TITLE
Into the Benthos and Back: What have Infauna Taught Us

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Because of death and gravity, the bottom of the sea is the memory of the ecosystem, where a record of all past events can be found as you move into deeper layers of sediment. Thus, benthos are primary indicators for environmental assessments. As hydrocarbon exploration and production moved to deeper waters, so did environmental studies. But there were only a few Gulf-wide surveys in the deepest parts of the Gulf of Mexico, and our understanding of deep-sea processes was based primarily on other regions of the world. The intensive focus on deep-sea response during and after the Deepwater Horizon (DWH) accident increased our knowledge dramatically. We learned that the deep sea is dynamic, fragile, and will take a long time to recover. There was a 50% loss of biodiversity within 9 km diameter around the DWH site, and a 10% within a 17 km of the site. But there is still much to learn. The deep-sea is a reservoir of biodiversity on Earth, but about 60% of Gulf of Mexico taxa are still unknown, which is a major hinderance to understanding the effects of oil spills. The northern Gulf of Mexico is dominated by Mississippi River outflow, but exactly how it drives deep-sea dynamics needs better resolution. Two outcomes of the last decade of research is that we know benthos diversity is a sensitive indicator of environmental change and damage, the surface sediments are the biologically active zones, and the natural process of sinking particles will eventually cover the damaged sediment leading to natural recovery. This "restoration in place" strategy must be confirmed by future monitoring and assessment studies.

INTRODUCTION

Oceans cover 71 percent of the Earth's surface. That means benthic (i.e., bottom) habitat also covers 71 percent of the Earth's surface, making benthos (i.e., bottom-dwelling organisms) members of the largest habitat on Earth. Yet, the National Oceanic and Atmospheric Administration (NOAA 2017) estimated that as much as 95 percent of the world's oceans and 99 percent of the ocean floor are unexplored. The lack of information about benthos in general, but the deep sea in particular, is problematic because benthos are sensitive bioindicators of disturbances, such as oil spills.

Benthos depend on the flux of organic material from the water column above, and deposited organic material is a main source of nutrition for a variety of suspension feeders, browsers, and deposit feeders. Because everything in the ocean dies and eventually falls to the bottom, there is a record of past events in the sediments. Thus, the benthic habitat is the "memory" of the ecosystem with a record of past and recent events. Benthos are often used as bioindicators of ecological conditions in environmental assessment studies because they are 1) relatively sedentary (meaning they cannot avoid deteriorating water and sediment quality conditions), 2) have relatively long life spans (which can indicate and integrate water and sediment quality conditions), 3) consist of different species that exhibit different tolerances to stress (because there are sensitive and tolerant functional groups), 4) are commercially important or are important food sources for economically or recreationally important species, and 5) have an important role in cycling nutrients and other chemicals between the sediments and the water column (Dauer 1993). There is a good ecological model, named succession theory, which predicts how benthic communities change when affected by organic enrichment (Pearson & Rosenberg 1978) or physical disturbance (Rhoads et al. 1978). Disturbed environments are characterized by a few, small, tolerant, surface-dwelling, and rapidly growing species (i.e., r-selected species); and healthier environments are characterized by many larger, sensitive, deeper-dwelling, and slower growing species (i.e., k-selected species). Thus, communities develop over time since a disturbance in an analogous way that communities change with distance from a source of pollution. The life-style and characteristics of benthos have led to the development of many schemes to create metrics of benthic community structure that can indicate environmental health, such as the nematode-copepod ratio [N:C] (Raffaelli and Mason 1981, the biotic index [BI] (Borja et al. 2000), and a benthic index of biotic integrity [BIBI] (Weisberg et al. 1997, LLanso et al., 2003, Morehead et al. 2008).

