    | 8 | 600       | 12.63 ± 0.68       | n/a              | -19.18 ± 0.77 | 9.40 ± 0.49 | 3.02 ± 0.02 |
| Conger conger (CCc)              | 1 | 1000      | 87                 | 1296             | -16.57    | 11.25     | 3.03 |
| Dentex macrophthalmus (Dm)       | 12| 200       | 13.67 ± 0.97       | 42.00 ± 8.96     | -18.67 ± 0.65 | 9.22 ± 0.45 | 3.38 ± 0.26 |
| Etmopterus spinax (Es)           | 3 | 1100      | 30.67 ± 1.17       | 121.00 ± 15.31   | -17.61 ± 0.05 | 8.83 ± 0.33 | 2.62 ± 0.20 |
| Galeus melastomus (Gm)           | 4 | 1100      | 33.53 ± 4.99       | 114.00 ± 39.62   | -17.75 ± 0.28 | 7.97 ± 0.13 | 2.42 ± 0.10 |
| Helicolenus dactylopterus (Hd)    | 14| 500, 600  | 17.50 ± 1.75       | 95.00 ± 29.99    | -17.99 ± 0.27 | 8.62 ± 0.29 | 3.08 ± 0.16 |
| Hoplostethus mediterraneus (Hm)  | 6 | 500, 600  | 16.00 ± 1.14       | 82.00 ± 5.66     | -17.49 ± 0.02 | 10.29 ± 0.67 | 3.04 ± 0.16 |
| Lepadottiris cavilone (Lc)       | 6 | 200       | 10.03 ± 0.60       | 14.00 ± 2.22     | -19.10 ± 0.25 | 8.35 ± 0.35 | 3.10 ± 0.25 |
| Lophius budogas (Lb)             | 2 | 1000      | 65.55 ± 21.14      | 37.00 ± 2.12     | -17.17 ± 0.18 | 9.80 ± 0.46 | 3.09 ± 0.01 |
| Macroramphosus scolopax (Ms)     | 5 | 200       | 9.08 ± 0.79        | 5.00 ± 1.48      | -20.85 ± 0.46 | 7.91 ± 0.36 | 4.48 ± 0.72 |
| Nezumia sp. (Ns)                 | 4 | 500       | n/a                | n/a              | -17.24 ± 0.30 | 10.76 ± 0.36 | 3.31 ± 0.07 |
| Nezumia sp. (Ns)                 | 9 | 1100      | 17.39 ± 1.44       | 14.33 ± 3.60     | -17.09 ± 0.24 | 11.36 ± 0.39 | 3.06 ± 0.09 |
| Decapod crustaceans              |   |           |                    |                  |           |           |     |
| Acanthephyra eximia (Ae)         | 11| 1400      | 12.04 ± 2.19       | 13.18 ± 7.49     | -17.59 ± 0.30 | 6.95 ± 0.64 | 2.90 ± 0.11 |
| Anistaeomorpha foliacea (Af)     | 3 | 1400      | 9.83 ± 1.53        | 5.36 ± 1.54      | -17.97 ± 0.87 | 7.68 ± 0.68 | 2.90 ± 0.06 |
| Anisteus antennatus (As)         | 6 | 600       | 12.02 ± 0.43       | 9.37 ± 0.95      | -18.54 ± 0.17 | 7.62 ± 0.14 | 2.87 ± 0.03 |
| Anisteus antennatus (As)         | 10| 1100, 1400| 11.50 ± 1.49       | 8.01 ± 3.66      | -18.09 ± 0.59 | 7.73 ± 0.46 | 2.84 ± 0.15 |
| Parapeneaeus longirostris (Pl)   | 7 | 200       | 10.17 ± 0.82       | 4.34 ± 1.18      | -18.45 ± 0.10 | 6.72 ± 0.19 | 2.94 ± 0.08 |
| Plesionika edwardsii (Pe)        | 3 | 200       | 11.37 ± 1.35       | 4.05 ± 0.56      | -17.72 ± 0.13 | 5.96 ± 0.24 | 2.66 ± 0.09 |
| Plesionika edwardsii (Pe)        | 3 | 600       | 13.43 ± 0.81       | 7.56 ± 0.66      | -17.66 ± 0.23 | 6.33 ± 0.30 | 2.74 ± 0.15 |
| Polycheles typophos (Pt)         | 3 | 1100, 1400| 6.93 ± 0.81        | 2.86 ± 0.58      | -16.38 ± 0.21 | 7.67 ± 0.18 | 3.36 ± 0.05 |
| POM                               |   |           |                    |                  |           |           |     |
| Shelf                             |   | surface   | 0-5                | n/a              | -24.32 ± 0.72 | 1.61 ± 0.92 | 9.42 ± 1.83 |
| Slope                             |   | bottom    | 60-250             | n/a              | -24.27 ± 1.24 | 1.09 ± 0.95 | 10.23 ± 3.34 |
| Deep basin                        |   | surface   | 0-5                | n/a              | -24.73 ± 0.82 | 1.26 ± 0.75 | 11.18 ± 2.29 |
|                                  |   | bottom    | 400-800            | n/a              | -25.45 ± 1.50 | 2.76 ± 0.85 | 15.19 ± 3.76 |
|                                  |   | bottom    | >1000              | n/a              | -23.91 ± 2.10 | 6.64 ± 2.96 | 10.25 ± 3.32 |

