Comparative feeding ecology of abyssal and hadal fishes through stomach content and amino acid isotope analysis

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A B S T R A C T

The snailfishes, family Liparidae (Scorpaeniformes), have found notable success in the hadal zone from ~6000–8200 m, comprising the dominant ichthyofauna in at least five trenches worldwide. Little is known about the biology of these deepest-living fishes, nor the factors that drive their success at hadal depths. Using recent collections from the Mariana Trench, Kermadec Trench, and neighboring abyssal plains, this study investigates the potential role of trophic ecology in structuring fish communities at the abyssal-hadal boundary. Stomach contents were analyzed from two species of hadal snailfishes, *Notoliparis kermadecensis* and a newly-discovered species from the Mariana Trench. Amphipods comprised the majority (Kermadec: 95.2%, Mariana: 97.4% index of relative importance) of stomach contents in both species. Decapod crustaceans, polychaetes (*N. kermadecensis* only), and remains of carrion (squid and fish) were minor dietary components. Diet analyses of abyssal species (families Macrouridae, Ophidiidae, Zoarcidae) collected from near the trenches and the literature are compared to those of the hadal liparids. Stomachs from abyssal fishes also contained amphipods, however macrourids had a higher trophic plasticity with a greater diversity of prey items, including larger proportions of carrion and fish remains; supporting previous findings. Suction-feeding predatory fishes like hadal liparids may find an advantage to descending into the trench – where amphipods are abundant. More generalist feeders and scavengers relying on carrion, such as macrourids, might not benefit from this nutritional advantage at hadal depths. Compound specific isotope analysis of amino acids was used to estimate trophic level of these species (5.3 ± 0.2 *Coryphaenoides armatus*, 5.2 ± 0.2 *C. yaquinae*, 4.6 ± 0.2 *Spectrunculus grandis*, 4.2 ± 0.2 *N. kermadecensis*, 4.4 ± 0.2 Mariana snailfish). Source amino acid δ^{15}N values were especially high in hadal liparids (8.0 ± 0.3‰ Kermadec, 6.7 ± 0.2‰ Mariana), suggesting a less surface-derived food source than seen in the scavenging abyssal macrourids, *C. armatus* (3.5 ± 0.3‰) and *C. yaquinae* (2.2 ± 0.3‰). These results are compared to bulk muscle tissue isotopic compositions. This study provides the first comprehensive examination of the feeding ecology of the ocean's deepest-living fishes and informs new understanding of trophic interactions and fish community structure in and near the hadal zone.

1. Introduction

The hadal zone consists of deep-sea trenches with depths ranging from 6000 to 11,000 m and houses a distinctly different community than the surrounding abyss with an apparently high level of endemism (Wolff, 1970; Jamieson et al., 2011c). The hadal community includes: amphipods, fishes, tanaids, isopods, cumaceans, decapods, echinoderms, nematodes, polychaetes, copepods, molluscs, foraminifera, and cnidarians (Wolff, 1958; Beliaev, 1989; Jamieson et al., 2009a, 2010). As on the abyssal plains (depths 4000–6000 m), most of the hadal community is supported by falling carrion and particulate organic

matter from the upper ocean (Angel, 1982). The processing of nutrients into the hadal food web is believed to be facilitated by an active heterotrophic psychrophilic and piezophilic microbial community (Zobell, 1952; Yayanos et al., 1981; Kato et al., 1997; Fang et al., 2002; Bartlett, 2003; Nunoura et al., 2015). Although there is evidence for chemosynthetic communities in deep-sea trenches their prevalence and importance in the hadal ecosystem is not yet characterized (Kobayashi et al., 1992; Fujikura et al., 1999; Fujiwara et al., 2001; Ohara et al., 2012). Current understanding of life in the hadal zone comes largely from trawl (Zenkevich and Bogoiavlenskii, 1953; Bruun et al., 1957; Svenska djuphavsexpeditionen, 1957) and free vehicle

camera and trap work (Jamieson et al., 2009b, 2009c; Søreide and Jamieson, 2013; Lacey et al., 2016), as well as a few ROV (Momma et al., 2004; Bowen et al., 2008, 2009) and manned submersible operations (Pérês, 1965; Forman, 2009; Gallo, et al., 2015). With the difficulty in observing and sampling this environment, the ecology of hadal organisms and their trophic relationships remain poorly understood.

Video observations, collections, and extrapolation from studies of shallower-living relatives of hadal taxa provide some information about trophic interactions in the hadal zone. *In situ* video collected by free-vehicle landers has allowed a glimpse of the feeding habits of hadal fauna such as: detritus-feeding holothurians (Jamieson et al., 2011b), scavenging isopods (Jamieson et al., 2012), predatory decapods (Jamieson et al., 2009a) and pardaliscid amphipods of the genus *Princaxelia* (Jamieson et al., 2011d), and lysianassoid amphipods, the most well-studied hadal animals. These amphipods are known to have morphological and chemosensory adaptations to carrion feeding (Dahl, 1979; Kaufmann, 1994; Hargrave et al., 1995; De Broyer et al., 2004) and scavenge and disperse bait rapidly at hadal depths (e.g. Hessler et al., 1978). Previous studies on the feeding ecology of hadal amphipods have found evidence for opportunistic scavenging, and a high degree of trophic flexibility, including adaptations to ingest large amounts of carrion and phytodetritus (Perrone et al., 2003; Blankenship and Levin, 2007; Kobayashi et al., 2012).

Even fewer data exists on the trophic ecology of hadal fishes. Video observations have shown the rapid consumption of bait by fishes such as macrourids at the abyssal-hadal boundary (Jamieson et al., 2011c). Liparids and ophidiids from the Japan, Kermadec, and Mariana trenches have been observed eating crustaceans (Jamieson et al., 2009b; Fujii et al., 2010; Linley et al., 2017). The present study focuses largely on liparids, a prominent endemic hadal group in at least five trenches (Japan, Kermadec, Kurile-Kamchatka, Mariana, Peru-Chile; Linley et al., 2016). Hadal liparids are small (≤30 cm) pink snailfishes that have been found as deep as 8145 m (Linley et al., 2016). This depth (~8200 m) is thought to be the lower limit for teleosts due to physiological constraints of pressure adaptation (Yancey et al., 2014). Nielsen (1964) reported stomach contents consisting mostly of amphipods in the liparid *Notoliparis kermadecensis* from the Kermadec Trench. However, the sample size in this description was small. Shallower-living snailfishes in the Kamchatka region are benthic feeders eating mostly crustaceans (Orlov and Tokranov, 2011). Many species of snailfishes from other localities also eat amphipods, which can make up as much as 88.8% of diet by numeric importance (Johnson, 1969; Falk-Petersen et al., 1988; Kobayashi and Hiyama, 1991; Labai et al., 2002, 2003; Glubokov, 2010; Jin et al. 2010; Cui et al., 2012).

