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Development of a Benthic Foraminifera Based Marine Biotic Index (Foram-AMBI) for the Gulf of Mexico: a Decision Support Tool

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Development of a Benthic Foraminifera Based Marine Biotic Index (Foram-AMBI) for the Gulf of Mexico: a Decision Support Tool.

by

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Introduction to the Gulf of Mexico and bioindicators

1.1 Introduction

The ocean plays a vital role in the global economy and associated activities account for up to 1.5 trillion US dollars in GDP per year. The ocean economy is expected to double by the year 2030 (OECD, 2016). The Organisation for Economic Cooperation and Development (OECD) projects over 40 million jobs will be created and based in ocean industry by 2030 (OECD, 2016). In addition to tangible resources like food, energy, minerals, and pharmaceuticals, the ocean also provides ecosystem services critical to human society like oxygen production and weather and climate regulation (Rayner et al., 2019). Both economic growth and ecosystem services are dependent on the sustainability of these ocean resources and the ability to manage them properly (Rayner et al., 2019). However, with rapid population growth and innovations in technology that allow for ultra-deep water exploration and exploitation of marine resources (>1500m), the risk of anthropogenic pollution increases coincidently (Murawski et al., 2020a). Increasing concern over the effects of anthropogenic pollution of important water bodies has generated a discussion amongst scientists, managers, and policy makers about effective ways to monitor and mitigate this impact.

Reference or pre-impact conditions are critical to monitoring programs of non-point source anthropogenic pollution as well as events like the Deepwater Horizon blowout because they provide quantitative values of ecological quality of a region at a specific moment in time (Cordes et al., 2016). Reference conditions are the basis of all monitoring programs and provide spatial and temporal comparability (Cordes et al., 2016) to assess the effectiveness of past and current management practices. Successful monitoring programs employ long-term monitoring with frequent sampling intervals in order to capture natural environmental variability (WFD, 2000/60/EC, Parker and Wiens, 2005, Cordes et al., 2016). Parker and Wiens (2005) introduced four possible ecological assumptions that incorporate natural environmental variability into impact and recovery models. They argue that without long-term monitoring in place, recovery of impacted systems may be prematurely established (Parker and Wiens, 2005). The development of a system-level benthic-management decision support tool for long-term biomonitoring is essential for the protection of one of North America's largest economic assets, the Gulf of Mexico.

1.2 Setting

The Gulf of Mexico is a semi-enclosed basin surrounded by continental landmass on three sides and an indispensable asset to the countries that border it. The Gulf is comprised of the Exclusive Economic Zones of Cuba, Mexico, and the United States of America. The value of the Gulf of Mexico's ecosystem-based goods and services to these three countries is estimated to be around 700 billion US dollars per year (Shepard et al., 2013). The United States relies on the Gulf of Mexico for over 90% of its marine derived oil production and up to 20% of its total oil production (Murawski et al., 2020a). The Gulf of Mexico is also home to one of the most productive fisheries in the world, accounting for over 25% of the United States commercial fish landings and 40% of recreational fish landings (NMFS, 2017; Chesney et al., 2000). One reason for this is the diverse breadth of habitats that occur in the Gulf including mangroves, marshes, river mouths, reefs, deepwater, and continental shelf and slope environments (Ward et al., 2017). Due to its semi-enclosed nature, the Gulf of Mexico receives freshwater discharge from 33 major rivers and is the recipient of watershed drainage from Canada, Cuba, Guatemala, Mexico, and the United States (Kumpf et al., 1999; EPA, 2015). Watershed drainage is one of the leading causes of chronic anthropogenic stress and hypoxia on the coastal ecosystems of the Gulf of Mexico (EPA, 2015; Rabalais et al., 1999; 2009). Nitrogen and phosphorous sources are primarily from agriculture, wastewater treatment, atmospheric deposition, and urban runoff (EPA, 2015). The loss of wetlands due to conversion into agricultural lands and the channelization of the Mississippi River have led to nutrient loading, which causes eutrophication resulting in hypoxic dead zones in the Gulf of Mexico (EPA, 2015; Rabalais et al., 1999; 2009).

In addition to chronic stressors, like eutrophication and hypoxia, the Gulf of Mexico is faced with other acute anthropogenic stressors like oil spills. Two of the largest oil spills ever took place in the Gulf, the IXTOC-1 oil spill in 1979, and the Deepwater Horizon oil spill in 2010. The IXTOC-1 blowout leaked over 130 million US gallons of oil over a ten-month period at a depth of 50 meters in the Bay of Campeche introducing polycyclic aromatic hydrocarbons to the sediment in the form of marine oil snow (Farrington, 1980; Jernelöv and Lindén, 1981; Daly, 2014; Ruiz-Fernandez et al., 2016). The dispersant, Corexit 9527, was administered to the area and oil was transported by prevailing currents to the northwest (Farrington, 1980; Jernelöv and Lindén, 1981; Boehm and Flest, 1982). In April 2010, the largest accidental oil spill occurred in the Northern Gulf of Mexico when the Macondo well drilled by the Deepwater Horizon platform blew out at 1,500 meters water depth and introduced 210 million gallons of crude oil into the surrounding environment over an 87-day window (US Department of Interior, 2010; Atlas and Hazen, 2011; Kujawinski et al., 2011). The Deepwater Horizon oil spill caused a Marine Oil

Snow Sedimentation and Flocculent Accumulation (MOSSFA) event, which occurred when marine snow, oil, biopolymers from phytoplankton, and sedimentary particulates from the Mississippi River amalgamated and settled to the seafloor depositing polycyclic aromatic hydrocarbons (Daly et al., 2016; Passow et al., 2012; Passow and Ziervogel, 2016; Romero et al., 2015). This was found to be an acute stressor to benthic communities like meio- and macro-invertebrates, which decreased in diversity by -38% and -54% respectively, as well as benthic foraminiferal communities, which decreased in density by 80-93% (Montagna et al., 2013; 2017; Schwing et al., 2015; Washburn et al., 2016; 2017). Benthic meio- and macro-fauna are especially important to benthic pelagic coupling of the food web in deep-sea environments because of their low trophic levels and the bottom-up effect this has on commercially important fish (Griffiths et al., 2017; Fisher et al., 2016)

After the 2010 Deepwater Horizon oil spill in the northern Gulf of Mexico, the Natural Resource Damage Assessment (NRDA) Restoration Program was left with the challenge of parsing out the complexities of the Gulf of Mexico ecosystem and establishing baselines in order to accurately assign value to and assess immediate and long-term damage (NRC, 2012; Shepard et al., 2013). This process, which has historically been done only for shallow-water events, unveiled the lack of deep-water baselines and reference conditions in the Gulf of Mexico and demonstrated the need for ecosystem-based management and monitoring (NRC, 2012; Shepard et al., 2013; Parker and Wiens, 2005). The ecosystem-based approach takes into consideration the beneficial services and resources provided by the functioning of an ecosystem (NRC, 2012). Ecosystem services include direct services and goods sourced from the ecosystem (e.g. seafood and petroleum), regulating services (e.g. flood control, climate regulation), cultural services (e.g.

recreation), and supporting services (e.g. primary production, nutrient cycling) (Shepard et al, 2013).

Since the development of ultra-deep water (>1500 m) oil exploration and production, over half of the US supply of marine-derived petroleum is now sourced from wells deeper than 1500 meters (Murawski et al., 2020a). Ultra-deep petroleum exploration and production comes with challenges like dealing with high pressures, strong currents, and low temperatures, which makes oil spill response and clean up in these environments equally as difficult and is a big reason why the Deepwater Horizon oil spill at 1,500 meters was so catastrophic (Murawski et al., 2020a). The development of environmental reference conditions and an understanding of the Gulf of Mexico ecosystem vulnerability through long-term monitoring will help minimize the risk in case of another ultra-deep water blowout and help disentangle the acute effects from chronic, long-term ecosystem changes (Shepard et al., 2013; Nelson & Grubesic, 2018; Murawski et al., 2020a).

In an effort to address concerns about the degradation of water resources and the increasing threat of anthropogenic pollution, the European Union (EU) established the European Water Framework Directive (WFD, 2000/60/EC). The goals of this directive are to prevent water resources from further deterioration and to restore eco-regions that do not meet the established standards (WFD, 2000/60/EC). It requires that regional water bodies be regularly evaluated on their Ecological Quality Statuses (EcoQS). The abundance of biological components (benthos, phytoplankton, fish) in concert with physicochemical attributes are used to calculate the EcoQS, which is split into five categories: High, Good, Moderate, Poor, and Bad (Borja et al., 2003). This directive has resulted in the development of a number of biotic indices that can qualitatively and quantitatively evaluate environmental impact on soft-bottom habitats (Borja et al., 2016).

1.3 Marine Biotic Indices

The most widely used of the environmental biotic indices is AZTI's Marine Biotic Index (AMBI), which was developed by Borja et al. (2000) to characterize benthic habitat by employing the relative abundances of macroinvertebrates in response to organic matter enrichment and assigning them to ecological groups: Sensitive, Indifferent, 3rd-order opportunists, 2nd-order opportunists, 3rd-order opportunists (I-V). The development of these Ecological Groups was based off of the ecological models of Hily and Glèmarec (1981). Benthic fauna are ideal bio-indicators because of their rapid responses to environmental change, their relatively non-motile life mode, their varying sensitivities to water/sediment quality, and their essential roles in water-sediment nutrient cycling and carbon preservation/degradation as well as the trophic transfer of organic matter (Danovaro et al., 2008). Benthic faunal distribution occurs along a continuum of environmental gradients, implying species-specific sensitivity, allowing for qualitative monitoring of these communities (Lindroth et al., 1971; Pearson & Rosenberg, 1978). In this case, community is defined by Mills (1969) as an assemblage of organisms occurring in a particular environment presumably interacting with each other and with the environment and separable from other communities by means of ecological survey. In Borja's (2000) AMBI index, organic matter enrichment is used as a proxy for pollution because organic matter input accompanies long-term contamination as well as contamination events and is a major control of benthic community composition according to the Pearson-Rosenberg paradigm (Pearson & Rosenberg, 1978; Scott et al., 2001; Romano et al., 2009). Ultimately, an increase in the organic deposition is accompanied by abiotic factors such as hypoxia, physicochemical changes in the sediment, and inorganic deposition at the sediment-water interface resulting in a more stressed benthic habitat thus changing the community structure to one with more tolerant species

(Pearson & Stanley, 1979). The resulting AMBI coefficient is directly related to the EcoQS ratings defined by Borja et al. (2003). For example, if the dominant Ecological Group is mainly composed by opportunistic species, then it is more affected. When the dominant Ecological Group is Group I (AMBI = 0-1.2) then the EcoQS is considered undisturbed (Tran et al., 2018). If the dominant Ecological Group is Group II (AMBI=1.3-3.3) then the EcoQS is slightly disturbed (Tran et al., 2018). If the AMBI is dominated by a combination of Group II-IV (AMBI=3.4-5.0) then the EcoQS is considered moderately disturbed (Tran et al., 2018). Finally, when the assemblage is dominated by Group V (AMBI=5.0-6) then the EcoQS is considered heavily disturbed (Tran et al., 2018). The AMBI has become one of the most widely used biotic indices across the world because of the database of classified species and how easily reproducible it is across different geographic regions. (Borja et al., 2003; Tran et al., 2018; Santibañez-Aguascalientes et al., 2018)

Traditionally, macroinvertebrates have been used to apply the AMBI (Borja et al., 2000, 2003; Muxica et al., 2005; Salas et al., 2004; Carvalho et al., 2006; Teixeira et al., 2012). However, meiofauna, such as benthic foraminifera, have proven to be robust indicators of environmental quality (Alve, 1995; Armynot du Chatelet et al., 2004, 2011; Alve et al., 2016; Culver and Buzas, 1995; Jorissen et al., 2018). Benthic foraminifera (BF) are advantageous in many circumstances because of their high biodiversity (>4,000 spp.), species-specific ecological niches, and high abundance in all marine and transitional environments providing a reliable database for statistical analysis even when restricted to small sample volumes (Alve, 1995; Scott et al., 2001; Martinez-Colón et al., 2009). In order to determine how and why foraminifera are optimal indicators of abiotic factors affecting the ambient environment, their biology and preservation must be examined.

1.4 Benthic Foraminifera

The taxon name, foraminifera, translates directly to "pore-bearers". Foraminifera are single-celled amoeboid protists that secrete or assemble calcareous, organic, or agglutinated tests (Sen Gupta, 1999). These tests have many pores to allow the cell to extend multipurpose granuloreticulopodia into the surrounding environment for feeding, locomotion, growth, as well as reproduction (Sandon, 1934). A wide variety of feeding methods are available through the use of pseudopodia including herbivory, carnivory, deposit feeding, suspension feeding, parasitism, and even autotrophism/mixotrophism through symbiotic relationships with algae (Goldstein, 1994, 1999; Todd, 1965; Leutenegger, 1984). The most common method is deposit feeding; so many foraminiferal assemblages rely on labile organic matter and its heterotrophic microbial consumers (Martins et al., 2015).

Since foraminifera are ubiquitous throughout nearly all marine environments and depths, they have several modes of life: epifaunal, infaunal, and planktonic. This study will focus on the benthic (infaunal, epifaunal) forms. Epifaunal foraminifera can be fixo-sessile (i.e., encrusting or sessile), semi-sessile (i.e., temporarily motile), or vagile (i.e. motile or permanently motile) whereas infaunal forms are all vagile (Sen Gupta, 1999; Mateu-Vicens et al., 2014). Mode of life and motility play large roles on the ecological response of benthic species to stress conditions (e.g. pollutants, organic matter, hypoxia). For example, vagile foraminifera are able to migrate away from unfavorable environmental conditions (Martins et al., 2015; Platon and Sen Gupta, 2001). Studies have found that epifaunal foraminifera have a higher average velocity of motion than infaunal foraminifera and that there is a correlation between velocity and the number of pores (Kitazato, 1988). The implication here is that epifaunal foraminifera are able to emigrate from stressed environments at a faster rate than infaunal foraminifera.

Calcareous foraminifera secrete their tests by the assimilation of calcium carbonate from the surrounding environment. Some suborders like Robertinina use aragonite to build their tests leaving them more susceptible to dissolution, while most others use either high-Mg or low-Mg calcite (Bandy, 1954; Hohenegger and Piller, 1975). Some species secrete hyaline tests, which are often perforate, low-Mg calcite, and lamellar layers are added to each chamber with the addition of each chamber. Others build porcelaneous tests, which are mainly imperforate and composed of high Mg-calcite crystals. The geochemistry of foraminiferal tests allow for the recording of the immediate surrounding environment (Bard, 1988; McCorkle et al., 1990; Mackensen et al., 1993; Anand et al., 2003) For example, Schwing et al. (2018) found that stable carbon isotope ratios of benthic foraminifera tests can be used as an indicator for marine oil snow sedimentation and flocculent accumulation (MOSSFA).