Benthos are classified in different ways, which can depend on locomotion, location, functional group, size, and phylogeny. Epifauna are typically larger and mobile and can crawl along the surface of sediments. Infauna are typically smaller and live in the sediment. Macrofauna are larger (greater than 0.3 or 0.5 mm in size) than meiofauna (from 0.042 or 0.062 mm to macrofauna size). There are also more phyla in the meiofauna than the macrofauna, and meiofauna mostly have direct development (i.e., laying eggs directly to the sediment or larvae emerging directly from eggs) compared to macrofauna that mostly have larval dispersal stages, so the difference isn't based on size alone. There have been many ways to categorize benthic feeding guilds or functional groups (Fauchald and Jumars 1979), but I prefer the simple surface versus subsurface feeder scheme (Tenore et al. 2006). Benthic studies should include both meiofauna and macrofauna because they represent different lifestyles, functional groups, trophic levels, and phyla.

When the Deepwater Horizon (DWH) blowout occurred in April 2010, it was inevitable that benthic metrics would be used to assess damage to the bottom of the ocean because of the importance of benthos as indicators of ecosystem health. Ecosystem health is assessed by determining if indicators of ecological conditions are in an acceptable range; and integrity is acceptable when biological diversity, species composition, structural redundancy, and functional processes are comparable to that of natural habitats in the same region (Montagna et al. 2013). Diversity is the key indicator of ecological integrity. The purpose here is to review the studies providing benthic assessments of ecosystem health of the deep sea after the DWH blowout and provide guidance on how this information can be used to assess recovery and assess future spills.

METHODS

Sealing the leaking wellhead took several months beginning 12 July 2010 and finishing on 17 September 2010. Three cruises were completed to assess the effects of the DWH oil spill on benthic communities: from 16 September through 30 October 2010 (two to four months after capping), from 23 May through 11 June 2011 (10 to 11 months after capping), and from 29 May through 28 June 2014 (45 to 47 months after capping). The 2010 cruise focused on the areal extent of the spill and sampled 179 stations (Montagna and Arismendez 2019), while the 2011 and 2014 cruises focused on temporal changes at 34 stations with 20 in the impact zone and 14 in the non-impact zone (Reuscher et al. 2017). Initially, only 58 stations were analyzed for macrofauna (Montagna et al. 2013) but eventually 116 stations were analyzed for macrofauna distribution (Reuscher et al. 2020).

Sediment samples were collected using a Bowers and Connelly MAXI corer manufactured by OSIL, which collects 12 10-cm inner diameter cores with each deployment (or drop). Detailed methods are provided in Montagna et al. (2013, 2017), and here is a brief description. Sediment was preserved for macrofauna or meiofauna analyses with 5% formalin, and for chemical, geological, or toxicological analyses by freezing. Macrofauna were extracted on a 0.3 mm sieve and identified to family level. Meiofauna were extracted on a 0.042 mm sieve and identified to higher taxonomic levels, ranging from order to phylum. Higher taxonomic levels have proved to be reliable surrogates for species level identification for the assessment of oil pollution impacts on benthic infauna communities (Somerfield and Clarke 1995, Gomez Gesteira et al. 2003). The chemical contaminant measurements were made by contractors and the data are publicly available (see acknowledgements). Sediment toxicity was measured using the Microtox® system where the bioluminescent bacterium, *Vibrio fischeri*, is exposed to the sediment and reduced fluorescence indicates toxicity (Montagna and Arismendez 2019). Toxicity is reported as effect concentration 50 (EC50), which is the concentration of sediment required to elicit a negative 50% effect relative to a control sample.

Principal components analyses (PCA) was used to classify the biological and environmental variables, which was log transformed and then normalized to a mean o zero and standard deviation of 1. The sample scores from the PCA were interpolated using Kriging geostatistical techniques to construct maps of the oils spill footprint (Montagna et al. 2013).

