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FEATURE ARTICLE

Impacts of the first deep-sea seafloor massive sulfide mining excavation tests on benthic communities

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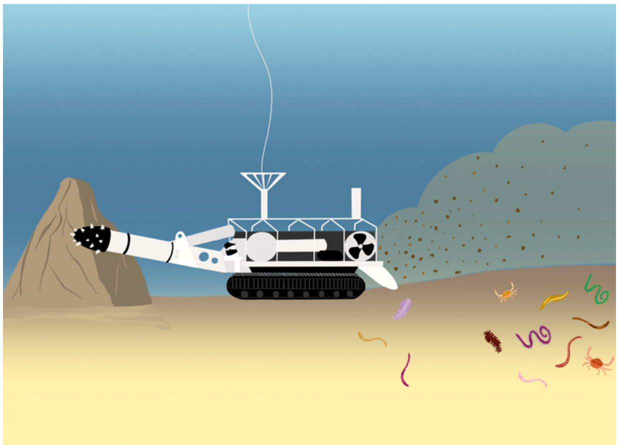
ABSTRACT: Japan undertook the first ever tests of deep-sea seafloor massive sulfide (SMS) excavation in 2017 in the Okinawa Trough. This study examines infauna from several nearby stations before and up to 3 yr after disturbance. Distance from excavation, current direction, seafloor topography, and modeled and observed deposition were used to classify the level of impact of each station. Metal concentrations were analyzed, as were nanofauna (2–32 µm), meiofauna (32–300 µm), and macrofauna (>300 µm). Elevated Cd, Pb, Hg, Zn, Fe, and Cu were confirmed as indicators of sedimentation from the SMS extraction. Benthic communities appeared altered by the disturbance test, with different size classes showing different levels of response and recovery. Nanofaunal and meiofaunal abundances appeared to take several weeks to show impacts from the disturbance and may have returned to pre-test levels within 1 yr, but changes to nematode community structure persisted longer. In contrast, macrofaunal abundances and diversity appeared to decrease immediately, and possibly remained depressed compared to pre-test levels at impacted sites at least 3 yr later. In addition, meiofaunal nematode:copepod ratio and macrofaunal percent composition of polychaetes, along with several nematode taxa, may serve as useful bioindicators of SMS mining. The small scale of disturbance requires caution when extrapolating to full-scale mining, but these results suggest current direction and topography greatly influence the extent of mining impacts; in addition, several metals may be useful for identifying the mining footprint. Biological results indicate that larger macroinfauna may be less resistant and resilient to mining impacts than smaller meiofauna and impacts from even small-scale mining activities may persist for at least 3 yr.



Impacts from sediment deposition created by deep-sea mining on seafloor ecosystems and their spatial/temporal extent remain largely unknown.

Graphic: Giun Yee Soong

KEY WORDS: Deep-sea mining · Seafloor massive sulfides · Environmental impact assessment · Benthic communities · Heavy metals · Macrofauna · Meiofauna · Nanofauna

1. INTRODUCTION

First-of-their-kind mining tests on deep-sea seafloor massive sulfides (SMS) were recently undertaken by the Japanese government's Ministry of Economy, Trade, and Industry (METI), led by the Japan Organization for Metals and Energy Security (JOGMEC) (Matsui et al. 2018, Okamoto et al. 2019).

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The goals of these experiments included testing mining technologies, creating environmental baselines, and exploring impacts to the surrounding deep-sea environment. Results from these experiments will help to identify and predict some of the impacts of future deep-sea mining on the seafloor and in the water column before commercial operations take place, and assist with both minimizing damages from exploitation and guiding regulations to include best practices.

Deep-sea mining (DSM) has been discussed for over 50 years (Brooks 1968, Auburn 1970), but the difficulty in working at these remote habitats combined with high operational costs, fluctuating metal prices, and uncertainty with pioneering operations has resulted in few real-world examples of DSM tests. Much of what is currently known on DSM impacts comes from modeling (Rolinski et al. 2001, Coulin et al. 2017, Gillard et al. 2019) and expert surveys (Washburn et al. 2019). What we have learned to date on actual DSM impacts comes from mining-simulation disturbance tests on manganese nodules and benthic trawling of seamount tops. Small-scale commercial test mining and disturbance tests in nodule-rich areas generally resulted in large decreases in both density and diversity of infauna (Borowski 2001, Jones et al. 2017, Vonnahme et al. 2020). Likewise, demersal trawling upon seamounts is generally associated with decreases in benthic density and diversity (Gollner et al. 2017, Clark et al. 2019, Goode et al. 2020), although there is evidence that smaller organisms may be more tolerant (George 2013). Impacts on nodule and seamount habitats can persist for at least decades; however, as these habitats can take millions of years or longer to form (Hein et al. 2000, 2013), it is likely that humans will never witness true recovery. Deep-sea mining-test experiments in the Japanese EEZ have focused on the mining of seafloor massive sulfides (SMS) associated with hydrothermal vents (Matsui et al. 2018, Okamoto et al. 2019), cobalt-rich ferromanganese crusts atop several seamounts (A. Suzuki, J. Minatoya, T. Fukuhara, H. Yokooka and others unpubl.), and methane hydrates (Konno et al. 2017), providing crucial information on relatively unknown habitats.

This study is the first to explore impacts to benthic communities by test mining of SMS. Mining of SMS will likely result in many of the same impacts as other deep-sea resources, from removing habitat to creating sediment plumes to introducing light and noise (Washburn et al. 2019). Also, while formation of habitats associated with SMS may take less time than other deep-sea mineral resources, SMS with sufficient size for mining to be economically viable still take several thousand years or longer of hydrothermal activity to form (Strens & Cann 1986, Petersen et al. 2016, Andersen et al. 2017), suggesting recovery will not occur on time scales relevant to humans. However, there are reasons to believe that exploitation of SMS may result in different types and levels of impact compared to other deep-sea habitats. Hydrothermal vents are generally more dynamic than abyssal seafloor or seamounts (e.g. high temperatures, high concentrations of dissolved metals, and variable concentrations of these metals in the surrounding seafloor over both space and time) which may have caused communities here to be more resistant and resilient to environmental change (Gollner et al. 2017, Washburn et al. 2019). Thus, mining of SMS may impact benthic communities (e.g. abundance and diversity) less than mining in other habitats. SMS are also associated with areas of less than a few km^2 but can require removal of many meters of sediment and substrate (Gwyther 2008, Van Dover et al. 2018), similar to some land-based mining activities. This results in a much smaller spatial extent of impacts but focused on one particular location for a much longer period of time compared to other deep-sea resources. Active hydrothermal vents host unique communities of endemic chemosynthetic organisms (Van Dover 2000), and thus have been designated as vulnerable marine ecosystems (FAO 2009, Van Dover et al. 2018), but little is known about habitats and communities associated with inactive hydrothermal vents (Van Dover 2019) which are much more likely to be mined.

This paper explores benthic infaunal communities associated with an inactive SMS in the Okinawa Trough where small-scale excavations of sulfide ore occurred, the first in the world. The Izena Cauldron in the Okinawa Trough is targeted for SMS mining by Japan, particularly due to the high Au and Ag contents of minerals here (Nakamura et al. 1989, Urabe 1989, Halbach et al. 1993). In January of 2017, an excavator was used to remove material from Jade site within the Izena Cauldron, and sediment samples were collected immediately before and at several points after this mining 'test'. Sediment metal concentrations and infaunal communities (i.e. nanofauna, meiofauna, and macrofauna) were examined at several locations between ~10–60 m from the disturbance site both upstream and downstream of the prevailing seafloor current. The following null hypotheses were examined: (1) there were no impacts of the disturbance test on sediment metal concentrations; (2) there were no impacts of the disturbance test on nanofaunal, meiofaunal, or macrofaunal abundance, diversity, and community structure; (3) if impacts were observed, there was no evidence of recovery for biological communities for at least 3 yr following the disturbance test; and (4) if impacts were observed, there were no differences in response among nanofauna, meiofauna, and macrofauna. These results can help to (1) understand how mining of deep-sea SMS will alter the surrounding seafloor; (2) explore what the data from different benthic components can tell us about the spatial extent and magnitude of mining impacts; and (3) focus efforts on mitigation strategies to limit the impacts of these alterations.