Benthic foraminifera are ideal for the characterization of benthic habitats due to their universal presence among all marine environments, high preservation potential, high abundance and biodiversity (>4,000 spp.), and short life span (weeks to years) (Lee et al., 1991, Goldstein, 1999). Zalesny et al. (1959) documented the effects of pollution on benthic foraminiferal distribution in Santa Monica Bay, California. Shortly after, Resig (1960) and Watkins (1961) suggested a correlation between the abundance of benthic foraminifera and pollution and proposed their use as bioindicators. Since these landmark studies, a multitude of papers have been published regarding the effects of environmental stressors on foraminifera and how they can be used to identify stressed regions (e.g., Boltovskoy and Wright, 1976; Alve, 1995; Culver and Buzas, 1995; Yanko et al., 1999; Martínez-Colón et al., 2009; Armynot du Châtelet et al., 2010; Frontalini and Coccioni, 2011; Schwing et al., 2018). While pollution can dictate foraminiferal ecology, natural abiotic factors play the biggest role geographically in the distribution and diversity (Donnici et al., 1997). Abiotic gradients include salinity, pH, temperature, substrate, light, dissolved oxygen, and nutrients. Shelford's Law of Tolerance (Fig.1) states that the abundance of a species and its resulting success is dependent on optimum levels of environmental gradients (Shelford, 1931). Foraminiferal species have varying optimal ranges for each gradient and as a result, individual species ranges are determined by the subdivision of key environmental factor(s) into overlapping intervals (Hohenegger, 2000). Correspondence to these factor(s) is characterized by the location, distribution form, and abundance of species along the gradient known as the coenocline (Hohenegger, 2000).

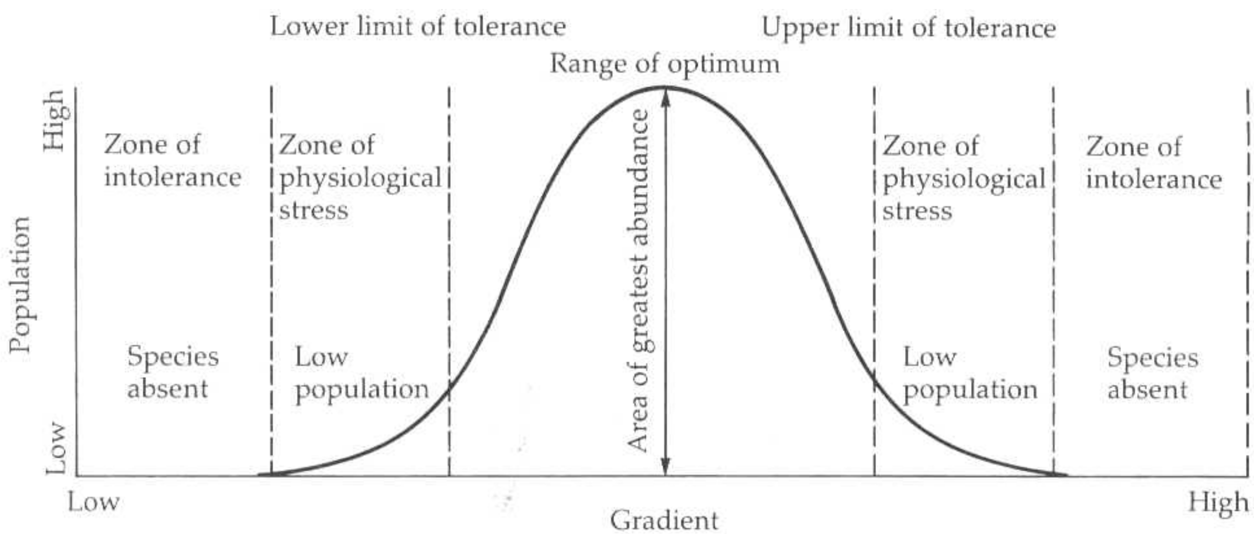


Figure 1. Shelford's Law of Tolerance diagram shows how population abundance is affected by varying levels or concentrations of an environmental parameter (Shelford, 1931).

The availability of oxygen and organic flux to the sea floor are considered the most important abiotic factors controlling foraminiferal community structure (Miller and Lohmann, 1982; Bernhard, 1986; Bernhard and Sen Gupta, 1999; Kaiho, 1994, 1999; Van der Zwaan et al., 1999; Donnici and Barbero, 2002; Panieri, 2006). Jorissen et al. (1995) presented the TROX model (Fig. 2), which is based on the premise that foraminiferal fauna have certain critical oxygen requirements as well as quantitative and qualitative food requirements. This model shows that in highly oligotrophic regions, all deposited labile organic material is almost immediately metabolized at the sediment-water interface. Only epifaunal and shallow infaunal foraminifera can be found in these areas. In eutrophic regions (right portion of Fig. 2), organic flux increases and metabolizable carbon reaches deeper in the sediments, due to bioturbation, leading to the presence of epifaunal as well as shallow and deep infaunal species. When conditions are fully eutrophic the major control of infaunal penetration depth switches from food availability to oxygen availability (Jorissen et al., 1995). Under anoxic conditions, negative geotaxis caused by external stimuli, like hydrogen sulfide, becomes the main control of microhabitat depth (Duijnstee et al., 2003).

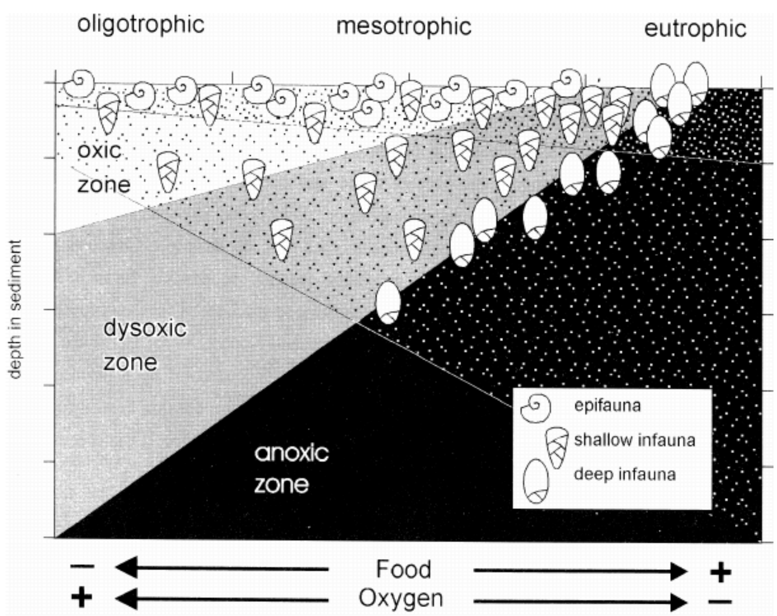


Figure 2. TROX model proposed by Jorissen et al. (1999) relating oxygen concentration to organic flux and foraminiferal morphotypes.

Foraminiferal assemblages are representative of the environment in which they live and prove useful as bioindicators of environmental change (Murray, 2002). As previously discussed, oxygen and organic matter flux are two parameters that are closely intertwined. Cannariato et al. (1999) used foraminifera as tracers of bottom-water oxygenation in the Santa Barbara Basin over a 60,000-year period. Throughout this time, the benthic foraminiferal assemblages transitioned from those typical of oxic conditions to those typical of hypoxic conditions, with suboxic assemblages between the two extremes (Cannariato et al., 1999). These faunal changes were interpreted as reflecting the expansion and contraction of the Oxygen Minimum Zone in response to climatic changes that lead to changes in ocean surface production and basin ventilation (Cannariato et al., 1999).

Benthic foraminiferal ecological distributions can be used to detect areas of contamination and pollution as well as eutrophication. With increasing proximity to the point source, there are changes in assemblage as well as a reduction in species diversity along with the dominance of opportunistic taxa (Frontalini and Coccioni, 2011). There are also higher percentages of abnormal, pyritized specimens and dwarf assemblages associated with increased contamination (Alve, 1991; Geslin et al., 2002; Frontalini and Coccioni, 2008). Abnormalities in assemblages can be calculated with the Foraminiferal Abnormality Index (FAI) and can be used to monitor heavy metal contamination in water bodies (Frontalini and Coccioni, 2008) as well as the effect of aquaculture on foraminifera (Debenay et al., 2009). In 2011, the Foraminiferal Index of Environmental Impact (FIEI) was used to evaluate the pollution status of stations after the Bohai oil spill (Lei et al, 2015). This index identifies areas of contamination using opportunistic taxa as indicators of petroleum, specifically polycyclic aromatic hydrocarbons (PAH) (Lei et al., 2015). In the aftermath of oil spills, foraminifera experience a reduction in diversity and density and the development of morphological abnormalities (Lei et al., 2015; Schwing et al., 2015). The PEB index, which is a proxy for hypoxia, is the combined percentages of three species of foraminifera in the assemblage (*Protononion atlanticum*, *Epistominella vitrea*, *Buliminella morgani*), and was used to characterize hypoxia of the northwestern Gulf of Mexico (Osterman, 2003). Thus, benthic foraminifera have a rich history of being successful bioindicators of anthropogenic pollution.

1.5 Foram-AMBI

In 2016, the first application of foraminifera to the AMBI was carried out by Alve et al. (2016) in the northeastern Atlantic basin following the protocol set forth by the Foraminiferal Biomonitoring (FOBIMO) working group, which standardized methodology to establish foraminifera as bioindicators (Schönfeld et al., 2012). This study assigned 128 species to respective ecological groups (I-V), calibrated the foraminifera-AMBI (Foram-AMBI) based on the relative abundance of these groups, and then validated the Foram-AMBI based on independent validation sites comparing them with Shannon diversity (H') and total organic carbon (TOC). This first attempt was followed by Jorissen et al. (2018) for the Mediterranean Sea who assigned, calibrated, and validated the Foram -AMBI in this region. This study addressed the difficulties in the assignment process in great detail including dealing with datasets that had different methods of organic matter quantification, having to parse out synonymic taxa, using thanatocoenoses as well as biocoenoses, and addressing substrate type similarities and differences amongst sites (Jorissen et al., 2018).

The Foram-AMBI is calibrated by assigning species into five groups (I-V) ranging from most sensitive to most tolerant of organic matter enrichment depending on the distribution form of the abundance to organic matter ratio (Fig. 3) (Alve et al., 2016). Romano et al., (2009) showed that a positive correlation exists between TOC and anthropogenic pollution (heavy metals, chemical pollutants) and describes organic enrichment as an accurate proxy to quantify pollution. Group I species are considered "sensitive" and occur in unpolluted oligotrophic conditions. When organic enrichment increases the species abundance decreases. Group II are the "indifferent" foraminifera that disappear in the case of increased organic matter. Group III is comprised of "tolerant" or "3rd-order" opportunists because they display a tolerance to the first stages of organic enrichment and increased abundance towards slightly closer to the point source. Group IV are the "2nd-order" opportunists that show an increase in abundance towards the point source but are absent at the reference station. Finally, Group V are the "1st-order" opportunists whose abundance increases with proximity to the point source (Alve et al., 2016; Jorissen et al., 2018). After the assignment of species into groups I-V, then the Foram-AMBI is calculated for independent foraminiferal assemblages in the same geographic region. These index values are validated by the comparing them to environmental gradients and how closely they correlate to the values of the initial calibration of the index. These values can be used to compare EcoQS with other sub-regions in the same basin. Alve et al. (2016) used the Foram-AMBI in their study to define the EcoQS in the Northeast Atlantic, Arctic fjord, and continental slope and shelf environments. The Foram-AMBI calibration was validated by two independent data sets from the Norwegian Skagerrak coast, which confirmed a good correlation between TOC, diversity and the Foram-AMBI (Alve et al., 2016).

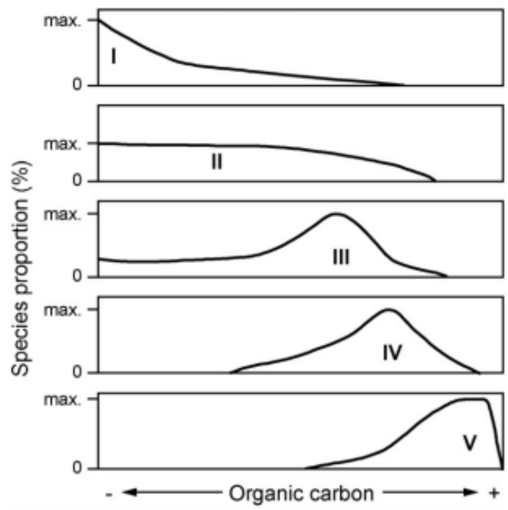


Figure 3. Characteristic distribution forms (Ecological Group I-V) of relative abundance correlated to the organic carbon gradient (Alve et al., 2016).

Jorissen et al. (2018) compiled 15 foraminiferal data sets from previous publications to test the Foram-AMBI in the Mediterranean using biocoenoses and thanatocoenoses. Even though FOBIMO mandates the use of live foraminifera in Foram-AMBI assemblage studies, Jorissen et al. (2018) supplemented their data set with total assemblages that can provide past environmental information. Jorissen et al. (2018) explain in depth how species are assigned if they don't exactly match one of the aforementioned distribution forms. Sediment grain size must be taken into account when analyzing foraminiferal response to TOC gradients. This is to avoid falsely concluding that a decline in a species is due to increased pollution when the grain size is actually what controls the species distribution. Clay sediments will often have a higher TOC and higher pollution resistant taxa, while sandy sediments display lower TOC values and higher pollution sensitive species (Jorissen et al., 2018).

The Foram-AMBI comes with its limitations and challenges. It is difficult to parse out anthropogenic pollution from natural eutrophication. If both exist at a site, foraminifera will often exhibit a "multiple stressors" response, which explains differences in faunal composition at sites with comparable organic enrichment (Jorissen et al., 2018). Another challenge that both Alve et al. (2016) and Jorissen et al. (2018) faced was taxonomic inconsistencies between datasets. Both studies attempted to populate the Foram-AMBI with outside datasets that were inconsistent methodologically and taxonomically. Unfortunately, not all foraminiferal studies include plates or SEM images so species that were just labeled by genus were not considered in the index. This can be avoided by using a dataset such as the one developed by the Gulf of Mexico Research Initiative funded, Center for Integrated Modeling and Analysis of Gulf Excosystems (C-IMAGE) that is consistent in taxonomy and sampling methods.

This study builds upon the efforts of Alve et al. (2016) and Jorissen et al. (2018) by developing and calibrating a Foram-AMBI for the Gulf of Mexico. As previously established, the Gulf of Mexico is an economically critical water body, that faces unique chronic and acute anthropogenic stressors including large water discharges from 33 major rivers and the presence of the petroleum industry in both exploration and production (Kumpf et al., 1999; EPA, 2015; Murawski 2020). This study has assigned benthic foraminifera to ecological groups in a master list that can be used by monitoring programs to determine EcoQS of benthic habitats to establish new reference conditions throughout the Gulf of Mexico in the case of future gas or oil spills and for the monitoring of chronic anthropogenic pollution. Using foraminifera to calculate a Foram-AMBI allows for the comparison of AMBI values between benthic fauna (macroinvertebrates, meioinvertebrates, benthic foraminifera) to determine how these different benthic organisms reflect environmental stress (Alve et al., 2016). The Foram-AMBI has significant potential as a standardized decision support tool that is sensitive to chronic and acute stressors and is useful in its analysis of ecological health beyond the basic diversity indices.

1.6 References

Adams, C. M., Hernandez, E., and Cato, J. C., 2004. The economic significance of the Gulf of Mexico related to population, income, employment, minerals, fisheries and shipping. Ocean & Coastal Management, 47(11-12), 565-580.

Alve, E., 1991. Benthic foraminifera in sediment cores reflecting heavy metal pollution in Sorfjord, western Norway. The Journal of Foraminiferal Research, 21(1), 1-19.