n/a denotes data not available.

**Stable Isotopes Analysis**

SIA was conducted on 86 fish and 46 crustacean specimens as well as 77 POM samples (Table 1). White muscle tissue for SIA was dissected from the dorsal musculature of fishes and from the abdominal segment of the crustaceans. Samples were rinsed with deionized water, frozen, and lyophilized for 48 h. Freeze-dried specimens were homogenized using a mortar and pestle, weighed, and shipped to the Stable Isotope Facility at Cornell University (USA) for SIA analysis. The isotopic composition of organic carbon and nitrogen was determined by the analysis of CO₂ and N₂ continuous-flow produced by combustion on a Carlo
Erba NC2500 connected on-line to a DeltaV isotope ratio mass spectrometer coupled with a ConFlo III interface.

Measured stable isotope ratios are reported in the δ-notation, i.e., as the deviation in per mill (‰) from the international standards:

\[
\delta X_{\text{Sample}} = \frac{R_{\text{Sample}} - 1}{R_{\text{Standard}} - 1} \times 10^6
\]

where, \( R \) represents the \(^{15}\text{N}/^{14}\text{N} \) or \(^{13}\text{C}/^{12}\text{C} \) ratio. Stable isotope data are expressed relative to international standards of Vienna PeeDee belemnite and atmospheric N, for carbon and nitrogen, respectively. The analytical precision for the in-house standard was ± 0.04‰ \[1σ\] for both \( \delta^{13}C \) and \( \delta^{15}N \). The C/N ratios of fishes and crustaceans in this study were low (species mean C/N ranged between 2.33 – 4.48; where in 97% of individuals C/N< 4.0, see Supplementary Figures 1, 2), suggesting that lipids did not significantly affect the \( \delta^{13}C \) interpretation (Post et al., 2007). Therefore, all data analyses were performed on uncorrected \( \delta^{13}C \) values. To determine if the isotopic signatures of POM samples changed with depth, we used collection depth to classify POM samples as epipelagic (0 – 200 m), mesopelagic (200 – 800 m), or bathypelagic (>800 m).

Data Analysis

We estimated and compared modal trophic position (TP) and 95% credibility interval (i.e., 95% of modeled estimates of TP) for each species using the R package tRophicPosition (version 0.7.7; Quezada-Romegialli et al., 2018) according to the following equation:

\[
\delta^{15}N_{\text{C}} = \delta^{15}N_{\text{B}} + \Delta N(TP - \lambda)
\]

Where \( \delta^{15}N \) corresponds to the nitrogen stable isotope value from the consumer that the TP is estimated, \( \delta^{15}N_{\text{B}} \) represents the nitrogen stable isotope value of the baseline consumer; \( \Delta N \) corresponds to the trophic discrimination factor (TDF) for nitrogen and \( \lambda \) the TP from baseline consumer. We used the average \( \delta^{15}N \) in zooplankton (3.9 ± 1.8‰) measured by Koppelmann et al. (2009) in the Eastern Levantine Basin as the baseline \( \lambda=2 \) using the oneBaseline model option and applied the trophic discrimination factor &xutri;\(^{15}N\) of 3.15 ± 1.28‰, which was previously used to calculate the trophic level of meso- and bathypelagic fish (Valls et al., 2014; Richards et al., 2018).