Studies of the trophic ecology of abyssal species (depths 4000–6000 m) are also limited, making comparisons to hadal taxa difficult. Macrourids are thought to have broad generalist diets as determined from stomach contents analysis and stable isotopic composition (e.g. Drazen et al., 2008). Other abyssal species are less well-studied. However, in a recent expedition, the ophidiid *Bassozetus* sp. was

observed feeding on amphipods in the Kermadec Trench (Linley et al., 2017). Further details on *in situ* observations and depth distributions of the fish community at the abyssal-hadal boundary are provided by Linley et al. (2017). Based on these studies, we hypothesized that hadal liparids would have a more specific predatory feeding strategy, while abyssal species such as macrourids might be more generalist opportunistic feeders.

Much of our current understanding of hadal fishes comes from baited trap and camera studies, which create an artificial food-fall. Although this mimics a natural process, it could bias our view of the community's normal feeding ecology. The bait provides a food source for both fishes and their prey, and the interactions observed in this setting may not fully reflect what happens on a routine basis at depth. Further, video observations and stomach contents provide only a brief 'snapshot' view of diet. Multiple approaches are therefore needed to advance the understanding of trophic ecology in the hadal zone.

Stable isotope analysis has been a useful tool in investigating the longer-term feeding ecology of many organisms (e.g. Peterson and Fry, 1987). Traditionally, this involves comparing differences in bulk tissue (generally white muscle) nitrogen isotopic composition, which display an ~2–4‰ ^{15}N enrichment in consumer relative to prey for each increasing trophic level (e.g. Post, 2002). This technique has been used to study four hadal lysianassoid amphipod morphotypes in the Kermadec and Tonga trenches. Bulk $\delta^{15}N$ values in these amphipods ranged from 7.9 to 13.8‰ (Blankenship and Levin, 2007). Interpreting results of nitrogen (and carbon) isotope analysis requires information about the isotopic compositions of organisms at the base of the food web. The isotopic compositions at the base of the hadal food web have not been well-characterized. Therefore, in this study, amino acid compound-specific nitrogen isotope analysis (AA-CSIA, e.g. Popp et al., 2007; Choy et al., 2012) was used to investigate the trophic level of abyssal and hadal fishes. In this newer technique, the $\delta^{15}N$ values of certain "trophic" amino acids, that fractionate with each trophic level (up to ~7‰ relative to source amino acids), are compared to those of "source" amino acids, that maintain relatively consistent $\delta^{15}N$ values throughout the megafaunal food web, to estimate a trophic position (McClelland and Montoya, 2002; Popp et al., 2007; Chikaraishi et al., 2009; Hannides et al., 2009). Source amino acid values are known to change with depth in small, slowly settling particles, with increasing $\delta^{15}N$ values at greater depths (McCarthy et al., 2007; Hannides et al., 2009). Consequently, source amino acid $\delta^{15}N$ values can also provide information about the origin of nitrogen in an animal's food.

The aims of this study were three-fold: 1) characterize the diet of the hadal snailfish through stomach contents analysis and compare it to the diets of abyssal species documented in the literature; 2) compare trophic positions of abyssal and hadal fishes using compound specific isotope analysis of individual amino acids; and 3) explore the role of trophic interactions in structuring fish depth zonation at the abyssal-hadal boundary.

Table 1

Collection Information (n=number of stomachs analyzed, n*=number of stomachs with prey items present). Ranges presented: Depth indicates capture depth. SL: Standard length, measured fresh with fish mass. Sex indicates number of individuals F: female, M: male, J: juvenile. Others were not sexed due to damage. Zoarcidae Gen et spp. includes individuals of the genera *Pachycara* and *Pyrolycus*.

Trench	Species	n	n*	Depth (m)	SL (cm)	Mass (g)	Sex (F, M, J)
Kermadec	*Notoliparis kermadecensis*	38	37	6456–7554	12.9–29	13.6–230	18, 12, 3
	Coryphaenoides armatus	4	2	3569–5112	50.6–78.6	576 –1930	1, 2, 0
	Spectrunculus grandis	10	6	3569–3865	26.9–43.9	106–532	0, 3, 5
	Zoarcidae Gen et spp.	3	3	4817–4989	42–46.8	460–660	1, 2, 0
Mariana	Liparidae sp. nov.	29	29	6898–7966	10.5–28.8	8 –160	14, 5, 7
	Coryphaenoides yaquinae	1	1	6081	23	40	0, 0, 1

2. Materials & methods

Fishes were collected using baited traps on cruises to the Kermadec and Mariana trenches in April-May and November-December of 2014 respectively (Table 1). Traps, described elsewhere (Linley et al., 2016), were baited with mackerel (in nylon mesh to prevent feeding) and squid. Each captured fish was measured and weighed fresh. Sex was determined visually during dissections at sea. Further information on these collections including site maps can be found in Linley et al. (2016).

2.1. Stomach contents

Stomachs were dissected shipboard, weighed fresh, and preserved in 10% buffered formaldehyde. In the lab, stomachs were weighed whole, then contents were removed and weighed. While whole, each stomach was roughly scored on a fullness scale of 0–4, 0 indicating an empty stomach, 1- less than half full, 2- half full, 3- more than half full, and 4- full. Fullness scores included the contribution of digestive mucus. Contents were sorted to discernible taxon and digestive state, a 1–4 index; 1- an undigested prey item, 2- some soft parts digested, 3- most soft parts digested with skeleton intact, and 4- items that were very digested, with only a few hard parts remaining. A separate analysis of stomach contents using only items of the higher digestive states was conducted to investigate potential trap effects. If the composition of prey items eaten most recently, when the artificial trap environment was introduced, differed greatly from more highly digested items, this would demonstrate a bias of sampling technique. Prey items of each taxon were grouped by digestive state, counted, weighed, and photographed.

Compositions of stomach contents are presented using four metrics. Percent frequency of occurrence (%F) shows the percentage of stomachs that had a certain prey type present. Percent numerical importance (%N) gives the proportion of a prey group compared to the total number of prey items examined for each species. Percent weight (%W) shows the gravimetric importance of a given prey group in relation to the total weight of all prey. These three indices were also used to generate an index of relative importance (IRI), which sums the %N and %W multiplied by the %F (Pinkas, 1971). These values were totaled for all items and a %IRI is presented. Further analyses were conducted using the statistical programming platform R (R Core Team, 2013). The nonparametric Kruskal-Wallis test was used for statistical comparisons due to small sample sizes. Cumulative prey curves were generated using the R package vegan (random, 5000 permutations, Oksanen et al., 2016) to investigate sampling thoroughness. A model (Lomolino) was constructed to estimate the maximum number of prey items for each species. Composition of stomach contents between families were compared using analysis of similarities (ANOSIM) in vegan. Principal components analysis (PCA) plots were made in R using the prcomp function to investigate differences in prey composition between species (%N). Additional figures were produced using the R package ggplot2 (Wickam, 2009).

