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Trophic interactions of megafauna in the Mariana and Kermadec trenches inferred from stable isotope analysis

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ABSTRACT

Hadal trenches house distinct ecosystems but we know little about their sources of nutrition or trophic structures. We evaluated megafaunal food web structure and nutritional sources in the Kermadec and Mariana trenches using carbon and nitrogen stable isotope analysis ($\delta^{15}N$ and $\delta^{13}C$ values) of bulk tissues and proteinaceous individual amino acids (AAs). In the Kermadec Trench, bulk $\delta^{15}N$ values ranged from 5.8‰ in trench sediment to 17.5‰ in tissues of the supergiant amphipod, *Allicela gigantea*. $\delta^{15}N$ values of detritivores were much higher than those of sediments (by 7.5‰ more). The $\delta^{13}C$ values ranged from −21.4‰ in sediments to −17.3‰ in the brittle star, *Ophiolimna* sp., and did not co-vary with $\delta^{15}N$ values. In the Mariana Trench, only bait-attending fauna and surface sediments were available for analysis. Mariana Trench fishes, amphipods, and sediments had slightly lower $\delta^{15}N$ values than those from the Kermadec Trench, possibly because the Mariana Trench lies under more oligotrophic surface waters. We found evidence for multiple food inputs to the system in each trench, namely substantially higher $\delta^{15}N$ values in detritivores relative to sediment and high variability in $\delta^{13}C$ values. Trophic levels determined from isotopic analysis of individual AAs in the Kermadec Trench ranged from three for detritivores to five for fishes. Source AA $\delta^{15}N$ values were variable (range of ~7.0‰ in average $\delta^{15}N$ source AA values), with much of this variation occurring in small amphipods. For the other fauna sampled, there was a significant increase in $\delta^{15}N$ source AA values with increasing collection depth. This increase could reflect larger amounts of highly microbially reworked organic matter with increasing depth or sporadic input from turbidity flows. Although further sampling across a broader faunal diversity will be required to understand these food webs, our results provide new insights into hadal trophic interactions and suggest that trench food webs are very dynamic.

1. Introduction

The ocean's hadal zone, 6,000 to 11,000 m, encompasses the greatest depths of the world's oceans. Trenches, which are formed at tectonic subduction zones, make up the majority of hadal habitats (Jamieson, 2015). This zone represents <1% of the global seafloor area but about 45% of the oceanic depth range (Jamieson, 2015). Trenches are dynamic habitats in which geological activity can cause earthquakes, triggering turbidity flows that carry organic matter and even benthic biomass downslope (Fukao, 1979; Oguri et al., 2013). For example, observations following the Tohoku-Oki Earthquake (2011) in the Japan Trench showed that benthic megafauna were absent and dead organisms were observed along trench axes, suggesting that both burial of organisms and the episodic delivery of organic matter to greater water depths are part of life in trenches (Fukao, 1979; Oguri et al., 2013).

Trophic interactions, food-web structure, and nutritional inputs of an environment are crucial to ecosystem function. Comprehensive ecosystem models require thorough understanding of how energy moves through a community and the production potential at different trophic levels (e.g. Choy et al., 2016). Yet, for hadal ecosystems, many of these

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trophic interactions remain unknown. We can assume that nutrient inputs to trenches likely share similarities with the abyssal plains in several respects. For instance, abyssal ecosystems are ultimately dependent upon sinking particulate organic matter (POM) produced in overlying, sunlit waters (Smith et al., 2008, 2018). Important nutrient inputs in the deep sea also come from sinking labile organic matter such as carrion (Amon et al., 2017; Drazen et al., 2012) and, for plains adjacent to continental margins, the lateral delivery of organic matter, including from terrestrial origin (Santschi and Rowe, 2008; Smith et al., 2001). Trench organisms are also likely to rely on such nutrient inputs as indicated in earlier investigations (Wolff, 1960). More recent video observations from trenches near continental margins found terrestrial plant debris (Gallo et al., 2015). Additionally, some hadal trenches harbor cold seeps with chemosynthetic communities (Ohara et al., 2012), which can provide a source of nutrition independent from photosynthesis. Perhaps most importantly, the geologically-active nature and v-shaped topography of trenches are predicted to facilitate substantial downslope transport of organic materials through turbidity flows (Ichino et al., 2015; Itou, 2000). Increasing faunal biomass with depth in trenches suggests that downslope transport is a significant food source (Beliaev, 1989; Danovaro et al., 2002; Jamieson et al., 2010; Leduc et al., 2016; Schmidt and Martínez Arbizu, 2015). However, the relative importance of these nutrient inputs and their spatial and temporal variability are not resolved.

Current knowledge of trophic interactions and food web structure in trenches comes mainly from *in situ* video observations collected by baited free-vehicle landers. Video cameras have provided valuable behavioral observations of hadal organisms, including holothurians feeding on detrital matter (Jamieson et al., 2011a), lysianassoid amphipods scavenging (e.g. Jamieson et al., 2011b), predatory amphipods (genus *Princaxelia*) feeding on other amphipods (Jamieson et al., 2012), and snailfishes (Liparidae) feeding on amphipods drawn to bait (Fujii et al., 2010; Linley et al., 2016). Such observations give valuable insight into the feeding habits of hadal organisms. However, baited cameras create artificial feeding environments and observations of natural feeding interactions are rare in the deep sea. Quantifying predator-prey relationships can also be done directly through stomach content analysis (e.g. in hadal fishes; Gerringer et al., 2017), but these only reflect the animal's most recent meals. For many hadal organisms, from holothurians to amphipods, stomach contents are difficult to identify because feeding involves biting, tearing, or fine-scale particle selection.

Trophic structure and food web function can also be studied through stable isotope analysis in whole animals or their tissues. Unlike other methods, stable isotopic compositions integrate feeding history over longer periods of time and can provide broad information on trophic relationships (Peterson and Fry, 1987). Further, compound-specific isotopic analysis of amino acids (AA-CSIA)—analyzing multiple individual amino acids—can augment bulk tissue isotope analysis. $\delta^{15}N$ values of source amino acids remain similar with each increasing metazoan trophic level, serving as indicators of basal sources of nutrition; in contrast, $\delta^{15}N$ values of trophic amino acids fractionate considerably with each trophic level (~4–8‰) (Ohkouchi et al., 2017; Popp et al., 2007). Together, these source and trophic amino acid isotopic compositions can be used to calculate trophic level normalized to $\delta^{15}N$ values at the base of the food web using the difference between trophic and source amino acid $\delta^{15}N$ values (Chikaraishi et al., 2009; McClelland and Montoya, 2002).

A few studies have used stable isotope analysis to characterize trophic interactions in trenches, focusing on detailed analysis within phylogenetic groups. Blankenship and Levin (2007) showed that scavenging amphipods from the Tonga and Kermadec trenches have a wider range of $\delta^{15}N$ and $\delta^{13}C$ values compared to those of the Porcupine Abyssal Plain, suggesting extreme trophic diversity. Gerringer et al. (2017) used both stomach content analysis and AA-CSIA to estimate trophic position of hadal liparids from the Mariana and Kermadec trenches and compared these to the estimated values for fishes from neighboring abyssal plains. These analyses showed that amphipods form the substantial portion (>95%) of the diet of hadal liparids compared to much more diverse diets and higher trophic levels in fishes from the neighboring abyssal plains. Although these studies give insights into hadal food-webs, a broader analysis encompassing a greater diversity of taxa is needed to understand full hadal ecosystem function.

Trench food-webs likely vary with depth because of depth-related shifts in faunal communities (e.g. Jamieson, 2015; Jamieson et al., 2011b; Linley et al., 2017; Wolff, 1959). These transitions are believed to involve pressure-related constraints (Tyler and Young, 1998; Vinogradova, 1997; Yancey et al., 2014) but could also result from changes in competitors or predator communities (Jamieson et al., 2011b; Wolff, 1959). Below ~8,200 m, fishes and large shrimps are believed to be absent (Jamieson et al., 2009; Yancey et al., 2014), reducing predation pressure on the deepest amphipod communities. This community shift from the upper hadal to lower hadal zone (Jamieson, 2015) would shift food-web structure.