2. MATERIALS AND METHODS

2.1. Mining test, sample design and collection

The test site for this disturbance experiment simulating the mining of deep-sea hydrothermal polymetallic sulfides was located in the Okinawa Trough, to the southwest of mainland Japan, on an inactive SMS at the hydrothermally active Jade site in the Izena Cauldron. This area of active hydrothermal venting was discovered in 1988 at ~1400 m depth in the northeastern section of the Izena Cauldron (Halbach et al. 1989, Fig. 1A). The Jade site houses a

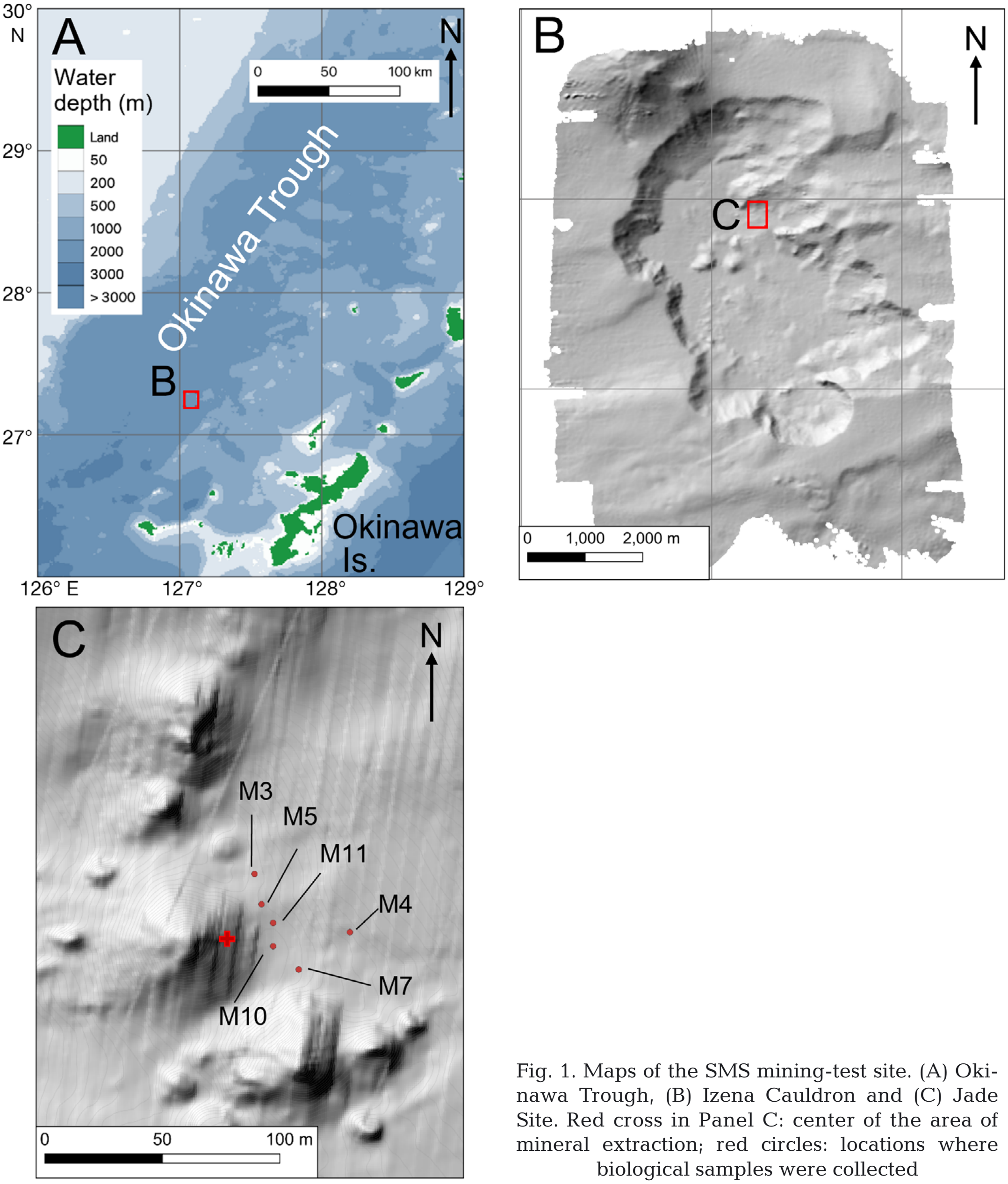

Fig. 1. Maps of the SMS mining-test site. (A) Okinawa Trough, (B) Izena Cauldron and (C) Jade Site. Red cross in Panel C: center of the area of mineral extraction; red circles: locations where biological samples were collected

'black smoker' chimney, which emits fluids at ~320°C, as well as other chimneys and mounds emitting fluids up to 220°C (Halbach et al. 1989, Nakamura et al. 1989, Tanaka et al. 1990). The disturbance site sits at ~1600 m depth to the west of previously explored active vents in the area (Ishibashi et al. 2014) with the closest known active vent ~200 m to the SW of the disturbance site itself. Sulfide ore and chimneys here are primarily composed of anhydrite, gypsum, sphalerite, barite, galena, and amorphous silica which are rich in Zn, Pb, and Ba. Chalcopyrite, rich in Cu and Fe, is also common (Nakashima et al. 1995).

The disturbance test for the SMS mining simulation was conducted on an inactive sulfide mound in the above area at a depth of ~1500 m (Fig. 1C). The disturbance experiment took place on January 12, 2017, with excavations occurring over the course of approximately 6 h. It is estimated that a total of 8.58 m^3 of seafloor were directly removed by excavation over an area of 26 m^2, which was calculated by estimating the depth of the excavated areas from seafloor photographs. During this time, the excavator itself was lifted and lowered to the seabed 9 times. The excavator is equipped with 2 crawlers, each ~1.925 m^3, and ROV images show that the depth of burial of each crawler was approximately one-third its height. The lowering and lifting of the excavator were estimated to displace approximately 1.28 m^3 of material each time (~0.33 m buried × 3.85 m long × 0.5 m wide × 2 crawlers) for a total of 11.5 m^3 material removed over 9 lifts (Matsui et al. 2018). While the total volume of minerals and sediment disturbed is estimated at 20.2 m^3, material disturbed by excavator liftings would likely sink in large chunks close to the retrieval site and would thus have less impact on the surrounding environment compared to the fine excavated material released into the water column.

For environmental and biological analyses, pushcore samples with an inner diameter of 8.2 cm were collected by the remotely operated vehicle (ROV) 'Kaiko' and its support ship the R/V 'Kairei' at 6 stations (M3, M4, M5, M7, M10, and M11) (Fig. 1C). Samples were collected 1–2 wk before (except M5, where samples were collected 5 mo before) the test (i.e. 'pre-test'), 2 wk after the test, 6 mo after the test, and one, 2, and 3 yr following the test. However, due to logistical constraints, samples were not collected, or some laboratory analyses were not able to be performed on specific locations at specific time points. The 2 closest stations to the disturbance site, Stations M10 and M11, were not sampled before the experiment as they were purposely chosen post-disturbance due to observations of sedimentation following the experiment. Stations M3 and M10 were not sampled 1 yr after the test experiment or beyond, and Station M11 was not sampled 1 yr following the experiment (Table 1).

2.2. Sediment-characteristic analyses

Sediment characteristics examined included: total organic carbon (TOC), total organic nitrogen (TON), median and average grain size, and $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$ as well as sediment concentrations of several metals including Cd, Pb, As, Hg, Mn, Zn, Fe, Cu, and Al. Biological components examined included: nanofauna (2–32 µm), meiofauna (32–300 µm), and macrofauna (>300 µm). Six cores were collected at each station and time point. Three cores were used for sediment-characterisic analyses, and three cores were used for biological analyses. Sediment-characteristic or biological analyses were performed on core samples sliced at 0–0.5, 0.5–1, 1–2, 2–3, and 3–5 cm depth intervals. Because of the large volume of material required for

Table 1. Stations sampled for environmental and infaunal analyses for the SMS mining-test experiment, as well as time periods when environmental and biological samples were collected for each location. Distance, direction, and time periods are in relation to the disturbance test. Depth is in meters with only values for the Ones digit provided. Values in the Tens, Hundreds, and Thousands digits were identical for all stations (not shown as they are proprietary information). X denotes times when samples were collected and n.d. denotes times when no data are available

Stn	Distance (m)	Direction	Impact Category	Depth (m)	Time periods					
					Pre	2 Weeks	6 Months	1 Year	2 Years	3 Years
M3	30	N – NE	Moderate	7	X	X	X	n.d.	n.d.	n.d.
M4	55	E	Unimpacted	1	X	X	X	X	X	X
M5	20	NE	Moderate	6	X	X	X	X	X	X
M7	30	SE	Heavy	6	X	X	X	X	X	X
M10	15	E – SE	Heavy	7	n.d.	X	X	n.d.	n.d.	n.d.
M11	15	E – NE	Moderate	7	n.d.	X	X	n.d.	X	X

the many physical and chemical analyses performed, sliced samples from the 3 sediment-characteristic cores (e.g. 0–0.5 cm) were mixed together resulting in only 1 replicate mud mixture per station/time and sediment depth, while each of the 3 biological cores was treated as a replicate. The division of cores for different analyses is shown in Fig. S1 in Supplement 1 at www.int-articles/suppl/m712p001_supp1.pdf.

For TOC and TON, ~5 g wet weight of the mud mixture for each sliced sample was placed in plastic bags and frozen. The remaining mud mixture was stored at 4°C and used for isotope, grain size, and metal analyses. Total carbon and TON were measured with a CHN analyzer and inorganic carbon by phosphate-nitrogen purge and coulometer detection method using standard protocols, with TOC being TC – IC (Nishida et al. 2015), analytical errors based on replicate analyses were within 1% for all analyses. Grain size was measured using a Horiba LA-950 laser diffraction particle size distribution analyzer with standard protocols. Stable isotope ratios were measured using a stable isotope ratio mass spectrometer and standard analytical methods (Onishi et al. 2018). Isotopic values are expressed using δ notation in per mille deviation (‰) from international reference materials (Vienna Pee Dee Belemnite for $\delta^{13}C$, atmospheric N for $\delta^{15}N$, and Cañon Diablo troilite for $\delta^{34}S$). Analytical errors associated with the overall process of these determinations were less than ±0.2, ±0.3, and ±0.3‰ for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ isotopic compositions, respectively. To measure metals, 200 mg of dry sediment was digested in ultrapure HF and HNO_3 before being diluted to 100 ml with Milli-Q water. Then Cd, Pb, Mn, Zn, Fe, and Cu were quantified using an ICP optical emission spectrometer (ICP-OES, CAP740 Duo, Thermo-Scientific), As was quantified using an atomic absorption spectrometer (AA280FS, Varian) equipped with an Agilent VGA 77 continuous-flow vapor generation assembly, and Hg was quantified using a mercury analyzer RA-3420 (Nippon Instruments Corporation) using standard protocols. For metals, a reference rock standard (JSO-1, Geological Survey of Japan) was used to calibrate samples as well as standard solutions prepared from pure elemental standard solutions (Wako Pure Chemical Industry Ltd., Osaka, Japan). Analytical error for metals was estimated to be <10% for each elemental analysis.