2.2. Isotope analysis

At sea, white muscle samples were collected from the anterior portion of the epaxial muscle and flash frozen in liquid nitrogen. Tissues were stored at −80 °C prior to preparation and lyophilized and ground for analysis. Roughly mid-size individuals from representative habitat depths of each species were selected. Bulk muscle tissue nitrogen and carbon isotope analyses were conducted with a mass spectrometer (DeltaXP) coupled with an elemental combustion system (Costech ECS 4010, MAT Conflo IV, ThermoFinnigan). Replicate measurements of individual samples were consistent within 0.12‰ for carbon (range 0.07–0.14‰) and 0.14‰ for nitrogen (range 0–0.21‰). Sample preparation for CSIA-AA followed methods detailed in

Hannides et al. (2009) and Choy et al. (2012). The method involves: acid hydrolysis with 6 N HCl, filtration and cation exchange chromatography, esterification of the carboxyl terminus with isopropanol and acetyl chloride, trifluoroacetylation of amine groups with methylene chloride and trifluoroacetyl anhydride, solvent extraction, and redissolvation in ethyl acetate. $\delta^{15}N$ values of individual amino acids were measured using a Delta V Plus mass spectrometer/Trace GC (gas chromatograph) with a GCC III combustion interface. Samples were analyzed in triplicate and measurements normalized to co-injected reference compounds norleucine and aminoadipic acid of known isotopic composition. When coelution of other compounds confounded norleucine and aminoadipic acid values, measurements were regressed against a suite of pure amino acids with known $\delta^{15}N$ values prepared in the same process and analyzed before and after every triplicate series of sample measurements. Instrumental accuracy averaged 0.4 ± 0.3‰ (range 0.02–1.0‰). Standard deviations of $\delta^{15}N$ values between triplicate runs ranged from 0.02 to 0.9‰ with an average of 0.3 ± 0.2‰ for individual amino acids used in trophic position calculations. All $\delta^{15}N$ values are presented in reference to atmospheric N_2.

Trophic positions were estimated according to the methods described by Chikaraishi et al. (2009) using the following equation, based on the update for fishes by Bradley et al. (2015).

$$\text{Trophic Position} = \frac{\delta^{15}N_{trophicAAs} - \delta^{15}N_{sourceAAs} - 3.86}{5.46} + 1$$

Weighted means (by error, e.g., Hayes et al., 1990) of source amino acids (lysine, phenylalanine) are compared to trophic amino acids (alanine, leucine, glutamic acid) as these were the most consistent measurements and according to the recommendations of Bradley et al. (2015). Glycine was excluded from the source amino acid calculations, contrary to the methods of Bradley et al. (2015), due to the co-elution of an unknown compound that could have confounded values. Beta (3.86 ± 0.23) and TDF (trophic discrimination factor, 5.46 ± 0.13) values for this equation were calculated using weighted mean differences between data-derived values of Bradley et al. (2015) considering the omission of glycine.

3. Results

3.1. Stomach contents of hadal liparids

Collection information is presented in Table 1 with individual sample details in Supplementary Table 1. The newly-discovered species of hadal liparid from the Mariana Trench, currently being described, will hereafter be referred to as the Mariana liparid or Mariana snailfish (further details in Linley et al., 2016). Thirty-eight *Notoliparis kermadecensis* stomachs were examined, 37 of which had prey items. Prey was present in all 29 Mariana liparid stomachs examined. Prey accumulation curves, used to evaluate sampling sufficiency, (Fig. 1) showed that the number of prey categories was likely beginning to plateau for both trenches, more so for the Mariana snailfish. A model (Lomolino) estimated asymptotes for these curves at 12.3 prey categories for the *N. kermadecensis* and 8.7 prey categories for the Mariana snailfish.

Large amounts of digestive mucus were present in each stomach. Mucus was not included in prey item weight. Prey items and their composition of total stomach contents by %F, %N, %W, and %IRI are presented in Table 2. Fish remains include bone, eye lenses, scales, and vertebrae. Crustacean remains include digested exoskeleton pieces that could not be identified to a more specific taxon. Unidentified remains included crustacean or squid eggs found in one Mariana snailfish stomach. All stomach contents data can be found in Supplementary Table 2.

Amphipods were by far the most numerically and gravimetrically important prey item. Every liparid with prey in its stomach had eaten at least one amphipod. As many as 378 amphipods were found in one
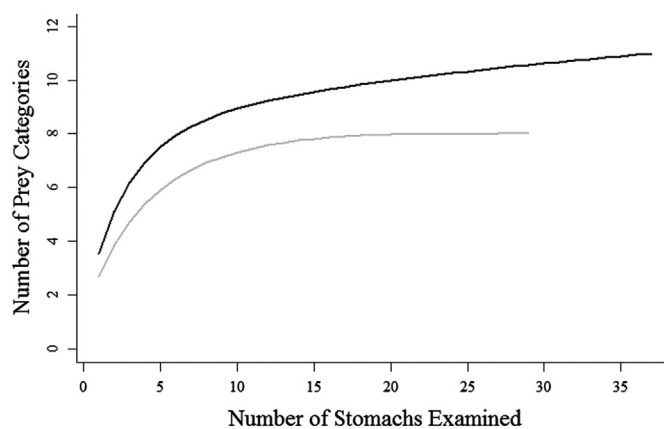
Fig. 1. Prey accumulation curves for hadal liparids. Includes broad prey categories- brooding amphipods, amphipods with nematode parasites, and *Princaxelia* classified as amphipods. Mariana liparid in light grey, *N. kermadecensis* in black.

capturing decapods *in situ*. Most of the decapods were present as highly digested (state 4) remains, though whole individuals up to 8.5 cm total length were found in a few fish.

Polychaete remains (of at least 20 individuals) were found in stomachs of liparids from the Kermadec Trench. Polychaetes are a relatively common, diverse, and characteristic hadal fauna (Kirkegaard, 1956; Jamieson, 2015). These remains are believed to be scale worms of the family *Polynoidae, Pholoidae,* or *Sigalionidae*. Members of the family *Polynoidae* are the most common hadal polychaete (Kirkegaard, 1956; Paterson et al., 2009). Polynoids have recently been imaged in the Kermadec Trench (Jamieson, 2015), and were collected on the same cruise as the liparids in this study (Shank et al. unpublished data), making this the most likely identification. *Sigalionidae* have also been found in trenches, but *Pholoidae* are not known to occur at hadal depths (Paterson et al., 2009). Notably, no polychaetes were found in the Mariana species, although their remains were present in 51.35% of stomachs from *Notoliparis kermadecensis*.

Fish remains were found relatively frequently (29.73% of Kermadec snailfish, 20.69% of Mariana snailfish stomachs). Remains included large scales of what appeared to be several species (possibly Melamphaeidae or Bathylagidae or very digested scales of large fishes), portions of eye lens, small vertebrae, and other bones. Some pieces of tissue from bait squid were found in the hadal liparid stomachs (18.42% of *N. kermadecensis* and 10.34% of Mariana snailfish), but other species of squid were also found. Three species were identified from beaks: *Onychoteuthis* sp., *Walvisteuthis* sp., and *Magnapinna* sp., the last a deep-sea benthopelagic squid (Vecchione and Young, 2006).