In addition to shifts in community structures, nutritional sources for the food web could also vary with depth. Organic matter is likely to be funneled towards greater depths in the trench axis through lateral advection and sinking and by seismically induced turbidity flows which would bring large amounts of sediment and organic matter to the trench axis (Ichino et al., 2015). These flows may include buried and more refractory organic matter; however, limited data suggests there are higher levels of labile markers such as chlorophyll a at hadal depths. These labile markers indicate that lighter detrital material could be resuspended and transported downslope as well (Wenzhöfer et al., 2016). In the upper ocean (<1,000 m), nitrogen isotope values of organic particles, including individual amino acid $\delta^{15}N$ values, increase with increasing depth due to microbial processing (Altabet et al., 1991; Gloeckler et al., 2018; Hannides et al., 2013; Mayor et al., 2014; McCarthy et al., 2007; Saino and Hattori, 1980). Microbial processing may also vary with depth in trench habitats.

To investigate trophic interactions in hadal trenches, we examined the bulk isotopic composition and AA-CSIA of megafauna in the Mariana and Kermadec trenches. Our goals were to 1) describe trophic positions and relationships in the Kermadec and Mariana trenches, 2) explore how two hadal food webs compared to one another given their contrasting environmental conditions, 3) evaluate organic matter sources to the trench ecosystem and their depth dependence, and 4) compare hadal organisms to those from the surrounding abyssal plains to better characterize the role of trenches in the broader deep sea.

2. Materials/methods

2.1. Study sites

This study focused on organisms in the Mariana and Kermadec trenches, located in the western Pacific Ocean (Fig. 1). The Mariana Trench is located approximately 200 km east of the Mariana Islands, extending south of Guam. The trench is 2,550 km long and averages 69 km wide, housing the deepest location on earth at ~10,984 m (Stewart and Jamieson, 2019). The trench is part of the Izu-Bonin-Mariana subduction system and forms where the western edge of the Pacific Plate subducts under the Mariana Plate (Fryer et al., 2003). Surface waters over the trench are oligotrophic, with satellite-derived primary production of ~120 mg C m^{-2} d^{-1} (over our study site, integrated over the previous year; Linley et al., 2017). The Kermadec Trench runs roughly north-south extending about 1,000 km north—from New Zealand to the Louisville Seamount Chain (Fig. 1). It is the fifth deepest trench, reaching 10,177 m (Jamieson, 2015; Stewart and Jamieson, 2019). This trench is part of the Kermadec-Tonga subduction system and a product of the western edge of the Pacific Plate subducting under the Australian Plate. It is located to the south of the South Pacific Subtropical Gyre province, and is considerably more eutrophic relative to the Mariana Trench, with estimated primary production values ranging from 420 to

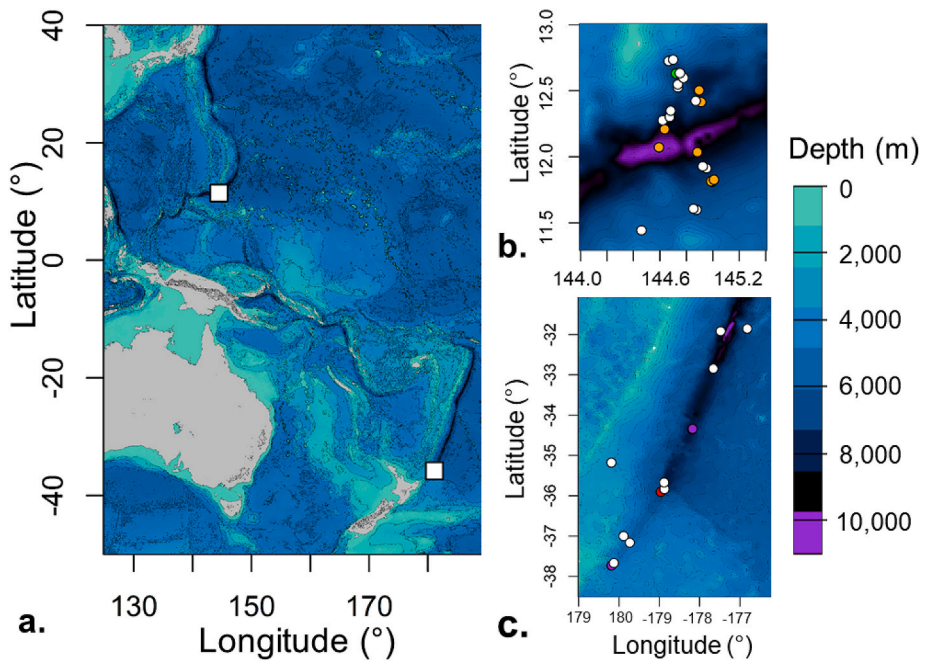

Fig. 1. **(a)** Map of the Kermadec (south) and Mariana (north) trenches with expedition locations. Sampling sites in the Mariana Trench (**b**) and Kermadec Trench (**c**) are shown. Point color indicates gear type: large traps (TR) in white, *HROV Nereus* collections in purple, elevator lander collections in red, small trap (WT) in orange, and sediment corer (CR) in green. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

640 mg C m^{-2} d^{-1} (Linley et al., 2017).

2.2. Sample collection and preparation

Samples were collected in April–May 2014 in the Kermadec Trench and November–December 2014 in the Mariana Trench. In both trenches, baited traps (large 1 cm mesh traps with PVC tube traps inside them, for amphipods; described fully in Linley et al., 2016) were used to capture motile fauna such as amphipods, shrimp, and fishes. We covered bait with mesh to minimize amphipods feeding in the traps. In the Kermadec Trench, other benthic invertebrates, such as holothurians, were collected by the *ROV Nereus* via a slurp gun or manipulator arm (more detail can be found in Nunnally et al., 2016). Sediment cores (0–1 cm surface sediment) were collected from both trenches, by ROV in the Kermadec Trench (6.35 cm internal diameter) and by a free-vehicle coring respirometer (9.5 cm internal diameter, 1 h after landing) in the Mariana Trench.

At sea, samples were processed in a 4 °C cold room or on ice. Holothurian and sea anemone body walls were collected, and supergiant amphipods, shrimp, and fishes were dissected for muscle tissue. Tissues were flash frozen in liquid nitrogen and transferred to a −80 °C freezer. The disk (with gut removed) and arms of the brittle star were frozen at −80 °C for isotope analysis. Amphipods were frozen in liquid nitrogen individually or in small groups for smaller specimens (<1.5 cm). Samples were shipped on dry ice to the lab and stored at −80 °C until analysis in 2015–2017. Sediment cores were sliced into centimeter intervals with a 95% ethanol-cleaned stainless-steel slicer. Sediments were then wrapped in pre-combusted aluminum foil, placed in a sterile plastic bag and stored at −20 °C.

In the laboratory onshore, all lyssianasid amphipods were carefully prepared for isotope analysis. Small amphipods were analyzed in batches of 20 or more (Table S2 and S3). Chitin has lower $\delta^{15}N$ values than muscle and other soft tissues (Schimmelmann, 2011; Søreide and Nygård, 2012). In light of this, we dissected amphipods under a microscope, separating chitinous exoskeleton from internal soft tissues. To get enough material for stable isotope analysis from smaller amphipods (<1.5 cm), tissues from ~5 individuals of similar size with the same morphology, trap, and site location were combined (~0.4 mg). Care was also taken to remove the gut contents prior to isotopic analysis. To test the effects of sample preparation on isotopic composition, samples of chitinous exoskeleton with no internal soft tissues/gut contents were analyzed for a subset of samples.

Samples were lyophilized then homogenized to a fine powder using a mortar and pestle. Then, lyssianasid amphipods and other samples with high C:N molar ratios were lipid extracted using sonication in hexane (HPLC grade or better) three times to ensure that all remaining lipids were removed (Tables S2 and S3). After lipid extraction, all samples were dried at 60 °C. Echinoderms and some amphipods were acidified in 1 M HCl for 24 h to remove carbonate and then dried overnight (60 °C). For sediments, reported $\delta^{15}N$ values are from unacidified samples, and $\delta^{13}C$ from acidified samples.

2.3. Isotope analysis

Whole organism or bulk tissue and sediment isotope analysis was conducted using a mass spectrometer (Delta^{Plus}XP) coupled with an elemental combustion system (Costech ECS 4010, MAT Conflo IV, ThermoFinnigan). Isotope values are reported in δ-notation relative to international standards: atmospheric N_2 (AIR) for $\delta^{15}N$, and Vienna Pee Dee Belemnite (V-PDB) for $\delta^{13}C$. Using in-house reference materials analyzed every 10 samples (NIST reference materials, glycine and tuna tissue homogenate), accuracy and precision were within 0.2%.

