1 **IN SITU RESPIRATION MEASUREMENTS OF MEGAFUAUANA IN THE KERMADEC TRENCH**

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10 **Abstract**

11 The aim of this paper is to measure metabolic rates of megafauna living in depths greater than 6000m.
12 Echinoderms, actinarians and a polychaete were captured by remotely operated vehicle (ROV) and inserted
13 into respiration chambers in situ at depths of 4049m, 7140m and 8074m in the region of the Kermadec Trench
14 SW Pacific Ocean. Hadal research has moved into a new frontier as technological improvements now allow for
15 a meticulous investigation of trench ecology in depths greater than 6000 meters. The development of an in
16 situ respirometer for use in these studies was deployed in the Kermadec Trench to obtain the first ever rates of
17 basal metabolic rates of hadal megafauna. Typical deep-sea experiments of individual animal physiology must
18 deal with covarying factors of pressure, temperature, light and food supply in this study investigated the
19 effects of pressure and increased food supply on overall animal metabolism. In the Kermadec Trench,
20 holothurian respiration rates (n=4), 0.079 ± 0.011 (mean $\pm$ SE) $\mu\text{mol-O}_2 \text{ g}^{-1} \text{ h}^{-1}$, were higher than those captured
21 at abyssal depths (n=2), $0.018 \pm 0.002 \mu\text{mol-O}_2 \text{ g}^{-1} \text{ h}^{-1}$, in the same region ($p < 0.001$). When Q_{10} adjusted to a
22 common temperature of 2.5 °C trench holothurian respiration rates ranged between 0.068 and 0.119 $\mu\text{mol-O}_2$
23 $\text{g}^{-1} \text{ h}^{-1}$. Anemone respiration rates were remarkably similar between abyssal and hadal specimens, 0.110 and
24 0.111 $\mu\text{mol-O}_2 \text{ g}^{-1} \text{ h}^{-1}$, respectively. Our results on echinoderm respiration when corrected for temperature
25 and mass fall below the slope regression when compared with other in situ measurements at shallower ocean
26 depths.

27 **Keywords**

28 Respiration; hadal; holothurian; polychaete; anemone; Kermadec Trench; in situ; metabolism.

29

30 1. Introduction

31 Hadal (depths greater than 6,000 meters) investigations have focused primarily on examining the
32 diversity and in some cases relative abundances of the trench fauna. These studies have identified
33 declines in diversity and suggest that meiofaunal (Danovaro *et al.*, 2002; George and Higgins, 1979;
34 Itoh *et al.*, 2011), megafaunal (Beliaev, 1989) and holothurian (Hansen, 1956; Wolff, 1960)
35 abundances increase with depth. The trenches represent the deepest ~45% of the oceans and
36 increasing hydrostatic pressures may limit the species that have evolved the capacity to live there.
37 Increased abundance of some taxa may exist due to increased food supply from channeling of
38 detritus, downslope transport of sediment, and turbidity flows from seismic activity (reviewed in
39 Jamieson *et al.*, 2010). Indeed accumulation of organic carbon in trenches may actually increase as
40 the distance from surface production increases (Ichino *et al.*, 2015; Itou, 2000; Oguri *et al.*, 2013).

41
42 Little research has yet begun to explore hadal food webs and carbon budgets. An important
43 component of such research is understanding trophic linkages. The diets of a few fauna such as
44 amphipods have been investigated with a variety of biomarkers (Blankenship and Levin, 2007;
45 Kobayashi *et al.*, 2012; Perrone *et al.*, 2003). Another important component is determining the
46 energy usage or metabolic rates of individual taxa or faunal groups. Metabolic rates can be used to
47 construct models of the flow of energy and materials in an ecosystem (Childress and Thuesen, 1992;
48 Christiansen *et al.*, 2001; Smith, 1992; Smith *et al.*, 2001) . In the deep sea, energetic demands can be
49 extrapolated from data on shallow living animals by using models of the mass and temperature
50 dependence of metabolic rate (Mahaut *et al.*, 1995), or they are based on a handful of measurements
51 of representative taxa (Ambrose *et al.*, 2001; Piepenburg and Schmid, 1996; Smith, 1992; Smith *et al.*,
52 2001). It remains unclear whether the energetic demands/metabolism of the trench fauna can be
53 extrapolated from work on shallower living animals.