Alve, E., 1995. Benthic foraminiferal responses to estuarine pollution: a review. Journal of Foraminiferal Research, 25(3), 190-203.

Alve, E., Korsun, S., Schönfeld, J., Dijkstra, N., Golikova, E., Hess, S., Panieri, G., 2016. Foram-AMBI: A sensitivity index based on benthic foraminiferal faunas from North-East Atlantic and Arctic fjords, continental shelves and slopes. Marine Micropaleontology, 122, 1-12.

Anand, P., Elderfield, H., & Conte, M. H., 2003. Calibration of Mg/Ca thermometry in planktonic foraminifera from a sediment trap time series. Paleoceanography, 18(2).

Armynot du Châtelet E.A., Debenay J.P., Soulard R., 2004. Foraminiferal proxies for pollution monitoring in moderately polluted harbors. Environ Pollut .127, 27–40.

Armynot du Châtelet, E., Gebhardt, K., Langer, M., 2011. Coastal pollution monitoring: Foraminifera as tracers of environmental perturbation in the port of Boulogne-sur-Mer (Northern France). Neues Jahrbuch für Geologie und Paläontologie. 262. 91–116.

Atlas, R.M. and Hazen, T.C., 2011. Oil biodegradation and bioremediation: a tale of the two worst spills in US history.

Bandy, O. L., 1956. Ecology of foraminifera in northeastern Gulf of Mexico. Geological Survey Professional Paper, 274, 179-204.

Bard, E., 1988. Correction of accelerator mass spectrometry 14C ages measured in planktonic foraminifera: paleoceanographic implications. Paleoceanography, 3(6), 635-645.

Bernhard, J. M., 1986. Characteristic assemblages and morphologies of benthic foraminifera from anoxic, organic-rich deposits; Jurassic through Holocene. Journal of Foraminiferal Research, 16(3), 207-215.

Bernhard, J. M., & Sen Gupta, B. K., 1999. Foraminifera of oxygen-depleted environments. In Modern foraminifera, 201-216.

Boehm, P.D. and Fiest, D.L., 1982. Subsurface distributions of petroleum from an offshore well blowout. The Ixtoc I blowout, Bay of Campeche. Environmental Science & Technology, 16(2), pp.67-74.

Boltovskoy, E., & Wright, R. C., 2013. Recent foraminifera. Springer Science & Business Media.

Borja, A., Elliott, M., Andersen, J. H., Berg, T., Carstensen, J., Halpern, B. S., Rodriguez-Ezpeleta, N., 2016. Overview of integrative assessment of marine systems: the ecosystem approach in practice. Frontiers in Marine Science, 3, 20.

Borja, A., Franco, J., Pérez, V., 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. Marine pollution bulletin, 40(12), 1100-1114.

Borja, A., Muxika, I., Franco, J., 2003. The application of a Marine Biotic Index to different impact sources affecting soft-bottom benthic communities along European coasts. Marine Pollution Bulletin, 46(7), 835-845.

Cannariato, K. G., Kennett, J. P., & Behl, R. J., 1999. Biotic response to late Quaternary rapid climate switches in Santa Barbara Basin: Ecological and evolutionary implications.Geology, 27(1), 63-66.

Carvalho, S., Gaspar, M. B., Moura, A., Vale, C., Attunes, P., Gil, O., Falcao, M., 2006. The use of the marine biotic index AMBI in the assessment of the ecological status of the Óbidos lagoon (Portugal). Marine Pollution Bulletin, 52(11), 1414-1424.

Chesney, E. J., Baltz, D. M., & Thomas, R. G., 2000. Louisiana estuarine and coastal fisheries and habitats: perspectives from a fish's eye view. Ecological Applications, 10(2), 350-366.

Cordes, E. E., Jones, D. O., Schlacher, T. A., Amon, D. J., Bernardino, A. F., Brooke, S., Gates, A. R., 2016. Environmental impacts of the deep-water oil and gas industry: a review to guide management strategies. Frontiers in Environmental Science, 4, 58.

Culver, S. J., & Buzas, M. A., 1995. The effects of anthropogenic habitat disturbance, habitat destruction, and global warming on shallow marine benthic foraminifera. The Journal of Foraminiferal Research, 25(3), 204-211.

Daly, K.L., Passow, U., Chanton, J. and Hollander, D., 2016. Assessing the impacts of oil-associated marine snow formation and sedimentation during and after the Deepwater Horizon oil spill. Anthropocene, 13, pp.18-33.

Danovaro, R., Gambi, C., Dell'Anno, A., Corinaldesi, C., Fraschetti, S., Vanreusel, A., ... & Gooday, A. J., 2008. Exponential decline of deep-sea ecosystem functioning linked to benthic biodiversity loss. Current Biology, 18(1), 1-8.

Debenay, J. P., Patrona, L. D., & Goguenheim, H., 2009. Colonization of coastal environments by foraminifera: insight from shrimp ponds in New Caledonia (SW Pacific). The Journal of Foraminiferal Research, 39(4), 249-266.

Donnici, S., & Barbero, R. S., 2002. The benthic foraminiferal communities of the northern Adriatic continental shelf. Marine Micropaleontology, 44(3-4), 93-123.

Donnici, S., Barbero, R. S., & Taroni, G., 1997. Living benthic foraminifera in the Lagoon of Venice (Italy): population dynamics and its significance. Micropaleontology, 440-454.

Duijnstee, I. A. P., Ernst, S. R., & Van der Zwaan, G. J., 2003. Effect of anoxia on the vertical migration of benthic foraminifera. Marine Ecology Progress Series, 246, 85-94.

EPA Hypoxia Task Force, 2015. Mississippi River/Gulf of Mexico Watershed Nutrient Task Force 2015 Report to Congress.

European Commission, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. J. Eur. Communities, 2000.

Farrington, 1980. NOAA Ship RESEARCHER/Contract Vessel PIERCE Cruise to IXTOC-I Oil Spill: Overview and Integrative Data Assessment and Interpretation. National Oceanic and Atmospheric Administration

Fisher, C. R., Montagna, P. A., and Sutton, T. T., 2016. How did the Deepwater Horizon oil spill impact deep-sea ecosystems?. Oceanography, 29(3), 182-195.

Frontalini, F., & Coccioni, R., 2008. Benthic foraminifera for heavy metal pollution monitoring: a case study from the central Adriatic Sea coast of Italy. Estuarine, Coastal and Shelf Science, 76(2), 404-417.

Frontalini, F., & Coccioni, R., 2011. Benthic foraminifera as bioindicators of pollution: a review of Italian research over the last three decades. Revue de micropaléontologie, 54(2), 115-127.

Geslin, E., Debenay, J. P., Duleba, W., & Bonetti, C., 2002. Morphological abnormalities of foraminiferal tests in Brazilian environments: comparison between polluted and non-polluted areas. Marine Micropaleontology, 45(2), 151-168.

Glemarec, M., Hily, C., 1981. Perturbations approves à la macrofaune benthique de la baie de Concarneau par les effluents urbains et portuaires. Oecologica Applicata, 2, 139-150.

Goldstein, S. T., 1999. Foraminifera: a biological overview. In Modern foraminifera (pp. 37-55). Springer, Dordrecht.

Goldstein, S. T., 1999. Foraminifera: a biological overview. In Modern foraminifera (pp. 37-55). Springer, Dordrecht.

Goldstein, S. T., & Corliss, B. H., 1994. Deposit feeding in selected deep-sea and shallow-water benthic foraminifera. Deep Sea Research Part I: Oceanographic Research Papers, 41(2), 229-241.

Griffiths, J. R., Kadin, M., Nascimento, F. J., Tamelander, T., Törnroos, A., Bonaglia, S., and Kotta, J., 2017. The importance of benthic–pelagic coupling for marine ecosystem functioning in a changing world. Global change biology, 23(6), 2179-2196.

Hohenegger, J., 2000. Coenoclines of larger foraminifera. Micropaleontology, 46, 127-151.

Hohenegger, J., & Piller, W., 1975. Wandstrukturen und Grossgliederung der Foraminiferen. Sitzungsberichten der Österreiche Akademie der Wissenschaften Mathematisch-naturwissenschaftliche Klasse, Abteilung I, 184, 67-96.

Jernelöv, A. and Lindén, O., 1981. Ixtoc I: a case study of the world's largest oil spill. Ambio, pp.299-306.

Jorissen, F. J., 1999. Benthic foraminiferal microhabitats below the sediment-water interface. In Modern Foraminifera (pp. 161-179). Springer, Dordrecht.

Jorissen, F. J., de Stigter, H. C., & Widmark, J. G., 1995. A conceptual model explaining benthic foraminiferal microhabitats. Marine micropaleontology, 26(1-4), 3-15.

Jorissen, F., Nardelli, M. P., Almogi-Labin, A., Barras, C., Bergamin, L., Bicchi, E., & Romano, E., 2018. Developing Foram-AMBI for biomonitoring in the Mediterranean: Species assignments to ecological categories. Marine Micropaleontology, 140, 33-45.

Kaiho, K., 1994. Benthic foraminiferal dissolved-oxygen index and dissolved-oxygen levels in the modern ocean. Geology,22(8), 719-722.

Kaiho, K., 1999. Effect of organic carbon flux and dissolved oxygen on the benthic foraminiferal oxygen index (BFOI). Marine Micropaleontology, 37(1), 67-76.

Kitazato, H., 1988. Locomotion of some benthic foraminifera in and on sediments. The Journal of Foraminiferal Research,18(4), 344-349.

Kujawinski, E.B., Kido Soule, M.C., Valentine, D.L., Boysen, A.K., Longnecker, K. and Redmond, M.C., 2011. Fate of dispersants associated with the Deepwater Horizon oil spill. Environmental science & technology, 45(4), pp.1298-1306.

Kumpf, H., Steidinger, K., & Sherman, K., 1999. The Gulf of Mexico large marine ecosystem: assessment, sustainability, and management.

Lee, J.J., Faber, W.W., Anderson, O.R., Pawlowski, J., 1991. Life-cycles of foraminifera. Biology of Foraminifera. Academic Press, London, 285-334.

Lei, Y. L., Li, T. G., Bi, H., Cui, W. L., Song, W. P., Li, J. Y., & Li, C. C., 2015. Responses of benthic foraminifera to the 2011 oil spill in the Bohai Sea, PR China. Marine pollution bulletin, 96(1-2), 245-260.

Leutenegger, S., 1984. Symbiosis in benthic foraminifera; specificity and host adaptations. The Journal of Foraminiferal Research, 14(1), 16-35.

Lindroth, A., 1971. On biocoenoses; coenotypes and coenoclines. Thalassia jugosl, 7, 185–194.

Mackensen, A., Hubberten, H. W., Bickert, T., Fischer, G., & Fütterer, D. K., 1993. The δ13C in benthic foraminiferal tests of Fontbotia wuellerstorfi (Schwager) relative to the δ13C of dissolved inorganic carbon in southern ocean deep water: implications for glacial ocean circulation models. Paleoceanography, 8(5), 587-610.

Martínez-Colón, M., Hallock, P., & Green-Ruíz, C., 2009. Strategies for using shallow-water benthic foraminifers as bioindicators of potentially toxic elements: a review. The Journal of Foraminiferal Research, 39(4), 278-299.

Martins, M.V.A., Silva, F., Laut, L. L., Frontalini, F., Clemente, I. M., Miranda, P., Dias, J. M. A., 2015. Response of benthic foraminifera to organic matter quantity and quality and bioavailable concentrations of metals in Aveiro Lagoon (Portugal). PLoS One, 10(2), e0118077.

Mateu-Vicens, G., Khokhlova, A., & Sebastián-Pastor, T., 2014. Epiphytic foraminiferal indices as bioindicators in Mediterranean seagrass meadows. Journal of Foraminiferal Research, 44(3), 325-339.

McCorkle, D. C., Keigwin, L. D., Corliss, B. H., & Emerson, S. R., 1990. The influence of microhabitats on the carbon isotopic composition of deep-sea benthic foraminifera. Paleoceanography, 5(2), 161-185.

Miller, K. G., & Lohmann, G. P., 1982. Environmental distribution of Recent benthic foraminifera on the northeast United States continental slope. Geological Society of America Bulletin, 93(3), 200-206.

Mills, E.I., 1969. The community concept in marine zoology, with comments on continua and instability in some marine communities: a review. J. Fish. Res. Bd. Can. 26, 1415-1428.

Montagna, P. A., Baguley, J. G., Cooksey, C., & Hyland, J. L., 2017. Persistent impacts to the deep soft-bottom benthos one year after the Deepwater Horizon event. Integrated environmental assessment and management, 13(2), 342-351.

Montagna, P. A., Baguley, J. G., Cooksey, C., Hartwell, I., Hyde, L. J., Hyland, J. L., ... & Rhodes, A. C., 2013. Deep-sea benthic footprint of the Deepwater Horizon blowout. PloS one, 8(8).

Murawski, S. A., Ainsworth, C. H., Gilbert, S., Hollander, D. J., Paris, C. B., Schlüter, M., & Wetzel, D. L., 2020. Summary of Progress on Major Research Issues: Deep-Sea Oil Spills. In Deep Oil Spills, 584-594. Springer, Cham.

Murray, J. W., & Alve, E., 2002. Benthic foraminifera as indicators of environmental change: estuaries, shelf and upper slope, 59-90.

Muxika, I., Borja, A., & Bonne, W., 2005. The suitability of the marine biotic index (AMBI) to new impact sources along European coasts. Ecological indicators, 5(1), 19-31.

National Marine Fisheries Service, 2017. Fisheries Economics of the United States, 2015. U.S. Dept. of Commerce, NOAA Tech. Memo. NMFS-F/SPO-170, 247p.

National Research Council, 2012. Approaches for Ecosystem Services Valuation for the Gulf of Mexico After the Deepwater Horizon Oil Spill: Interim Report. Washington, DC: The National Academies Press, https://doi.org/10.17226/13141.

Nelson, J. R., Grubesic, T. H., 2018. Oil spill modeling: Risk, spatial vulnerability, and impact assessment. Progress in Physical Geography: Earth and Environment, 42(1), 112–127, https://doi.org/10.1177/0309133317744737.

OECD, 2016. The Ocean Economy in 2030. Paris: OECD Publishing.

Osterman, L. E., 2003. Benthic foraminifers from the continental shelf and slope of the Gulf of Mexico: an indicator of shelf hypoxia. Estuarine, Coastal and Shelf Science, 58(1), 17-35.

Panieri, G., 2006. Foraminiferal response to an active methane seep environment: a case study from the Adriatic Sea. Marine Micropaleontology, 61(1-3), 116-130.

Parker, K. R., Wiens, J. A., 2005. Assessing recovery following environmental accidents: environmental variation, ecological assumptions, and strategies. Ecological Applications, 15(6), 2037-2051.

Passow, U. and Ziervogel, K., 2016. Marine snow sedimented oil released during the Deepwater Horizon spill. Oceanography, 29(3), pp.118-125.

Passow, U., Ziervogel, K., Asper, V. and Diercks, A., 2012. Marine snow formation in the aftermath of the Deepwater Horizon oil spill in the Gulf of Mexico. Environmental Research Letters, 7(3), p.035301.

Pearson, T.H., Rosenberg, R., 1978. Macrobenthic Succession in Relation to Organic Enrichment and Pollution of the Marine Environment. Oceanography and Marine Biology—An Annual Review, 16, 229-311.