RESULTS/DISCUSSION

From 20 April to 15 July 2010, between 3.19 million barrels (DWH NRDA Trustees 2016) and 6.2 million barrels (Griffiths 2012) of oil was released from the Macondo 252 wellhead into the Gulf of Mexico. That is enough energy to fuel the world for 40 minutes. This was the first large release of hydrocarbons at great depths (Peterson et al. 2012). Release of hydrocarbons at extreme pressure and temperature combined with the use of nearly 2 million gallons of dispersant resulted in a massive underwater plume (Socolofsky et al. 2011). Some of the plume impinged on the bottom around 1000 m in depth, but some of the finely dispersed droplets and emulsions were deposited to the bottom via a Marine Oiled Snow (MOS) (Daly et al. 2016). This impingement, fallout, and MOS contaminated a very large area of the deep-sea bottom, was estimated to be 4 – 31% of all the oil spilled after the DWH event and covered an area of about 3,200 km^2 (Valentine et al. 2014). Other studies estimated up to 57% of the oil remained in the deep (Passow and Hetland 2016), and covered areas up to 24,000 km^2 (Chanton et al. 2014). This amount of oil contamination in the deep seafloor. Because oil reached the bottom, there was great concern that there would be benthic effects (Peterson et al. 2012).

The initial cap stack was put in place and closed on 15 July 2010, which essentially shut off the oil leaking from the wellhead (USCG 2011). Initially, 658 sediment samples were collected through 20 September 2010 to determine if there was oil on the bottom, and if it was at toxic concentrations (UAC 2010). Based on U.S. Environmental Protection Agency (EPA) standards, it was concluded that only 12 samples exceeded EPA Aquatic Life Chronic Benchmarks for polycyclic aromatic hydrocarbons (PAH), and 2 samples exceeded EPA Aquatic Life Acute Benchmarks for PAH. These standards are based on responses of aquatic life in shallow water ecosystems.

Toxic responses found toxic responses in sediments from 24 of 179 station locations, and toxicity could be detected at PAH concentrations as low as 140 $\mu g\ kg^{-1}$ (i.e., ppb) (Montagna and

Arismendez 2019) (Fig. 1). Microtox sediment toxicity was found within 50 km of the DWH wellhead site, and mainly along the depth contours ranging from the southwest to the northeast.

Sediment toxicity benchmarks for benthic invertebrates at the DWH site were developed for taxa richness from 37 of the stations from the same sampling set (Balthis et al., 2017). Likelihood of impacts to benthic macrofauna and meiofauna communities is low (< 20%) at total PAH concentrations of < 4000 µg kg^{-1} for both macrofauna and meiofauna, high (> 80%) at concentrations greater than 24000 µg kg^{-1} for macrofauna, and 25000 µg kg^{-1} for meiofauna. Both the bacteria and invertebrate studies detected much more sediment toxicity than predicted by the EPA aquatic life standards.

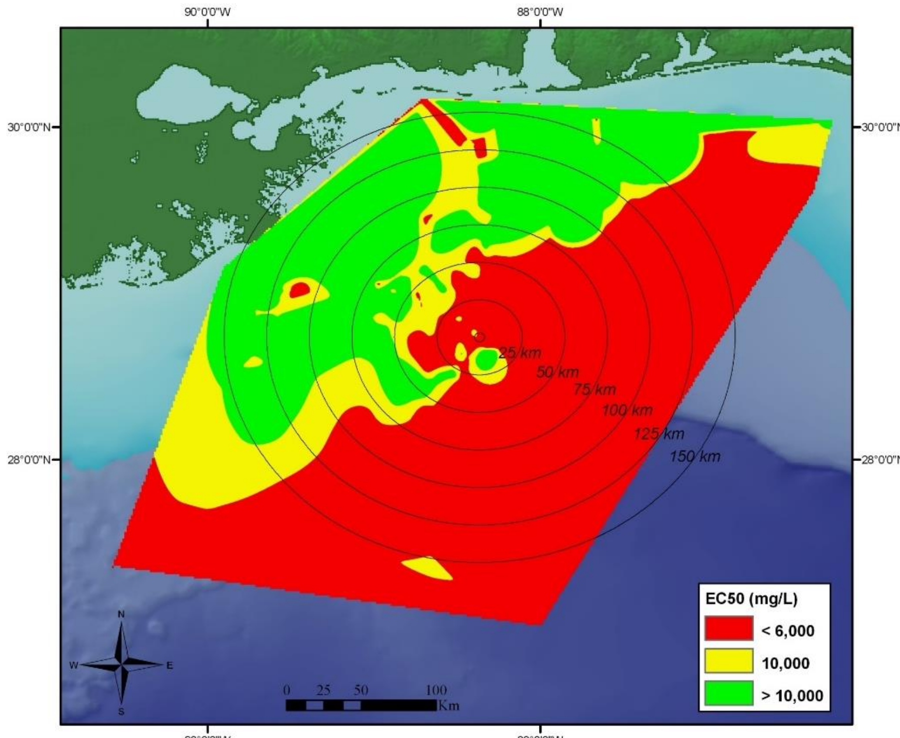

Figure 1. Spatial distribution of sediment microbial toxicity (EC50) in Fall 2010. Modified from Montagna and Arismendez (2019).