Least-squares linear regression analysis was conducted for each species to explore the relationship between fish length and the \( \delta^{13}C \) and \( \delta^{15}N \) values. Spatial variation in \( \delta^{13}C \) and \( \delta^{15}N \) of both fishes and crustaceans was investigated using least-squares linear regression between stable isotopic values and depth. All statistical analyses were performed in R v. 4.0.5 (R Core Team, 2020).

The trophic breadth of each species (n ≥5) and trophic similarity among species were assessed by calculating Standard Ellipse Area (SEA) using the R package SIBER v. 2.1.6 (Jackson et al., 2011; Jackson and Parnell, 2021). Size-corrected SEAs (SEAc) and Bayesian estimate of SEAs (SEAb) were calculated for each species, which adjusts for underestimation of ellipse area at small sample sizes and allows for inter-study comparison of ellipse sizes (Jackson et al., 2011). Fish and crustacean community metrics were calculated based on Layman et al. (2007).

Bayesian mixing models were applied using R package MixSIAR v. 3.1.12 (Stock et al., 2018; Stock et al., 2021) to estimate the relative contribution of shelf, slope, and deep-basin surface vs. bottom POM to each species. These models are sensitive to variable discrimination factors (Bond and Diamond, 2011; Olin et al., 2013), which may be influenced by diet (Caut et al., 2009), tissue type (Malpica-Cruz et al., 2012), temperature (Britton and Busst, 2018), and species-specific metabolic rates (Peccquier et al., 2010). Since the modeled species are predators and do not feed directly on POM, the total trophic fractionation per species was calculated as follows:

\[
\text{TEF}_{\text{species}} = \text{TEF}_{\text{TL}} \times (T_{\text{species}} - T_{\text{source}})
\]

Where, \( \text{TEF}_{\text{species}} \) is the total trophic fractionation in the consumer relative to its basal source, \( \text{TEF}_{\text{TL}} \) is the mean enrichment per trophic level, \( T_{\text{species}} \) is the trophic position of the consumer, and \( T_{\text{source}} \) is the trophic position of the source. To evaluate the contribution of the basal sources to the studied species, we have assigned to POM \( T_{\text{source}} \) = 1.0, since POM-\( \delta^{13}C \) values are compatible of the lowest size fraction (pico-phytoplankton) measured in the Western Mediterranean Sea (Hunt et al., 2017).

To calculate the enrichment factors from POM to the top consumers included in this study, we have used two discrimination factor levels — from POM to zooplankton, and from zooplankton to the studied species. Based on average \( \Delta^{13}C \) and \( \Delta^{15}N \) between POM to zooplankton measured in the Western Mediterranean Sea, we set the basal enrichment factors of 1.40 ± 1.15‰ for \( \delta^{15}N \) and 4.10 ± 1.63‰ for \( \delta^{13}C \) (Hunt et al., 2017). The enrichment from zooplankton to the studied species was done using discrimination factors of 3.15 ± 1.28‰ for \( \delta^{15}N \) and 0.97 ± 1.08‰ for \( \delta^{13}C \) (Sweeting et al., 2007), which have been previously used to study the trophic structure of meso- and bathypelagic fishes in the Gulf of Mexico (Richards et al., 2018) and in the Western Mediterranean Sea (Valls et al., 2014).

Each model was run with identical parameters (number of MCMC chains = 3; chain length = 300000; burn in = 200000; thin = 100), and model convergence was determined using Gelman-Rubin and Geweke diagnostic tests (Stock et al., 2018).

RESULTS

Stable Isotopes

Species-specific mean \( \delta^{13}C \) values ranged from -20.85 to -16.89‰ for fish and from -18.54 to -16.38‰ for crustaceans. Fish mean \( \delta^{13}C \) values differed by 3.96‰, separating the most depleted (Macrorhamphosus scolopax: -20.85 ± 0.46‰, ...
sampling depth of 200 m) and the most enriched species (Centrophorus granulosus and Conger conger: -16.89 and -16.57‰, respectively, sampling depth of ~1000 m) (Table 1 and Figure 2). Crustaceans species-specific mean δ¹³C varied by 2.18‰, where the most depleted species was Aristeus antennatus (-18.54 ± 0.17‰, sampling depth of 600 m) and the most enriched species was Polycheles typhlops (-16.38 ± 0.21‰, sampling depth of ~1400 m). Species-specific differences in δ¹³C and δ¹⁵N were significant for both fish (MANOVA, F₁₃,144 = 19.73, p< 0.001) and crustaceans (MANOVA, F₅,78 = 14.62, p< 0.001).