2.3. Biological analyses

Each sediment slice (e.g. 0–0.5, 0.5–1 cm) of each biological core was first divided in half, with half being used for microbial, nanofaunal, and genetic analyses and the other half for meiofaunal/macrofaunal taxonomy and biomass (Fig. S1). For the microbial/nanofaunal/genetic core half, first a 1 ml syringe was used to subsample each sediment slice of the 3 biological cores for nanofaunal counts. These nanofaunal samples were then preserved in 1% glutaraldehyde/2% formalin seawater and refrigerated at 4°C. In the laboratory, pyrophosphoric acid was added, nanofauna were separated using sonication, and DAPI (4′,6-diamidino-2-phenylindole 2 hydrochloride, final concentration 1 µg ml^{-1}) and Zdan black were added to the supernatant for staining. Nanofaunal count samples were filtered through a 0.8-µm-pore Nucleopore filter, and nanofaunal sized cells with a well-defined nucleus were counted by direct counting using an epifluorescence microscope with a blue excitation filter (Table S2a–f in Supplement 2 at www.int-res.com/articles/suppl/m712p001_supp2.xlsx).

For the meiofaunal/macrofaunal half of each biological core (~26.4 cm^2), each sediment slice was sieved on stacked 32, 300, 500, and 4000 µm sieves. Material from each sieve was preserved in 10% buffered formalin and stained with rose Bengal. Because of limited resources, material retained on the 32 µm sieve (i.e. meiofauna) was subsampled for community analyses. Meiofaunal samples were placed in test tubes, tubes were thoroughly mixed/stirred, and 1/8 of this volume was removed using a pipette for community identifications (equivalent to ~3.3 cm^2 sample area). Animals captured on the 300, 500 and 4000 µm sieves were collectively considered macrofauna. Meiofaunal and macrofaunal organisms were identified morphologically to class or order for most taxa and family for polychaetes (Table S2a–f). Meiofaunal nematodes from the entire material on the 32 µm sieve were identified by an outside specialist (Ryuta Yamamoto, Doris Japan Co., Ltd) to genus when possible (Table S2g) (Platt & Warwick 1983, Platt & Warwick 1988, Warwick et al. 1998). For all meiofauna (except nematodes identified to genus) and macrofauna, biomass was also estimated. Sizes of individuals were measured, and the amount of carbon present was calculated by first estimating the volume of each organism as a spheroid and converting this volume to carbon content with the conversion formula:

$$C = 4/3 \times a/2 \times b^2/2 \times \pi \times \alpha \times \beta \times \gamma \qquad (1)$$

where C = carbon content (pg), a = length of the individual, b = width of the individual at its widest point, α = is the specific gravity (1; Taniguchi 1986), β = dry weight:wet weight ratio (0.4; Nishizawa

1989), and γ = carbon wet weight:dry weight ratio (0.4; Takahashi & Hoskins 1978). Because only a subset of each taxon was measured, the total biomass of each taxon and size class (e.g. nematodes 32–300 µm in size or paraonids 500–4000 µm in size) was summed across all stations and time periods and divided by the total number of individuals to get one estimated biomass value per individual of a given taxon and size class (Table S2h–i).

2.4. Classification of stations into disturbance impact categories

Sample stations were divided into impact categories based on proximity to the disturbance site, current direction measured during the disturbance using an acoustic Doppler current profiler (ADCP), and topography, as well as observations of particle flux from sediment traps and sedimentation thickness from re-sedimentation markers. Station M4 was the furthest station sampled from the disturbance site, perpendicular to the prevailing current, and elevated (~5 m up a slope) compared to all other stations (Fig. 1C). It also had the lowest levels of particle flux measured via sediment traps and re-sedimentation thickness measured via re-sedimentation markers of all stations examined in this study (Matsui et al. 2018). Thus, Stn M4 was likely to be least affected by the excavation test and classified as a control or 'unimpacted' station. The classification of M4 as less impacted than other stations is also supported by a re-sedimentation model created for an ore lifting test at SMS in the Okinawa Trough which estimated that re-sedimentation thickness ≥ 2 mm remained within 50 m of discharge. Finally, actual measurements from an ore-lifting test in the area found increased sedimentation along an isobath and to the SE of the disturbance site, neither of which applied to M4 in this study (Okamoto et al. 2019).

Station M7 was further from the disturbance site than several other stations, but it was directly downstream of the prevailing current (which was to the southeast; Matsui et al. 2018), and along an isobath with all stations but M4. Re-sedimentation thickness was more than twice as high at this site compared to other stations examined (M7 = 5 mm, M5 = 2 mm, M4 = 1 mm; Matsui et al. 2018) and so M7 was designated as being 'heavily' impacted from the disturbance site. Station M3 was also further from the disturbance site than several other stations and was almost directly upstream of the prevailing current. However, peak particle flux during the disturbance test was 20 times greater at M3 compared to M4 (Matsui et al. 2018), and M3 was designated as being 'moderately' impacted. Station M5 was one of the closest stations to the disturbance but was perpendicular to the prevailing current. Because re-sedimentation thickness at M5 was also less than half of M7, M5 was designated as 'moderately' impacted. Station M10 was in the same direction as M7 but much closer to the disturbance site with visible signs of re-sedimentation; thus, M10 was designated as being 'heavily' impacted. Station M11 was also close to the disturbance site but perpendicular to the prevailing current; thus, M11 was designated as 'moderately' impacted (Table 1).

2.5. Sediment-characteristic and biological statistical analysis

Elevated concentrations of Cd, Pb, Hg, Zn, Fe, and Cu peaked in settling particles immediately after the disturbance test and decreased with time. These metals were derived from excavated minerals and sediments making them valid indicators to determine impacts from the disturbance test (Matsui et al. 2018). Concentrations of these 'mining-indicator metals' were examined in sediments to confirm assigned impact classification categories of stations and examine the amount of time metal concentrations would be useful as indicators of disturbance following the test. Metal concentrations of sediments were examined using principal component analysis (PCA) plots and similarity profile analysis (SIMPROF) in PRIMER 7 (Clarke & Gorley 2015). Sediment concentrations of the 6 mining indicator metals (i.e. Cd, Pb, Hg, Zn, Fe, and Cu) at each location and sampling period were first normalized. To explore natural temporal variability in communities, we examined TOC — a measure of food availability. Food availability heavily influences deep-sea benthic community abundance (Rex et al. 2006, Wei et al. 2010), along with diversity and community structure.

Abundances of nanofauna, meiofauna, and macrofauna, and the biomass and number of taxa for meiofauna and macrofauna, were summed among sediment sections within a core to get a total abundance, biomass, or taxa richness per core for analyses. Taxa captured on 300 µm or larger sieves but which are generally considered meiofauna (i.e. Foraminifera, Copepoda, Ostracoda, nauplius larva, and Nematoda) or sessile or mobile epifauna (i.e. Pycnogonida, Holothuroidea, and Ascidiacea) were excluded from macrofaunal analyses. The nematode:copepod ratio

(N:C ratio) and percentage of the community comprised of polychaetes (% Poly) were calculated by dividing the abundance of meiofaunal nematodes by the abundance of meiofaunal harpacticoids (harpacticoid copepods generally dominate deep-sea meiofaunal crustaceans; Coull & Bell 1979, Wilson & Ahyong 2015) or dividing the abundance of macrofaunal polychaetes by the total macrofaunal abundance and multiplying by 100, respectively. Nematodes are generally considered to be more tolerant than copepods to pollution, hence the creation and use of the N:C ratio (Raffaelli & Manson 1981). For cores with no meiofaunal copepods, the N:C ratio was calculated by dividing the nematode abundance by 1 individual (3 individuals 10 cm^{-2}), while for cores with no macrofauna whatsoever, the % Poly was set at 100% as both higher polychaete composition and complete lack of organisms are considered indicative of heavily impacted areas (Table S2a–f). Analyses were also performed on the top 0–0.5 cm section of sediment independently as this is the area most likely to be affected first and most strongly.