While changes in diversity and community structure are sensitive indicators of environmental change (Clarke 1993, Warwick 1993), it is known that benthic community analysis requires specialize knowledge, is labor intensive, and is time consuming (and the combination of the three makes it expensive) (Montagna et al. 2017). Also, data was needed immediately after the oil spill for the Natural Resource Damage Assessment (NRDA) scientific and legal activities. It was decided that the initial benthic community structure assessment would be based on only 58 of the 179 Stations sampled in 2010, so that data would be available for assessment within one year. The initial assessment was based on meiofauna, macrofauna, sediment chemistry (hydrocarbons and metals), and sediment physical characteristics (Montagna et al. 2013). The first problem to solve was how to draw conclusions based on a complex multivariate data set. The question asked was: "how much damage was done over what area?" Therefore, a spatially explicit dose-response approach was taken. The dose-response approach posits that indicators of biotic responses (i.e., decreases in sensitive biotic components and increases in tolerant biotic components) will be inversely correlated with indicators of contamination (i.e., concentrations of hydrocarbons and heavy metals associated with drilling and capping activities), and these differences in responses will decrease with distance away from the spill center until it is indistinguishable from background conditions. So, principal components (PC) analysis was used to reduce the multivariate data to a single new variable representing the oil spill footprint (Fig. 2A), which could then be plotted on a map (Fig. 2B).

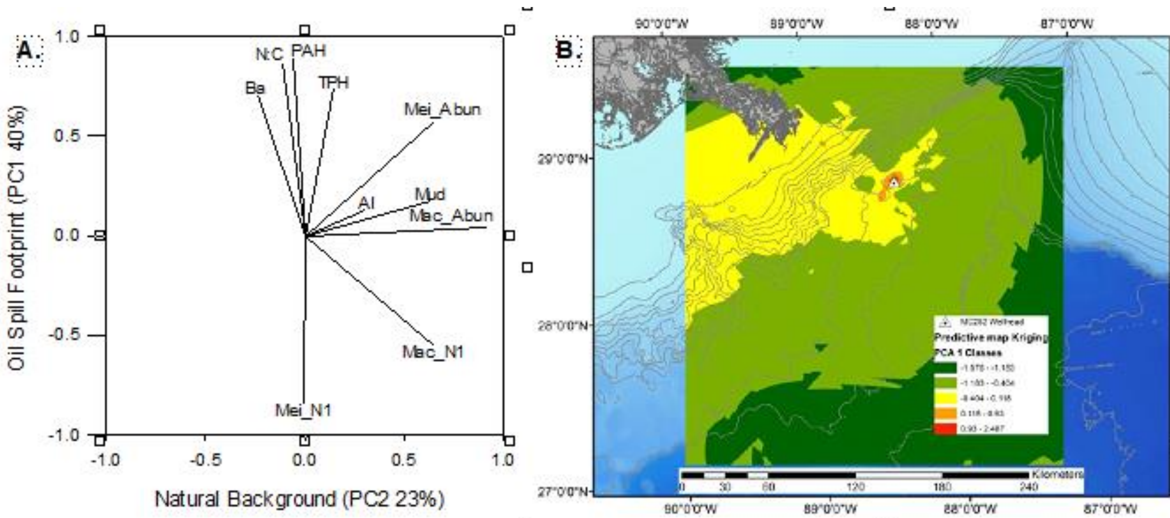

Figure 2. Oil spill footprint in the deep-sea based on 58 stations sampled in 2010. A) Variable reduction. Abbreviations: BA=barium, PAH=polycyclic aromatic hydrocarbons, TPH=total petroleum hydrocarbons, Mud=percent mud content of sediment, AL=aluminum),N:C=nematode to copepod ratio, Mei_Abun=meiofauna abundance, Mac_Abun=macrofauana abundance, Mac_N1=macrofauna Hill's N1 diversity index, and Mei_N1=meiofauna diversity index. B) Footprint based on kriged PC1 values. Modified from Montagna et al. (2013).