Samples for AA-CSIA were hydrolyzed and derivatized using procedures of Popp et al. (2007) and Hannides et al. (2009). Briefly, 5–10 mg of homogenized samples were hydrolyzed (6 N HCL, 150 °C, 70 min) and hydrolysate purified using low protein-binding filters (0.2 μm) and cation exchange chromatography. The purified hydrolysate was esterified (4:1 isopropanol:acetyl chloride, 110 °C, 15 min). The trifluoroacetyl and isopropyl ester derivatives were further purified using solvent extraction and stored at −20 °C for up to a month prior to isotope analysis.

The $\delta^{15}N$ values of individual amino acids were determined using isotope ratio monitoring gas chromatography-mass spectrometry (ThermoScientific Delta V Plus) interfaced to a Trace GC gas

chromatograph fitted with a 60 m BPX5 forte column (0.32 mm internal diameter with 1.0 μm film thickness; SGE, Inc.) through a GC-C III combustion furnace (980 °C), reduction furnace (650 °C), and a liquid nitrogen cold trap as previously described (Hannides et al., 2009). Prior to analysis, samples were dried and dissolved in an appropriate volume of ethyl acetate. Each sample was analyzed in at least triplicate, with norleucine and aminoadipic acid internal reference compounds co-injected in each run. A suite of 15 pure amino acids was also analyzed every three injections to provide an additional measure of instrument accuracy. The $\delta^{15}N$ values of all pure amino acid reference compounds were previously determined using the bulk tissue isotope technique described above and were used to normalize the sample $\delta^{15}N$ values. Nitrogen isotope values are reported in standard δ-notation relative to atmospheric N_2. For replicate injections of samples, amino acid $\delta^{15}N$ standard deviations ranged from 0.06‰ to 1.65‰ and averaged 0.44 ± 0.33‰.

Trophic positions of samples were estimated using Equation (1).

$$Trophic\ Position = \frac{\delta^{15}N_{trophic-AAs} - \delta^{15}N_{source-AAs} + \beta_{trophic-AAs/source-AAs}}{\Delta_{trophic-AAs/source-AAs}} + 1 \quad (1)$$

where $\beta_{trophic\text{-}AAs/source\text{-}AAs}$ is the difference in weighted mean $\delta^{15}N$ values of trophic (alanine, leucine, glutamic acid) and source (lysine, phenylalanine) amino acids in primary producers at the base of the food web and $\Delta_{trophic\text{-}AAs/source\text{-}AAs}$ is the trophic discrimination factor between this combination of amino acids. We adopted the $\beta_{trophic\text{-}AAs/source\text{-}AAs}$ (3.86 ± 0.23) and $\Delta_{trophic\text{-}AAs/source\text{-}AAs}$ (5.46 ± 0.13) values for this combination of amino acids as suggested by Bradley et al. (2015). We assume that the $\Delta_{trophic\text{-}AAs/source\text{-}AAs}$ of 5.46 ± 0.13, which is based on analyses of teleosts, is applicable to the diverse trench fauna. Uncertainty in trophic position was determined by propagation of errors (Jarman et al., 2017; Ohkouchi et al., 2017).

2.4. Statistical analysis

One of our goals was to compare the isotope values of the organisms between the Mariana and Kermadec trenches. Faunal diversity in the Mariana Trench was limited and species differed between the trenches so, for comparisons, we grouped animals into similar categories that included small amphipods, the supergiant amphipod (*Allicela gigantea*), rattail fishes, snailfishes, shrimp, and sediments. A two factor PERMANOVA with trench and organism type as fixed factors, and utilizing Euclidean distances, was employed to evaluate differences in each isotope separately using PRIMER version 6. In cases where a significant interaction was identified, pairwise post-hoc tests were conducted for each organism type between the two trenches. Linear least-squares regressions were performed between isotope values and depth with isotopic compositions as the dependent variable (Statistica version 13.3). A PERMANOVA test was also used to compare $\delta^{15}N$ values of source-AAs between abyssal and hadal depths (>6,500 m) in the Kermadec Trench.

3. Results

3.1. Bulk isotope analysis of amphipod chitin and soft tissues

We compared soft tissue and exoskeleton isotopic values of amphipods to investigate how chitin influences $\delta^{15}N$ values. The carbon isotopic composition of soft tissue and exoskeleton of amphipods was not significantly different (n = 15, paired *t*-test, p = 0.71). However, their exoskeleton $\delta^{15}N$ were significantly lower than their soft tissues (paired *t*-test, p = 0.001). C:N molar ratios of soft tissues ranged from 3.7 to 4.9 whereas those of exoskeleton ranged from 4.6 to 5.9 (Fig. 2a). The difference in $\delta^{15}N$ values was positively correlated to the C:N molar ratios of the exoskeleton ($p < 0.05$) but not to the C:N of soft tissues (Fig. 2b). In other words, when the C:N ratios of amphipod soft tissues and exoskeleton were similar, converging at values of about 5, their $\delta^{15}N$ values were also similar (Fig. 2b). However, when the C:N values of the exoskeleton rose to above this point their $\delta^{15}N$ values were increasingly lower than that of the soft tissues. Note that the molecular C:N ratio of pure chitin is 8 (Schimmelmann, 2011). For further analysis of the samples (sections below), we used only values from soft tissue where the C:N molar ratio was <5 to ensure there was minimal chitin contamination.

3.2. Bulk isotope analysis of Mariana and Kermadec trench fauna

To characterize the food web in both trenches, bulk isotope analysis was conducted on 51 samples from 17 taxa across 5 phyla and surface sediments in the Kermadec Trench (Table S2) and 37 samples from 9 taxa across 2 phyla and surface sediments in the Mariana Trench (Table S3).

In the Kermadec Trench, $\delta^{15}N$ values increased from sediments to fishes and other putative predators (Fig. 3a). There is a large (~7.5‰) difference between bulk $\delta^{15}N$ values of sediments and holothurians (*Elpidia glacialis kermadecensis*, *Abyssocucumis abyssorum* and *Bathyplotes* sp.), which feed on detritus. Detritivores had the lowest $\delta^{15}N$ value among the taxa analyzed. For amphipods, with the exception of the supergiant amphipod (*Allicela gigantea;* $\delta^{15}N$ = 17.5‰), $\delta^{15}N$ values were similar and slightly higher than those of detritivores. The hadal snailfish, *Notoliparis kermadecensis*, had $\delta^{15}N$ values that overlapped with those of co-occurring amphipods (*S. schellenbergi*; Fig. 3a). The other fishes that were sampled at abyssal depths had slightly higher $\delta^{15}N$ values than the hadal snailfish. The two anemones (abyssal/hadal Actinostolidae and abyssal Cerianthid) had similar $\delta^{15}N$ values to the abyssal fishes but with high intraspecific variability. The highest $\delta^{15}N$ values were found in the supergiant amphipod and the predatory polychaete worm (*Macellicephala* sp.) both from ~7,200 m.

The $\delta^{13}C$ values of Kermadec taxa were variable and there was no correlation to $\delta^{15}N$ values (Fig. 3a). Holothurians, which feed on detritus, had higher $\delta^{13}C$ values than the smaller amphipod taxa, comparable to those of the abyssal fishes *Coryphaenoides armatus* and *Spectrunculus grandis*. Amongst the megafauna the hadal snailfish had the lowest $\delta^{13}C$ value, much lower than amphipods (by ~1.3–2‰). The hadal ophiuroid (*Ophiolimna* sp.) and abyssal Cerianthid anemone had the highest values, ~1–2‰ higher than the holothurians, penaeid shrimp (*Benthiscymus* sp.) and Actinostolid anemones.

In the Mariana Trench, the $\delta^{15}N$ values of taxa increased from sediments towards fishes. This trend echoes that seen in the Kermadec Trench, however, only bait attending fauna were represented (Fig. 3b). The difference between the single sediment $\delta^{15}N$ value and the crustaceans is much smaller (~3.5‰) than the difference between sediments and holothurians in the Kermadec Trench. The large penaeid shrimp (6,068 m) had the lowest mean $\delta^{15}N$ values of all the crustaceans analyzed. The small amphipods' $\delta^{15}N$ values clustered together and were similar to the value for the abyssal rattail (*C. yaquinae*). The predatory hadal amphipod *Princaxelia* sp. and the Mariana snailfish (*Pseudoliparis swirei*) had $\delta^{15}N$ values about 1‰ higher than co-occurring amphipods (*S. schellenbergi* and *H. gigas*). As found in the Kermadec Trench, the supergiant amphipod, collected from hadal depths, had a considerably higher $\delta^{15}N$ value than the other amphipods and fishes. Similar to findings in the Kermadec Trench, $\delta^{13}C$ values were variable, particularly amongst the amphipod taxa, and did not covary with $\delta^{15}N$ values.