Species-specific mean δ¹⁵N values varied from 7.91 ± 0.36‰ (M. scolopax, 200 m depth) to 11.36 ± 0.39‰ (Nezumia sp., 1100 m depth) in fish and from 5.96 ± 0.24‰ (Plesionika edwardsii; 200 m depth) to 7.73 ± 0.46‰ (Aristeus antennatus; 1100 – 1400 m depth) in crustaceans. Fish mean δ¹⁵N values positively correlated with the δ¹³C values (r² = 0.6, p< 0.001, Figure 2 and Supplementary Figure 1) and varied among species (ANOVA, F₁₃,72 = 24.22, p< 0.001). Crustaceans, however, did not show this correlation between δ¹⁵N and δ¹³C (r² = 0.002, p > 0.05, Figure 2 and Supplementary Figure 2), observed in fish from similar depths. Due to limited spatial coverage within each species (the specimens of most species were sampled from the same depth), spatial variation could not be tested within each species, and therefore, spatial trends were tested by addressing all fish species together. Fish δ¹³C values positively varied with bottom depth (r² = 0.42; P< 0.01, Figure 3), where the most enriched samples were found at the continental rise (> 1000 m).
and the most depleted at the shallow slope (200 m) at the edge of the shelf. This pattern was less clear in the case of fish δ15N values (Figure 4), where species-specific mean values seem more variable in the continental rise (> 1000 m). Crustaceans mean δ15N values positively correlated with depth (r² = 0.76, p = 0.053, Figure 4), while their mean δ13C values showed no such correlation (Figure 3).

POM collected from depths ranging from 0 to 1135 m over the continental shelf, slope and rise exhibited a wide δ13C range (-27.72 to -21.36‰) and δ15N range (-3.25 to 12.76‰), with POM samples generally becoming more enriched in 15N and more depleted in 13C at bottom depths (Figure 5). Significant differences in POM δ13C and δ15N among the zones and depths were observed (MANOVA, F10,98 = 7.15, p< 0.001). POM-δ13C and C/N ratio exhibited a significant negative correlation (Pearson correlation, r = -0.566, p< 0.001, Supplementary Figure 3), which was not observed in POM-δ15N and C/N ratio.

**Trophic Position Estimates**

We used the average δ15N value of Eastern Levantine zooplankton (3.9 ± 1.8‰) obtained from Koppelmann et al. (2009) as a baseline (λ=2.0) for estimating species-specific TL. Based on the low δ15N values of zooplankton in the eastern Mediterranean, it was assumed that the primary food source, namely smaller zooplankton, phytoplankton and particles have a δ15N value around zero (Koppelmann et al., 2009). Large mesozooplankton (333-µm mesh size, upper water column) δ15N values in the EMS showed an enrichment trend across a west-east transect (SE Crete mean δ15N value ~2.0‰ and SE Cyprus mean δ15N value ~4.0‰, Koppelmann et al., 2009). Using these data to set the baseline,
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fish modal TL ranged from 3.17 (M. scolopax; 200 m depth) to 4.19 (C. conger; 1000 m depth), while the average modal TL of all fish species was 3.62 ± 0.31 (Figure 6). Crustaceans- δ¹⁵N values yielded modal TLs between 2.66 (P. edwardsii; 200-600 m depth) and 3.11 (A. antennatus; 600 – 1400 m), with an average of 2.94 ± 0.18 (Figure 6).

Of the species examined, only few enabled an estimation of ontogenetic effect (Figure 7). This is due to the low range of body size within individual species that were sampled in this study. Nevertheless, the crustaceans A. eximia ($r^2 = 0.82, p< 0.001$) and P. edwardsii ($r^2 = 0.72, p< 0.05$) exhibited a positive relationship between length and δ¹⁵N values. Size and δ¹³C values did not yield significant correlations. Positive relationship between size and δ¹⁵N values was also observed for the fish D. macrophthalmus ($r^2 = 0.59; p< 0.05$).