The new variable (PC1) defines the oil spill footprint because contaminants e.g., concentrations of polycyclic aromatic hydrocarbons (PAH), total petroleum hydrocarbon (TPH), and barium (Ba) have high values, and biological responses, e.g., low values of meiofauna and macrofauna diversity, and high values of nematode:copepod ratios (N:C) (Fig 2A). Thus, biotic response is inversely related to oil spill contaminants. The most severe relative reduction of faunal abundance and diversity extended to 3 km from the wellhead in all directions covering an area about 24 km^2 (Fig. 2B). Moderate impacts were observed up to 17 km towards the southwest and 8.5 km towards the northeast of the wellhead, covering an area 148 km^2.

Macrofauna diversity losses occurred primarily in the surface layers of sediment, between 0 and 3 cm beneath the surface (Washburn et al. 2017). However, macrofauna abundance increased in some samples close to the wellhead. The increase may indicate an early stage of succession following a disturbance or the presence of tolerant species (Washburn et al. 2017). Macrofauna community structure change was due to an increase in the abundance of opportunistic polychaetes of the family Dorvilleidae, genus Ophryotrocha at contaminated stations near the DWH wellhead. Many macrofauna taxa were bioindicators sensitive or tolerant to DWH contamination (Washburn et al. 2016) (Table 1). Crustacea taxa were sensitive to oil spill contaminants. Polychaete taxa varied in their sensitivity, but Dorvilleidae which is often associated with organic enrichment, was responsible for the largest amount of dissimilarity between stations close and far from the wellhead.

Table 1. Bioindicators of oil spill effects in the deep sea. Classification based relative abundances near(Impact) and far (Background) from the MC252 wellhead in 2010. Modified from Washburn et al.(2016).

Classification	Impact:Background	Class	Family
Sensitive	1:09	Polychaeta	Opheliidae
Sensitive	1:12	Scaphopoda	Scaphopoda
Sensitive	1:14	Cumacea	Nannastacidae
Sensitive	1:14	Tanaidacea	Typhlotanaidae
Sensitive	1:17	Amphipoda	Oedicerotidae
Tolerant	2:01	Polychaeta	Maldanidae
Tolerant	2:01	Polychaeta	Paraonidae
Tolerant	3:01	Bivalvia	Thyasiridae
Tolerant	3:01	Tanaidacea	Sphyrapidae
Tolerant	8:01	Polychaeta	Dorvilleidae

Meiofauna appear to be more sensitive indicators than macrofauna because there are higher numbers of tolerant nematodes relative to more sensitive copepods, thus high N:C ratios. This is a common response to pollution (Raffaelli and Mason 1981). In fact, when the footprint was reanalyzed using meiofauna community structure alone, it was larger (Baguley et al. 2015). The severe impact zone was 52 km^2, which is 2.1 times greater than the estimate when macrofauna are included, and the moderate impact zone was 406 km^2, which is 2.7 times greater than the estimate when macrofauna are included. One reason meiofauna may sensitive to oil deposition is that they live on the surface (Montagna et al. 2016) and are enriched by marine snow (van Eenennaam et al. 2019). In experimental, laboratory studies, meiofauna lack a toxic response to relatively low oil concentrations but are enriched by marine snow (Rohal et al. 2020).