Isotopic values of similar taxa (e.g. rattails or amphipods) were compared between the two trenches. Organisms of a taxa from the Kermadec Trench often had higher $\delta^{15}N$ values than those from the Mariana Trench (Fig. 4) but this was significant only for amphipods (PERMANOVA post-hoc pairwise test, $p < 0.05$). Rattail fishes, the supergiant amphipod, and shrimp also had higher $\delta^{15}N$ values in the Kermadec Trench, but with low sample sizes, these differences were not significant. In contrast to $\delta^{15}N$, $\delta^{13}C$ values of samples were lower in the

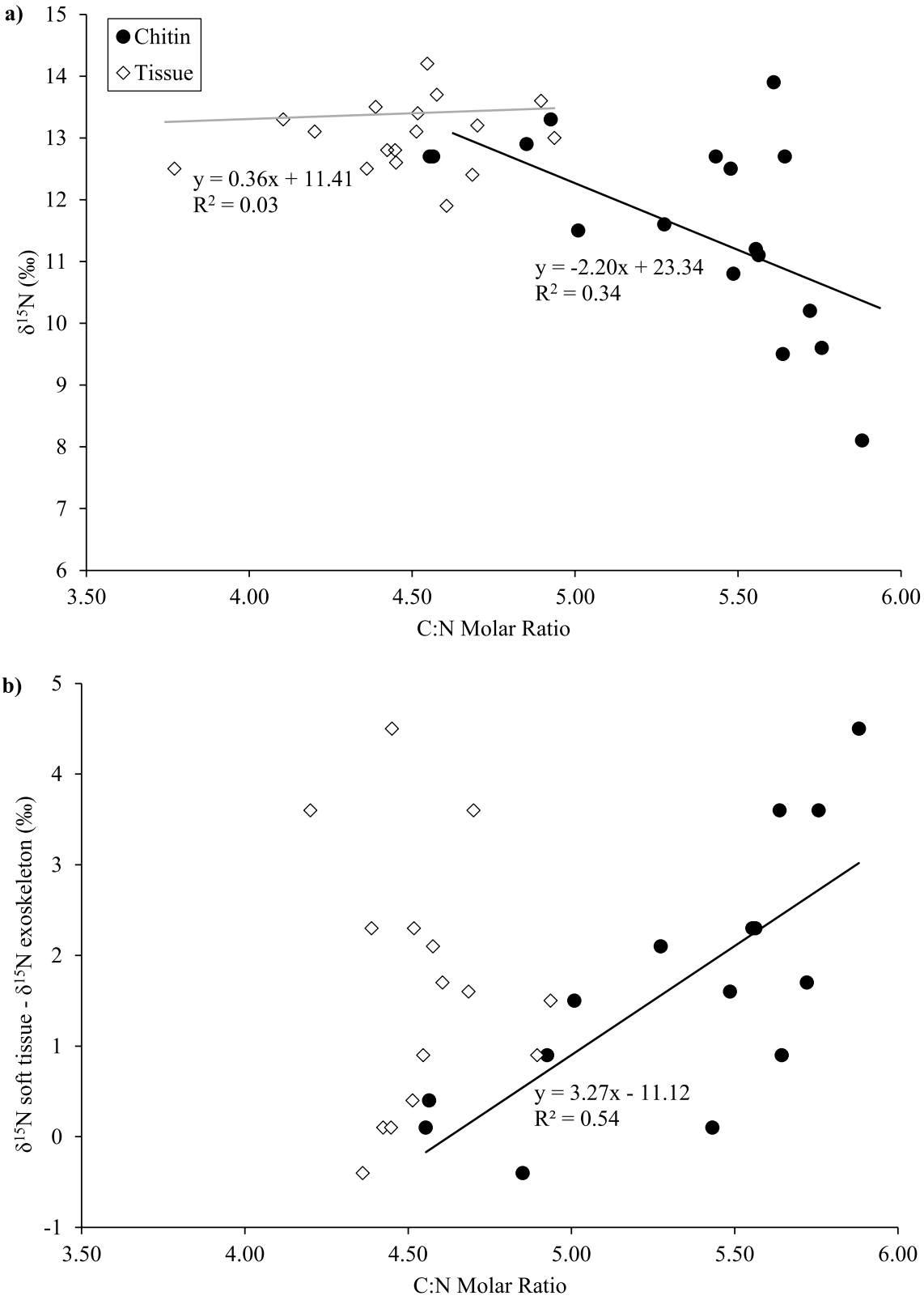

Fig. 2. a) $\delta^{15}N$ values of amphipod soft tissue and exoskeleton as a function of C:N ratio. Each sample has a pair of points. b) The carbon to nitrogen (C:N) molar ratio of amphipod exoskeleton and soft tissues and the difference in their $\delta^{15}N$ values. Points are paired horizontally (soft tissue and chitin for each amphipod) such that for any difference in isotopic values there are two points for the C:N ratios.

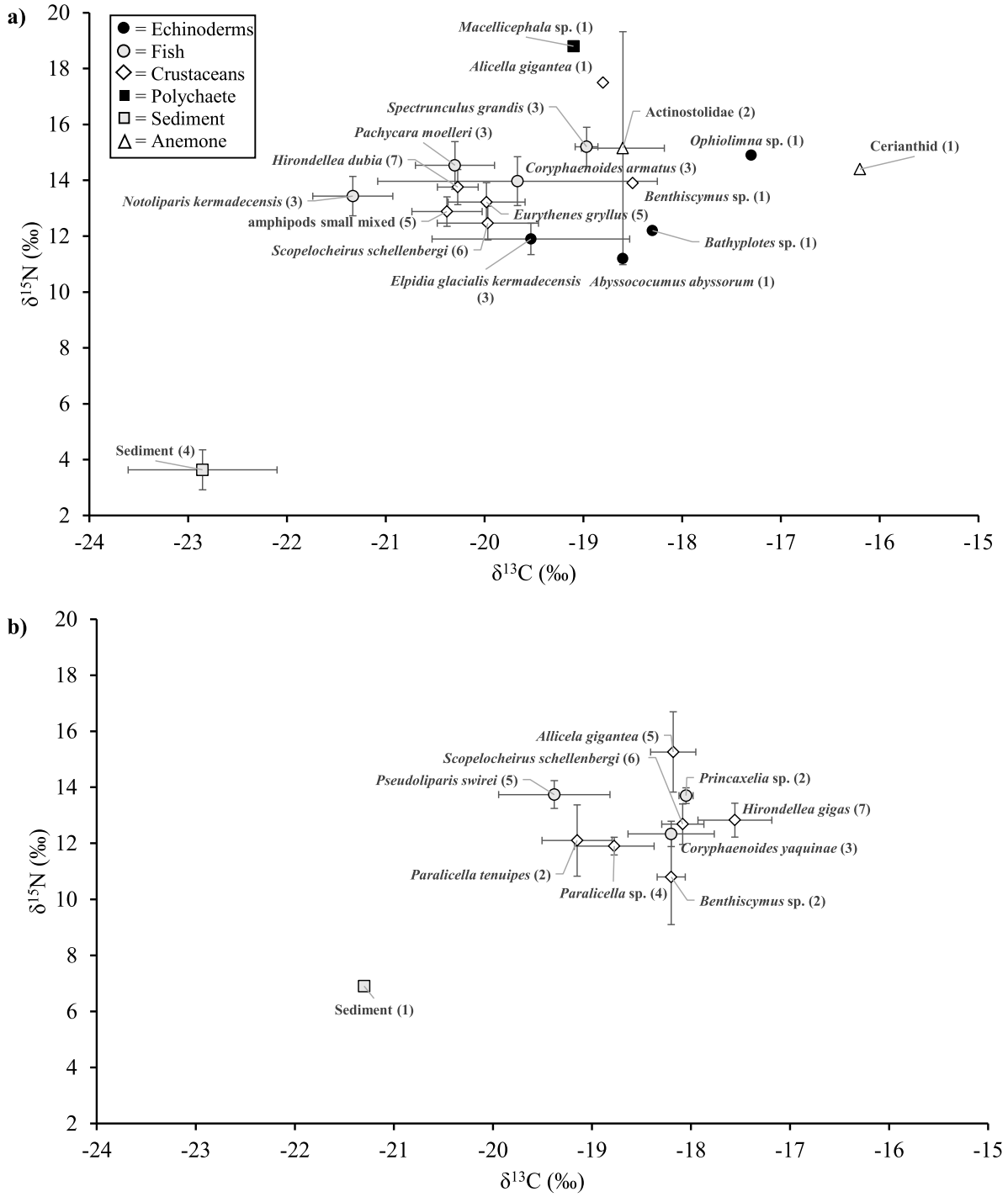

Fig. 3. Carbon and nitrogen isotopic compositions of sediment (0–1 cm) and megafauna from the Kermadec Trench (a) and Mariana Trench (b). Means and standard deviations are shown with sample sizes in parentheses.