A 2-way ANOVA was performed with impact category and sampling time as the independent variables and abundance (for nanofauna, meiofauna, and macrofauna), biomass and taxon richness (for meiofauna and macrofauna), meiofaunal N:C ratio and macrofaunal % Poly as dependent variables in R. Dependent variables (except % Poly) were first natural log-transformed. For analyses, the significance level was set at $\alpha = 0.05$. A significant interaction term was used to indicate that communities responded differently to the disturbance test among impact categories, and thus that communities may have been impacted. Due to unequal sample sizes among impact categories and the small number of samples at many time periods, non-parametric analyses were used to test for differences in abundances, biomass, and richness among impact categories at each time to examine significant interaction terms in R version 4.1.2. A Kruskal-Wallis test, package 'stats' (R Core Team 2013), was used to compare each variable across impact categories (unimpacted, moderately impacted, heavily impacted) in each time period (pre-test, 2 wk, 6 mo, 1 yr, 2 yr, and 3 yr after). A power analysis was also performed in R 'pwr' (Champely 2020). Power analysis showed that 3 categories with 3 replicates per category (the number for the unimpacted category) only resulted in 0.7 probability of finding a genuine difference, even if the disturbance represented the strongest effect size possible, which is 1, using a significance level of 0.1. Thus, while 0.05 was considered significant, 2-way ANOVA interaction terms and 1-way non-parametric ANOVAs with a p-value < 0.1 were still explored with pairwise comparisons. For pairwise post-hoc comparisons among impact categories, the Dunn test (Dunn 1964), package 'PMCMR' (Pohlert 2016), was used. Low faunal abundances per core made statistical examination of patterns in abundance and richness across sediment depths uninformative. In general, patterns observed in surface sediments matched those for the entire core in this study.

For multivariate analyses, a PCA was performed in Primer using the 6 indicator metals as independent variables. Finally, non-metric multidimensional scaling (nMDS) plots and similarity percentage (SIMPER) analysis were performed on meiofaunal and macrofaunal phyla/classes as well as meiofaunal nematode genera, with abundances first square-root transformed. To identify nematode genera for use as possible future indicators of mining impacts or unimpacted areas, cores within the nMDS cluster identified with SIMPROF that included all samples from station M4, as well as all pre-test samples, were considered representative of 'unimpacted' (n = 51) nematode communities, while all other cores were considered 'impacted' (n = 30). A 1-way ANOVA was then performed on nematode taxa found in more than 10 cores (13 taxa total) after being ln-transformed. If abundances were significantly higher in the unimpacted stations with more than twice as many individuals per core in unimpacted cores compared to impacted cores, then the taxon was classified as 'sensitive' to mining disturbance and possibly indicative of undisturbed conditions; if abundances were significantly higher in the impacted stations, then the genus was considered 'tolerant' to mining disturbance and a possible indicator of deep-sea mining.

3. RESULTS

3.1. Impacts of the disturbance experiment to sediment characteristics

When examining sediment concentrations of metals considered indicators of the disturbance test (i.e. Cd, Pb, Hg, Zn, Fe, and Cu), multivariate analyses indicated that there were differences among impact categories. The 2 data points collected at Stn M10 (within 15 m and downstream of the test site) at 2 wk and 6 mo were very different to the other locations and grouped on the far left of the PCA in Fig. 2, with higher values of these metals (sometimes an order of magnitude) than any other location/time (Fig. S2 in

Supplement 1). Besides M10, stations and time points were largely grouped into 4 clusters supported by SIMPROF. The cluster on the left was comprised of samples at Station M7 collected 2 wk to 1 yr after and had the highest metal concentrations, supporting the classification of this site as 'heavily' impacted. The cluster in the middle was comprised of Stations M5 and M11 after 2 wk and 6 mo, as well as Station M7 after 2 and 3 yr, supporting the classification of M11 with M5 as 'moderately impacted'. The cluster on the right included all pre-test samples, except at Station M7 and all time points for Station M4, supporting classification of M4 and pre-test samples as 'unimpacted'. The placement of samples from M5 and M11 in this unimpacted cluster 1 and 2 yr after combined with the placement of samples from M7 in the moderately impacted cluster 2 and 3 yr after also suggests different levels of impact between these 2 groups over time. The 4th group was comprised of M7 pre-test and moderate stations 3 yr after, suggesting it also represented relatively unimpacted conditions. Finally, Station M3 was grouped with the unimpacted cluster 2 wk after but in a group of its own at 6 mo (Fig. 2). In the PCA analysis, the first principal component explained 80% of all variability in metals across samples, while the second principal component explained ~10%, indicating high correlations among concentrations of these indicator metals.

Since sediment TOC would likely not increase from sediment deposited by the mining test, variability of TOC over time was used to estimate natural temporal variability. Sediment TOC ranged ~1.2–2.7% among stations but differed by ≤0.3% among all time points for any given station, suggesting little temporal variability at the sites examined. Sediment TOC was also lowest at the unimpacted Station M4 for all time points, suggesting that decreases in abundance at impacted sites compared to the unimpacted site were due to impacts from the mining test rather than changes in food supply (Fig. S3 in Supplement 1). No discernable patterns were observed among impact categories and time periods for TON, isotope analyses, and sediment grain size.

3.2. Infaunal abundance and biomass responses to the mining experiment

3.2.1. Nanofauna

The 2-way ANOVA for nanofaunal counts found a significant interaction effect between impact cate-

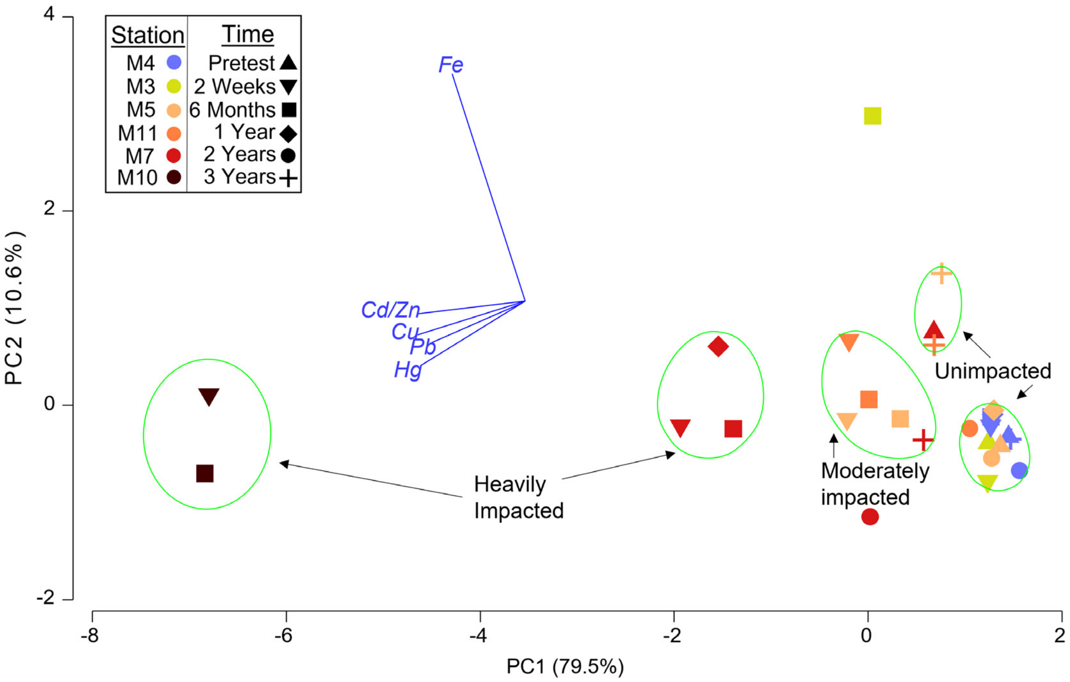

Fig. 2. Principal Component analysis (PCA) plots using the 6 'mining indicator' metals (Cd, Pb, Hg, Zn, Fe, and Cu) as independent variables. Different colors denote different stations and impact categories (blue: unimpacted, yellow/orange: moderately impacted, red/dark red: heavily impacted) while different shapes denote different time periods. The relationship of each metal to the principal components is shown in blue. Green outlines around stations: significant clusters identified using SIMPROF

gory and time period ($F_{10,63} = 2.55$; $p = 0.012$), meaning that impact categories behaved differently over time, as expected if the communities responded differently to the disturbance test at different sites. Nanofaunal abundances were significantly lower at the unimpacted station compared to other locations pre-test and were not different at other time points (Table S1 in Supplement 1). However, the unimpacted Station M4 had a >100% increase in abundance at 2 wk while moderately and heavily impacted stations had little change. Furthermore, heavily and moderately impacted stations had a 50–100% decrease from 2 wk to 6 mo after, while the unimpacted station remained the same. The biggest difference occurred 1 yr after, with the unimpacted and moderately impacted stations having a 3-fold increase in abundance, while the heavily impacted station increased by ~100%. By 2 yr nanofaunal abundances at all stations had decreased to similar levels with similar decreases to 3 yr (Fig. 3). The percentage of nanofaunal abundance found in the surface 0.5 cm of sediment ranged between ~40 and 65% of the unimpacted and moderately impacted stations at all time periods. However, it was below 30% at the heavily impacted Stations M7 and M10 2 wk after and quickly rebounded.