Pearson, T.H., Stanley, S.O., 1979. Comparative measurement of the redox potential of marine sediments as a rapid means of assessing the effect of organic pollution. Mar. Biol., 53, 371-379.

Platon, E., Sen Gupta, B. K., 2001. Benthic Foraminiferal Communities in Oxygen-Depleted Environments of the Louisiana Continental. Coastal Hypoxia: Consequences for Living Resources and Ecosystems, 58, 147.

Rabalais, N. N., 1999. Characterization of Hypoxia: Topic 1, Report for the Integrated Assessment on Hypoxia in the Gulf of Mexico (No. 15). US Department of Commerce, National Oceanic and Atmospheric Administration, Coastal Ocean Program.

Rabalais, N. N., Turner, R. E., Díaz, R. J., Justić, D. 2009. Global change and eutrophication of coastal waters. ICES Journal of Marine Science, 66(7), 1528-1537.

Rayner R., Jolly C., and Gouldman C., 2019. Ocean Observing and the Blue Economy. Front. Mar. Sci. 6:330. doi: 10.3389/fmars.2019.00330

Resig, J.M., 1960. Foraminiferal ecology around ocean outfalls off southern California. In Proceedings of the First International Conference on Waste Disposal in the Marine Environment, 104-121.

Romano, E., Bergamin, L., Finoia, M. G., Celia Magno, M., Ausili, A., Gabellini, M., 2009. The effects of human impact on benthic foraminifera in the Augusta harbour (Sicily, Italy).Integrated Coastal Zone Management. Blackwell Publishing Ltd., Chichester, UK, 97-115.

Romero, I.C., Schwing, P.T., Brooks, G.R., Larson, R.A., Hastings, D.W., Ellis, G., Goddard, E.A. and Hollander, D.J., 2015. Hydrocarbons in deep-sea sediments following the 2010 Deepwater Horizon blowout in the northeast Gulf of Mexico. PLoS One, 10(5), p.e0128371.

Ruiz-Fernández, A. C., Portela, J. M. B., Sericano, J. L., Sanchez-Cabeza, J. A., Espinosa, L. F., Cardoso-Mohedano, J. G., ... & Tinoco, J. A. G., 2016. Coexisting sea-based and landbased sources of contamination by PAHs in the continental shelf sediments of Coatzacoalcos River discharge area (Gulf of Mexico). Chemosphere, 144, 591-598.

Salas, F., Neto, J. M., Borja, A., & Marques, J. C., 2004. Evaluation of the applicability of a marine biotic index to characterize the status of estuarine ecosystems: the case of Mondego estuary (Portugal). Ecological indicators, 4(3), 215-225.

Sandon, H., 1934. Pseudopodial movements of foraminifera. Nature, 133(3368), 761.

Santibañez-Aguascalientes, N. A., Borja, Á., Kuk-Dzul, J. G., Montero-Muñoz, J. L., & Ardisson, P. L., 2018. Assessing benthic ecological status under impoverished faunal situations: A case study from the southern Gulf of Mexico. Ecological indicators, 91, 679-688.

Schönfeld, J., Alve, E., Geslin, E., Jorissen, F., & Korsun, S., Spezzaferri, S. Abramovich, S., Almogi-Labin, A., Châtelet, E., Barras, C., Bergamin, L., Bicchi, E., Bouchet, V., Cearreta, A., & Bella, L., & Dijkstra, N., Disaró, S., Ferraro, L., Frontalini, F., Tsujimoto, A., 2012. The FOBIMO (FOraminiferal BIo-MOnitoring) initiative—Towards a standardised protocol for soft-bottom benthic foraminiferal monitoring studies. Marine Micropaleontology. 94-95. 1-13. 10.1016/j.marmicro.2012.06.001.

Schwing, P. T., O'Malley, B. J., & Hollander, D. J., 2018. Resilience of benthic foraminifera in the northern Gulf of Mexico following the Deepwater Horizon event (2011–2015). Ecological indicators, 84, 753-764.

Schwing, P. T., Romero, I. C., Brooks, G. R., Hastings, D. W., Larson, R. A., & Hollander, D. J., 2015. A decline in benthic foraminifera following the Deepwater Horizon event in the northeastern Gulf of Mexico. PLoS One, 10(3), e0120565.

Scott, D., Medioli, F.S., Schafer, C.T., 2001. Monitoring in Coastal Environments Using Foraminifera and Thecamoebian Indicators. 10.1017/CBO9780511546020.

Sen Gupta, B., 1999. Modern foraminifera. B. K. S. Gupta (Ed.). Dordrecht: Kluwer Academic Publishers.

Shelford, V. E., 1931. Some concepts of bioecology. Ecology, 12(3), 455-467.

Shepard, A. N., Valentine, J. F., D'Elia, C. F., Yoskowitz, D. W., & Dismukes, D. E., 2013. Economic impact of Gulf of Mexico ecosystem goods and services and integration into restoration decision-making. Gulf of Mexico Science, 31(1), 2.

Teixeira, H., Weisberg, S. B., Borja, A., Ranasinghe, J. A., Cadien, D. B., Velarde, R. G., Ritter, K. J., 2012. Calibration and validation of the AZTI's Marine Biotic Index (AMBI) for Southern California marine bays. Ecological Indicators, 12(1), 84-95.

Todd, R., 1965. A new Rosalina (Foraminifera) parasitic on a bivalve. In Deep Sea Research and Oceanographic Abstracts 12(6), 831-837.

Tran, T. T., Le, H. D., & Ngo, X. Q., 2018. Comparison of the Shannon-wiener, Ambi, and M-AMBI index for Assessing Sediment Ecological Quality in Organic Shrimp Farming Ponds, Nam Can District, Ca Mau Province.

US Department of Interior, 2010. U.S. scientific teams refine estimates of oil flow from BP's well prior to capping. Retrieved December 16, 2010, from http://www.doi.gov/news/pressreleases/US-Scientific-Teams-Refine-Estimates-of-Oil-Flow-from-BP-Well-Prior-to-Capping.cfm.

Van der Zwaan, G. J., Duijnstee, I. A. P., Den Dulk, M., Ernst, S. R., Jannink, N. T., & Kouwenhoven, T. J., 1999. Benthic foraminifers: proxies or problems?: a review of paleocological concepts. Earth-Science Reviews, 46(1-4), 213-236.

Ward, C. H., & Tunnell, J. W., 2017. Habitats and biota of the Gulf of Mexico: an overview. In Habitats and Biota of the Gulf of Mexico: Before the Deepwater Horizon Oil Spill (pp. 1-54). Springer, New York, NY.

Washburn, T. W., Reuscher, M. G., Montagna, P. A., Cooksey, C., & Hyland, J. L., 2017. Macrobenthic community structure in the deep Gulf of Mexico one year after the Deepwater Horizon blowout. Deep Sea Research Part I: Oceanographic Research Papers, 127, 21-30.

Washburn, T., Rhodes, A. C., & Montagna, P. A., 2016. Benthic taxa as potential indicators of a deep-sea oil spill. Ecological Indicators, 71, 587-597.

Watkins, J.G., 1961. Foraminiferal ecology around the Orange County, California, ocean sewer outfall. Micropaleontology, 199-206.

Yanko, V., Arnold, A. J., & Parker, W. C., 1999. Effects of marine pollution on benthic foraminifera. In Modern foraminifera, 217-235.

Zalesny, E.R., 1959. Foraminiferal ecology of Santa Monica Bay, California. Micropaleontology, 5(1), 101-126.

Chapter 2

Development of a benthic foraminifera based Marine Biotic Index (Foram-AMBI) for the Gulf of Mexico: a decision support tool

Abstract

The Gulf of Mexico (GoM) is an economically important region (e.g. oil and gas, fisheries) and with the expansion of oil drilling, harmful algal bloom events, oil blowouts, dead zones, anthropogenic eutrophication and contaminant loading, it is important that the ecological quality statuses (EcoQS) of different localities in the Gulf are closely monitored. The EcoQS, as implemented by the European Water Framework Directive, is an effective tool for monitoring ecological health and developing reference conditions. One such index used to define EcoQS is the AZTI Marine Biotic Index (AMBI), which pairs species abundance with environmental stressors. Benthic foraminifera are ideal specimens to populate the index due to their varying environmental sensitivities among species, preservation potential, and high diversity and abundance in nearly every marine environment. To calculate the benthic foraminiferal AMBI (Foram-AMBI), species are assigned to one of five groups ranging from sensitive (I) to first-order opportunists (V) based on their correlation to total organic matter and sediment grain size. This study constructs a Foram-AMBI from benthic foraminifera assemblages collected at 59 sites throughout the GoM. This approach provides reference EcoQS for the GoM and will satisfy the need for widespread geospatial coverage in the case of future natural or anthropogenic

disturbances. This study provides a master assignment list of 155 species and identification plates of 44 of those species from a depth range of 42m-2975m. The development of a GoM Foram-AMBI encourages collaborative partnerships between academic scientists and living resource managers throughout the GoM to operationalize, refine and implement the Foram-AMBI as a decision support tool.

2.1 Introduction

In a world with rapid population growth, economically and ecologically important bodies of water face threats caused by anthropogenic contamination promoted by the increasing worlwide energy demand and advancement in technologies, that allow for deepwater and ultra-deepwater petroleum exploration (Cordes et al., 2016; Murawski et al, 2020a). The growing concern about the possible adverse effects of global change on the marine environment has generated discussions amongst scientists, managers, and policy makers about effective ways to monitor and mitigate those impacts. In order to understand how contamination affects the natural marine world, local to regional baseline studies and long-term biomonitoring programs must be established (Cordes et al, 2016).

The framework of all monitoring and management programs relies on the establishment of reference conditions. Such conditions are critical to monitoring programs of non-point source anthropogenic contamination as well as events like the Deepwater Horizon (DWH) blowout because they provide reference points of ecological health from which temporal changes can be assessed (Cordes et al, 2016). Parker and Wiens (2005) highlighted the need of incorporating natural environmental variability into impact and recovery environmental models, and stressed that without long-term monitoring in place, recovery of impacted systems may be prematurely identified. The development of a system-level benthic-management decision support tool for long-term biomonitoring is essential for the protection of one of North America's largest economic assets, the Gulf of Mexico (GoM).

The GoM is home to the Exclusive Economic Zones (EEZ) of Cuba, Mexico, and the USA, and the economic value of the GoM is indispensable to these countries that surround it. The ecosystem-based goods and services that stem from the Gulf account for over 700 billion dollars per year in combined Gross Domestic Product (Shepard et al, 2013). After the 2010 DWH oil spill in the northern GoM, the Natural Resource Damage Assessment (NRDA) Restoration Program was left with the challenge of parsing out the complexities of the GoM ecosystem and establishing baselines in order to quantitatively assess and assign value to immediate and long-term damage (NRC, 2012; Shepard et al., 2013). This process, which has historically been done only for shallow-water events, unveiled the lack of deep-water baselines and reference conditions in the GoM and demonstrated the need for ecosystem-based management and monitoring (NRC, 2012; Shepard et al., 2013; Parker & Wiens, 2005). The ecosystem-based approach takes into consideration the beneficial services and resources provided by the functioning of an ecosystem (NRC, 2012). Ecosystem services include direct services and goods sourced from the ecosystem (e.g. seafood and petroleum), regulating services (e.g. flood control, climate regulation), cultural services (e.g. recreation), and supporting services (e.g. primary production, nutrient cycling) (Shepard et al, 2013). Since the development of ultra-deep water (>1500m) oil exploration and production, over half of the US supply of marine-derived petroleum is now sourced from wells deeper than 1500 meters (Murawski et al., 2020a). In addition to acute stressors (e.g. oil spills, hurricanes), the GoM also faces chronic stressors, like the degradation of water quality or eutrophication of coastal environments leading to dead zones (Rabalais et al., 1999; 2009). The development of environmental reference conditions and an understanding of the GoM ecosystem vulnerability will help minimize the risk during another ultra-deep water blowout and help disentangle the acute effects from chronic, long-term ecosystem changes (Shepard et al., 2013; Nelson & Grubesic, 2017; Murawski et al, 2020a).

In an effort to address concerns about the degradation of water resources, the European Union (EU) established the European Water Framework Directive (WFD, 2000/60/EC). The goals of this directive are to prevent water resources from further deterioration and to restore eco-regions that do not meet the established standards (WFD, 2000/60/EC). It requires that regional water bodies be regularly evaluated on their ecological quality statuses (EcoQS). The abundance of biological components (benthos, phytoplankton, fish) in concert with physicochemical attributes are used to calculate the EcoQS, which is split into five categories according to Borja et al. (2003):

$0 < \text{AMBI} \leq 1.2$ (High status or “unpolluted”)

$1.2 < \text{AMBI} \leq 3.3$ (Good status or “slightly polluted”)

$3.3 < \text{AMBI} \leq 4.3$ (Moderate status or “polluted”)

$4.3 < \text{AMBI} \leq 5.5$ (Poor status or “transition to heavily polluted”)

$5.5 < \text{AMBI} \leq 7$ (Bad status or “heavily polluted”)

This directive has resulted in the development of a number of biotic indices that can qualitatively and quantitatively evaluate environmental impact on soft-bottom habitats (Borja et al., 2016).

The most successful and widely used of biotic indices is AZTI's Marine Biotic Index (AMBI), which was developed by Borja et al. (2000) to characterize benthic habitat by employing the relative abundances of macroinvertebrates in response to organic matter enrichment and assigning them to ecological groups: Sensitive, Indifferent, 3rd-order opportunists, 2nd-order opportunists, 3rd-order opportunists (I-V). The classifications of these ecological groups were originally determined by the research of Glemerac and Hily (1981). Benthic faunal distribution occurs along a continuum of environmental gradients, implying species-specific sensitivity, allowing for qualitative monitoring of these communities (Lindroth et al., 1971; Pearson & Rosenberg, 1978). In this case, community is defined by Mills (1969) as an assemblage of organisms occurring in a particular environment presumably interacting with each other and with the environment and separable from other communities by means of ecological survey. In Borja's (2000) AMBI index, organic matter enrichment is used as a proxy for contamination because organic matter input accompanies long-term contamination as well as contamination events and is a major control of benthic community composition according to the Pearson-Rosenberg paradigm (Pearson & Rosenberg, 1978; Scott et al., 2001; Romano et al., 2009). Ultimately, an increase in the organic deposition is accompanied by abiotic factors such as hypoxia, physicochemical changes in the sediment, and inorganic deposition at the sediment-water interface resulting in a more stressed benthic habitat thus changing the community structure to one with more tolerant species (Pearson & Stanley, 1979). The resulting AMBI coefficient is directly related to the EcoQS ratings defined by Borja et al. (2003). For example, if the dominant ecological group is comprised of mainly opportunistic species, then the habitat is evaluated as being more affected.