A small number of other prey items were found, including a few calanoid copepods and ostracods in stomachs of *N. kermadecensis*. Both of these groups have been recorded at hadal depths (Vinogradova, 1962; Jamieson, 2015). Rocks were found in about a third of stomachs from both hadal liparids. Rocks were typically small; likely debris ingested during suction feeding or compacted sediment from digested amphipod guts. Some prey items could not be identified due to

Kermadec liparid stomach (minimum 1), with as many as 226 in one Mariana liparid stomach (minimum 19). The average number of amphipods found in each stomach was 96.59 ± 71.07 for *N. kermadecensis* and 71.07 ± 52.85 for the Mariana snailfish. Predatory amphipods of the genus *Princaxelia* were also found, albeit infrequently. Lysianassoid amphipods were not identified to higher taxonomic resolution, as morphological classifications are complex and likely in need of revision (Ritchie et al., 2015). Probable morphotypes from these depths in the Mariana and Kermadec trenches include *Bathycallisoma (Scopelocheirus) schellenbergi, Hirondellea dubia,* and *H. gigas* (e.g. France, 1993; Blankenship et al., 2006; Ritchie et al., 2015).

Decapod remains were found in the majority of liparid stomachs from both trenches (62.16% of *N. kermadecensis* with food and 58.62% of the Mariana liparid). Decapods had not previously been found in the stomachs of hadal liparids, and snailfish have not yet been seen

Table 2

above) Hadal liparid prey tables for all digestive states. From 37 Kermadec and 29 Mariana liparids with prey remains in their stomachs. Sample size (n) indicates the total number of prey items examined or the total weight of all prey items. **below)** Hadal liparid prey tables showing highly digested items (digestive states 3 and 4) only.

	Kermadec Trench				Mariana Trench			
	%F	%N	%W	%IRI	%F	%N	%W	%IRI
Amphipods (Lysianassidae)	100	95.48	84.63	95.73	100	97.26	87.66	87.74
Amphipods (Pardaliscidae)	2.70	0.03	0.40	0.01	3.45	0.05	0.38	0.01
Amphipods with Nematodes	48.65	1.14	0.77	0.49	3.45	0.05	0.00	0.00
Brooding Amphipods	0.00	0.00	0.00	0.00	6.90	0.15	0.19	0.01
Copepods	2.70	0.05	0.37	0.01	0.00	0.00	0.00	0.00
Crustacean Remains	8.11	0.08	0.40	0.02	13.79	0.20	3.19	0.25
Decapods	62.16	0.57	3.31	1.28	58.62	0.83	3.93	1.47
Fish Remains	29.73	1.54	1.97	0.55	20.69	0.68	0.18	0.09
Ostracods	2.70	0.08	0.01	0.00	0.00	0.00	0.00	0.00
Polychaetes	51.35	0.57	5.08	1.54	0.00	0.00	0.00	0.00
Squid	18.92	0.16	2.28	0.25	27.59	0.54	2.51	0.44
Unidentified	21.62	0.30	0.79	0.13	17.24	0.24	1.97	0.20
	n=37	n=3692	117.8 g		n=29	n=2046	110.4 g	

	Kermadec Trench				Mariana Trench			
	%F	%N	%W	%IRI	%F	%N	%W	%IRI
Amphipods (Lysianassidae)	91.89	91.81	70.98	89.97	89.66	94.44	75.10	91.43
Amphipods with Nematodes	2.70	0.17	0.01	0.00	3.45	0.14	0.01	0.00
Crustacean Remains	8.11	0.25	1.01	0.06	13.79	0.56	6.89	0.62
Decapods	62.16	1.74	8.42	3.80	58.62	2.36	15.16	6.18
Fish Remains	29.73	3.39	4.17	1.35	20.69	1.94	0.38	0.29
Polychaetes	51.35	1.65	12.83	4.47	0.00	0.00	0.00	0.00
Squid	5.41	0.17	0.64	0.03	3.45	0.14	0.06	0.00
Unidentified	18.92	0.83	1.95	0.32	6.90	0.28	3.43	0.15
	n=37	n=1209	46.3 g		n=29	n=720	51.2 g	

advanced digestion. Unidentified material made up a very small portion of the hadal liparid stomach contents (0.12% IRI for *N. kermadecensis*, 0.20% IRI for the Mariana liparid).

Nematode parasites were found in 27.03% (17) of Kermadec liparid stomachs and 13.79% (6) of Mariana liparids. Amphipods with nematode parasites were more common in the Kermadec snailfish (47.37% of stomachs) than in the Mariana snailfish (3.45%). 42 total amphipods with nematodes in *N. kermadecensis* out of 3573 total amphipods were found, with only 1 of 1991 in the Mariana liparid. Details on nematode-parasitized amphipods collected from the Kermadec Trench concurrently with liparids in this study are provided by Leduc and Wilson (2016).

Analysis of prey items in greater digestive states alone (3 and 4) revealed that amphipods still comprised the overwhelming majority of both hadal liparid diets (Table 2). Highly digested amphipods were most likely consumed before the traps were deployed (maximum of ~20 h of bottom time before retrieval). Other prey items such as decapods, fish remains, and polychaetes (*N. kermadecensis* only), appear to be slightly more important dietary components when looking at only highly digested items (Table 2).

No significant trend was found between percent stomach fullness (mass of stomach content: mass of fish) and depth of capture for either trench. However, there were trends in the number of prey items seen with depth. When standardized to the total mass of the fish, individuals caught deeper in the Mariana Trench had more prey items in their stomachs (ANOVA, 26 df, F=8.10, p < 0.01). In the Kermadec Trench, there was no significant trend (33 df, F=2.74, p=0.108).

3.2. Stomach contents of abyssal species

Sample sizes of abyssal fish collections in this study were too small to categorize the complete diets of abyssal species from the Mariana and Kermadec regions (Table 1). Although stomachs were collected from ten *Spectrunculus grandis*, only six of these had any prey remains present. These remains were all amphipods, in very low numbers (1–6). A few Zoarcidae Gen et spp. stomachs contained amphipods, fish remains, and rocks, however these data are too scant to allow broad conclusions. We found a comparatively diverse collection of prey in *Coryphaenoides armatus*, with contributions from amphipods, fish remains, decapods, polychaetes, and squid (Table 3). One *Coryphaenoides yaquinae* contained a large number of amphipods, possibly a result of collection location, the individual's small size (juvenile, 23 cm standard length), or the artificial food-fall trap environment. Trematode parasites were found in one *Pachycara* sp. and one *Coryphaenoides armatus* from near the Kermadec Trench.

For a quantitative comparison of abyssal and hadal fish feeding, we chose the family Macrouridae, a common, often abundant, and relatively well-studied abyssal group, which have traditionally been considered characteristic abyssal species (e.g. Wilson and Waples, 1983). Although we acknowledge that this comparison likely under-appreciates the importance of other families in the deep abyssal community, especially ophidiids (Linley et al., 2017), the paucity of data limits their inclusion in a statistical assessment. Stomach contents data from Drazen et al. (2008) for *C. armatus* and *C. yaquinae* were compared to results from the present study. Small *C. armatus* (≤20 cm pre-anal fin length) were treated as a separate group from larger *C. armatus*, to account for ontogenetic changes in diet. The category crustacean remains includes euphausids, mysids, isopods, barnacles, tanaids, and galatheid crabs. The contents of stomachs from abyssal macrourids were significantly different than those of the hadal liparids (ANOSIM, Bray-Curtis dissimilarity, 999 permutations, by %N: R =0.7916, p=0.001, by %W: R =0.8749, p=0.001). Although the *C. armatus* and *C. yaquinae* were collected in a different season at a different location, the macrourids that were collected in the present study showed relatively similar results. We therefore believe this to be an appropriate comparison. Principal components analysis revealed that the high abundance of amphipods (high %N), lack of piscivory (low %N of fish remains), and low overall prey diversity in the liparids drove the majority of differences seen in diet between the two groups (Fig. 2). The hadal liparids had low PC1 scores and grouped closely along PC2 whereas the abyssal macrourids had higher PC1 scores and were overall more scattered along both principal axes.