3.2.2. Meiofauna

The 2-way ANOVA for total meiofaunal counts found a significant interaction effect between impact category and time period ($F_{10,63} = 2.76$; $p = 0.007$) as did the 2-way ANOVAs for meiofaunal nematode ($F_{10,63} = 2.98$; $p = 0.004$) and arthropod ($F_{10,63} = 2.22$; $p = 0.028$) abundances. No meiofaunal components were significantly different pre-test. Arthropod abundances were significantly higher in the unimpacted station 2 wk after, while nematodes and total meiofauna were not different. All meiofaunal components were significantly lower in the heavily impacted stations 6 mo after but not at 1 or 2 yr (Fig. 4, Table S1). The percent of meiofauna in the surface sediments showed the same patterns as communities throughout the sediment discussed above, except for 2 wk after when ~40% of individuals were in the 0–0.5 cm section in unimpacted and moderately impacted sites while ~15% were in these surface sediments at the heavily impacted sites.

Meiofaunal biomass had similar trends across impact categories and sampling times as abundance. Because only 1 biomass value was assigned for nematode individuals, nematode biomass mirrored abundance. There were 5 different arthropod taxa for which biomass estimates were calculated; however, results of pairwise analyses were still the same between arthropod abundances and biomass. When looking at biomass of all meiofauna, results were different from meiofaunal abundance 2 wk after, with biomass significantly higher in the unimpacted site (Table S1, Fig. S4 in Supplement 1).

3.2.3. Macrofauna

The 2-way ANOVA for macrofaunal counts also found a significant interaction effect between impact category and time period ($F_{10,63} = 1.99$; $p = 0.0495$) as did the ANOVA's for macrofaunal polychaetes ($F_{10,63} = 2.26$; $p = 0.025$) and non-polychaetes ($F_{10,63} = 2.46$; $p = 0.015$). Total macrofaunal and polychaete abundances were significantly different pre-test; however, this was due to moderate stations having lower abundances than unimpacted and heavily impacted stations with no difference between these two. Two weeks after the mining test, total macrofaunal and polychaete abundances decreased in the heavily impacted stations but were still not significantly different to unimpacted stations, while abundances of non-polychaete macrofauna were significantly higher in the unimpacted station compared to moderately and heavily impacted locations. After 6 mo, total macrofaunal abundances were significantly different between unimpacted and heavily im-

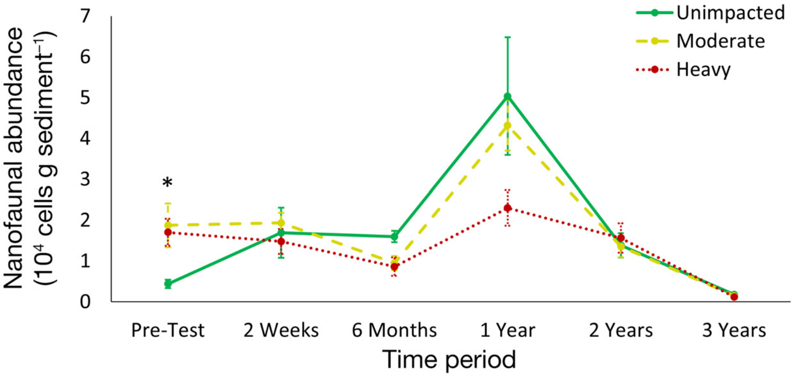

Fig. 3. Average nanofaunal abundances of all cores collected within an impact category and time period. The interaction term of the 2-way ANOVA (impact category × time period) was significant ($p = 0.012$). Error bars: SE; asterisks: significant pairwise differences ($p < 0.05$)

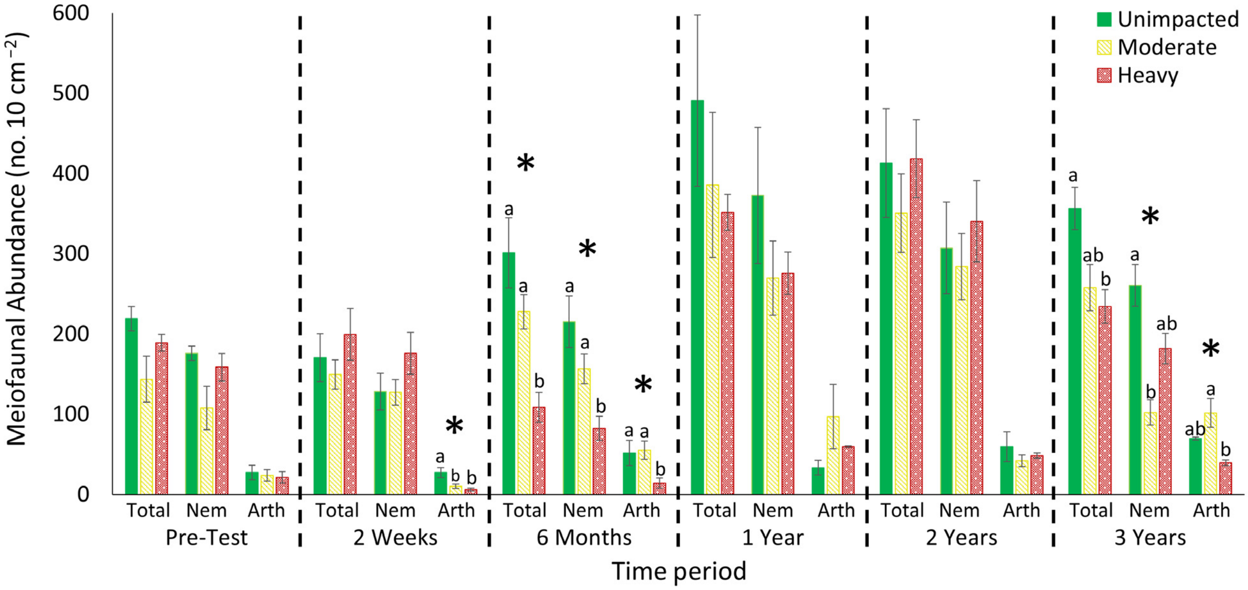

Fig. 4. Average counts for all meiofauna (Total), meiofaunal nematodes (Nem), and meiofaunal arthropods (Arth) for all replicate cores within an impact category for each time period. Error bars: SE; asterisks above a time period: significant difference among impact categories ($p < 0.05$); different letters above bars: significant pairwise differences; letters without an asterisk: analyses where the p-value for the ANOVA was between 0.05 – 0.1, but pairwise comparisons found significant differences

pacted stations, and total macrofaunal and non-polychaete abundances remained significantly higher in the unimpacted station compared to heavily and/or moderately impacted stations at least 3 yr after the disturbance test, except for 1 yr after when only 3 stations were examined. In contrast, polychaete abundances were not significantly different between unimpacted and heavily impacted categories throughout the study (Fig. 5, Table S1). The percentage of macrofauna in the surface sediments was significantly higher in the unimpacted station compared to moderately and heavily impacted stations 2 wk after; however, there were no differences in surface sediments at any other time point.

Macrofaunal biomass results were very different to macrofaunal abundance; however, this was likely due to extremely low abundances of very large animals (Fig. S5 in Supplement 1).

3.3. Infaunal diversity responses to the mining experiment

3.3.1. Meiofauna

Meiofauna were comprised of 12 higher taxa in this study. Nematodes were found in all samples while foraminiferans, harpacticoids, and nauplius larva were each found in over 80% of samples. There was no significant interaction term for meiofaunal richness ($F_{10,63}$ = 1.27; p = 0.268), and richness was higher at the unimpacted station compared to moderately and heavily impacted stations at all times (Fig. S6 in Supplement 1). The interaction term for the nematode: copepod ratio (N:C ratio) was not significant, but the p-value was less than 0.1 ($F_{10,63}$ = 1.94; 0.056). There was no difference in the N:C ratio among impact categories pre-test, while ratios were significantly higher in the heavily impacted stations compared to unimpacted station 2 wk after. By 6 mo after, N:C ratios were again similar among impact categories and remained similar throughout the rest of the study (Fig. 6). Finally, there was a significant interaction term for nematode genera richness. There were no significant differences in the number of nematode genera among impact categories pre-test, 2 wk, or 6 mo following the disturbance test. There were significantly more nematode genera in the unimpacted site compared to moderately and heavily impacted sites 1 and 2 yr after, while 3 yr after, the unimpacted site was only different from the heavily impacted site (Table S1 in Supplement 1).