Trophic Niche Breadth

Isotopic niche breadth, calculated using size-corrected standardized ellipse area SEAₐ (Supplementary Table 1 and Figure 8), was largest for the fish collected from the shallow continental slope C. caelorhincus (SEAₐ = 2.04), D. macrophthalmus (SEAₐ = 0.94) and M. scolopax (SEAₐ = 0.66), and for the shrimps A. antennatus (SEAₐ = 0.63) and A. eximia (SEAₐ = 0.56), both opportunistic carnivores. The smallest isotopic niche breadth belonged to the deep-water rose shrimp P. longirostris (SEAₐ = 0.07). Fish and crustacean assemblage metrics (Table 2A) showed a contrasting trend of isotopic niche size with depth, where fish isotopic niche size was largest in the shelf assemblage (SEAₐ = 1.81) and crustaceans isotopic niche size was largest in the deep assemblage (SEAₐ = 1.43). As a trophic guild (Table 2B), the fish showed higher convex hull area.
(TA = 7.37) than the crustaceans (TA = 0.81), indicating a larger trophic community width (Layman et al., 2007).

Bayesian Mixing Models

The results of the mixing models indicated variable sources sustaining the deep-sea fish and crustacean consumers included in this study (Figure 9). The contribution of deep bathypelagic POM was highest in the decapod crustaceans A. eximia (74.8 ± 10.8%), A. antennatus (63.3 ± 9.5%) and P. typhlops (43.1 ± 1.7%), and in the rattail fish Nezumia sp. (66.3 ± 8.0%). The contribution of pelagic POM to the deep bathypelagic fauna spanned 26 ± 4%, increasing in the slope, varying between 24 and 61% (including both the shelf and slope surface POM). Whereas, the models showed an increased contribution of benthic POM to the deep-sea fauna (Figures 9G–O). For example, the benthic contribution to the red shrimp A. antennatus was 20 ± 18.9% in the slope (Figure 9A) versus 63.3 ± 9.5% in the rise (Figure 9I). Therefore, the model results support a shift from pelagic to benthic source with increasing depth, i.e. with distance from shore.

DISCUSSION

This is the first attempt to elucidate the trophic ecology of deep-sea fish and crustacean species in the SEMS. The knowledge gained in this study provides insights into the main energy sources sustaining deep-sea food webs in one of the most oligotrophic, nutrient-improved marine basins, worldwide. However, insights gained in this study are not limited to the SEMS alone, and can be relevant to many oligotrophic basins with limited carbon and nutrient sources.

Our δ13C and δ15N values varied across fish species and as a function of depth, suggesting that depth and diet are controlling the trophic positions inferred from our stable isotope data. As expected, top predators such as the European conger eel C. conger, occupied the highest trophic position. The rattail Nezumia sp., a small macrourid fish that was collected from similar depths of >1000 m, yielded similar high δ13C values. Both species occupied a maximum trophic position of 4.14 – 4.19 (modal TL). Polunin et al. (2001) found similar trophic position of 4.4 for both the shark Centroscymnus coeleopis and Nezumia aequalis in the continental slope of the Balearic Islands. High δ13C values of Nezumia (11.09 ± 0.58‰ and 11.31‰) were also recorded by Fanelli and Cartes (2010) in the Archipelago of Cabrera (Algerian Basin) and by Papiol et al. (2013) in the Balearic Islands (Catalan Sea, West Mediterranean), respectively, and were attributed to the suprabenthic crustaceans and polychaetes that constitute the diet of this macrourid. Our TL data also agree well with that of benthic carnivorous fish from Bay of Banyuls-sur-Mer (northwest Mediterranean, France; Carlier et al., 2007). Among the fish, the lowest trophic position (3.17) was found in the snipefish M. scolopax, which feeds on hyperbenthic demersal zooplankton during daytime (Carpentieri et al., 2016). This was
also inferred from the results of the mixing models, indicating a relatively high contribution of mesopelagic POM to the diet of *M. scolopax*. Relatively to the fish, the bathybenthic crustaceans measured in this study occupied lower trophic positions — between 2.66 and 3.11, in agreement with the TL of deep benthic invertebrates of the Western Mediterranean [Carlier et al., 2007; Zorica et al., 2021].