54
55 Pelagic fish, crustaceans and cephalopod metabolism in the deep-sea is predicted to vary with light
56 levels, and thus depth to about 1000m, and the nature of predator prey interactions (Childress, 1995;
57 Seibel and Drazen, 2007). In the dimly lit and sparsely populated deep-sea, predators and prey do
58 not interact as frequently or over as large a distance, relaxing the need for locomotory capacity which
59 reduces metabolism. This argument is supported by the fact that sighted taxa exhibit depth-related
60 metabolic declines but non-visual groups such as holothurians do not (Hughes *et al.*, 2011; Seibel and
61 Drazen, 2007). In contrast, recent data on non-visual copepods found declines from the epipelagic to
62 the abyssopelagic (Ikeda *et al.*, 2006; Wilson *et al.*, 2013). The role of food supply in governing the
63 evolution of individual basal metabolic rates has largely been discounted (McClain *et al.*, 2012; Seibel
64 and Drazen, 2007) but a variety of animals respond to low rations by depressing routine metabolism
65 (Christiansen and Diel-Christiansen, 1993; Sullivan and Smith, 1982; Yang and Somero, 1993)
66 suggesting at least some effect intraspecifically. Past studies suggest that pressure does not greatly
67 affect metabolic rates (Belman and Gordon, 1979; Childress, 1977; Meek and Childress, 1973) though
68 there are clearly many examples of molecular adaptation to ensure cellular activity and the depth
69 limits of different groups are likely constrained by their physiological adaptations to pressure (Yancey
70 *et al.*, 2014). In previous studies it has been difficult to separate the covarying effects of food supply,
71 temperature, light, and pressure on metabolic rates. Thus in situ measurements of hadal respiration
72 rates could provide a test both for the effects of pressure and that of increased food availability,
73 while keeping other factors of light, and temperature constant.

74
75 Hadal ecological studies have entered a new era of investigation due to advances in submersible
76 vehicles and full-ocean depth rated instruments. The development of hadal Remotely Operated

77 Vehicles (ROV) such as Kaiko (Kyo *et al.*, 1995) and Nereus (Bowen *et al.*, 2009) have provided the
 78 ability to explore the hadal environment in a reactive manner and to precisely make measurements,
 79 take samples, and conduct experiments. Here, we use a full-ocean depth *in situ* respirometer,
 80 manipulated by Hybrid Remotely Operated Vehicle (HROV) Nereus, within the Kermadec Trench, to
 81 measure hadal animal metabolism for the first time. Our objective was to test echinoderms and
 82 abundant megafauna found commonly in the hadal realm for variations in overall respiration as
 83 depth and ostensibly food supply increased within the Kermadec Trench (Ichino *et al.*, 2015; Itou *et al.*,
 84 *et al.*, 2000)(Figure 1). Lack of suitable dives during the expedition did not provide us with the expected
 85 abundance of respiration measurements. However, we do compare our limited measurements to
 86 bathyal and abyssal species and provide data that can ultimately be used to compose hadal carbon
 87 budgets and food web models.
 88

89
 90 **Figure 1.** Map of study area. Inset shows the location of the study area
 to the North of New Zealand. Station details are given in Table 1.

91 **2. Materials and Methods**

92 **2.1 In Situ Respirometer**

93 The respirometer consisted of five main components that allow for in situ measurement of oxygen
94 consumption anywhere in the ocean. These were the pressure housing, junction box, Deep-Sea Power and
95 Light (DSPL) battery, umbilical/manifold and the chamber assembly (Figure 2A). The pressure housing, a
96 titanium cylinder, contained data recorders and a voltage regulator that modulated the flow rates of stirring
97 pumps. Adjustable flow rates allowed us to deploy the respirometer with both large (0.97 L) and small (0.60 L)
98 chambers that could be swapped out depending on the assumed target animal size. Tests measured total
99 volume turnover of less than one minute using an injected dye to calibrate homogenous but gentle mixing.
100 The junction box is an oil filled volume that incorporated the electrical and data feed of the pressure housing,
101 the power supply from the battery which was distributed through the umbilical cable. A 5 meter umbilical
102 cable served as the conduit for all functions necessary for the incubation of animals within the chamber
103 assembly. The long length allowed ample manipulative space for respirometer assembly after being placed
104 onto the elevator platform. The manifold dispersed data feeds and power to each oxygen optode/pump pair
105 that made up the instrument package of the individual incubation chambers. The chamber assembly consisted
106 of two parts, the collection chambers and the lid assembly. The incubation chambers were sealed once the
107 two halves are joined and locked together by the ROV. Viton o-rings were using on the chambers to ensure a
108 tight seal that would prevent leaks. Once assembled the oxygen optode (Aanderaa) recorded dissolved oxygen
109 and temperature values at specified intervals (30 seconds) and the submersible pump (SeaBird) ensured
110 adequate water flow to ensure a homogenous water volume. The respirometer was deployed using an
111 elevator which delivered the pressure housing, junction box, battery and the lid assembly to the seafloor. The
112 delivery of the in situ respirometer with the elevator eliminated extra ROV bottom time freeing it for more
113 dive tasks. The incubation chambers were carried to the seafloor on the instrument tray of the HROV Nereus.
114 Stainless steel trap doors with a spring mechanism were held open until an animal was deposited into a
115 chamber using the Nereus slurp gun. The traps doors were perforated with large holes that prevented animals
116 from reaching the optode or pumps when closed and also allowed the pump to circulate and mix the chamber
117 water.