Kermadec Trench compared to those from the Mariana Trench (Fig. 4; PERMANOVA, df = 6,1, $p < 0.01$). Within similar taxa, $\delta^{13}C$ values were significantly lower only in amphipods and the snailfishes (PERMANOVA post-hoc pairwise tests, $p < 0.01$ and $p < 0.05$ respectively).

The relationships between bulk isotope values and depth were examined across and within taxa where possible (Fig. 5). Across all samples in both trenches, there were no significant relationships between $\delta^{15}N$ values and depth ($p > 0.05$). In the Kermadec Trench, $\delta^{13}C$ values of all samples declined with increasing depth ($p < 0.01$, $r^2 = 0.12$) and in the Mariana Trench they increased with depth ($p < 0.01$, $r^2 = 0.19$). There was considerable scatter about these relationships (Fig. 5c and d). We also examined depth patterns in bulk isotope values for taxa with sufficient depth resolution (>3 samples across multiple depths). In the Kermadec Trench, *S. schellenbergi* showed a significant increase in $\delta^{15}N$ values with depth ($n = 6$, $p < 0.05$, $r^2 = 0.73$). For the Kermadec Trench sediment samples, $\delta^{15}N$ and $\delta^{13}C$ values increased with depth ($n = 4$, $r^2 = 0.50$ and 0.61 respectively) but the patterns were not significant ($p > 0.05$). Thus, with the exception of $\delta^{13}C$ values across

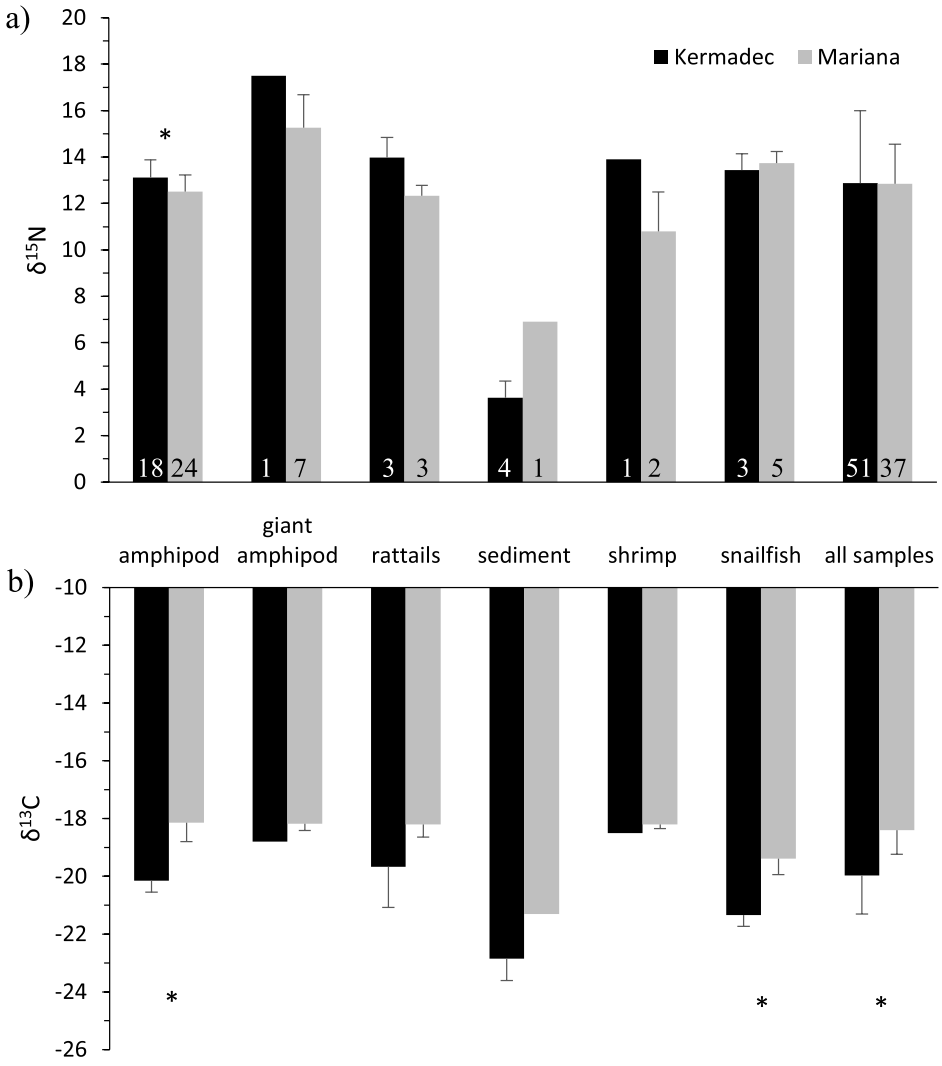

Fig. 4. Average a) nitrogen and b) carbon isotopic values (with standard deviation) for similar taxa between the Mariana (grey) and Kermadec (black) trenches. Numbers shown in the bars are sample sizes (identical for both nitrogen and carbon isotope values). *indicates a significant difference in mean values between trenches (PERMANOVA post-hoc pairwise test, $p < 0.05$).

all samples in the Kermadec Trench, bulk isotope values increased with increasing depth.

3.3. AA-CSIA

To quantify trophic position and evaluate nutritional sources, we applied AA-CSIA to samples from the Kermadec Trench where we had the broadest faunal sampling of animals from the hadal food web. We analyzed a subsample of taxa across feeding guilds (detritivores, scavengers, predators). $\delta^{15}N$ values of 16 individual amino acids were determined for 14 taxonomic groups across 3 phyla (see Supplementary Table S4). Estimated trophic positions (Fig. 6) ranged from 2.98 to 5.3. Deposit-feeding holothurians had the lowest trophic positions and the abyssal fish, *Coryphaenoides armatus* had the highest. *Notoliparis kermadecensis*, which had a similar bulk $\delta^{15}N$ value to amphipods, also had a similar estimated trophic position (4.16 ± 0.12) to amphipods (4.18 ± 0.12 *H. dubia* co-occurring at ~7,250 m; 4.16 ± 0.12 *Eurythenes gryllus* from 6,000 m. *Scopelocheirus schellenbergi* (8,000 m) had the highest trophic position estimate amongst all of the amphipods at 4.80 ± 0.15, surpassing even the supergiant amphipod, *A. gigantea* (4.51 ± 0.14) and the ophidiid fish, *Spectrunculus grandis* (juvenile; 4.63 ± 0.12). All amino acid $\delta^{15}N$ values were high in the supergiant amphipod, *A. gigantea* (Supplementary Table S4), explaining its high bulk value, yet relatively moderate trophic position.

No significant relationship between the $\delta^{15}N$ values of source-AAs and depth was found across the species (Fig. 7). The average source-AA (lysine and phenylalanine) $\delta^{15}N$ values of the Kermadec Trench organisms varied considerably between taxa, particularly the small amphipods, across the depths (Fig. 7). Excluding the small amphipods (white symbols on Fig. 7 except for *Benthiscymus* sp. and *A. gigantea*), there was a significant increase in $\delta^{15}N$ values of source-AAs with depth ($r^2 = 0.56$, $p < 0.05$) and greater average values in hadal organisms than in abyssal organisms ($p < 0.05$).