3.3.2. Meiofaunal nematodes

PERMANOVA results found a significant interaction term (Pseudo-$F_{10,63}$ = 2.42; p <0.001) between

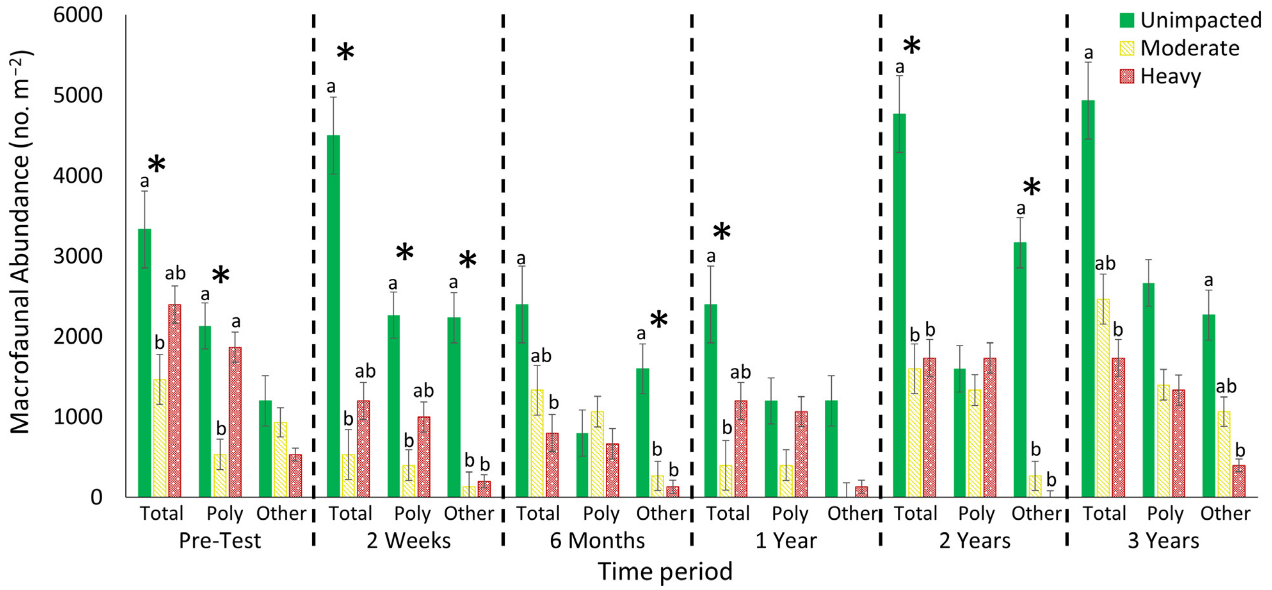

Fig. 5. Average counts for all macrofauna (Total), macrofaunal polychaetes (Poly), and macrofaunal non-polychaetes (Other) for all replicate cores within an impact category for each time period. Other details as in Fig. 4

time and impact category for genus-level nematode community structure. Pre-test, communities were not significantly different among impact categories, with all pre-test samples grouped together in one large cluster along with all other time periods for the unimpacted station in the nMDS plot, except M5 which was unique (Fig. 7). Two wk and 6 mo after, communities were significantly different between heavily impacted stations and other stations, although all stations (except M10) 2 wk after were also within this large 'unimpacted' cluster as were the moderately impacted stations 6 mo after. However, 1 and 2 yr after, moderately and heavily impacted communities were similar, and different to unimpacted communities, with all groups significantly different 3 yr after.

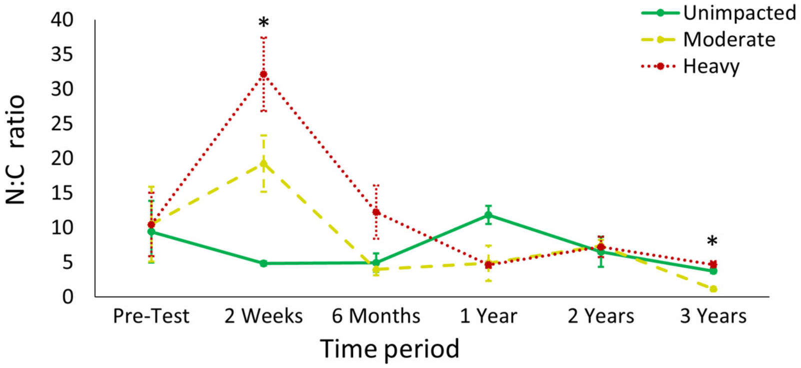

Fig. 6. The average nematode:copepod (N:C) ratio for all replicate cores within an impact category for each time period. The interaction term of the 2-way ANOVA (impact category × time period) was significant ($p = 0.021$). Error bars: SE; asterisks above a time period: significant pairwise differences ($p < 0.05$)

These differences were observable on the nMDS plot, with the 2 heavily impacted stations forming their own group 6 mo after the disturbance, while the heavily/moderately impacted stations formed separate groups 1 yr, 2 yr, and 3 yr after. PERMANOVA results were nearly identical for each time period using nematodes at the family level, although there was higher overlap among groups in nMDS space (Fig. S7 in Supplement 1).

Specific nematode taxa were explored as indicators of disturbance from SMS mining activities or undisturbed conditions using SIMPROF clusters. Communities in the large central group (Fig. 7) of the nMDS plot which included all cores from the unimpacted Station M4 and moderately impacted M3, all stations after 2 wk except M10, and moderately impacted stations M5 and M11 after 6 mo and 3 yr were considered representative of unimpacted conditions. Other SIMPROF groups were considered representative of impacted communities and included all M10 cores; M11 and M7 after 6 mo; M5, M11, and M7 after 1 and 2 yr, and M7 after 3 yr. Examining nematode genera only found in 10 or more cores, taxa which may serve as indicators of areas unimpacted by SMS mining include *Halalaimus*, *Molgolaimus*, *Sphaerolaimus*, *Oxystomina*, *Diplopeltoides*, *Halichoanolaimus*, *Pselionema*, and

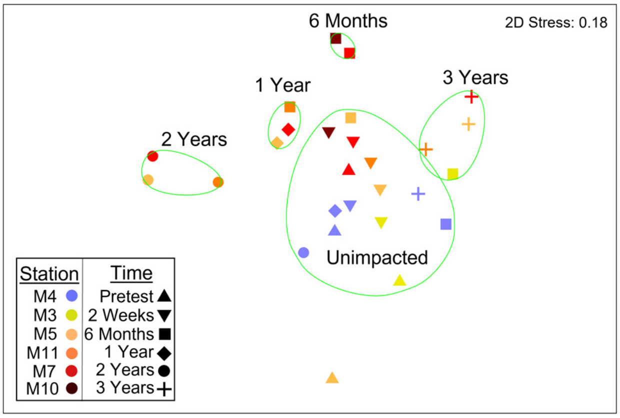

Fig. 7. nMDS plot of nematode genera. Different colors denote different stations and impact categories (blue = unimpacted, yellow/orange = moderately impacted, red/dark red = heavily impacted) while different shapes denote different time periods. Green outlines around stations: significant clusters identified using SIMPROF

Leptolaimus. High abundances of *Monhystera*, *Daptonema*, and *Desmodora* may be indicative of environments impacted by SMS mining, while *Desmoscolex* and *Actinonema* appear to be abundant in most locations (Table 2). *Monhystera* was responsible for the largest amount of similarity within communities at the moderately and heavily impacted sites 6 mo, 1 yr and 2 yr after, while *Molgolaimus* was responsible for the largest amount of similarity of communities within the 'unimpacted' cluster.

3.3.3. Macrofauna

There was a total of 28 macrofaunal taxa identified in this study: 2 mollusc classes, 4 crustacean orders, 19 polychaete families, and 3 other (Nemertea, Sipuncula, and Oligochaeta). No taxon was found in all samples, although polychaetes were present in 90% of samples. Nemerteans, sipunculans, oligochaetes, and gastropods were each only found in 3–4 cores, while each crustacean class was found in ~15–30 cores. Macrofaunal diversity was mostly dominated by polychaetes and crustaceans. The interaction term for macrofaunal taxa richness was not significant, but the p-value was <0.1 ($F_{10,63}$ = 1.93; p = 0.057), so pairwise comparisons were explored. Pre-test, richness was significantly higher in the unimpacted station compared to moderately impacted stations, but not different from the heavily impacted stations. There was also no difference between unimpacted and heavily impacted richness 2 wk to 1 yr after; however, 2 and 3 yr after, richness at the unimpacted station was significantly higher than the heavily impacted station (Fig. S8 in Supplement 1).

The percent of the macrofaunal community comprised of polychaetes (% Poly), like the meiofaunal N:C ratio, may serve as a useful indicator of deep-sea mining impacts. There was a significant interaction term for % Poly composition ($F_{10,63}$ = 2.28; p = 0.024) with no difference between unimpacted and either heavily or moderately impacted stations pre-test. The % Poly was significantly lower in the unimpacted vs heavily and moderately impacted stations 2 wk to 2 yr after disturbance. The heavily impacted station continued to have higher % Poly 3 yr after, while the moderately impacted stations were similar to unimpacted; however, differences were not significant (Fig. 8; Table S1).

4. DISCUSSION

4.1. Identifying areas impacted by SMS mining

Distance from the mining site is obviously critical in determining the extent of environmental impacts.