118 2.2 Animal Collection

119 All but the chambers of the respirometer were deployed on a hadal elevator (instrument package, battery, and
120 pressure housing) prior to HROV Nereus dives. Nereus, carrying the rosette of animal chambers, used its
121 suction sampler to collect suitable animals (echinoderms, anemones and polychaetes), place them in individual
122 chambers and close the trap-door lids as it proceeded towards the elevator site. At the elevator the
123 respirometer was assembled beginning experiments (Figure 2B) on the elevator, free from the motion and
124 activity of the ROV. The first 2-3 hours of each experiment were not used to allow the animals to adjust to the
125 chamber. Once regular oxygen declines began, data was considered suitable for analysis. Experiments ranged
126 in duration from 14 to 35 hrs. At the end of incubations animals were retrieved upon recovery of the elevator
127 at the surface. Collection of animals and experiments were performed between April 11 and May 4, 2014
128 (Table 1). Unfortunately the number of planned experiments was curtailed by the loss of the HROV Nereus on
129 May 9, 2016 due to catastrophic implosion of the vehicle at 10,000 meters.

130 Replicate measurements were made by dividing the linear consumption of oxygen into equal time segments
131 and calculating the resulting rate. Each in situ set of experiments were accompanied by a control experiment
132 that measured oxygen consumption in an empty incubation chamber. This value was used to account for
133 microbial respiration in the ambient bottom water.

Figure 2. A. The in-situ respirometer components. 1) The umbilical/manifold which consists of the cable connecting the junction box to the manifold and the optodes and pumps used to conduct the incubations. 2) The Deep-Sea Power and Light battery. 3) The chamber assembly carried by the ROV for collection of experimental animals. 4) The pressure housing that regulates power input to the pumps and records dissolved oxygen and temperature data from the optodes. 5) The junction box unifies power and data transmission and distributes to the manifold and pressure housing accordingly. **B.** The fully assembled respirometer as seen from the HROV Nereus in the Kermadec Trench.

2.3 Tissue Processing

Animals were weighed wet after recovery using a motion compensated scale and submerged in seawater to determine displacement volume using a graduated cylinder. Tissue samples were collected from each incubated organism by dissecting a large piece of the body wall of holothurians or the entire cross section for other animals and frozen in cryo-vials in liquid nitrogen and stored at -80°C. In the lab, tissue was weighed wet placed into pre-combusted tin foil wells and dried for 48 hours at 60°C to obtain dry weight. Samples were then combusted for 4 hours at 550° C and weighted for the remaining tissue as ash. Ash free dry weight was calculated by subtracting the remaining tissue from the dried value.

2.4 Mass specific respiration rates and Q_{10} adjustments for temperature

Respiration was calculated by dividing the change in dissolved oxygen ($\Delta[O_2]$) within the experimental water volume (V) by the time (T) of the incubation. All chamber volumes were corrected for the animal by assuming a density of 1 ml per gram. Mass specific respiration rates are then calculated by dividing by the mass of the animal (M) (Equation 1).

$$R = (\Delta[O_2] \times V) / (T \times M) \quad (\text{Eq. 1})$$

To compare hadal animal metabolism to values in the literature, we adjusted values to a common temperature of 2.5°C using the Q_{10} value of 2.15 as used in Hughes *et al.* (2011). None of our other experimental organisms

151 can boast the number of published studies to accomplish a similar analysis, thus 2.15 was used for Q_{10}
152 transformations on all experimental rates.