3.3. Isotope analysis

Compound-specific nitrogen isotope analysis of amino acids provided additional information about trophic ecology of these deepest-living fishes. $\delta^{15}N$ values of sixteen individual amino acids were determined for five species (n=3–4). Weighted means of $\delta^{15}N$ values for source amino acids, trophic amino acids, and resulting trophic position estimates are presented in Table 4. All $\delta^{15}N$ values for measured individual amino acids are available in the supplementary information (Supplementary Table 3).

Weighted means of $\delta^{15}N$ values of source amino acids were significantly different between species (Kruskal-Wallis rank sum test, 4 df, p=0.01); higher in hadal species (8.09 ± 0.75‰ Kermadec liparid, 6.11 ± 0.46‰ Mariana liparid) than for abyssal species (4.54 ± 1.01‰ *C. armatus*, 3.05 ± 0.66‰ *C. yaquinae*, 5.79 ± 0.46‰ *S. grandis*; Fig. 3). The ophidiid, *Spectrunculus grandis*, from the Kermadec collection had an intermediate source amino acid value of 5.79 ±

Table 3
Prey tables for abyssal species, including all digestive states. From collection of two *C. armatus*, one *C. yaquinae*, three zoarcids, and six *Spectrunculus grandis* with prey in stomachs.

	Coryphaenoides armatus				*Coryphaenoides yaquinae*			
	%F	**%N**	**%W**	**%IRI**	**%F**	**%N**	**%W**	**%IRI**
Amphipods	50.00	14.29	0.50	7.39	100.00	96.15	76.45	86.30
Crustacean Remains	50.00	0.00	11.65	5.83	0.00	0.00	0.00	0.00
Decapods	50.00	14.29	68.22	41.25	0.00	0.00	0.00	0.00
Fish Remains	50.00	28.57	0.04	14.30	100.00	2.88	2.00	2.44
Polychaete	0.00	0.00	0.00	0.00	100.00	0.96	21.55	11.26
Squid	50.00	14.29	0.11	7.20	0.00	0.00	0.00	0.00
Unidentified	50.00	28.57	19.49	24.03	0.00	0.00	0.00	0.00
	n=2	n=7	27.6 g		n=1	n=104	2.0 g	

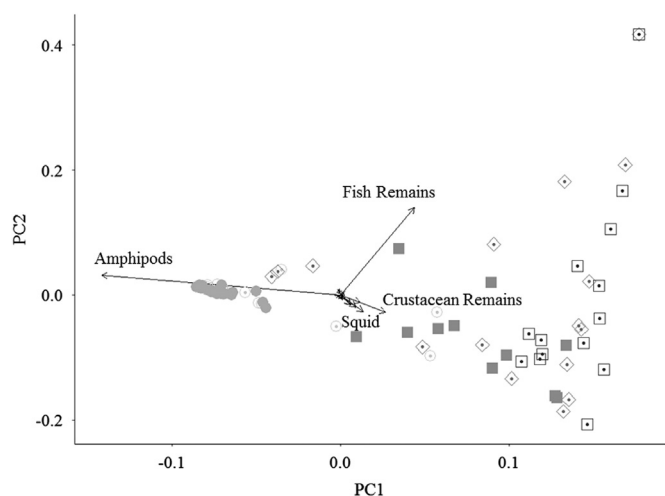|	Zoarcidae Gen et spp.				*Spectrunculus grandis*			
	%F	**%N**	**%W**	**%IRI**	**%F**	**%N**	**%W**	**%IRI**
Amphipods	100.00	98.92	96.10	99.16	100.00	100.00	100.00	100.00
Fish Remains	33.33	0.90	0.08	0.16	0.00	0.00	0.00	0.00
	n=3	n=558	32.8 g		n=6	n=16	0.37 g	

Fig. 2. Principal components analysis comparing diet composition of hadal liparids and abyssal macrourids. Macrourid data from Drazen et al., 2008. Loadings for most important drivers of differences indicated with labeled black arrows. Comparison based on relative numeric abundance (%N) for each individual (n=15 *C. armatus* large, 16 *C. armatus* small (≤20 cm pre-anal fin length), 11 *C. yaquinae*, 37 *N. kermadecensis*, 29 Mariana liparid). Excludes parasites. *Coryphaenoides armatus* (large - open squares, small – diamonds), *C. yaquinae* (closed squares), *Notoliparis kermadecensis* (open circles), Mariana liparid (closed circles) and *Spectrunculus grandis* (open triangles) shown.

Fig. 3. $\delta^{15}N$ values (weighted means) of source amino acids (lysine and phenylalanine) by capture depth. Error bars indicate standard deviations between three runs. Capture region and species labeled for each sample group. *Coryphaenoides armatus* (open squares), *C. yaquinae* (closed squares), *Notoliparis kermadecensis* (open circles), Mariana liparid (closed circles) and *Spectrunculus grandis* (open triangles) shown.

0.46‰ (marginally higher than macrourids, Kruskal-Wallis rank sum test, 1df, p=0.071, lower than liparids, p=0.087). Source amino acid values were higher in the Kermadec liparid than the Mariana liparid (p < 0.05). Bulk tissue $\delta^{15}N$ values ranged from 12.3 to 15.5‰ overall, with an average range of 1.1‰ between individuals of the same species (averages: *C. armatus* 13.6 ± 0.8‰; *C. yaquinae* 12.6 ± 0.8‰; Mariana liparid 13.7 ± 0.5‰; *N. kermadecensis* 13.4 ± 0.7‰; *S. grandis* 15.0 ± 0.8‰; Fig. 4).