Table 2. Bioindicator classification of nematode genera based on p-values from 1-way ANOVAs and abundance ratios between unimpacted and impacted cores performed on each taxon found in 10 or more cores. N: number of cores with taxon present; unimpacted mean: average abundance 10 cm^{-2} in all cores at stations/time periods unimpacted by the mining disturbance; impacted mean: average abundance 10 cm^{-2} in all impacted cores; c-p value: colonizer-persister (c-p) value from Bongers et al. (1991) classifying taxa as tolerant or persistent (with 1 being the most tolerant and 5 being the most persistent) to environmental changes from shallow-water studies (NA is not available). Green represents taxa classified as 'Sensitive', yellow represents 'Cosmopolitan', and red represents 'Tolerant to impacts of the SMS excavation test'

Genus	N	Bioindicator classification	p	Unimpacted mean (no. core^{-1})	Impacted mean (no. core^{-1})	c-p value
Molgolaimus	66	Sensitive	<0.0001	11.6	6.8	3
Halalaimus	46	Sensitive	<0.0001	3.9	0.3	4
Sphaerolaimus	22	Sensitive	0.0003	1	0	3
Oxystomina	11	Sensitive	0.008	0.3	0	4
Halichoanolaimus	13	Sensitive	0.011	0.7	0	NA
Diplopeltoides	15	Sensitive	0.016	0.6	0.1	NA
Pselionema	27	Sensitive	0.036	1	0.4	3
Leptolaimus	66	Cosmopolitan	0.042	6	4.6	2
Actinonema	70	Cosmopolitan	0.322	5.3	4.5	4
Desmoscolex	23	Cosmopolitan	0.579	1.7	1.2	4
Monhystera	75	Tolerant	<0.0001	5.7	22.9	2
Daptonema	14	Tolerant	<0.0001	0.1	5.7	2
Desmodora	34	Tolerant	0.005	0.9	2.3	2

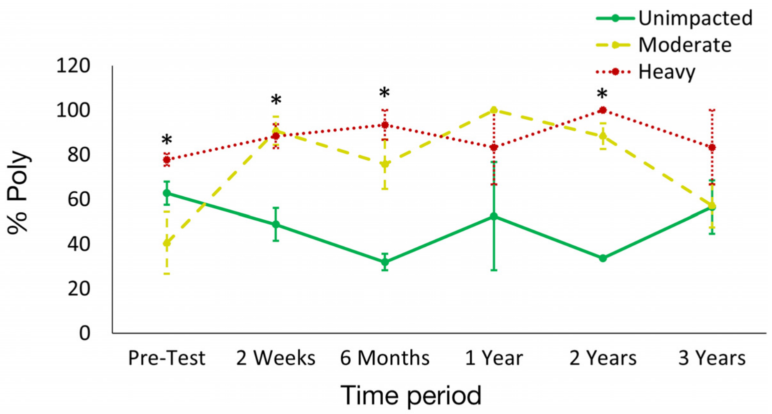

Fig. 8. Average percent of the macrofaunal community comprised of polychaetes (% Poly) for all replicate cores within an impact category for each time period. The interaction term of the 2-way ANOVA (impact category × time period) was significant ($p = 0.021$). Error bars: SE; asterisks above a time period: significant pairwise differences ($p < 0.05$)

Infaunal communities within 15–30 m of the disturbance site were heavily to moderately influenced by the experiment, while the one station > 50 m from the disturbance site appeared to be relatively unimpacted in this study; however, based on the asymmetrical observational data, impacts were likely more confined than this range in some directions and more extended in others. Current direction appears to be a large driver in the spatial extent of impacts from the disturbance test. Stations M5, M10, and M11 are all nearly equidistant from the disturbance site; however, M10 had much higher concentrations of indicator metals and was the only one of the 3 stations south, along the prevailing current. Furthermore, M7 was twice the distance from the disturbance site as some moderately impacted stations, but again was directly downstream of the disturbance site and had more than twice the re-sedimentation of the moderately impacted M5 station (Matsui et al. 2018). These results suggest that heavy impacts were almost entirely limited to areas down-current of the disturbance site, extending at least 30 m, while moderate impacts occurred in other directions and extended at least 30 m as well. A different SMS disturbance test in the area found the largest impacts to the southeast of the disturbance site (Okamoto et al. 2019). Sediment deposition from deep-sea disturbances in nodule areas was also focused in the direction of the prevailing current (Trueblood 1993, Sharma et al. 2001).

Seafloor topography (measured via AUV surveys at depth) likely plays a role in the spatial extent of impacts as well. Station M4 was the only unimpacted site and the furthest from the disturbance site, and it was 5 m shallower than all other stations (Fig. 1C). Stations located ~25 m west, ~50 m northwest, and ~50 m south of the disturbance site (where no biological samples were collected for this study) had no detectable re-sedimentation (Matsui et al. 2018), suggesting that impacts were isolated to an area from the north to southeast which fell along an isobath at 6–7 m. All stations with measured re-sedimentation were also on the same side (east) of a 10 m tall mound at the disturbance site, highlighting the importance of seafloor topography as a driver of the spatial extent of impacts from deep-sea mining (Fig. 1C). The lack of impacts at an elevation of as little as 5 m highlights the need to understand fine-scale topography when planning mining operations. Mitigation of impacts may thus include mining of areas that are situated in localized seafloor depressions.

Finally, the distance of re-sedimentation from mining may be much larger than the distance of increased metal fluxes, with 1 mm of re-sedimentation observed at stations in some directions > 100 m away from the mining test (Matsui et al. 2018). The ore lifting test in the Okinawa Trough found 4 mm of re-sedimentation 130 m from the disturbance site (Okamoto et al. 2019). There is a need to extend monitoring efforts beyond 50 m and in multiple directions to identify areas outside of the zone of mining influence, although the highly heterogeneous nature of SMS habitats will likely, unfortunately, introduce more confounding factors (such as nearby active venting or different sulfide structures) with additional distance between stations.

4.2. Metals as indicators of mining impacts and persistence

Elevated concentrations of Cd, Pb, Hg, Zn, Fe, and Cu appear to be useful indicators of mining of sulfides at the Jade site (Figs. 2 & S2). Material settling during this disturbance test had elevated concentrations of Cd, Pb, Hg, Zn, Fe, and Cu (Matsui et al. 2018), and we found higher concentrations with closer proximity to the disturbance site as well as along the prevailing seafloor current. Metal data grouped stations in the same manner as their designated impact categories using distance and direction from the disturbance site and local topography (i.e. highest metal concentrations in heavily impacted stations and lowest in the unimpacted station) (Figs. 2 & S2). While concentrations of these metals are naturally high in areas of hydrothermal venting, a previous study found sediments in the area contained maximum concentrations of Cu up to 8500 ppm, Zn up to 11200 ppm, Cd up to 115 ppm, Hg > 100 ppm, and Pb up to 9000 ppm (Marumo & Hattori 1999), several times lower than concentrations observed at Stations M7 and M10 in the present study.

While the above metals are useful to determine the extent of mining impacts immediately after activities have ceased, metal concentrations will likely decrease over time. Understanding temporal changes in metal concentrations after mining activities cease is thus extremely important. Elevated metal concentrations in surface sediments appeared to return to pre-disturbance levels at moderate stations within 1 yr while at the heavily impacted station concentrations had decreased to moderate levels after 2 yr but remained elevated at least 3 yr after the disturbance test. The one outlier was Station M3, which was the only impacted station within the unimpacted group 2 wk after, but was very different to other stations 6 mo after (Fig. 2). Due to its location in the opposite direction of the prevailing current during the test, one hypothesis is that re-sedimentation following the test may have resulted in delayed impacts here. Re-sedimentation from bottom currents should be explored in future mining tests well after mining activities cease.

It is hypothesized that metal oxidation was largely responsible for decreases in metal concentrations during this experiment. As sedimentation rates in the area are estimated to be < 1 mm yr^{-1} (Feng-ye & Yu-lan 1996, Xiong et al. 2005) metal removal by sedimentation burial is unlikely. Bioturbation is a possibility as the deeper sediments had higher metal concentrations in the later years. However, total concentrations of indicator metals throughout the surface 0–5 cm of sediment decreased 2–4 fold from immediately after the disturbance to 1–2 yr after the disturbance at all moderately and heavily impacted sites, likely driven by oxidation in surface sediments. These temporal changes highlight the possibility of rapid metal oxidation following sulfide mining, at least for small particles. Similar to our observations, particles of pyrrhotite and chalcopyrite had residence times of 1.5 and 11 mo, respectively, but for particles 1 µm in diameter. For particles ~10 µm in diameter, residence times were years to decades (Bilenker et al. 2016). Importantly, while the return of metal concentrations to pre-test levels may indicate that some recovery of environmental conditions has occurred, this is not to say that other alterations to the environment do not persist longer. Also, alterations in benthic communities, either due to previous increases in metals or other factors like sedimentation, may persist for longer periods of time.

4.3. Infaunal responses to the mining experiment and bioindicators

There were interesting patterns in benthic abundances and diversity following the SMS mining test. There were no significant differences in nanofaunal abundances, although the increase in abundances at the unimpacted site over time compared to other locations is an interesting result that should be further explored (Fig. 3). It is unclear if meiofaunal abundance and N:C ratio were altered at the moderately impacted sites (Figs. 4 & 6), although it appears that meiofaunal nematode diversity and macrofaunal abundance/diversity may have been (Figs. 5, 7 & 8). All components of the meiofaunal and macrofaunal community appear to have been altered at the heavily impacted sites (Figs. 4–8). Thus, macrofauna may be affected by deep-sea mining over a larger spatial extent than smaller organisms.

It may have taken several weeks to months for the mining test to alter nanofaunal and meiofaunal abundance (Figs. 3 & 4), and recovery of abundances largely occurred by 2 yr following the disturbance. On the other hand, the N:C ratio increased dramatically immediately after the test but returned to background numbers by 6 mo after. This suggests that meiofaunal copepods may be more quickly influenced by mining than meiofaunal nematodes. Often, organic enrichment or toxicity is associated with an increase in nematode abundances and/or a decrease in copepod abundances, meaning that higher N:C ratios may be associated with more polluted conditions (Raffaelli & Manson 1981, Baguley et al. 2015). Macrofaunal abundance and richness appear to have been altered more quickly, with recovery possibly taking 3+ yr (Figs. 5 & S7), while the percent of macrofauna comprised of polychaetes has potential as an indicator of mining disturbance (Fig. 8). Many polychaetes are often considered tolerant to pollution/stress, and high dominance of polychaetes can indicate environmental impacts from other activities such as offshore oil production (Andrade & Renaud 2011, Dauvin et al. 2016). These results suggest that mining impacts on nanofaunal and meiofaunal communities will not only be more limited spatially but also temporally compared to macrofaunal communities. Larger animals are often affected by disturbance more than smaller animals (Pearson & Rosenberg 1978); however, following natural disturbance at hydrothermal vents, macrofaunal communities may recover more quickly than meiofaunal communities, due to higher mobility (Gollner et al. 2017).