153 **3. Results**

154 The respirometer was deployed 9 times in the region of the Kermadec trench; one test site at ~1600m and
155 then at roughly 1000 m increments from 4000 to 10000 meters. Aside from the 1500 meter test site,
156 consistent changes in oxygen and temperature were seen with depth proceeding from the abyssal to the
157 deepest hadal sites (Table 1). Temperature within the Kermadec trench was 1.16 at 6000 meters and
158 increased to 1.83 at 10,000 meters via adiabatic heating (Jamieson *et al.*, 2010; Taira *et al.*, 2005). Dissolved
159 oxygen was highest (~205-207 μ M) at abyssal depths, with a regular but small amplitude decline to a depth of
160 9989 meters (171 μ M).

161 **Table 1.** Depth of elevator, bottom water dissolved oxygen (DO) and temperature (T), and sample data
162 obtained from 9 deployments of the in situ respirometer.

SITE	DEPLOYMENT DATE (GMT)	DEPTH (m)	[O ₂] (μ M)	T (°C)	Latitude (DM)	Longitude (DM)
1S	12-APR	1578	175	2.80	-37.2010865	178.882473
2T	13-APR	4049	207	1.32	-37.733695	179.81889
5T	21-APR	5025	205	1.07	-37.16967333	-179.73402
9T	1-MAY	6069	199	1.16	-35.91150333	-178.9644967
9Tb	2-MAY	6084	199	1.16	-35.91752333	-178.96607
11T	5-MAY	7140	192	1.33	-35.846925	-178.8788183
12T	6-MAY	8074	185	1.48	-34.34371167	-178.1748133
13T	7-MAY	9175	179	1.68	-32.85133	-177.6595567
14T	8-MAY	9989	171	1.83	-31.93094167	-177.289845

163

164 Specimens ranged in size from 1 to 53 grams wet weight (Table 2). Specimens captured at abyssal site were all
165 larger than the hadal specimens except for the ophiuroid. Holothurians captured at 7130 and 8074 meters for
166 respiration experiments were 2 grams wet weight. The polychaete and anemone were the largest animals
167 incubated at hadal depths, with masses of 6 and 4 grams respectively. Ash-free dry weight as a percentage of
168 wet weight ranged from 1.125 to 13.95 %. The range of water content among specimens was 55.9 to 90.4 %.

169 Respiration rates varied by taxa (Table 2). The highest mass specific rate was for the ophiuroid from 4000m
170 depth and the lowest rates were for the large holothuroids from this same station. The mass specific
171 respiration rates of the hadal holothurians were higher than those from the abyssal station but all of these
172 differences are largely driven by variations in body mass. Presentation of mass specific animal respiration as a
173 function of body mass clearly illustrates this point (Figure 3). Due to the limited amount of data comparisons
174 between depths cannot be made robustly. However, generally the respiration rates of the ophiuroid,
175 polychaete and abyssal anemones were higher than rates for the holothurians, accounting for the variation in
176 masses (Figure 3).

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179

180

181 **Table 2.** Masses and respiration rates (whole animal and mass specific – mean and standard error) of
182 abyssal and hadal organisms collected for incubation experiments. Values are corrected to a standard
183 temperature of 2.5 °C using a Q₁₀ value of 2.15 (see text).

Site	Phylum, Class	Taxon	Wet Weight (g)	% Water	Respiration Rate (μmol-O ₂ h ⁻¹)	2.5 °C	Mass specific Respiration Rate (μmol-O ₂ g ⁻¹ h ⁻¹)	2.5 °C
2T	Echinodermata, Holothuroidea	<i>Bathyplores</i> sp.	14	90.4	0.34 ± 0.017	0.38	0.025 ± 0.0006	0.027
	Echinodermata, Holothuroidea	<i>Abyssocucumis abyssorum</i>	53	86.1	0.57 ± 0.038	0.62	0.011 ± 0.0004	0.012
	Cnidaria, Anthozoa	Actinostolidae	24	81.8	1.40 ± 0.17	1.53	0.058 ± 0.007	0.064
	Cnidaria, Anthozoa	Hormathiidae	24	55.9	1.33 ± 0.029	1.45	0.055 ± 0.001	0.060
	Echinodermata, Ophiuroidea	<i>Ophiolimna</i> sp.	1	76.9	0.22 ± 0.015	0.25	0.22 ± 0.016	0.25
11T	Echinodermata, Holothuroidea	<i>Elpidia glacialis kermadecensis</i>	2	76.5	0.16 ± 0.077	0.17	0.078 ± 0.038	0.086
	Echinodermata, Holothuroidea	<i>Elpidia glacialis kermadecensis</i>	2	70.1	0.13 ± 0.038	0.14	0.063 ± 0.019	0.069
	Annelida, Polychaeta	<i>Macellicephala</i> sp.	6	68.5	0.60 ± 0.16	0.66	0.100 ± 0.027	0.110
12T	Echinodermata, Holothuroidea	<i>Elpidia glacialis kermadecensis</i>	2	67.9	0.13 ± 0.015	0.14	0.063 ± 0.007	0.068
	Echinodermata, Holothuroidea	<i>Elpidia glacialis kermadecensis</i>	2	74.1	0.22 ± 0.038	0.24	0.11 ± 0.019	0.12
	Cnidaria, Anthozoa	Actinostolidae	4	78.8	0.23 ± 0.017	0.25	0.058 ± 0.004	0.063