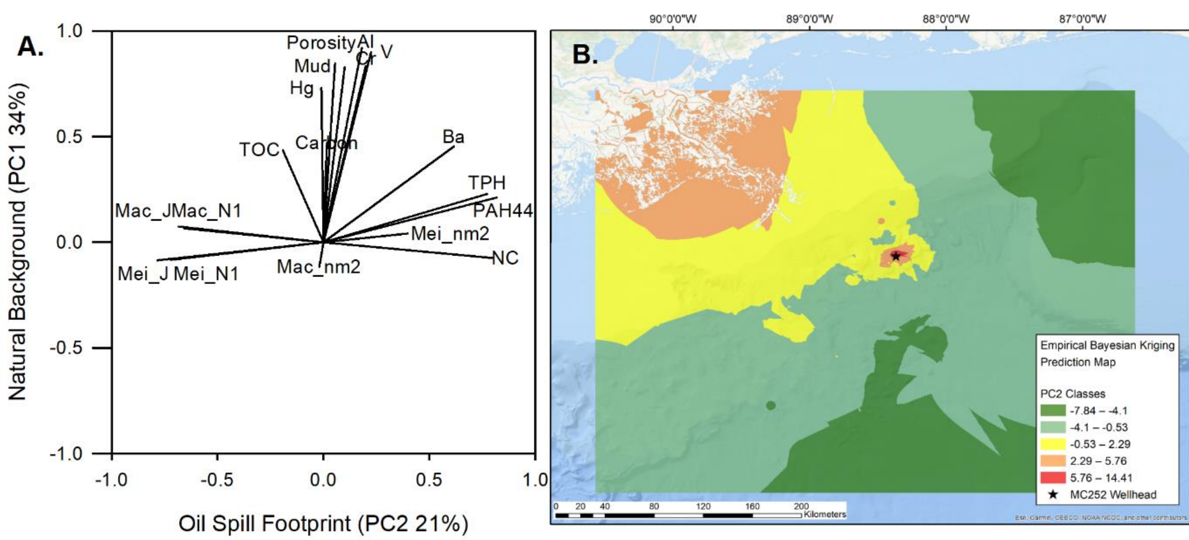

Figure 3. Oil spill footprint in the deep-sea based on 116 stations sampled in 2010. A) Variable reduction. Abbreviations: same as Fig.2 and Hg=mercury, V=vanadium, TOC=total organic carbon. B. Footprint based on kriged PC2 values. Modified from Reuscher et al. (2020).

By 2019, macrofauna from an additional 58 stations collected in 2010 were extracted from sediment and identified (Reuscher et al. 2020). This allows for a re-examination of the footprint to determine if there were any DWH effects to the northeast in DeSoto Canyon, or in shallower water. More environmental variables were added to help distinguish natural background from oil spill effects (Fig 4A), and this plus the greater number of background stations, caused the footprint variable to become the second principal component (PC2). This new analysis identified an area of approximately 263 km^2 around the wellhead as severely or moderately affected, which is 78% higher than the original estimate. The area that was severely damaged was approximately an area of 58 km^2, which is 142% higher than the original estimate. The addition of the new stations extended the area of the benthic footprint map to about twice as large as originally thought and improved the resolution of the spatial interpolation.

To test for trends over time, 20 stations in the severe and moderate zones were classified as the impact zone; and 14 stations farther away were classified as the non-impact zone, and these were re-sampled in 2011 and 2014. The footprint of the oil spill did not change from 2010 to 2011 (Washburn et al. 2017), and there was little evidence of recovery in 2014, four years after the spill (Reuscher et al. 2017). By 2014 there was a reduction in the concentration of total petroleum hydrocarbons (TPH), but it averaged 1166 ppm in the impact zone compared to 102 ppm in the non-impact zone (Fig. 3A). Although there is year-to-year variability in the total number of species (S), there were no changes in the diversity loss for either macrofauna (Fig. 3B), nor meiofauna (Fig. C). The trends for diversity in the impact and non-impact zones are parallel, indicating there has been no recovery of the diversity loss in the impact zone.