The δ15N values of the deep-sea decapods *A. eximia* and *P. edwardsii* increased significantly with length indicating an ontogenetic effect. Such trend was found in *A. eximia* from the Catalan Sea (Western Mediterranean), where gut content analysis indicated a dietary shift from scavenging and detrivory in small individuals to active predation in larger individuals [Cartes, 1993]. Smaller sized *A. eximia* were suggested to be better adapted to regions where resources are scarce [Thiel, 1983; Pérès, 1985]. Similar dietary preferences were observed in *P. edwardsii* [Cartes, 1993]. The lower δ15N that we measured in the smaller individuals of these decapods may indicate a detrivorous mode of feeding [Polunin et al., 2001]. We found a similar ontogenetic effect in the demersal fish *D. macrophthalmus*. A gut analysis of this spardin fish off Angola and Namibia (South Atlantic Ocean) supports our finding, as the smaller fish tended to feed almost exclusively on polychaetes and euphausiids, but the larger fish preferred fish prey [Kilongo et al., 2007].

In the fish species examined here, mean δ15N values spanned 3.45‰, about 1.1 TL, while in the crustacean species mean δ15N values spanned 1.77‰, about 0.6 TL (assuming trophic enrichment factor of 3.15‰). Our observed ranges of estimated trophic levels are in line with other studies examining Mediterranean (1.1 TL, Valls et al., 2014), Pacific (1.6 TL, Choy et al., 2015), and the Gulf of Mexico (0.62 TL, Richards et al., 2018). Different feeding strategies as well as different migration habits may explain wider range of δ15N [Shipley et al., 2017a; Richards et al., 2020]. Despite of the reliance on similar basal production, mesopelagic fishes from the Western Mediterranean were segregated by trophic position, between 2.9 for the small bristlemouth *Cyclotheta braueri* to 4.0 for the lanternfish *Lobianchia dofleini* [Valls et al., 2014], and bathyal fishes off the Balearic Islands appeared to be foraging over two to three full trophic levels [Polunin et al., 2001]. Our results support a much narrower trophic range for bathyal fish and bathybenthic decapod crustaceans in the SEMS. We attribute this narrow range to the ultra-oligotrophic state of the SEMS, resulting in limited carbon sources to sustain the deep-sea food webs, reflected by a general increase of δ13C in fish as function of bottom depth. This pattern could be driven by a number of factors including shifting production sources, or shifts in community composition and feeding strategies, and or switching from benthic to pelagic prey [Fanelli et al., 2011; Trueman et al., 2014]. For example, 13C became more depleted in individuals captured at greater depths in the deep-sea island slope system of the Exuma Sound, the Bahamas [Shipley et al., 2017b]. Inshore-to-offshore depletion in 13C values were also apparent in epipelagic fishes in the northern California Current, where copepods, gelatinous zooplankton, and nekton showed a significant linear decrease in δ13C with distance offshore [Miller et al., 2008]. Our results show a different trend of increase in fish δ13C as function of bottom depth, i.e., distance offshore.

The major carbon sources supporting deep-sea food webs are poorly defined, aside from oligotrophic open-ocean gyres, where sinking phytoplanktonic-POM is considered the main energy source [Shipley et al., 2017b]. This was observed by a narrow range of δ13C in meso- and bathypelagic predatory fishes in the Gulf of Mexico, indicating an exclusive epipelagic carbon source [Richards et al., 2018]. Differently, the results of our mixing-model show increased contribution of bathypelagic POM and a reduction in the epipelagic source with increasing depth, i.e., distance offshore. This pattern agrees well with the extremely low primary production and carbon flux from the surface open water of the SEMS to the deep-seafloor [Katz et al., 2020]. While most of the flux to the deep seabed is transported laterally.

Indeed, the majority of carbon supporting the species examined in this study is not derived from epipelagic sources. An alternative hypothesis is that the source of carbon in the deep-sea originates from the shelf. A significant proportion of neritic-derived primary production may be transported into deep-sea systems by currents [Suchanek et al., 1985; Sanchez-Vidal et al., 2012; Efrati et al., 2013], or via lateral transport [Fahl and Nöthig, 2007], and once assimilated into the food web, more enriched 13C values are to be expected [Polunin et al., 2001; Fanelli et al., 2011]. Katz et al. [2020] used deep-sea sediment traps in the Israeli Southeastern Mediterranean Sea and showed that lateral transport from the continental margin contributes the greatest fraction of particulate flux to the seafloor. Therefore, we suggest that lateral transport constitutes the main source of carbon to the
deep-sea food web in the Southeastern Mediterranean Sea. Our mixing model results and the relative decrease of C:N ratio in fish with increasing depth, indicating lower lipid content in deep-sea fish, further support a shift from pelagic to regenerated benthic carbon sources with depth. Given that the δ¹³C of POM slightly vary between the different foraging habitats, it is likely that this shift in δ¹³C occurs in the sediments water interface, by the enhanced bacterial regeneration of organic matter. This is likely driven by the high temperatures of the SEMS deep water near the seafloor (~13-14°C), compared to much lower temperatures of ~4°C at similar depths in most oceanic basins and despite the relatively low organic content of the SEMS sediments <1% (Ogrinc et al., 2007).