Trophic positions (Table 4) were estimated from the weighted means of $\delta^{15}N$ values of source and trophic amino acids according to Bradley et al. (2015) and were found to be significantly different between families (ANOVA, 4 df, F=17.41, p < 0.001). For hadal liparids, trophic levels were estimated at 4.15 ± 0.22 for *N. kermadecensis* and 4.48 ± 0.13 for the Mariana snailfish. Trophic level estimates were significantly higher for macrourids, 5.14 ± 0.01 for *Coryphaenoides armatus* and 5.08 ± 0.02 for *C. yaquinae*, than for liparids or *S. grandis* (Kruskal-Wallis rank sum test, 1 df, p < 0.05). The abyssal ophidiid, *Spectrunculus grandis* had an intermediate
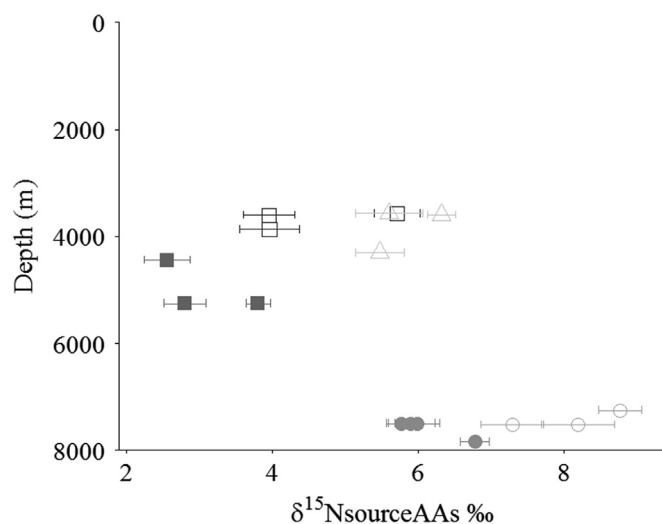
trophic level (4.65 ± 0.30). No significant difference was found in trophic positions between trenches for liparids (p=0.16), suggesting that the species play similar roles in their respective trenches.

Most of the weighted means of source and trophic amino acid $\delta^{15}N$ values were highly consistent between samples of the same species (Fig. 3). Trophic position estimates also varied little within species (Table 4). The $\delta^{15}N$ values of one *C. armatus* sample (#100367) did not cluster as closely as the other species. While the absolute $\delta^{15}N$ values of this sample differed, the estimated trophic position was very similar to the other two samples.

4. Discussion

4.1. Stomach contents

We describe the feeding habits of two hadal snailfishes from the Mariana Trench and Kermadec Trench. Both snailfishes seem to be predatory. Amphipods were the most abundant prey items in stomach contents of both species. This study is the first to document that hadal liparids also feed on decapods and polychaetes. The finding of decapods, large predatory amphipods (*Princaxelia*), and a few other

Table 4

AA-CSIA Results. *C. armatus*, *S. grandis*, and *N. kermadecensis* from the Kermadec Trench, *C. yaquinae* and the Mariana liparid. Depth indicates capture depth (in meters), with individual standard lengths (SL) from fresh measurements. Sample numbers indicate HADES collection information. Standard deviations of weighted means of $\delta^{15}N$ values (‰) for source (lysine, phenylalanine) and trophic (alanine, leucine, glutamic acid) amino acids and trophic positions are presented from three replicate measurements.

| Species	Sample #	Depth (m)	SL (cm)	$\delta^{15}N_{sourceAAs}$	$\delta^{15}N_{trophicAAs}$	Trophic Position
C. armatus	100038	3865	50.6	3.96 ± 0.41	30.40 ± 0.19	5.14 ± 0.17
	100363	3601	78.6	3.95 ± 0.36	30.34 ± 0.12	5.13 ± 0.11
	100367	3569	69.0	5.71 ± 0.31	32.26 ± 0.22	5.16 ± 0.18
C. yaquinae	200008	4441	42.6	2.55 ± 0.32	28.69 ± 0.25	5.08 ± 0.20
	200151	5255	30.6	2.80 ± 0.29	28.82 ± 0.20	5.06 ± 0.17
	200152	5255	77.3	3.80 ± 0.17	29.98 ± 0.18	5.09 ± 0.12
S. grandis	100060	4303	40.4	5.47 ± 0.33	27.44 ± 0.23	4.32 ± 0.19
	100377	3569	33.8	5.60 ± 0.46	30.78 ± 0.15	4.90 ± 0.15
	100364	3601	29.0	6.32 ± 0.19	30.51 ± 0.13	4.72 ± 0.11
N. kermadecensis	100175	7515	18.3	8.19 ± 0.50	28.85 ± 0.22	4.08 ± 0.20
	100310	7251	21.0	8.77 ± 0.30	28.89 ± 0.21	3.99 ± 0.17
	100171	7515	18.3	7.29 ± 0.43	29.75 ± 0.19	4.41 ± 0.17
Liparidae sp. nov.	200039	7497	21.0	5.99 ± 0.31	29.81 ± 0.27	4.66 ± 0.20
	200070	7841	17.2	6.78 ± 0.20	29.11 ± 0.35	4.38 ± 0.17
	200033	7495	12.6	5.89 ± 0.33	28.76 ± 0.22	4.48 ± 0.18
	200041	7497	10.5	5.77 ± 0.18	28.15 ± 0.29	4.39 ± 0.15
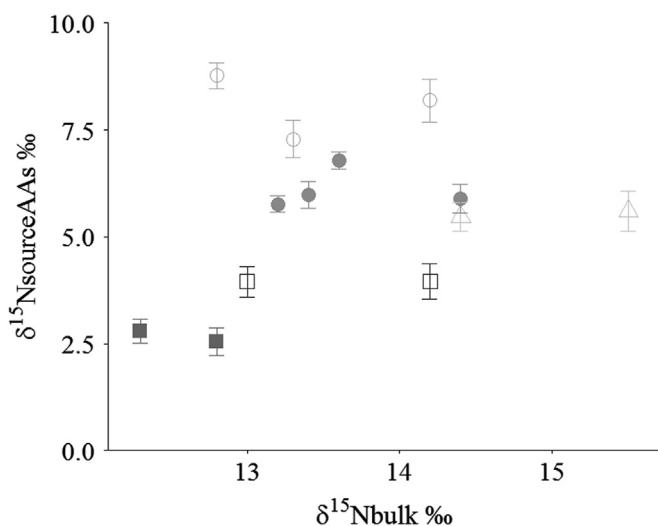
Fig. 4. Source amino acid $\delta^{15}N$ values (weighted means of lysine and phenylalanine) compared to those of bulk tissue from the same individuals. Error bars indicate standard deviation between triplicate runs. *Coryphaenoides armatus* (open squares), *C. yaquinae* (closed squares), *Notoliparis kermadecensis* (open circles), Mariana liparid (closed circles) and *Spectrunculus grandis* (open triangles) shown.

large lysianassoid amphipods (up to 7.6 cm in size) in the diet of hadal snailfishes suggests that liparids can catch fast-swimming animals and are the top predators known in both trenches. The hadal liparids from both trenches have a highly developed and strongly muscularized pharyngeal jaw apparatus to facilitate processing of large, live prey (Gerringer and Linley, unpublished data). Polychaetes were present in stomachs of *Notoliparis kermadecensis* from the Kermadec Trench although they were not found in the Mariana liparid. It is possible that they are not common and/or not present at hadal depths in the Mariana Trench, that they are present but not eaten by liparids, or that they are present and eaten but missed in this collection. The published literature conflicts on whether or not these polychaetes have been collected from the Mariana Trench (Kirkegaard, 1956; Paterson et al., 2009; Gallo et al., 2015; Jamieson, 2015).