Traditionally, macroinvertebrate data have been used to populate the AMBI (Borja et al., 2000, 2003; Muxica et al., 2003, 2005; Salas et al., 2004; Carvalho et al., 2006; Teixeira et al., 2012). However, meiofauna, such as benthic foraminifera, have proven to be robust indicators of environmental quality (Alve, 1995; Armynot du Chatelet et al., 2004, 2011; Alve et al., 2016;

Jorissen et al., 2018). Benthic foraminifera (BF) are advantageous in many circumstances because of their high biodiversity (>4,000 extant spp.), species-specific ecological niches, and high abundance among all marine and transitional marine environments providing a reliable database for statistical analysis even when restricted to small sample volumes (Alve, 1995; Scott et al., 2001; Martinez-Colón et al., 2009; Prazeres et al., 2019). Benthic foraminifera construct calcareous or agglutinated tests or shells that are well preserved in the sediment records allowing for environmental paleo-reconstructions. The application of the foraminiferal-based AMBI was carried out by Alve et al. (2016) in the northeastern Atlantic basin following the protocol set forth by the Foraminiferal Biomonitoring (FOBIMO) working group, which recommended standardized sampling methodologies to be used in foraminiferal biomonitoring (Schönfeld et al., 2012). This study assigned 128 species to respective ecological groups (I-V), calibrated the foraminifera-AMBI (Foram-AMBI) based on the relative abundance of these groups, and then validated the Foram-AMBI based on independent variables such as the Shannon diversity (H') and total organic carbon (TOC). This first attempt was followed by Jorissen et al. (2018) for the Mediterranean Sea to assign, calibrate, and validate the Foram-AMBI in this region. Jorissen et al. (2018) addressed the difficulties in the assignment process in great detail including dealing with datasets that had different methods of organic matter quantification, having to parse out synonymic taxa, using thanatocoenoses as well as biocoenoses, and addressing substrate type similarities and differences amongst sites (Jorissen et al., 2018).

This study builds upon the efforts of Alve et al. (2016) and Jorissen et al. (2018) by developing and calibrating a Foram-AMBI for the Gulf of Mexico. It seeks to assign benthic foraminifera to ecological groups that can be used by monitoring programs to determine EcoQS of benthic habitats to establish new reference conditions throughout the Gulf of Mexico in the case of future gas or oil spills and for the monitoring of anthropogenic contamination. Results have tested the efficacy of the Foram-AMBI as a standardized biomonitoring tool in the Gulf of Mexico and demonstrated its sensitivity to environmental stress.

2.2 Methods

Sample Collection

Fifty-nine sediment samples were collected throughout the GoM from 2015 to 2017 aboard the R/V Weatherbird II and B/O Justo Sierra (Fig. 4, Table 1). Sediment cores (10 cm diameter) were retrieved at 45 sites, using an Ocean Instruments MC-800 multicorer and an Oktopus MC-08-12; and surface samples were taken from 14 sites, using a Shipek grab sampler.

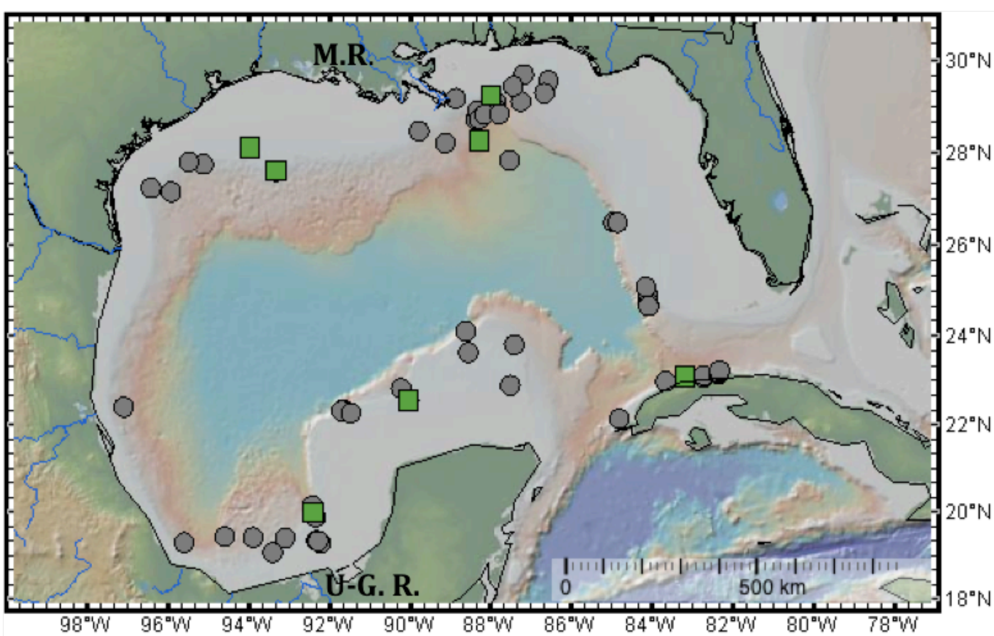


Figure 4. Map of 59 sites in the GoM used to calibrate (circles) and validate (squares) the GoM Foram-AMBI. (M.R.= Mississippi River; U-G. R.= Usumacinta-Grijalva River)

Table 1. List of station name, sample type, latitude, longitude, date of collection and water depth of the sediment samples taken throughout the Gulf of Mexico.

Station	Type	Latitude	Longitude	Date	Depth (m)
Abkatun	Multi-core	19.314	-92.208	Aug. 2015	50
DSH08	Multi-core	29.122	-87.869	Aug. 2015	1123
DSH10	Multi-core	28.976	-87.868	Aug. 2015	1490
DWH01	Multi-core	28.745	-88.381	Aug. 2015	1580
IXN250	Multi-core	19.907	-92.337	Aug. 2015	779
IXN500	Multi-core	20.008	-92.387	Aug. 2015	1240
IXN750	Multi-core	20.170	-92.420	Aug. 2015	1647
IXTOC1A	Multi-core	19.370	-92.317	Aug. 2015	60
IXW250	Multi-core	19.430	-93.095	Aug. 2015	583
IXW500	Multi-core	19.444	-93.889	Aug. 2015	1010
IXW750	Multi-core	19.459	-94.585	Aug. 2015	1440
LT3	Multi-core	19.356	-92.276	Aug. 2015	51
MC01	Multi-core	28.937	-88.337	May. 2018	1703
MC04	Multi-core	29.304	-86.677	Aug. 2015	407
MC09	Multi-core	28.288	-88.287	May. 2018	2272
MC12	Multi-core	28.759	-88.292	May. 2018	2179
MC14	Multi-core	28.851	-88.159	May. 2018	1946
MC16	Multi-core	28.865	-87.797	May. 2018	2381
MC24	Multi-core	27.863	-87.541	May. 2018	2975
MV02	Multi-core	28.494	-89.779	Aug. 2015	550
PCB06	Multi-core	29.133	-87.261	Aug. 2016	1023
SL1040	Multi-core	29.196	-88.869	Aug. 2017	59
SL1-150	Multi-core	24.916	-84.117	Jul. 2017	255
SL1460	Multi-core	29.456	-87.451	Aug. 2015	235
SL1-500	Multi-core	24.734	-84.102	Jul. 2017	1014
SL1-750	Multi-core	24.680	-84.099	Jul. 2017	1564
SL1-80	Multi-core	25.095	-84.165	Jul. 2017	135
SL20-150	Shipek Grab	27.626	-93.305	Aug. 2016	293
SL20-40	Shipek Grab	28.129	-93.958	Aug. 2016	67
SL21-150	Shipek Grab	27.782	-95.112	Aug. 2016	357
SL21-40	Shipek Grab	27.829	-95.479	Aug. 2016	83
SL22-150	Shipek Grab	27.184	-95.917	Aug. 2016	311
SL22-40	Shipek Grab	27.266	-96.413	Aug. 2016	42
SL26-750	Multi-core	22.412	-97.089	Aug. 2015	1499

Table 1 (continued)

Station	Type	Latitude	Longitude	Date	Depth (m)
SL28-750	Multi-core	19.324	-95.591	Aug. 2015	1564
SL30A-250	Multi-core	19.092	-93.402	Aug. 2015	496
SL33-150	Shipek Grab	22.362	-91.659	Sep. 2015	453
SL33-200	Multi-core	22.331	-91.702	Aug. 2015	391
SL33-60	Shipek Grab	22.284	-91.468	Sep. 2015	100
SL34-100	Shipek Grab	22.835	-90.223	Sep. 2015	200
SL34-40	Shipek Grab	22.562	-90.031	Sep. 2015	73
SL35-150	Shipek Grab	24.114	-88.627	Aug. 2016	260
SL35-60	Shipek Grab	23.640	-88.565	Aug. 2016	134
SL36-150	Shipek Grab	23.817	-87.427	Aug. 2016	302
SL36-20	Shipek Grab	22.903	-87.526	Aug. 2016	55
SL37-250	Multi-core	22.151	-84.826	May. 2017	530
SL40-750	Multi-core	23.002	-83.68	May. 2017	1490
SL41-750	Multi-core	23.084	-83.196	May. 2017	1511
SL43-500	Multi-core	23.073	-82.746	May. 2017	1120
SL43-750	Multi-core	23.129	-82.732	May. 2017	1512
SL44-150	Multi-core	23.156	-82.369	May. 2017	316
SL44-500	Multi-core	23.196	-82.364	May. 2017	970
SL44-750	Multi-core	23.237	-82.344	May. 2017	1475
SL7-150	Multi-core	29.568	-86.578	Aug. 2015	284
SL8-100	Multi-core	29.701	-87.192	Aug. 2015	210
SL9-150	Multi-core	29.247	-87.998	Aug. 2015	287
SW01	Multi-core	28.238	-89.131	Aug. 2015	1131
WFS1	Multi-core	26.526	-84.973	Sep. 2015	1587
WFS1-500	Multi-core	26.514	-84.869	Jul. 2017	986

Sample Processing

All cores designated for benthic foraminiferal analysis were extruded at 2 mm increments with a threaded-rod extrusion device (Schwing et al., 2016) and stained with Rose Bengal solution for 24 hours (Bernhard et al., 2006). After 24 hours, the sub-samples were washed through a 63-μm sieve and the remaining fraction was oven dried at 32 ˚C (Osterman et al., 2003). The top 1 cm (0-2 mm, 2-4 mm, 4-6 mm, 6-8 mm, and 8-10 mm increments) of each core was used for stained BF identification and f-AMBI calibration (Schönfeld et al, 2012). As there was no way to discern the top 1 cm from the 14 grab samples, the entire sample from each site was stained, washed, and dried with the same methodology as the core sub-samples before prior to faunal analysis. From each site, 300 stained foraminifera were identified to species level using the following taxonomic references: d'Orbigny (1826); d'Orbigny (1839); Williamson (1858); Jones and Parker (1860); Brady (1878); Brady (1879); Brady (1884); Cushman (1922); Cushman (1927); Stewart and Stewart (1930); Phleger and Parker (1951); Parker et al (1953); Parker (1954).

Total organic matter (TOM) was measured using the loss on ignition (LOI) method (Deanet al., 1974; Heiri et al. 2001). One gram of sediment sample was dried, weighed, and homogenized prior to combustion in a muffle furnace for four hours at 550 ˚C. Data is represented as a TOM percentage (%).

Grain size was determined by sieving and settling-tube techniques (Folk, 1964), and laser particle analyzer following the standard operating protocol for the Malvern Mastersizer 2000. Data is represented as percent sand (%).

Foram-AMBI Calibration

The Gulf of Mexico Foram-AMBI was calibrated by first assigning as many species as possible to one of the five ecological groups defined by Borja (2000) in the initial AMBI review. These five ecological groups are defined as follows (Fig. 3):

The following descriptions of ecological groups are cited from Jorissen et al. (2018).

Group I (Sensitive species): present at their highest abundance at unimpacted, oligotrophic sites with low sedimentary organic matter content. The abundance of these species tends to decline with increasing organic enrichment. They are completely absent at highly impacted sites.

Group II (Indifferent Species) present at most levels of organic enrichment, except for highly enriched or polluted areas. They never display a clear trend in response to the enrichment gradient and are never dominant in the assemblage hence being deemed "Indifferent".

Group III (Third-order opportunists) display tolerance to the first stages of organic enrichment. They can be found at sites with low organic matter but are most abundant where there is moderate organic enrichment. They cannot tolerate extreme organic enrichment and disappear at these sites.

Group IV (Second-order opportunists): minimal or absent at reference sites with low organic matter. They exhibit a clear positive relation to organic enrichment and occur at maximum abundance between Groups IV and V.

Group V (First-order opportunists): comprised of highly opportunistic species that have adapted to thrive in conditions that most other species could not survive. This could be due to adaptations in species' metabolic pathways that allow them to survive in hypoxic conditions (Jeffreys et al., 2015). These species are minimal or absent at the reference sites and dominate the assemblages at maximum organic enrichment before azoic levels are reached.

This study closely followed the assignment protocols of both Alve et al. (2016) and Jorissen et al. (2018). Fifty-two calibration sites throughout the GoM were used to produce plots of the relative abundance of each species versus TOM percentage at each site. Sedimentary substrate was also taken into account to avoid species assignment based on grain size gradient rather than TOM gradient, following the recommendations of Jorissen et al. (2018). It is necessary to account for grain size because fine-grain sediments (< 63 μm) naturally retain higher proportions of TOM due to the adsorption of organic compounds onto the clay particles and, due to tortuosity, limited oxygen diffusion and more limited organic matter degradation (Kennedy et al., 2002). Sandy sediments have very low and potentially less reliable TOM values and were only scrutinized in the case of any outliers. Species were individually and independently assigned to ecological groups based on their response to TOM reflected in the plots (Fig. 3) by four co-authors who were familiar with (or involved in) the FOBIMO protocol and Foram-AMBI development. A meeting was then held to discuss any varying assignments and reach a consensus on final assignments based on these calibration plots and in some cases referring to species ecological preferences reported in the literature. Species that occurred in fewer than three sites and/or species that did not make up more than 1% of the assemblage at any site were left unassigned. The aim of this methodology was to assign as many species as possible because if left unassigned, the species effectively acts as a Group I due to the weighting of ecological groups in the AMBI equation (Equation 1).

Foram-AMBI={(0 * %EGI) + (1.5 * %EGII) + (3 * %EGIII) + (4.5 * %EGIV) + (6 * %EGV)}/100

(Equation 1)

Once species were assigned, the relative abundances of each Ecological Group (I-V) was inserted in the Foram-AMBI equation and a single coefficient was derived for each site (Borja et al., 2000; Alve et al., 2016; Jorissen et al., 2018).

A heat map of the Foram-AMBI values was generated using the Ocean Data View visualization and mapping software.

Foram-AMBI Validation

After the initial assignment of each species to ecological groups, based on the 52 calibration sites and their respective TOM concentrations, the set of assignments was independently tested on seven sites (MC09, SL20-150, SL41-750, SL34-40, IXN500, SL20-40, and SL9-150) that were not included in the calibration; following methods described in Alve et al., (2016). These sites were chosen with respect to geographical coverage in and varying water depths in the Gulf of Mexico. This study followed the quality assurance threshold of Borja and Muxica (2005) that requires at least 80% of species at each validation site be assigned. If greater than 20% remains unassigned then the calibration set is not considered significantly robust.

Using a jackknife approach, each species calibration graph was individually analyzed by removing one site at a time and scrutinizing whether the species assignment would change with the removal of any one site. This analysis was performed in order to produce and report a Foram-AMBI value for the calibration sites (n=52) while avoiding any circular bias.

Following the validation methods described in Alve et al. (2016), the Foram-AMBI values were calculated for all 59 sites and correlated with: 1) TOM as the environmental forcing element used to assign ecological groups (TOM) and 2) the Shannon (H') index, which measures species diversity (Shannon & Weaver, 1963). The Shannon index was calculated using the PAST (PaleoStatistics) software suite and linear regressions were performed using Microsoft Excel.