Since the carbon signature of primary producers can significantly vary between macroalgae and different phytoplankton groups (Fanelli et al., 2011; Gроссowicz et al., 2019), food webs that show a linear relationship between δ¹³N and δ¹³C values are suggestive of a single food source (Polumin et al., 2001; Carlier et al., 2007). Generally weak δ¹³C–δ¹³N correlations were found in deep-sea macrozooplankton and micronekton off the Catalan slope likely due to the consumption of different kinds of sinking particles (e.g. marine snow, phytodetritus). Multiple recycling of POM constituted an enrichment effect on the δ¹³C and δ¹⁵N values of deep-sea macrozooplankton and micronekton (Fanelli et al., 2011). Our results yielded significant positive correlation between fish δ¹⁵N and δ¹³C values, further supporting a single food source.

Previous studies have shown that δ¹³C fractionation is less than 1.0‰ for each trophic position. The Δ¹³C between the mean water column POM-δ¹³C (-24.13 ± 1.56‰) and fish/crustaceans δ¹³C (-18.10 ± 0.93‰) of the SEMS amounted to 6.04‰ (equal to at least six trophic positions), and therefore, cannot be attributed to trophic enrichment alone, but could be partly linked to the regeneration of benthic carbon sources, which may be relevant for detritus feeding animals, including all of the crustacean and some of the fish studied here. Moreover, our δ¹³C-C/N data support the potential effect of microbially degraded phyto-detritus resulting in higher isotopic values of nitrogen and carbon in deep benthic food webs compared with pelagic food webs (Papiol et al., 2013; Romero-Romero et al., 2021).

Deep-sea ecosystems are subjected to exacerbating anthropogenic stressors, including overfishing, chemical pollution, mining, dumping, litter, plastics, and climate change (Davies et al., 2007). In oligotrophic environments such as the ultra-oligotrophic SEMS, deep-sea ecosystems are further vulnerable to reduced food availability (Kröncke et al., 2003). Regeneration of benthic carbon sources, supported by this study, provides oligotrophic deep-sea food webs with a greater ability to endure carbon limitation. Benthic carbon sources originating in lateral transport from the shallow shelf to the deep-sea, as indicated here, may hold important implications for marine spatial planning and the establishment of Marine Protected Areas (MPAs) in the SEMS exclusive economic zones (EEZ), promoting the extension of protected areas from the shelf to the deep-sea in a continuum rather than the establishment of MPAs that are disconnected from the continental shelf and slope. Furthermore, lateral transport from the shelf to the deep sea may carry detrimental implications to the ecosystem via pollutant accumulation and biomagnification (Liu et al., 2020). This is particularly important in marginal seas that are prone to anthropogenic pollution (Kim et al., 2019; Shoham-Frider et al., 2020). Continuous studies should be undertaken to further unveil the implications of lateral transport and benthic carbon regeneration to deep-sea food webs.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in: Guy-Haim, Tamar; Stern, Nir; Sisma-Ventura, Guy (2022): Bulk stable isotopes of deep sea fish and crustaceans from the Southeastern Mediterranean Sea. PANGAEA, https://doi.org/10.1594/PANGAEA.945321.

ETHICS STATEMENT

The animal study was reviewed and approved by Israel National Institute of Oceanography Ethics Committee.

AUTHOR CONTRIBUTIONS

GS-V and TG-H conceived this study. GS-V, NS, and TG-H collected the data. NS provided species identification and measurements. GS-V and TG-H analyzed and modeled the stable isotope data. All co-authors contributed substantially to drafting the manuscript, and approved the final submitted manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.857179/full#supplementary-material