4. Discussion

4.1. Sources of nutrition to hadal food webs

We used results of carbon and nitrogen isotope analysis to evaluate nutritional sources in the Mariana and Kermadec trenches. We found slightly higher $\delta^{15}N$ values in organisms from the Kermadec Trench compared to those in the Mariana Trench that suggests differences in the isotopic composition of primary production in surface waters. Over the Mariana Trench, nitrogen fixation is the dominant mechanism of

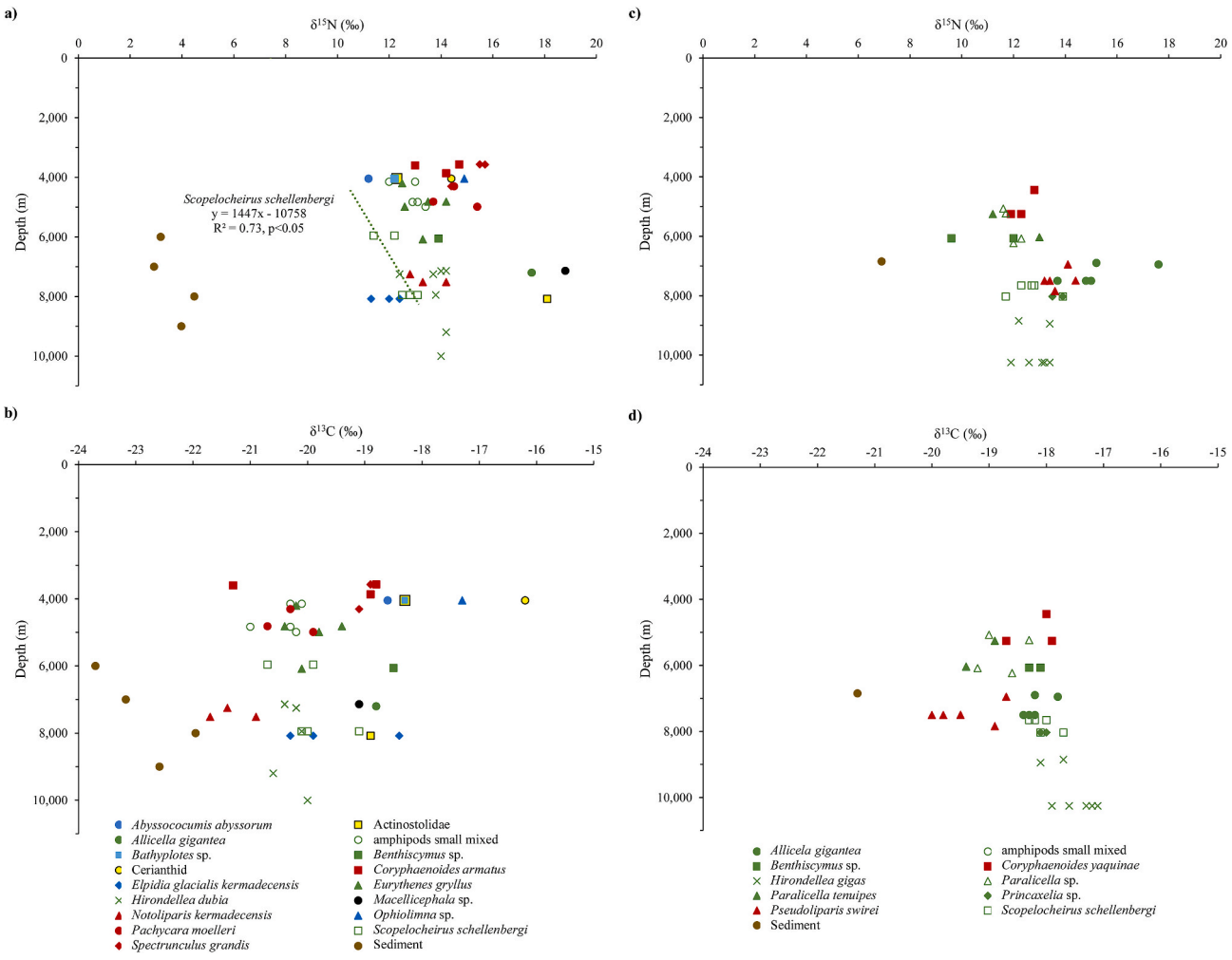

Fig. 5. Isotope values by taxa and depth in the Kermadec (a, b) and Mariana (c, d) trenches. Relationships between isotope values and depth for specific taxa are shown in each panel where significant. Brown = Sediment, Green = Crustaceans, Red = Fish, Blue = Echinoderms, and Yellow = Anemones. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

nutrient supply to the euphotic zone (Checkley and Miller, 1989; Montoya et al., 2002). Nitrogen fixation is known to produce biomass with low $\delta^{15}N$ values, which can range between −1‰ and −2‰ (Montoya et al., 2002). In contrast, the Kermadec Trench, particularly its southern end near New Zealand, is under more eutrophic waters (Linley et al., 2017). These inputs of upwelled nitrate have higher $\delta^{15}N$ values (Bury et al., 2001), which is reflected in $\delta^{15}N$ values in primary producers. Although terrestrial inputs in trenches have not yet been fully characterized, terrestrial inputs of organic matter may also explain the higher $\delta^{15}N$ values found in the Kermadec Trench food web (Xu et al., 2018). This trench is close to a continental landmass and may have more terrestrial organic material entering via downslope transport (Linley et al., 2017). Significant amounts of pine pollen grains were found in Kermadec Trench sediments, and this pollen was ingested by sediment protists (Leduc and Rowden, 2017). Pine pollens have relatively higher $\delta^{15}N$ values (~3.4‰) compared to biomass produced by nitrogen fixation (Masclaux et al., 2013) and are known to sink when water fills their air bladders (Davis and Brubaker, 1973). Also in support of the importance of terrestrial inputs to the Kermadec Trench is our finding of low sediment $\delta^{13}C$ values (−22.9‰; Fig. 5) that may arise from a mix of phytodetritus with values of −20‰ to −22‰ and plant material as found in New Zealand waters of −26‰ to −29‰ (Leduc et al., 2020; McLeod and Wing, 2007).

The lack of correlation between $\delta^{13}C$ and $\delta^{15}N$ values is further support that the trenches have a diversity of nutritional sources. In other habitats such correlation is expected if a single important source of nutrition dominates as has been observed in some bathyal and abyssal habitats (Drazen et al., 2008; Iken et al., 2001; Polunin et al., 2001). This occurs because with each trophic step $\delta^{13}C$ and $\delta^{15}N$ values increase due to isotope fractionation from a common isotopic baseline (Peterson and Fry, 1987).

The large disparity between the $\delta^{15}N$ values of detritivores and sediment values suggests that detritivores are feeding selectively. Large differences in $\delta^{15}N$ values of detritivores and sediment have been observed in other deep-sea habitats as well (e.g. Drazen et al., 2008; Leduc et al., 2020; Romero-Romero et al., 2016). If detritivores were feeding indiscriminately on the organic matter in these sediments then their $\delta^{15}N$ values should be ~3‰ higher than this sediment (Peterson and Fry, 1987), rather than the ~7‰ difference observed in the Kermadec Trench (Fig. 2). Particulate organic matter (POM) on most sea beds is usually composed of a variety of living and dead microorganisms, molts, and amorphous aggregates (Sokolova, 1997) which all can serve as food sources for surface deposit-feeders (Jumars et al., 1990; Miller et al., 2000). Holothurians favor sediments with high organic matter concentrations (Navarro et al., 2013) and they are known to be selective feeders based on gut pigment analysis (Wigham et al., 2003). Given this behavior, some of the POM selected by the hadal detritivores could have higher $\delta^{15}N$ values compared to average sedimentary POM, which would then result in the higher than expected values for these animals. We also calculated a trophic position of 3 for hadal detritivores