184

185 Comparisons were made to the other existing in situ respiration measurements available in the literature
186 (Hughes *et al.*, 2011; Smith, 1983) and generally the rates were similar to those we made at abyssal and hadal
187 depths (Figure 3). For the ophiuroids our abyssal animal, while having a relatively high rate compared to the
188 other animals we tested, has a considerably lower respiration rate compared to bathyal species of similar size
189 (Smith, 1983). Respiration of the abyssal holothuroids is similar to other abyssal holothuroids (Hughes *et al.*,
190 2011). However, the four hadal holothurian measurements are somewhat lower than those for bathyal
191 holothurians if one compares deviations from the regression between respiration and mass for all the
192 holothurians (Figure 3).

Figure 3. In situ respiration rates as a function of animal mass for abyssal and hadal megafauna including other in situ measurements from the literature (Smith 1983; Hughes et al. 2011). All values are standardized to 2.5 °C. Regressions are for all data (solid line), all ophiuroids (orange line) and all holothuroids (blue line).

4. Discussion

Though it is not possible to evaluate our original hypotheses about the effects of depth on the respiration rates of benthic invertebrates in the Kermadec trench, this is the first study to measure the respiration rates of hadal animals (to ~8000m) and one of only a few to measure abyssal animal respiration rates. The data can be compared to measurements of other deep sea megafauna. An excellent study using a similar in situ ROV operated respiration system measured ophiuroid and holothurian respiration at abyssal depths in the North Atlantic (Hughes *et al.*, 2011). Our measurements of hadal and abyssal species are quite similar. This general observation is in keeping with the finding that the movement levels or activity of a hadal holothurian was similar to shallower taxa (Jamieson *et al.*, 2011). Measurements of an ophiuroid, *Ophiophtalamus normani*, respiration made by Smith (1983) was much higher than our single measurement of an ophiuroid at 4049 meters on the abyssal plain. Given that nutritional status (e.g. Brockington and Clarke, 2001), growth (e.g. Fraser *et al.*, 2004), and other factors can affect respiration rates in echinoderms, it is premature to speculate as to the reasons for these differences.

Expanding on the work of Hughes *et al.* (2011) we plotted our echinoderm respiration rates against capture depth (Figure 4). Like the Hughes *et al.* (2011) echinoderm data our rates were normalized for a standard 15 grams wet weight and for a temperature of 12°C. Our abyssal rates fall below the regression line but are comparable to the rates reported by Hughes *et al.* (2011). Interestingly of the

215 rates that fall below the line at abyssal depths, the two values closest to the regression line belong to
 216 ophiuroids. However the Hughes *et al.* (2011) value came from a much larger specimen than ours, 8.3
 217 grams compared to 1 gram.

218
 219 **Figure 4.** Echinoderm metabolic rate (V_{O_2}) as a function of Capture Depth. Data comes from the
 220 collated data and new rates as reported in Hughes *et al.* (2011) (Historic) and those generated by this
 221 study (HADES). Data from our work was transformed to be comparable by standardizing to a
 222 echinoderm class specific weight of 15 grams and for a temperature of 12°C.

223 Anemones are rarely used as organisms for respiration experiments in the deep-sea thus few
 224 comparisons can be made. Ex situ incubations of anemones recovered in a trawl from 477 meters in the
 225 Gulf of Mexico and incubated at 8.5°C (Nunnally unpublished data) when corrected for temperature of
 226 2.5 °C had respiration rates of 0.049 and 0.051 $\mu\text{mol-O}_2 \text{ g}^{-1} \text{ h}^{-1}$ at 111 and 140 grams, slightly lower than
 227 those measured in situ in this study.