Some fish remains and squid beaks were found in stomachs of both hadal liparid species, however, we do not believe squid and fish are captured as live prey. Fish and squid remains found in this study appeared to be from species not known from the hadal zone, further suggesting that these fishes were consumed as carrion. The relative sizes of the squid and the snailfishes also indicate that these remains were ingested after dying. The morphology of the hadal liparid jaw, with small palatine teeth, also makes it unlikely that hadal snailfishes are catching live fish prey (Nielsen, 1964). Many of the squid and fish remains were found surrounded by, even thoroughly embedded with, large numbers of amphipods. The hadal snailfishes were likely targeting swarms of amphipods that were feeding on squid or fish remains. This behavior has been seen several times in video observations by Linley et al. (2017), who also suggest that ingestion of carrion is relatively infrequent and probably incidental in hadal liparids.

Prey accumulation curves suggest that our sampling effort likely captured much of the diversity of the diet of the Mariana Trench liparids. However, our limited number of specimens probably prevented us from describing the complete diet of the Kermadec Trench liparids. Trap effects have not confounded the overall trends described in this study. Amphipods also made up the majority of highly digested prey items. High proportions of amphipods were also found in both the *N. kermadecensis* holotype (10 amphipods) and paratype (16 amphipods) collected by trawl (not by trap) on the *Galathea* expedition (Nielsen, 1964).

Amphipod abundance increases with depth in the hadal zone (Jamieson, 2015), suggesting a potential advantage to predatory fishes descending into the trench. The increase in number of prey items with

depth of capture in the diet of the Mariana liparid could suggest that in the Mariana Trench amphipods were increasingly abundant or available with increasing depth. It is possible that the Mariana amphipod distributions or availability contribute to the relatively deeper distribution of the Mariana liparid (Linley et al., 2016), but greater sample sizes across the full depth range of these species are needed before firm conclusions can be reached.

Our principal components analysis of stomach content composition supports the hypothesis that there are indeed differences in feeding habits between the abyssal macrourids and hadal liparids. According to stomach contents analysis, hadal liparids are more selective predators, clustering tightly in the principal components analysis. The high degree of scatter in the macrourid data show that these abyssal species have varied generalist diets and rely heavily on carrion and squid (Drazen et al., 2008). The few macrourid individuals available for stomach contents analysis in this study support this trend. With limited numbers of species and individuals available, we were not able to fully characterize the diets of abyssal species for comparison to the hadal community.

Fortunately, a few existing studies allow for qualitative comparison of hadal fish feeding ecology to that of other abyssal families. Crustacean, gastropod, polychaete, and detritus remains were observed in radiographs of three specimens of *S. grandis* (Uiblein et al., 2008). A more detailed stomach contents analysis of *S. grandis* (n=9, 2000–2500 m collection depth), found a mixed diet (actinozoans, polychaetes, amphipods, tanaids, mysids, euphausids, decapods, cephalopods, echinoderms, chateognaths, and fish fragments), with the largest contributions from epibenthic fauna (Mauchline and Gordon, 1984). Very few studies exist on the feeding ecology of zoarcids (e.g. Ferry, 1997), which have representative species at upper hadal depths (Linley et al., 2016). One deep-sea zoarcid, *Lycodes atlanticus*, was found to rely on benthic invertebrates, with a diet of sponges, polychaetes, gastropods, pycnogonids, ostracods, isopods, amphipods, and ophiuroids (n=34, 723–2251 m collection depth, Sedberry and Musick, 1978). There are other abyssal fish taxa that reach near-hadal depths that could have potentially colonized trenches. One such group are the Chlorophthalmoids (Order Aulopiformes), including the deep-sea tripodfishes, Ipnopidae. *Bathypterois longipes, B. grallator,* and *B. phenax* stomach contents were found to contain mostly crustaceans (copepods, amphipods, decapods, ostracods, and mysids, collection depths 1239 – 5345 m) suggesting that these are epibenthic crustacean feeders (Crabtree et al., 1991). Other members of this order are believed to rely on the benthic food web, with polychaetes, bivalves, and copepods making up the majority of their stomach contents (*Ipnops murrayi*, n=43, 1239–4539 m collection depths, Crabtree et al., 1991). These results suggest a potentially higher diversity of diet components than seen in hadal liparids. It is possible that these abyssal species with less-specialized feeding have had less selective pressure to descend far into hadal trenches.

Investigations of synaphobranchid eel diet showed carrion to be of significant importance, while amphipods did not contribute (Merrett and Domanski, 1985; Jones and Breen, 2014). This tendency towards scavenging is supported by a functional morphology analysis of *Synaphobranchus brevidorsalis* and *Ilyophis brunneus* (Eagderi, et al., 2016), shallower representatives of abyssal genera. Synaphobranchids at abyssal depths are thought to rely on a diet of largely dead or dying pelagic species and have been shown to wait for larger scavengers to tear carrion, making it more accessible (Jamieson et al., 2011a). While benthic biomass of small crustaceans such as amphipods may increase in the hadal zone, the occurrence of large carrion falls would not be a function of depth (Linley et al., 2017). Perhaps this fact and the lack of large scavengers such as sharks have resulted in little selective pressure for these eels to colonize the hadal zone. Future study on abyssal ophidiids and zoarcids, and other deep abyssal taxa (Synaphobranchidae, Ipnopidae) will be needed to fully characterize this relationship.

4.2. Isotope analysis

The $\delta^{15}N$ values of source amino acids can be used to trace origins of nutrient input to the hadal zone. This input can be thought of in two major pathways; first – sinking particles that slowly descend to hadal depths and organic material that accumulates through downslope transport due to trench topography and seismic activity, and second – organisms that rapidly sink after death in overlying waters. Both sources ultimately come from the euphotic zone, however processing times and mechanisms are distinct for each. This has important implications for the isotopic compositions of source amino acids from the two pathways. Large carrion falls will sink quickly, carrying with them the isotopic compositions of the feeding depth of the carrion. We can therefore consider input from carrion as derived from the euphotic zone with source amino acid $\delta^{15}N$ values that are comparatively low representing the primary producers at the base of the food web (Hannides et al., 2009). Lower source amino acid $\delta^{15}N$ values in the macrourids are consistent with a more upper ocean-derived food source, with larger input of fast-sinking carrion. These lower values support the reliance on fast-sinking carrion found previously (Drazen et al., 2008). Conversely, small particles will be reprocessed as they sink, becoming increasingly enriched in ^{15}N with depth through the multiple microbial trophic interactions that occur throughout this long descent (Hannides et al., 2013). Those organisms that are more closely tied to the benthic food web (that consume detritivores or their primary and secondary predators) will have higher source amino acid $\delta^{15}N$ values reflecting that relationship. Particles that have followed the slow sinking pathway become the primary base of the benthic food web, although this is not always the case if there are significant inputs of rapidly sinking larger particles (e.g. after a spring bloom) which could have lower source amino acid $\delta^{15}N$ values (McCarthy et al., 2007). The liparids with higher weighted mean source amino acid $\delta^{15}N$ values appear more directly connected to the benthic hadal food web, where nutrient input is primarily sinking particles.