2.3 Results

Calibration

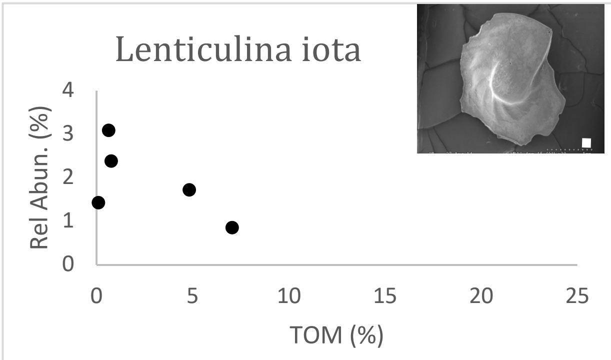
A total of 239 species were identified from 52 calibration sites across the GoM. Out of these 239 species, 155 (65%) were assigned to the five ecological groups defined by Jorissen et al. (2018). The remaining 84 species (35%) were not assigned because they were either found at less than three sites or accounted for less than 1% total relative abundance at all sites. Because of the rarity and relatively low abundance of these 84 species, they would not have had a strong influence on Foram-AMBI values. Of the 155 assigned species, 21 were assigned to EG I (sensitive), 50 were assigned to EG II (indifferent), 36 were assigned to EG III (3rd -order opportunists), 29 were assigned to EG IV (2nd-order opportunists), and 19 were assigned to EG V (1st-order opportunists)(Table 2).

Table 2. Comparison of taxa assignments to Ecological Groups between the Gulf of Mexico, the Northeast Atlantic (Alve et al., 2016) and the Mediterranean Sea (Jorissen et al., 2018).

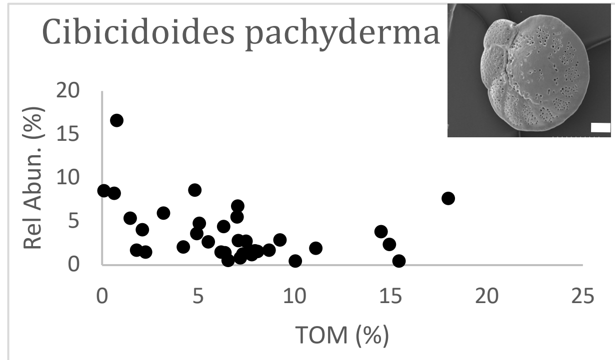
	Gulf of Mexico	**NE Atlantic**	**Mediterranean**
Taxa identified	239	419	493
Taxa assigned	155	128	199
EG I	21	65	79
EG II	50	33	60
EG III	36	27	46
EG IV	29	1	12
EG V	19	2	2

Table 3. The final assignment (fa) list of the 155 species assigned to Ecological Groups.

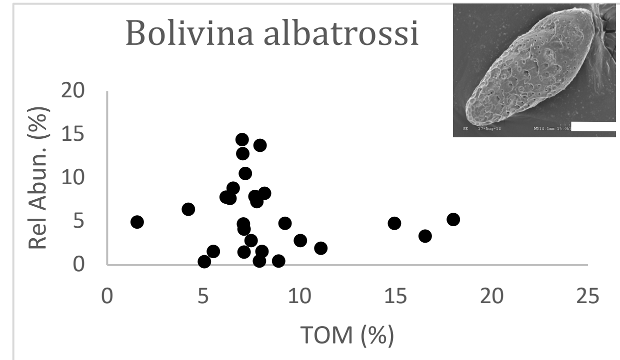
Species	**fa**	**Species**	**fa**	**Species**	**fa**
Ammodiscus anguillae	3	*Epistominella vitrea*	3	*Pullenia quinqueloba*	2
Ammoglobigerina globigeriniformis	4	*Eponides turgidus*	3	*Pyramidulina comatula*	3
Ammolagena clavata	1	*Fissurina marginata*	2	*Pyrgo murrhyna*	2
Ammonia beccarii	5	*Fissurina radiata*	4	*Pyrgo nasutus*	4
Amphicoryna hirsuta	4	*Fursenkoina complanata*	4	*Pyrgoella sphaera*	1
Amphicoryna scalaris	3	*Fursenkoina mexicana*	4	*Quinqueloculina auberiana*	1
Angulogerina bella	2	*Fursenkoina pontoni*	5	*Quinqueloculina bosciana*	2
Anomalinoides colligerus	2	*Gaudryina pyramidata*	2	*Quinqueloculina impressa*	2
Astacolus crepidulus	1	*Gavelinopsis translucens*	3	*Quinqueloculina laevigata*	2
Asterigerina carinata	1	*Globobulimina affinis*	4	*Quinqueloculina lamarckiana*	2
Bifarina decorata	4	*Globocassidulina subglobosum*	3	*Quinqueloculina polygona*	2
Bolivina albatrossi	3	*Glomospira charoides*	5	*Quinqueloculina tropicalis*	1
Bolivina lowmani	3	*Glomospira gordialis*	3	*Reophax agglutinatus*	5
Bolivina ordinaria	4	*Gyroidina altiformis*	2	*Reophax scorpiurus*	3
Bolivina striatula	3	*Gyroidina soldanii*	4	*Reussella spinulosa*	3
Bolivina subaenariensis	2	*Gyroidinoides regularis*	4	*Reussoolina laevis*	4
Bolivina subspinescens	2	*Haplophragmoides fragile*	3	*Robertinoides bradyi*	1
Bolivinellina translucens	3	*Haynesina germanica*	5	*Rosalina concinna*	2
Brizalina spathulata	2	*Hoeglundina elegans*	2	*Rosalina globularis*	2
Bulimina aculeata	3	*Hormosina globulifera*	5	*Rutherfordoides rotundata*	3
Bulimina alazanensis	2	*Hyperammina elongata*	5	*Sagrinella durandii*	2
Bulimina marginata	2	*Hyperammina laevigata*	3	*Seabrookia earlandi*	4
Bulimina striata mexicana	3	*Ioanella tumidula*	4	*Sigmoilinita tenuis*	5
Buliminella elegantissima	5	*Karerriella bradyi*	4	*Sigmoilopsis schlumbergeri*	2
Buliminella morgani	5	*Karrerulina conversa*	5	*Siphogenerina striata*	3
Buzasina ringens	4	*Laevidentalina communis*	1	*Siphonina bradyana*	3
Cancris auriculus	5	*Lagena striata*	2	*Sphaeroidina bulloides*	3
Cassidulina carinata	2	*Lagenammina difflugiformis*	4	*Spiroplectinella filiformis*	4
Cassidulina crassa	4	*Laticarinina pauperata*	5	*Subreophax monile*	3
Cassidulina laevigata	3	*Lenticulina calcar*	2	*Testulosiphon indivisus*	3
Cassidulina neoteretis	4	*Lenticulina convergens*	2	*Textularia agglutinans*	4
Cassidulina reniforme	2	*Lenticulina cultrata*	3	*Textularia mayori*	5
Chilostomela oolina	5	*Lenticulina iota*	1	*Tretomphalus atlanticus*	2
Cibicides io	2	*Lenticulina vortex*	1	*Trifarina bradyi*	3
Cibicides mollis	1	*Marginulopsis marginulinoides*	2	*Triloculina linneiana*	1
Cibicides refulgens	1	*Melonis barleeanus*	2	*Triloculina oblonga*	2
Cibicides tenuimargo	3	*Melonis pompiloides*	1	*Triloculina trigonula*	3
Cibicidoides bradyi	2	*Neoconorbina terquemi*	1	*Trochammina inflata*	4
Cibicidoides kullenbergi	1	*Neolenticulina peregrina*	2	*Trochammina ochracea*	5
Cibicidoides pachyderma	2	*Nonionella atlantica*	3	*Trochammina rotaliformis*	5
Cibicidoides robertsonianas	1	*Nonionella turgida*	2	*Uvigerina canariensis*	4
Cibicidoides subhaidingerii	2	*Nouria polymorphinoides*	4	*Uvigerina flintii*	2
Cibicidoides wuellerstorfi	2	*Nuttalides rugosus*	4	*Uvigerina laevis*	2
Cornuspira involvens	1	*Oridorsalis tenerus*	2	*Uvigerina peregrina*	5
Cribrostomoides bradyi	3	*Osangularia culter*	2	*Uvigerina pigmaea*	4
Cribrostomoides nitidum	4	*Paracassidulina minuta*	3	*Valvulineria minuta*	3
Cribrostomoides subglobosum	3	*Peneroplis pertusus*	1	*Veleroninoides jeffreysii*	4
Cymbaloporetta squammosa	1	*Peneroplis planatus*	1	*Veleroninoides wiesneri*	3
Eggerella bradyi	2	*Planulina ariminensis*	2	*Verneuilina advena*	2
Elphidium discoidale	2	*Procerolagena gracilis*	2	*Virgulina tessellata*	3
Elphidium excavatum	4	*Procerolagena gracillima*	2	*Wiesnerella auriculata*	2
Epistominella exigua	4	*Pullenia bulloides*	2		



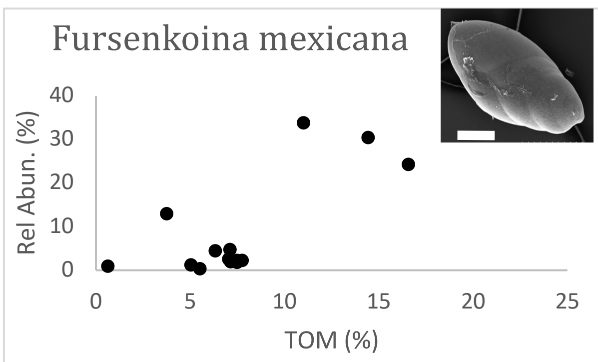
a.) Group I: Sensitive



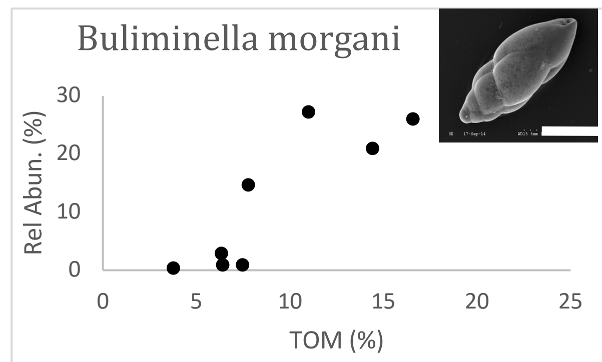
b.) Group II: Indifferent



c.) Group III: Third-order



d.) Group IV: Second-order



e.) Group V: First-order

Figure 5. Examples of distribution patterns within each Ecological Group (I-V). Each plot represents the relative abundance of the species as a function of the Total Organic Matter (TOM) gradient. Note the different scales on the y-axes. Scale bars represent 100 μm.

Species assigned to Ecological Group I mainly consisted of epifaunal species like *Lenticulina iota* (Fig. 5a), *Cibicidoides kullenbergi*, *Peneroplis pertusus,* and *Neoconorbina terquemi* that thrive in oligotrophic settings. *Ammolagena clavata* is an agglutinated suspension feeder, so it is expected that this species would exist in areas with moderate organic matter flux, however it was an interesting that this species shows a Group I pattern. However, these attached epilithic species have been discovered to be well adapted to oligotrophic conditions (Waśkowska & Kaminski, 2019). One reason for this is that *A. clavata* colonizes mostly dead epifaunal benthic foraminifera and is able to feed on remains of organic matter in the shell (Waśkowska, 2014). *A. clavata* has also been found to exclusively live in high diversity assemblages (Waśkowska, 2014), which in general are not representative of polluted environments. Thus, *A. clavata* was assigned to Ecological Group I.

Fifty species were assigned to Ecological Group II; the most assignments of all five Groups. A species was considered to belong to Group II if it was abundant at low TOM percentages and present at high TOM percentages but at much lower frequencies. *Cibicidoides pachyderma* (Fig. 5b) was a clear example of this. The assignments of Group II's in this study aligned well with previous research. For example, *Eggerella-Oridorsalis* assemblages had previously been identified as low-productivity indicators in the central Pacific (Burke et al. 1993). However, Sen Gupta et al. (1997) found the assemblage also tolerant to intermediate organic flux. Taking both studies into account in addition to the relative abundance versus TOM trends observed in this study, both species were assigned to Group II.

Thirty-six species were assigned to Ecological Group III. *Bolivina albatrossi* (Fig. 5c) is a prime example of a third-order opportunist that thrives at intermediate levels of organic flux. *Epistominella vitrea* followed a Group II curve excluding the data point from site SL1040.

However, this lone data point in concert with the findings of Ernst et al. (2005) support the assignment of *E. vitrea* to Group III. Ernst et al., (2005) found that *E. vitrea* responded positively to a pulse of organic matter input, but a sustained flux declined their standing stock confirming the Group III assignment.

Group IV species are generally accessory indicators of contamination, or second-order opportunists, existing just outside the influence of extreme pollution by the region and/or point-source. Twenty-nine foraminiferal species from this study were assigned to this group. *Fursenkoina mexicana* (Fig. 5d) is a species that increases with organic matter content, but only to a threshold and there is a clear decrease in abundance when overwhelmed. One problematic assignment in this study was *E. excavatum*. It did not occur at very high relative abundances (maximum: 1.55%) and was only found at 5 sites. Its trend along the TOM gradient may classify it as a Group III species but due to its presence at IXTOC1A where the organic matter percentage is high (16.57% TOM) and its previous use as an indicator of anthropogenic pollution (Armynot du Chatelet et al., 2011) it was assigned to Group IV.

The final group, Group V, consists of the first-order opportunists. These species are the most resistant to organic enrichment, hypoxia, and anthropogenic influences. The trend of *Buliminella morgani* vs the TOM content (Fig. 5e) was representative of this group, increasing with organic content and has repeatedly been identified as a strong indicator of hypoxia in the Gulf of Mexico. It is also one of the main components of the PEB index (Osterman, 2003), which is a foraminifera-based index used to identify hypoxic zones. Other Group V species included *Ammonia beccarii,* which is used as a prominent bioindicator for anthropogenic contamination (McCrone & Schafer, 1966; Donnici, 2012). A group V species that was difficult to assign was *H. germanica* since it had a general positive trend with organic matter content but was only present at a few sampling sites. This foraminifer is a well-studied species that is highly opportunistic and tolerant to differing forms of anthropogenic pollution (heavy metals, hydrocarbons, organic matter) leading to a Group V assignment (Stubbles et al., 1993; Alve & Murray, 1994; Armynot du Chatelet et al., 2004; Frontalini et al., 2009).

Validation

The percentage of unassigned species in the validation sites ranged from 0.96% to 10.17%, which is well below the quality assurance threshold of 20% unassigned designated by previous AMBI studies (Borja & Muxica, 2005; Alve, 2016; Table 4). Because of the low number of unassigned species, the calibration sites were considered viable to characterize the species assignments and the Foram-AMBI values of the validation sites.

Table 4. Percentage of unassigned benthic foraminifera species present at the seven validation sites.