228 The measured rate of the large polynoid polychaete captured and incubated by the in situ respirometer
 229 was comparable with in situ experiments of pelagic polychaetes (Thuesen and Childress, 1993).
 230 Thuesen and Childress (1993) measured the respiration rates of five pelagic mid-water polychaete
 231 species captured at 1 ATM and incubated at 5°C. Their results showed a high respiration rate of 3.928
 232 $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$ for *Tomopteris pacifica* and a low of 0.068 $\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$ for *Poebius meseres*. Our value

of $0.094 \mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$ is near the median of their 5 measured species. However the biomasses of the three pelagic species measured were much smaller than the benthic polynoid we captured, 0.02 to 1.2 grams compared to 6 grams. The largest of which had the lowest oxygen consumption rate. Predator-prey dynamic in the pelagic realm would dictate a fast moving 'errant' polychaete which would have a nominally high metabolism. Although very little is known of the life history and ecology of the polynoid polychaete in our study, it is relatively large and was observed moving quickly over the sediments from ROV Nereus and baited lander footage. These observations suggest that it is a carnivore and would thus have a functional need for high activity and an elevated metabolic rate.

The estimation of megafaunal density in trenches is based on historical expeditions using a combination of grabs, trawls and photographs and is incredibly difficult to do (Beliaev, 1966; Beliaev and Brueggeman, 1989; Lemche *et al.*, 1976). Recent modelling efforts have sought to correlate biological information with geological morphology of the Kermadec Trench in an effort to predict benthic biomass (Ichino *et al.*, 2015) but this does not differentiate amongst size classes. The relationship of bacterial biomass to that of the various size classes of benthic metazoan fauna is also unknown in trenches, making it perilous to predict megafauna biomass based on total benthic biomass. For that reason, we used historical estimates of megafaunal density and biomass (most commonly holothurians) to estimate the total amount of carbon respired by megafauna at 7 and 8 kilometers within the Kermadec Trench. Trawl records of megafaunal biomass (Beliaev, 1966; Beliaev and Brueggeman, 1989) in the Kermadec range from 0.4 to 0.6 g m^{-2} . Estimated biomass from the photographic evidence of Lemche *et al.* (1976), which surveyed an estimated areas of $6,000 \text{ m}^2$ in the Palau, New Britain, Bougainville and New Hebrides trenches, was equivalent to 1.0 g m^{-2} . Using these estimates of megafaunal biomass and our calculated holothurian respiration rates adjusted for mass (which among our subjects was always 2 g wet weight) and a suitable respiratory quotient of 0.8 (Piepenburg and Schmid, 1996) we estimated megafaunal carbon turnover due to respiration at these depth in the Kermadec Trench ranged between 0.02 and $0.5 \text{ mg-C m}^{-2} \text{ y}^{-1}$. Our crude estimate encompasses the estimates of ophiuroid and holothurian communities of $0.029 \text{ mg-C m}^{-2} \text{ y}^{-1}$ (Ruhl *et al.*, 2013) and $0.47 \text{ mg-C m}^{-2} \text{ y}^{-1}$ Smith (1983) made at shallower depths of 4.1-4.8 and 1.3 km respectively. Our estimates of total community respiration are necessarily lacking because the megafaunal density estimates are based on one study from 40 years earlier (Lemche *et al.*, 1976). Ruhl *et al.* (2013) clearly demonstrates that this is a major caveat as taxa dominance can play a role in total respiration, likely making our values underestimates as we have included only holothurians in our community estimate. Still it is surprising that hadal megafaunal community respiration would be similar to that at much shallower depths.

5. Conclusions

Prior food web models of deep-sea habitats have been defined by the amount of information they lack (Rowe *et al.*, 2008). Carbon utilization that occurs due to the respiration of fish and megafauna is a consistently lacking variable. Some work has provided in situ rates of fish (Drazen and Seibel, 2007; Drazen and Yeh, 2012; Drazen *et al.*, 2011; Drazen, 2002; Smith and Hessler, 1974) and invertebrate respiration (Hughes *et al.*, 2011; Smith, 1983; Wilson *et al.*, 2013) including this study that helps close this gap in our knowledge. The current works provides data that brings us closer to estimates of megafauna carbon demand though clearly better estimates of their density within trenches are needed.

273 As there are likely few biological pathways for carbon to leave the trench, the continued study of
274 trenches must seek to include a reliable estimate of biomass and metabolic rates for the epibenthic and
275 infaunal communities to determine the overall mass of carbon sequestered in biological forms.

276

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