The underlying mechanisms behind specific nematode taxa being useful indicators of pristine or impacted locations are less clear. Much of this uncertainty may be due to the almost complete lack of toxicological tests on deep-sea animals in general, making it necessary to examine shallow-water studies. Nematode genera *Monhystera*, *Daptonema*, and *Desmodora* are all possible indicators of areas impacted by the mining disturbance test, while taxa including *Molgolaimus*, *Halalaimus*, and *Sphaerolaimus* appeared indicative of unimpacted areas in this study (Table 2). Other studies have also found that *Halalaimus* is likely a genus sensitive to stress in shallow waters, while *Daptonema* and *Monhystera* are indicative of stressful conditions (Bongers et al. 1991, Danovaro et al. 1995, Semprucci et al. 2015). However, classifications in this study do not always match current literature. For example, *Molgolaimus* and *Oxystomina* have been considered indicators of stressful conditions in shallow-water systems, while *Desmoscolex* and *Desmodora* have been considered indicators of good environmental quality (Mirto et al. 2002, Semprucci & Balsamo 2012), the opposite of results from the present study. Thus, the taxa classifications in our study should be treated as a starting point, with future work needed concerning toxicological studies of specific nematode genera to confirm their use as bioindicators of deep-sea mining.

4.4. SMS mining impacts on benthos: sedimentation, toxicity, habitat alteration, and future directions

Likely one of the most immediate impacts from deep-sea mining on the nearby benthic communities will be the re-sedimentation of, and burial by, material disturbed during mining (Gwyther 2008, Boschen et al. 2013, Washburn et al. 2019). Unfortunately, re-sedimentation markers were not deployed or did not work at Stations M3, M10, and M11 while sedimentation traps were only deployed at Stations M3 and M4. However, re-sedimentation from this 6 h test resulted in burial of some areas at least 0.5 cm thick (at M7) (Matsui et al. 2018). Burial at rates of ~2 cm mo^{-1}, especially fine sediment, can greatly decrease infaunal densities (Turk & Risk 1981), while deeper communities may be more susceptible to burial (Gallucci & Kawaratani 1975). Unfortunately, thresholds of sensitivity to turbidity, sedimentation, and burial are currently based on data from shallow-water ecosystems which experience sedimentation rates orders of magnitude higher than the deep sea (Smith et al. 2020), and even at these shallow environments, effects of sedimentation and thresholds of impacts are relatively unknown (Airoldi 2003). Re-sedimentation has also been suggested to increase organic carbon on the seafloor (Sharma et al. 2001, Ingole et al. 2005), further complicating the projection of mining impacts.

Another likely impact of deep-sea mining on nearby benthic communities is toxicity from heavy-metal deposition (Gwyther 2008, Narita et al. 2015, Washburn et al. 2019). The fact that nanofauna, meiofauna, and macrofauna behaved differently among moderately and heavily impacted stations may suggest different susceptibilities to toxicity amongst the different benthic-community components from mining that should be explored in future studies. There is evidence to suggest that toxicity from metals released by mining may cause limited harm to the surrounding communities in SMS habitats. The addition of sulfide can ameliorate metal toxicity by forming metal-sulfide complexes and precipitates (Edgcomb et al. 2004). Several benthic animals associated with hydrothermal vents use metallothionien-like proteins to help in detoxification as well (McMullin et al. 2000). Likewise, the actual concentrations of metals calculated for sediments in this study likely do not represent the level of risks posed to communities (Chapman et al. 1998, Simpson & Batley 2007, Simpson & Spadaro 2016) since metals in sulfides often have low bioavailability (Morse 1994, Lee et al. 2000, USEPA 2005). However, these sulfides may transform into forms with higher bioavailability through oxidation, dissolution, and partitioning to food sources such as particulate organic carbon (Morse & Luther 1999, Campana et al. 2012, Simpson et al. 2012). There have been some experiments testing metal toxicity of sulfides to communities (e.g. Simpson & Batley 2007, Fuchida et al. 2017), but these have concentrated on metal concentrations in pelagic or bottom waters rather than pore-waters and sediments. Thus, research is needed to understand (1) the bioavailability of sulfide metals to infaunal communities, (2) how concentrations of metals in sediments deposited from mining will affect these communities and (3) the extent of these effects. While the potential toxicity of material released into the water column from deep-sea mining remains largely unknown, deep-sea mining experts consider toxicity from the mining of sulfides to be one of the greatest risks (Washburn et al. 2019).

While the SMS mound targeted for test mining in this study was inactive, the Jade site includes several active hydrothermal vents (Halbach et al. 1989). Drilling activity at an inactive deposit in another field within the Okinawa Trough, resulted in reactivation of hydrothermal activity there, in essence creating hydrothermal vent habitat (Kawagucci et al. 2013). This resulted in the appearance of microbial mats and vent-endemic species and converted soft-sediment habitat to hardened substrate for at least 3 yr following disturbance (Nakajima et al. 2015), all of which would greatly impact infaunal communities. In this study, mining activities did not appear to reactivate hydrothermal activity in the area, although substrate was only removed up to ~1 m in depth.

The findings from this study are unfortunately limited by the low numbers of sampling sites and times. The main results and conclusions in this paper are well-supported by the data acquired; however, these data are not sufficient to fully explain natural spatial or temporal variability, nor completely represent the benthic communities sampled for some components (e.g. the small core size was not able to characterize macrofaunal communities as evidenced by their very low abundances). Deep-sea benthic communities are well known to be highly heterogenous over both space and time. In order to estimate the impacts of mining in their entirety, we recommend sampling around any disturbance site in a classic 'bulls-eye' pattern. This entails collecting samples along transects at several distances from the disturbance (e.g. 20, 50, 100 m) in multiple directions (e.g. north, south, east, and west). The inclusion of more replicates per site (e.g. 5) and more pre-test sampling would also aid in capturing natural spatial and temporal variability. This of course requires additional time and expense for both collection and processing, and truly adequate sampling designs are often not logistically possible given limited resources. Hopefully our work will help provide a template for using current direction and topography so that future studies are designed to use available resources in the most efficient way possible. In spite of the lack of adequate baseline data to fully describe natural variability, the impacts of the SMS mining trial were still large enough to demonstrate significant differences.

5. CONCLUSIONS

First-of-their-kind experiments involving mining activities of SMS are taking place in the Okinawa Trough under the auspices of the government of Japan. At an inactive sulfide mound, removal of a small amount of substrate over a short time frame resulted in physical, chemical, and biological impacts within at least 30–40 m of the disturbance site. Physical impacts of mining included re-sedimentation, while chemical impacts included increased concentrations of metals: Cd, Pb, Hg, Zn, Fe, and Cu. Current direction and slope appeared to influence the extent of impacts. While elevated metal concentrations may be used to determine the spatial extent of mining impacts during mining and immediately after activities cease, they may not show the full extent of the footprint within 1–2 yr.

Benthic communities of different size classes were impacted differently by the mining experiment. Nanofaunal abundances were not significantly altered, although they did increase at the unimpacted site after the mining test compared to impacted locations. Meiofaunal arthropod abundances and nematode community structure appeared to be impacted by the mining experiment almost immediately after it took place, while observable changes to meiofaunal nematode abundances did not occur until several weeks later. Meiofaunal abundance and nanofaunal communities may have largely recovered within 1–2 yr, but nematode community structure remained altered at the heavily impacted site 3 yr later. In contrast, macrofaunal communities appeared to be impacted almost immediately after the mining test with impacts possibly persisting at 3 yr, even at moderately impacted sites.

Results from this mining disturbance test suggest that while toxicity from heavy metals may be an important risk from SMS mining, re-sedimentation will likely have a larger areal footprint. It also suggests that larger benthic components are more susceptible to impacts from mining. Finally, it reinforces the need for extensive sampling, both spatially and temporally, to distinguish impacts of deep-sea mining from natural variability at highly heterogeneous sulfide habitats. Future studies needed include examining natural spatial and temporal variability at SMS of both environmental and biological characteristics, studying the extent to which currents and seafloor slope affect re-sedimentation following deep-sea mining activities, determining the bioavailability of various metals released during SMS mining, testing the tolerance of deep-sea infaunal communities to different heavy metals and sediment burial, exploring species-level changes in benthic communities caused by deep-sea mining, and determining mechanisms of community recovery. Our research will aid in guiding the design of future studies by showing the importance of current direction and topography on mining impacts, providing data on metals likely to be elevated from sulfide mining, and showing that impacts will likely need to be monitored beyond 50 m. As nematode community structure and macrofauna are affected for longer periods of time than other benthic metrics, a focus on these parameters in the future may help to determine when full recovery has occurred.

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