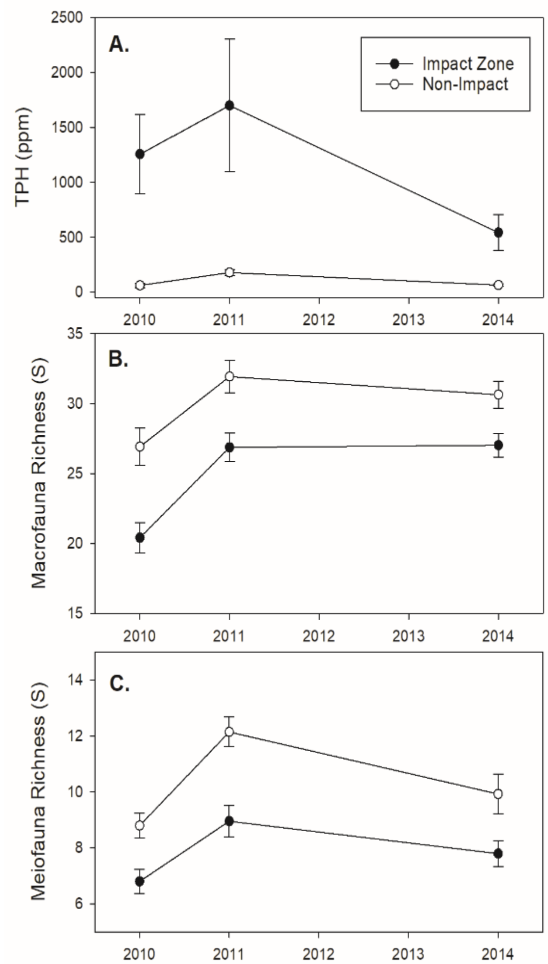

Figure 4. Temporal trends in the impact zone and non-impact zone. Mean and standard error. A) Total petroleum hydrocarbons (TPH). B) Macrofauna diversity. C) Meiofauna diversity. Modified from Reuscher et al. 2017.

Recovery of the deep-sea benthos will likely take a long time. The benthos is mostly distributed in the top 10 cm in Gulf of Mexico deep-sea sediments (Montagna et al. 2016). So, once the oil is capped with 10 cm of fresh sediment, via marine snow and deposition, the area will likely return to full functioning. For example, a biogeochemical signal from the 1979 Ixtoc-1 blowout and oil spill could be found in the top 2.4 to 2.8 cm of sediment in near the wellhead in the Bay of Campeche off the coast of Mexico in 2015 (Rohal et al. 2020). Trends of macrofauna diversity, and the N:C ratio with sediment depth indicate the benthic community has not yet recovered from the Ixtoc oil spill after 36 years. Extrapolating the sedimentation rate, the Bay of Campeche will not likely recover for another 97 years, for a total of 143 years. Because of proximity to the Mississippi River, sedimentation rates in the northern Gulf of Mexico are much higher, ranging from 0.1 – 0.3 cm/y

(Brooks et al. 2015). Based on this rate, it will take 50 years to deposit 10 cm of sediment in the DWH oil spill area.

CONCLUSIONS

We learned that the deep sea is dynamic, fragile, and will take a long time to recover. Benthos metrics, especially diversity, are sensitive indicators of environmental change and ecological health. Damage was mainly a loss of biodiversity. But there is still much to learn. The deep-sea is a reservoir of biodiversity on Earth, but about 60% of Gulf of Mexico taxa are still unknown, which is a major hinderance to understanding the effects of oil spills.

The footprint of an oil spill can be defined by high concentrations of oil and drilling mud indicators, and low diversity of meiofauna and macrofauna. There was a wide-spread oil footprint on the deep-sea bottom consistent with deep-sea DWH plume trajectories and deposition of marine oiled snow events. The losses of biodiversity were mainly in surface sediments indicating that the benthos were smothered by sinking oil and marine oiled snow. Measuring benthic indicators can be used to assess the impact of oil spills anywhere in the world.

Recovery from the oil spill will occur when diversity of the surface sediments is restored. This might be aided by outflow from the Mississippi River, and primary production in the ocean surface waters. We know the surface sediments of all bottom habitats are the biologically active zones. The surface sediments are also damaged by deposition of oil onto the sediment because of smothering and toxicity. In the deep sea, the natural process of sinking particles will eventually cover the damaged sediment leading to natural recovery, but this will take decades or even a century or more. This "restoration in place" theory must be confirmed by future monitoring and assessment studies.

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