Site	**Percent Unassigned (%)**
MC09	0.96
SL20150	9.43
SL41750	10.17
SL3440	8
IXN500	2.78
SL2040	3.03
SL9150	2.15

To determine if the calibration sites were suitable to use for Foram-AMBI calculation, each assignment graph was analyzed by individually removing one data point corresponding to a site at a time (jackknifing approach) and determining if any one data point would change the assignment of each species. There were 14 species assignments that were affected by the removal of a specific site. However, six of these species, *Ammoglobigerina globigeriniformis* (Lei et al., 2011)*, Buliminella elegantissima* (Eichler et al. 2015)*, Cibicidoides kullenbergi* (Woodruff et al., 1992)*, Elphidium excavatum* (Rotstigen, 2009)*, Globobulimina affinis* (Schmiedl et al., 1998), and *Karrerulina conversa* (Bindiu et al., 2011) have shown to be either indicators of low or high TOM input. Therefore, these assignments were not considered affected and were defaulted into their original categories based on previously published literature. *Astacolus crepidulus, Buzasina ringens, Hormosina globulifera, Karreriella bradyi,* and *Nuttalides rugosus* accounted for less than 3% relative abundance at any given site and were therefore insignificant in the Foram-AMBI calculation. The species *Quinqueloculina auberiana, Quinqueloculina tropicalis,* and *Siphogenerina striata* were the only three species to significantly change assignment. The assignments for these species changed by one group; *Q. auberiana* from Group I to Group II, *Q. tropicalis* from Group I to Group II, and *S. striata* from Group III to Group II. The average changes of the Foram-AMBI in the sites where these species were found were: +0.04 for *Q. tropicalis* at eight sites, +0.03 for *Q. auberiana* at the five sites, and-0.04 for *S. striata* at six sites. The most extreme Foram-AMBI changes ranged from +0.15 (site SL40-750) to -0.1 (site SL33-60). This approach also provides an uncertainty term for the Foram-AMBI scores. Seeing as this only marginally affects the Foram-AMBI calculation (maximum of <3% margin of uncertainty) it was deemed appropriate to calculate the Foram-AMBI for all of the sites.

The 59 Foram-AMBI values (Table 5, Fig. 6) ranged from 0.77-4.57. According to the Ecological Quality Status designations of Borja et al. (2003), there were two “unpolluted” sites, 50 “slightly polluted” sites, five “polluted” sites, and two transitional heavily polluted sites. TOM correlated positively with Foram-AMBI values (Table 6) ($r= 0.64$, $p=3 \times 10^{-8}$). Shannon diversity was negatively, but still significantly correlated to Foram-AMBI values ($r=-0.26$ $p=0.04$).

Table 5. Foram-AMBI values, Shannon diversity values, and Ecological Quality Status (EcoQS) for 59 sites in the GoM.

Site	Foram-AMBI	Shannon (H')	EcoQS
Abkatun	4.15	1.9	Polluted
DSH08	3.11	3.16	Slightly polluted
DSH10	3	3.14	Slightly polluted
DWH01	3.27	3.2	Slightly polluted
IXN250	2.87	2.76	Slightly polluted
IXN500	3.06	2.79	Slightly polluted
IXN750	3.24	2.37	Slightly polluted
IXTOC1A	4.42	2.01	Trans. heavily polluted
IXW250	3.22	3.3	Slightly polluted
IXW500	3.15	3.35	Slightly polluted
IXW750	3.11	2.9	Slightly polluted
LT3	4.57	1.9	Trans. heavily polluted
MC01	3.83	2.54	Polluted
MC04	2.63	3.05	Slightly polluted
MC09	2.72	2.92	Slightly polluted

Table 5 (continued)

Site	Foram-AMBI	Shannon (H')	EcoQS
MC12	2.67	2.82	Slightly polluted
MC14	3.03	2.92	Slightly polluted
MC16	2.7	2.97	Slightly polluted
MC24	1.91	3.04	Slightly polluted
MV02	3.43	2.59	Polluted
PCB06	2.67	3.1	Slightly polluted
SL1040	3.46	2.4	Polluted
SL1-150	1.52	3.09	Slightly polluted
SL14-60	2.22	3.06	Slightly polluted
SL1-500	2.21	3.55	Slightly polluted
SL1-750	2.85	3.36	Slightly polluted
SL1-80	0.77	2.73	Unpolluted
SL20-150	2.46	2.72	Slightly polluted
SL20-40	2.68	2.61	Slightly polluted
SL21-150	2.32	2.58	Slightly polluted
SL21-40	2.51	2.81	Slightly polluted
SL22-150	2.4	2.69	Slightly polluted
SL22-40	2.5	2.82	Slightly polluted
SL26-750	2.87	2.94	Slightly polluted
SL28-750	2.82	3.06	Slightly polluted
SL30A-250	3.27	3.04	Slightly polluted
SL33-150	2.37	2.89	Slightly polluted

Table 5 (continued)

Site	Foram-AMBI	Shannon (H')	EcoQS
SL33-200	2.82	3.11	Slightly polluted
SL33-60	2.02	2.43	Slightly polluted
SL34-100	1.88	3.02	Slightly polluted
SL34-40	2.52	1.97	Slightly polluted
SL35-150	1.71	2.61	Slightly polluted
SL35-60	1.61	2.98	Slightly polluted
SL36-150	1.46	2.78	Slightly polluted
SL36-20	0.58	2.38	Unpolluted
SL37-250	2.18	2.87	Slightly polluted
SL40-750	2.16	2.14	Slightly polluted
SL41-750	1.83	2.94	Slightly polluted
SL43-500	2.17	2.55	Slightly polluted
SL43-750	1.27	3.03	Slightly polluted
SL44-150	2.21	3.18	Slightly polluted
SL44-500	2.69	3.04	Slightly polluted
SL44-750	3.2	2.65	Slightly polluted
SL7-150	2.55	3.21	Slightly polluted
SL8-100	2.32	3.25	Slightly polluted
SL9-150	2.55	3.1	Slightly polluted
SW01	3.42	2.92	Polluted
WFS1	1.66	3.08	Slightly polluted
WFS1500	1.91	3.01	Slightly polluted

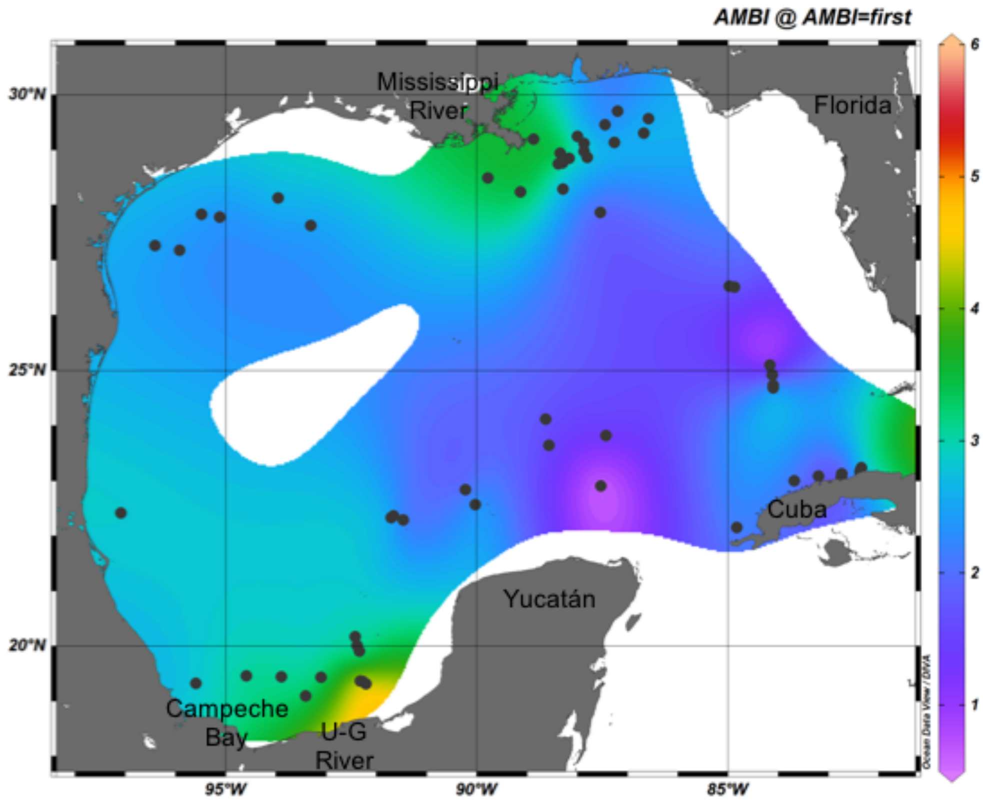


Figure 6. Heat map of the 59 Foram-AMBI values across the GoM (Table 5).

Table 6. Correlation values for the linear regression of Shannon diversity (H')_and f-AMBI values and TOM and f-AMBI values.

	r	**p**
H':Foram-AMBI	-0.262	0.040
TOM:Foram-AMBI	0.643	3.91E-08

2.4 Discussion

The worldwide expansion and implementation of marine legislation such as the Clean Water Act (USA, Australia, Canada), Europe's Water Framework Directive (WFD, 2000/60/EC), and Marine Strategic Framework Directive (MSFD, 2008/56/EC) has demonstrated the need for a standardized biological index that is easily determined, accurately measured, sensitive to multiple stressors, cost efficient, easily communicable, and demonstrative of spatial and temporal trends (Rees et al., 2006). The Foram-AMBI meets these criteria and will improve as more species and additional marine environments (e.g., estuaries) are added to the database and additional, local calibrations are constructed. The aim of this study was to adapt the Foram-AMBI developed by Alve et al. (2016) and Jorissen et al. (2018) as a decision support tool for the GoM and to further understand its vulnerability to various stressors. The Foram-AMBI provides a standardized value of EcoQS that allows for direct comparison to the macroinvertebrate AMBI, parsing out dynamics between trophic level responses to pollution. The present study offers a preliminary Foram-AMBI master species list of 155 benthic foraminifera species assignments in the GoM.

Compared to the initial Foram-AMBI studies in the northeast Atlantic Ocean (Alve et al., 2016) and the Mediterranean Sea (Jorissen et al., 2018), the Gulf of Mexico had a more even distribution among ecological groups including a higher number of Group IV and Group V species (Table 2.). This could be due to a higher abundance of opportunistic taxa in the GoM. However, Alve et al. (2016) noted that the low number of Groups IV and V in the NE Atlantic could be the result of datasets lacking sediment organic matter data. Jorissen et al. (2018) discussed several limitations stemming from the use of published datasets from different laboratories and that the methodologies for organic matter and grain size determination differed between studies, leading to inconsistencies in the data. Also, because their datasets included assemblages that were identified by different laboratories and lacked identification plates, synonymy between species was also identified as a primary challenge (Alve et al. 2016; Jorissen et al, 2018). As reported by Jorissen et al. (2018) another limitation was that not all of the published datasets used in their study contained stained (living) foraminifera, so thanatocoenoses (live + dead assemblages) had to be used for some sites (Jorissen et al., 2018). In order to address these concerns, taxonomy in the present study was done by one working group using previously agreed upon methods and taxonomical references. The samples were collected, stained, identified, quantified for organic matter percentage, and analyzed for grain size by the same working group to avoid any discrepancies in methodology or taxonomy. Plates of type specimens for most species identified in this study are available in the supplementary material.

One limitation of this study was the use of total organic matter as the analog for anthropogenic pollution. Even though organic matter has been deemed an apt proxy for pollution (Pearson & Rosenberg, 1978; Scott et al., 2001; Romano et al., 2009), there is still difficulty in separating natural organic enrichment from anthropogenic organic enrichment, which should be a focus of future studies. However, as a decision-support tool, organic matter determination is easy, quick, and readily adaptable for monitoring programs that may not have funds or the instruments to quantify other types of pollutants such as hydrocarbons or heavy metals (Rees et al., 2006). In future studies, the Foram-AMBI methodology can be adapted for different environmental parameters like polycyclic aromatic hydrocarbon concentration, heavy metals, and polychlorinated biphenyls (Aylagas et al., 2017; Borja, 2018). Future work could also develop the Foram-AMBI using thanatocoenoses to recreate pre-industrial environmental baselines from recent historical (20th-21st centuries) sediment core records.

Diversity is generally directly related to the ecological health of a benthic ecosystem (Bouchet et al., 2012, 2018). The GoM has a more evenly distributed number of species in each group compared to the northeast Atlantic and the Mediterranean (e.g. higher number of species in EG IV and V). The correlation of the Foram-AMBI values to the H' diversity index was inversely related, as expected, but only to a small degree (Table 6). This may be related to the relatively high amount of opportunist foraminifera identified in the GoM and the possibility of sites with a high diversity of Group IV and V species. For example, this was observed at site SL9-150 where 10 of the 42 species identified at that site were assigned to Groups IV and V. The Foram-AMBI is able to identify these intricacies and avoid mislabeling a diverse assemblage of opportunists with a Good Ecological Quality Status (EcoQS).

After the Deepwater Horizon oil spill in 2010, the establishment of reference conditions was identified as a need for the GoM in order to prepare and minimize risk for future spills (Shepard et al., 2013; Cordes et al, 2016). Schwing et al. (2018) determined the resiliency of benthic foraminifera up to five years after the blowout and established new reference conditions in terms of diversity and density. The Foram-AMBI expands on this study by taking into account the species that made up the assemblages and their ecological groups. The Foram-AMBI value at the Deepwater Horizon site (DWH01; 3.27; "slightly polluted") is one of the higher values identified in this study (Table 5, Fig. 6). Two general areas in the GoM with concentrations of high Foram-AMBI values include the Campeche Bay and the Mississippi River delta (Table 5, Fig. 6). The two highest Foram-AMBI values in the study, with EcoQS of transitional to heavy pollution (IXTOC1A; 4.42, LT3; 4.57), are both located in the Southern GoM. One possible explanation for these higher values could be riverine influence, with the input of fine-grained sediments and dissolved nutrients, as these areas are the two largest fluvial basins in the GoM.

The Usumacinta River and Grijalva River form the largest fluvial basin in Mexico with a water discharge of 2,678 m3s-1 into the southern GoM (Munoz-Salinas & Castillo, 2015). The Mississippi River has a water discharge of 16,806 m3 s−1 into the northern GoM and forms the largest fluvial basin in North America- draining 40% of the continental U.S.A. (Waterson & Canuel, 2008). Riverine outflows tend to discharge nutrient-enriched water, polluted with agricultural and industrial contaminants (Mitsch et al., 2001). The eutrophication caused by the Mississippi outflow has resulted in the largest hypoxic zone in the USA, located off of the Louisiana coast (Rabalais et al., 1999). An alternate explanation for high Foram-AMBI values may be related to oil rig density, which is high in Campeche Bay and the Mississippi delta (Murawski et al., 2020b). Mojtahid et al. (2006) found that proximity to an oil rig can have a negative effect on benthic foraminiferal communities due to the introduction of hydrocarbons into the environment from oiled drill muds. Another interesting Foram-AMBI area to note is the southeastern GoM near the northwestern coast of Cuba. The majority of the sample sites along the northwestern shelf and slope of Cuba had very low Foram-AMBI values ranging from 1.27 to 2.18 (Table 5, Fig. 6). The collections north of Havana, the largest city in Cuba, had values of 2.21, 2.69, and 3.20 (Table 5, Fig. 6). These relative higher values may be indicative of anthropogenic influence originating from the population center of Havana, demonstrating the sensitivity of the Foram-AMBI in identifying regions of human influence.