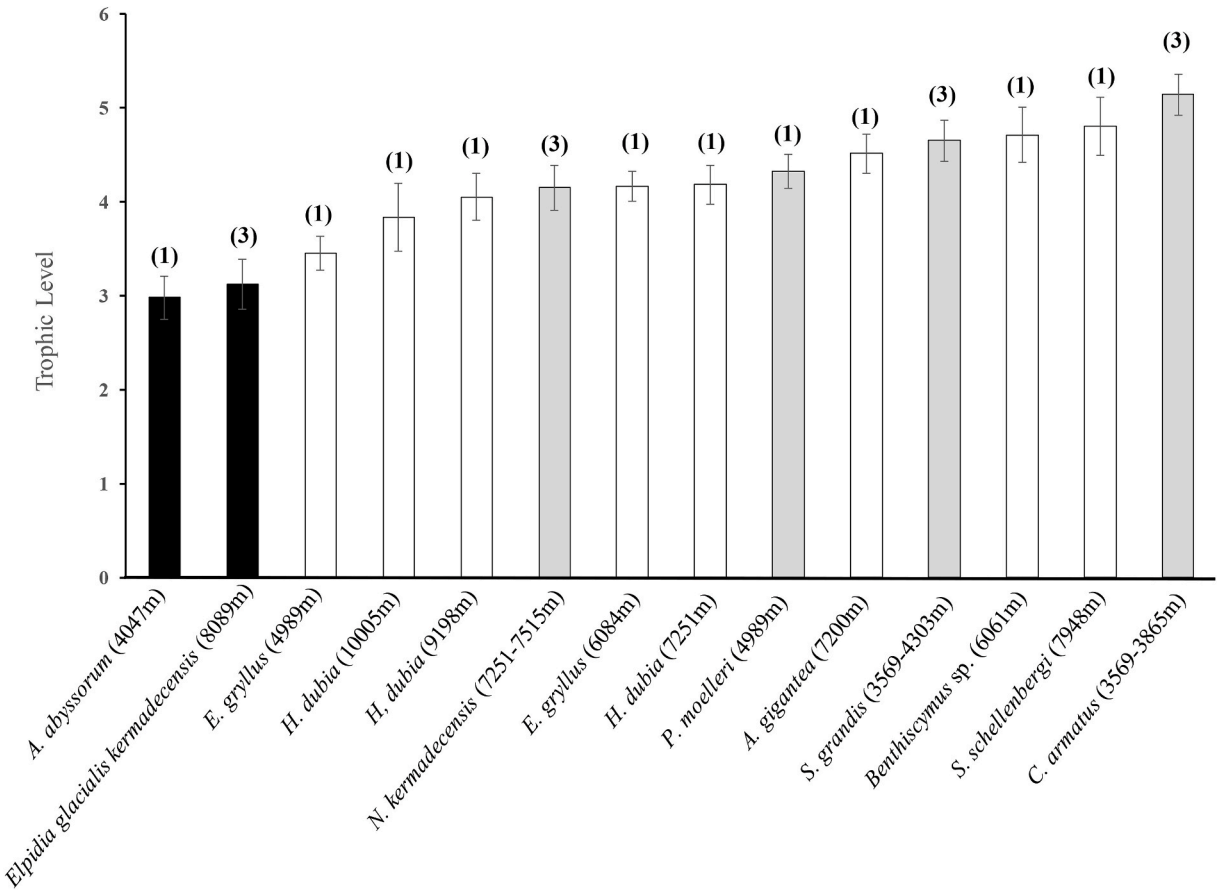

Fig. 6. Trophic levels calculated using AA-CSIA for samples from the Kermadec Trench. Numbers in parenthesis above each bar show sample size and numbers in parenthesis after taxa names represent depths of collection. Fishes are in grey, crustaceans in white, and echinoderms in black. Trophic levels were calculated using equation (1). Uncertainty are standard deviation calculated by propagating all errors.

(*Abyssococumus abyssorum* & *Elpidia glacialis kermadecensis*) based on AA-CSIA, which is expected for a detritivore feeding on a mix of phytoplankton and zooplankton remains at depth. Other studies have shown that the $\delta^{15}N$ value of detritus increases with depth due to microbial degradation and addition of fecal pellets to particle flux (Hannides et al., 2020; Ohkouchi et al., 2017). It is also possible that deposit feeders cultivate gut bacteria (e.g. Amaro et al., 2012) that add a trophic step but CSIA estimated trophic positions are not available for sediments to evaluate this. Additional isotopic data on sinking detritus might help us understand how it contributes to the diet of hadal detritivores. Our results suggest that bulk sediment POM $\delta^{15}N$ values integrated from 0 to 1 cm do not reflect all sources of nutrition for detritivores in the Kermadec Trench.

There was a wide range of source-AA $\delta^{15}N$ values in small amphipods in the Kermadec Trench suggesting that there are variable sources of organic matter at all depths. Amphipods are opportunistic scavengers which can rely on many types of organic matter for nutrition, ranging from infauna, to carrion falls (Blankenship and Levin, 2007), to possibly wooden debris (Kobayashi et al., 2012). Sporadic turbidity flows triggered by earthquakes resuspend older sediment. If that resuspended POM is utilized, it could lead to variability in source-AA $\delta^{15}N$ values in organisms along trench axes. Furthermore, lateral or downslope transport could bring material from neighboring regions that have differing isotopic compositions. The same is true of amphipods feeding on carrion, including large epipelagic fishes that may have migrated through the region before sinking. Starvation effects may also explain some of the variability in the source-AA $\delta^{15}N$ values observed in the amphipods. Starvation can lead to higher $\delta^{15}N$ values (Doi et al., 2017) and amphipods may feed sporadically (Hargrave et al., 1994; Smith and Baldwin, 1982). Blankenship and Levin (2007) examined amphipod diet using isotope analyses and DNA markers and found high levels of variation in inferred diet, some of which was explained by ontogeny, a factor not controlled for here. Lastly, cold seeps harboring chemosynthetic ecosystems have been found in the Mariana Trench to 5,622 m (Ohara et al., 2012). Such habitats might provide a localized source of nutrition (Cordes et al., 2010; MacAvoy et al., 2002), that are depleted in ^{15}N relative to photosynthetic organisms (Pinti and Hashizume, 2001). Unfortunately, this study was not able to sample phytodetritus (e.g. from sediment traps) or collect other potential nutritional sources to this food web to provide potential isotopic endmembers. Such samples are key targets for future hadal studies.

The other taxa—fishes, holothurians and larger crustaceans—exhibit an increase in source-AA $\delta^{15}N$ values with depth which could suggest increasing amounts of microbially reworked organic matter contributing to hadal food webs with increasing depth. In pelagic environments, $\delta^{15}N$ source-AA values of organisms increase with depth (Gloeckler et al., 2018; Hannides et al., 2013). The depth-related increase in values has been attributed to an increase in suspended particles forming the base of mesopelagic food webs. $\delta^{15}N$ source AA values in the small, slowly settling suspended particles increased as a result of microbial reworking (Hannides et al., 2013, 2020). Downslope transport through turbidity flows or topographic funneling may introduce older microbially-reworked organic matter to greater depths in trenches that is consumed directly by holothurians, affecting $\delta^{15}N$ values. The larger crustaceans and fishes, which feed on varying quantities of carrion and scavenging amphipods (Drazen et al., 2008; Gerringer et al., 2017; Linley et al., 2017), may integrate the isotopic variability in trophic sources, in contrast to small amphipods which appear to have high levels