The ophidiid, *S. grandis*, had higher $\delta^{15}N$ values in source amino acids relative to the abyssal macrourid, *C. armatus*, collected from a similar depth in the same region, suggesting a less surface-derived food source. It is therefore likely that *S. grandis* is more closely dependent on the benthic food web, supported by the few stomach contents data available in the present study and in the literature (Mauchline and Gordon, 1984; Uiblein et al., 2008). The source amino acid values may be lower than in the liparids because *S. grandis* occasionally feeds on carrion, as seen in some video observations (Janßen et al., 2000; Henriques et al., 2002; Kemp et al., 2006; Cousins et al., 2013). Source amino acid $\delta^{15}N$ values from *N. kermadecensis* collected from the Kermadec Trench were slightly higher than those of the Mariana Trench liparid. The $\delta^{15}N$ values of the isotopic baseline may therefore be higher in waters overlying the Kermadec Trench. This could be a result of differences in sources of nitrate (with different $\delta^{15}N$ values) to waters in these environments, the extent of nitrate utilization by phytoplankton in these regions (Waser et al., 1998; Sigman et al., 2009), or nitrogen fixation dominating the nitrogen source of phytoplankton (Montoya et al., 2002; Hannides et al., 2009).

Our results also suggest that conclusions from bulk tissue N isotope measurements should be drawn cautiously for hadal organisms. It is well documented that shifts in source amino acid isotope values can strongly influence the interpretation of bulk tissue $\delta^{15}N$ values (e.g. Hannides et al., 2009; Choy et al., 2015; Nielsen et al., 2015). In our study, fishes with the highest bulk tissue $\delta^{15}N$ values (*N. kermadecensis*, Mariana liparid; Fig. 4) did not have the highest trophic positions, highlighting the limitations of drawing conclusions from bulk tissue alone. Amino acid specific analyses show that the higher bulk tissue $\delta^{15}N$ values in hadal liparids are due to high values in the 'isotopic baseline,' rather than trophic position alone. These results further demonstrate the value of the compound-specific method for isotope measurements of individual amino acids, particularly in

systems like hadal trenches, where the base of the food web is extremely difficult to determine and therefore is poorly characterized.

This study provides the first trophic position estimates for fishes from the hadal zone. Based on trophic position (~4) and stomach contents, liparids are likely the top predators below the abyssal-hadal boundary. Our results suggest that hadal amphipods are at approximately trophic level three, which follows previous analysis, although there is a large degree of variation in amphipod diet (Blankenship and Levin, 2007). The trophic level of liparids is also relatively high, partially due to input from predatory crustaceans. High trophic levels in macrourids support previous findings that documented macrourid reliance on small fishes and squid, as well as large carrion falls (Drazen et al., 2008). The higher source amino acid value in one *C. armatus* could suggest a smaller contribution of upper-ocean carrion for that individual.

4.3. Fishes in the hadal food web

Our findings on the feeding ecology of hadal fishes contribute to a new understanding of trophic interactions in the deepest seas. Where present (e.g. Kermadec, Mariana, Japan, Peru-Chile trenches), hadal snailfishes are the top known predators of the upper hadal zone. These fishes suction feed on mainly amphipods, though they also catch predatory crustaceans such as decapods and *Princaxelia* amphipods. Some hadal liparids will also eat polychaetes. Hadal amphipods are known to have extremely diverse prey, including carrion, urochordates, ascidians, salps, diatoms, detritus, polychaetes, and copepods (Blankenship and Levin, 2007) and are even known to cannibalize one another in an artificial trap environment (Ingram and Hessler, 1983). The amphipods obviously make up an important part of the hadal food web, (Blankenship and Levin, 2007) and clearly are the most important prey of hadal snailfishes.

We are a long way from a complete understanding of the energetic pathways at work in the hadal zone. Deep-sea trenches are still relatively unexplored, and undiscovered species and interactions probably outnumber the known. Our understanding of the hadal community is heavily biased by gear type, as the difficulties and high costs of sampling at such high hydrostatic pressures favor the use of free-vehicle cameras and traps and the study of bait-attending fauna (e.g. Jamieson et al., 2011c). Even if we had a thorough understanding of hadal community structure, construction of a hadal food web is not straightforward. We found evidence for variation in trophic interactions between trenches, such as a higher prey diversity in liparids from the Kermadec Trench as compared to those in the Mariana Trench. A common hypothesis for inter-trench variability is that productivity of surface waters overlying the trench will affect the community below. The extent of this relationship and the effects of downslope funneling and organic matter accumulation due to trench topography have yet to be fully characterized (e.g. Itou et al., 2000; Ichino et al., 2015). Further, the depth-related changes in community structure, present in most groups, mean that trophic interactions at 6000 m are likely different than those at 9000 m in the same trench. This has been demonstrated in some hadal amphipods (Blankenship and Levin, 2007), but likely spans to other taxa as well. For example, the probable lack of fishes below ~8200 m (Yancey, et al., 2014) would of course lead to a very different food web in the lower reaches of the trench than that seen in the upper hadal zone. More investigation of these trophic relationships and on processes in the environments overlying hadal trenches will be needed to understand the hadal food web and the role of trenches in global biogeochemical cycling.

5. Conclusions

This study provides the first in depth investigation of the trophic ecology of fish species in the hadal zone, using multiple approaches. Hadal liparids are clearly predatory, relying heavily on amphipods as a

food source, as seen in stomach contents analysis. This is supported by video observations of hadal liparids *in situ*, which showed high numbers of predatory feeding events in hadal liparids (Fujii et al., 2010; Jamieson et al., 2009b; Linley et al., 2016). High $\delta^{15}N$ values of source amino acids also suggest that hadal liparids are closely tied to the benthic food web. Macrourids from the abyss near the Kermadec and Mariana trenches displayed a high degree of trophic plasticity (stomach contents analysis diversity, in agreement with previous findings), a close linkage to the pelagic food web (lower $\delta^{15}N$ values of source amino acids), and a high trophic level of > 5, further suggesting the importance of both carrion and live fish and squid to their diet. Although more research is needed on other abyssal groups (Ophidiidae, Zoarcidae, Synaphobranchidae) our results demonstrate differences in feeding strategy between characteristic abyssal species (Family Macrouridae) and dominant endemic hadal species (Family Liparidae).

Trophic interactions may be important evolutionary drivers of depth zonation patterns in abyssal and hadal fishes. At the upper edges of the trench, the hadal fish community resembles that found on the abyssal plain, with macrourids, ophidiids, zoarcids, and synapho-branchids. From depths around 6500–8200 m, in a number of trenches, however, the fish fauna seems to shift to a dominance by the family Liparidae (Jamieson et al., 2011c; Linley et al., 2016). While scavenging and piscivorous fishes do not extend far into the hadal zone, suction-feeding predatory fishes are dominant. This community shift at the upper edges of the trench may, in part, relate to a difference in trophic strategy. The increased amphipod biomass in the hadal zone compared to the abyss may provide little benefit for macrourids and synaphobranchids to descend to hadal depths, but large advantage for suction-feeding fishes such as liparids. This may be one of the reasons why liparids are so notably successful in many hadal trenches.

Contributors

AJJ, TDL, MEG, and JCD collected specimens. MEG and JCD analyzed stomach contents. MEG and BNP conducted and interpreted isotope analyses. All authors contributed to the writing and editing of the manuscript and the discussion of ideas therein, and have approved this version for submission.

Acknowledgments

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.dsr.2017.01.003.

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