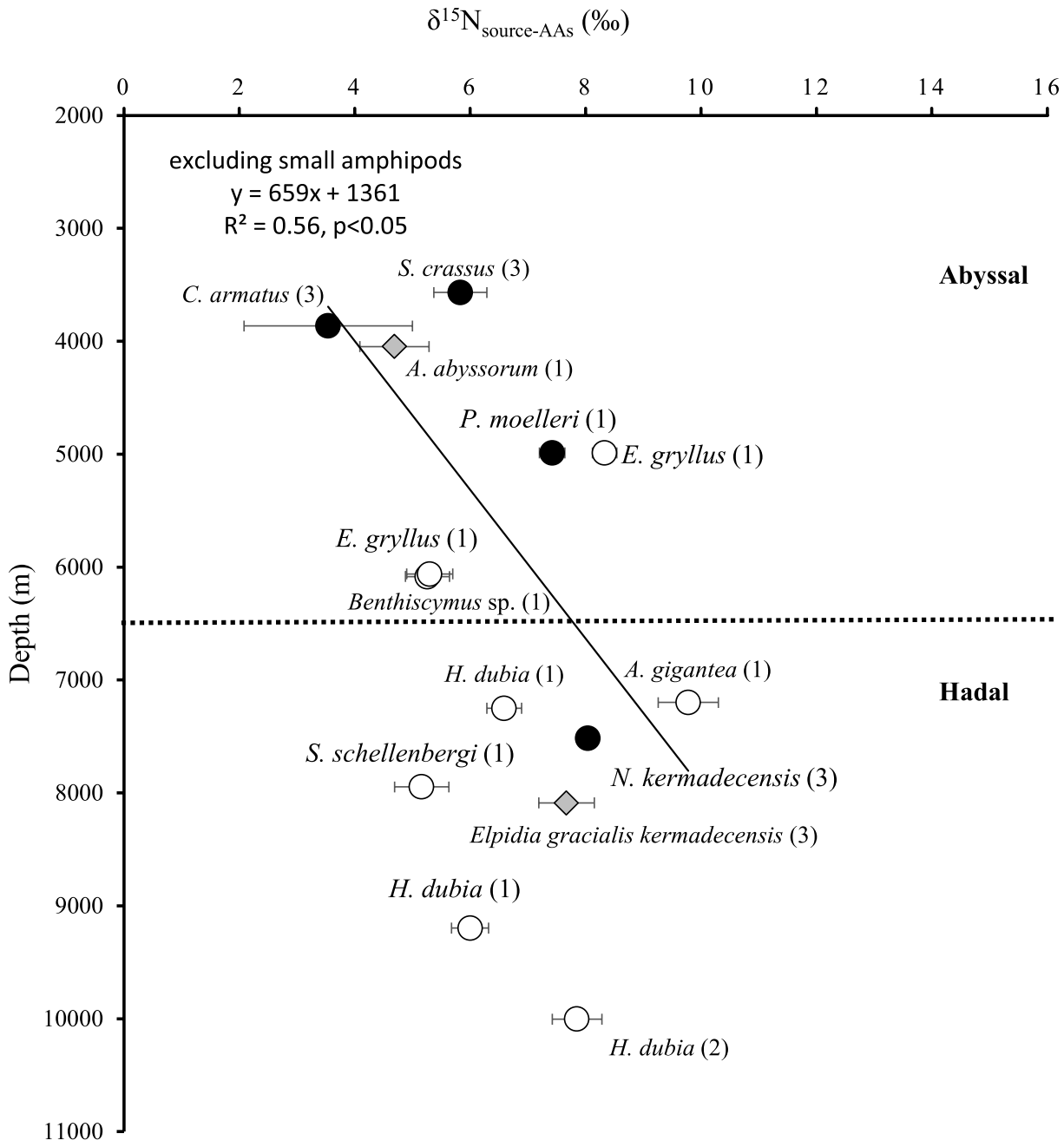

Fig. 7. The $\delta^{15}N$ values for source amino acids (lysine and phenylalanine) of megafauna selected from the Kermadec Trench with depth of collection. Means and standard errors are shown with sample sizes in parentheses. The relationship between source amino acid $\delta^{15}N$ values and depth excluding small amphipods (all white symbols except those for *Benthiscymus* sp. and *A. gigantea*) is shown. A dotted line is drawn at 6,500 m in depth to mark the boundary between abyssal and hadal samples for the Kermadec trench, a depth previously shown to be associated with a significant community transition (Jamieson et al., 2011b). Fishes are in black, crustaceans in white, and echinoderms in grey.

of variation in nutritional sources (see paragraph above). Further sampling of a broader swath of taxa, particularly of deposit feeders, is required to fully address this hypothesis.

4.2. Trophic levels and food web connections

AA-CSIA of megafauna from the Kermadec Trench suggests that differing organic matter sources likely result in variable isotopic baselines that confound interpretation of trophic levels based on bulk tissue isotope analyses. In our study, the range of bulk $\delta^{15}N$ values were compressed (11.2–18.8‰), with only a ~3.5‰ difference between detritivores and fishes. However, AA-CSIA revealed differences in trophic position between holothurians (TP ~ 3), amphipods, liparids, juvenile cusk eels, eelpouts (TP ~ 4), and rattails (TP ~ 5). Similar disconnects between bulk isotope values and trophic level were found by Choy et al. (2012) for mid-water fishes. Differences between bulk and AA-CSIA values were also evident for the supergiant amphipod, *Allicela gigantea*, which had a very high bulk $\delta^{15}N$ value, but a CSIA derived trophic position of 4. The very high bulk isotope and source AA $\delta^{15}N$ values for *A. gigantea* could result from starvation effects as discussed above. Fasting or nutritional restriction can lead to variable, but generally higher bulk tissue $\delta^{15}N$ values across a variety of taxa (Doi et al., 2017; Haubert et al., 2005; Varela et al., 2015). We speculate that effects of fasting would lead to isotopic fractionation in both trophic and

source AAs as we see here, although no studies to date have rigorously tested this assumption at the level of amino acids. It is possible that *A. gigantea* may feed infrequently between carrion sources so that trapped specimens had not eaten for some time. It is important to note that our knowledge of the feeding habits of *A. gigantea* is limited to video observations of scavenging (Jamieson et al., 2013) and a few stomach content analyses that found only the bait used in traps (De Broyer and Thurston, 1987).

Our data suggest that hadal snailfishes gain substantial nutrition from amphipod chitin and/or the material in amphipod digestive tracts. Bulk isotope $\delta^{15}N$ values show that liparids from the Kermadec Trench (*N. kermadecensis*) nearly overlap those of the amphipods (various taxa). Further analysis from AA-CSIA also indicate that *N. kermadecensis* hold a nearly identical trophic position (TP = 4) to *H. dubia* from a similar depth range (~7,000 m). This was not expected, given stomach content analysis by Gerringer et al. (2017), which found that amphipods form a substantial portion of the diet of hadal liparids (84–88% by mass, with amphipods found in 100% of analyzed stomachs), and thus should have higher bulk $\delta^{15}N$ values and AA-CSIA trophic position. In addition, *in situ* video observations of hadal liparids show the fish suction feeding on smaller amphipods which were attracted to bait (Fujii et al., 2010; Linley et al., 2016). Although the diet studies by Gerringer et al. (2017) were conducted on snailfishes captured in baited traps in which amphipods are readily available, it is unlikely that trap effects influenced results. Analysis of the stomach contents that included only digested remains, excluding freshly consumed amphipods, still revealed that amphipods were the dominant prey (71–75% by mass). Hadal liparids do feed on organisms other than amphipods, including decapods and polychaetes (*N. kermadecensis* only) (Gerringer et al., 2017). Our results may suggest that trench liparids feed on a greater diversity of prey than originally thought. Alternatively, the similarity in trophic position between snailfishes and amphipods may mean that liparids actively break down and assimilate nitrogen from the chitin in the amphipods. Chitin has a $\delta^{15}N$ value about 3‰ lower than soft tissues (Schimmelmann, 2011; Søreide and Nygård, 2012). Many studies (e.g. Gutowska et al., 2004; Lindsay, 1984; Sugita and Ito, 2006) describe chitin digestion in fishes but, the functional role of chitinase in snailfishes is unknown. Finally, the snailfishes may have similar bulk isotope values to co-occurring small amphipods because so much of the amphipod body can be stomach contents. If snailfishes eat amphipods that are filled with semi-digested carrion—which has a lower $\delta^{15}N$ value than the amphipod's own tissues—then the amphipod specimens we examined with gut contents removed do not tell the full story. Some lysianassoid amphipods (e.g. *S. schellenbergi* and *E. gryllus*) are known to store large amounts of food within their extensive gut capacity (Blankenship and Levin, 2007), from 20 to 40% of body weight in *E. gryllus* of similar size to the hadal amphipods sampled here (Hargrave et al., 1994) and as much as 10–30% of their own dry mass in the related species *Orchomenella pinguis* (Sainte-Marie et al., 1989). The overlapping $\delta^{15}N$ values between liparids and amphipods could mean that liparids acquire high concentration of nitrogen from amphipod gut contents with lower $\delta^{15}N$ values in addition to digesting the amphipod tissues themselves. Many species consume amphipods at bait, a feeding mode termed necrophagivory (Drazen and Sutton, 2017), that becomes more important than scavenging itself at hadal depths (Linley et al., 2017).

5. Conclusions

This study is the first to describe trophic interactions in the Mariana and Kermadec trenches incorporating echinoderms, crustaceans, and fishes. Isotopic values suggested that both trenches are strongly connected to surface production. Small differences in $\delta^{15}N$ values of hadal organisms between the two trenches were likely the result of differing nutrient dynamics above each trench. Nutrient sources to the trench are complex which was evident in the variability in amphipod source-AA $\delta^{15}N$ values, and suggest that amphipods may consume infauna, carrion, terrestrial plant debris, older material from turbidity flows, or even seep-derived organic material. The increase in source-AA $\delta^{15}N$ values for other taxa with increasing depth also suggest that more microbially-reworked organic matter from downslope transport may become more important with depth. A large difference exists in $\delta^{15}N$ values between trench sediment and detritivores, suggesting that these animals are selective when feeding on sediments that contain complex mixtures of organic material and microbes. Clearly, hadal food webs are complex and require further study to evaluate general patterns, the importance of many microhabitats and variations between trenches. Importantly, future work will need to sample a diversity of fauna in relatively good condition and from discrete habitats within trenches. This will require targeted sampling from ROVs and/or manned submersibles in conjunction with the continued use of baited landers.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsr.2020.103360.

Author's Contributors

JCD, DJM and BNP conceived of the study. JCD, MEG and EG collected the samples. AKT, MEG, JCD and EG processed and analyzed the samples in the laboratory. AKT and JCD led the analysis of the data and writing. All authors contributed to the discussion and interpretation of the ideas presented and the editing of the manuscript.

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