There is a need for a standardized decision support tools in the GoM that are sensitive to chronic and acute stressors. With the significant increase in ultra-deep water (>1,500 m) petroleum exploration, there is increased potential for an ultra-deep water spill (Murawski et al., 2020a). Thus, the establishment of ultra-deep water baselines and reference conditions is critical to assess environmental value and subsequently environmental impact (Shepard et al., 2013;

Cordes et al., 2016). The majority of the sites included in this study are shallower than 1500 m depth, and future work should include additional sites and better spatial coverage in areas deeper than 1500 m to address this need. The Foram-AMBI has significant potential as a decision support tool that is useful in its analysis of ecological health beyond the basic diversity indices. The Foram-AMBI can now be operationalized and provides an opportunity for living resource managers throughout the GoM to implement the assignments established in this study. This study assigned 65% of species from 52 calibration sites from a depth range of (42m-2975m) and over 90% of species from seven validation sites that were chosen from a wide spatial and depth range (67m-2272m) and established environmental reference conditions across the GoM demonstrating the GoM-wide applicability and value to ecosystem-based management of the Foram-AMBI.

2.5 Conclusions

1. This study developed and tested the efficacy of the Foram-AMBI as a standardized biomonitoring tool in the Gulf of Mexico and demonstrated its sensitivity to environmental stressors. The Foram-AMBI was able to identify the Mississippi River basin (NGoM), the Usumacinta-Grijalva River basin (SGoM), and Havana Bay (SEGoM) as regions of environmental stress. This may be due to fluvial influence, eutrophication, continentally-derived contaminants, oil rig density, or likely a combination of these factors.

2. A master species assignment list of 155 benthic foraminifera and an identification plate of 44 of these species were generated for easy adaptability as a managerial decision-support tool. These assignments can be implemented in biomonitoring programs as temporal and spatial trackers of ecological quality. As a result of this study, post-Deepwater Horizon reference

conditions have been recorded throughout the Gulf of Mexico in the form of Foram-AMBI values in case of future impact events.

3. The AMBI is used as a standardized metric to define Ecological Quality Statuses in European bodies of water and introducing it in the Gulf of Mexico allows for inter-regional comparisons of ecological quality status. Foraminifera may have different ecological preferences as well as species endemism that may vary in different regions. This study fits into the global effort of the Foraminiferal Biomonitoring Group (FOBIMO) to standardize and construct foraminifera-based indices for regulatory and managerial purposes (Schönfeld et al., 2012). Additionally, the introduction of the Foram-AMBI adds the unique ability to compare how different benthic organisms deal with environmental stress.

4. The Foram-AMBI is an appropriate suitor to provide benthic ecological health data across U.S. bodies of water. This is important in the establishment of environmental baseline studies for environmental impact assessments as well as the ability to properly evaluate natural resource damage from events like oil spills.

2.6 Supplementary Identification Plates

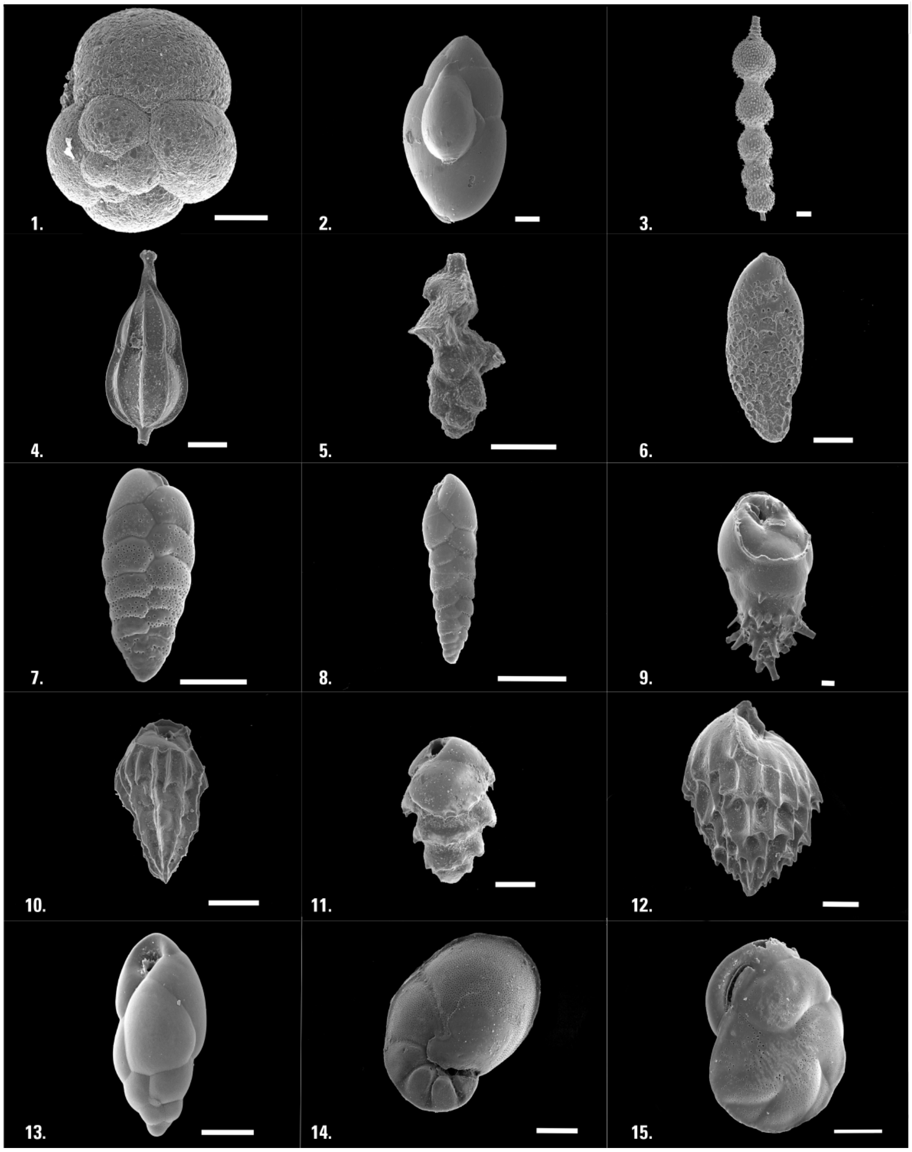


Figure 7. Scanning electron micrographs of foraminifera found in this study. Scale bars equal 100 µm. (1) Ammoglobigerina globigeriniformis. (2) Ammolagena clavata attached to Globobulimina affinis. (3) Amphicoryna hirsuta. (4) Amphicoryna scalaris. (5) Angulogerina bella. (6) Bolivina albatrossi. (7) Bolivina lowmani. (8) Bolivinellina translucens. (9) Bulimina aculeata. (10) Bulimina alazanensis (11) Bulimina marginata. (12) Bulimina striata mexicana. (13) Buliminella morgani (14) Cancris auriculus. (15) Cassidulina laevigata.

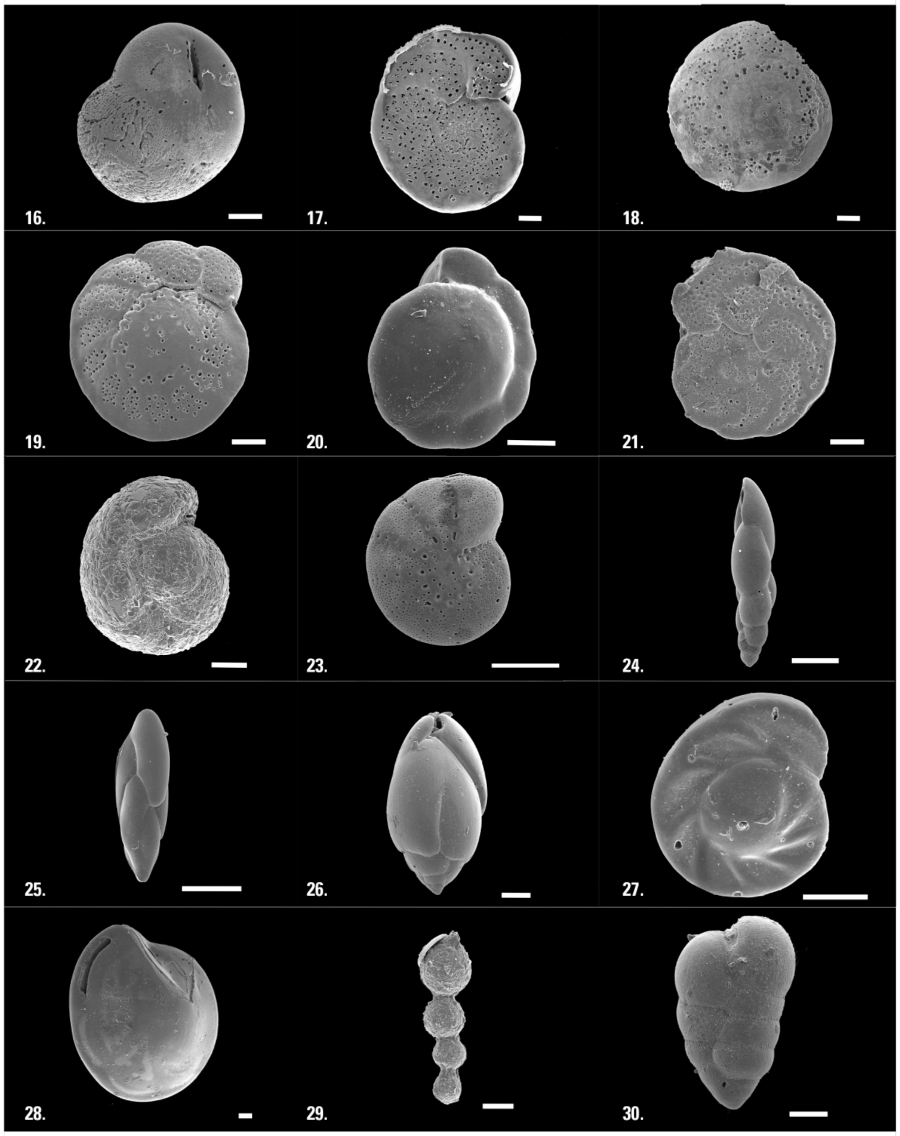


Figure 8. Scanning electron micrographs of foraminifera found in this study. Scale bars equal 100 µm. (16) Cassidulina reniforme. (17) Cibicides refulgens. (18) Cibicidoides kullenbergi. (19) Cibicidoides pachyderma. (20) Cibicidoides robertsonianas. (21) Cibicidoides wuellerstorfi. (22) Cribrostomoides subglobosum. (23) Elphidium discoidale. (24) Fursenkoina complanata. (25) Fursenkoina mexicana. (26) Globobulimina affinis. (27) Gyroidina altiformis. (28) Hoeglundina elegans. (29) Hormosina globulifera. (30) Karreriella bradyi.

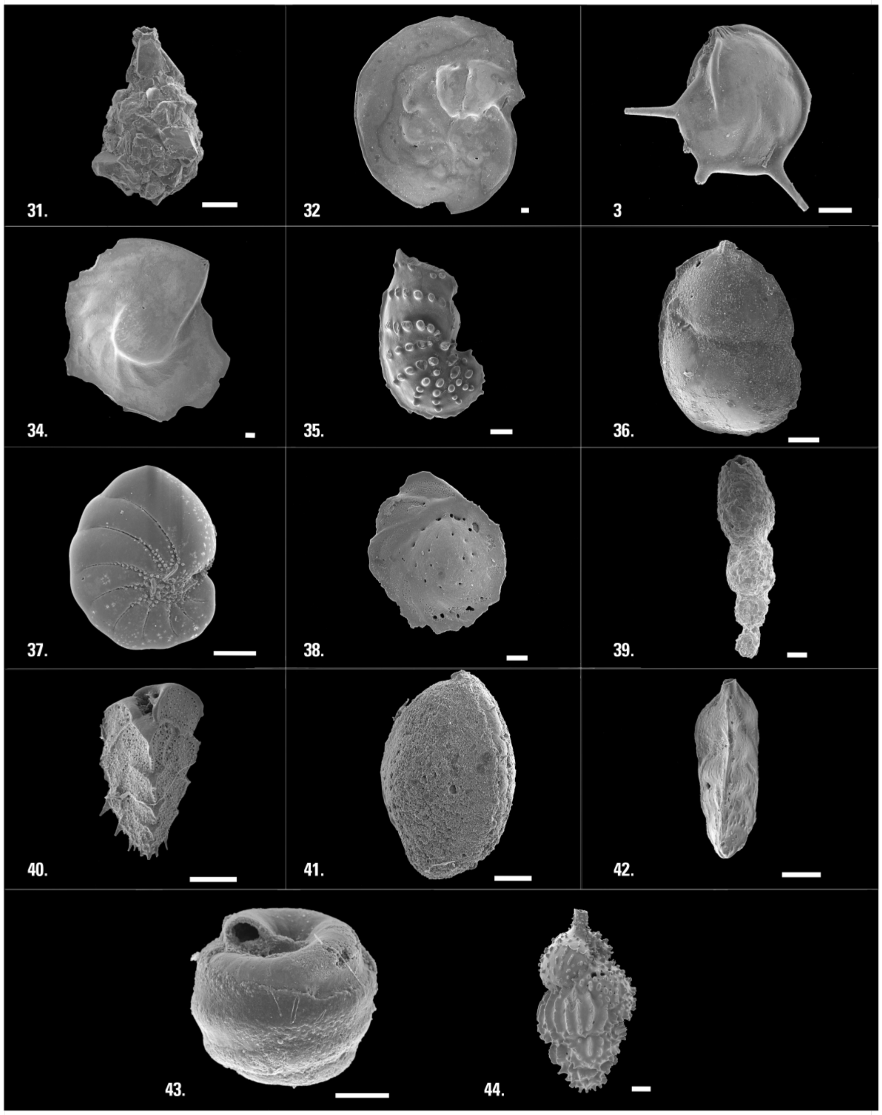


Figure 9. Scanning electron micrographs of foraminifera found in this study. Scale bars equal 100 μm. (31) Lagenammina difflugiformis. (32) Laticarinina pauperata. (33) Lenticulina calcar. (34) Lenticulina iota. (35) Marginulinopsis marginulinoides. (36) Neolenticulina peregrina. (37) Nonionella atlantica. (38) Osangularia culter. (39) Reophax scorpiurus. (40) Reussella spinulosa. (41) Sigmoilopsis schlumbergeri. (42) Trifarina bradyi. (43) Glomospira charoides. (44) Uvigerina peregrina.

2.7 References

Alve, E. 1995. Benthic foraminiferal responses to estuarine pollution: a review. Journal of Foraminiferal Research, 25(3), 190-203.

Alve, E., and Murray, J.W. 1994. Ecology and taphonomy of benthic foraminifera in a temperate mesotidal inlet. Journal of Foraminiferal Research, 24, 18-27.

Alve, E., Korsun, S., Schönfeld, J., Dijkstra, N., Golikova, E., Hess, S., Panieri, G. 2016. Foram-AMBI: A sensitivity index based on benthic foraminiferal faunas from North-East Atlantic and Arctic fjords, continental shelves and slopes. Marine Micropaleontology, 122, 1-12.

Armynot du Châtelet E.A., Debenay J.P., Soulard R. 2004. Foraminiferal proxies for pollution monitoring in moderately polluted harbors. Environ Pollut .127, 27–40.

Armynot du Châtelet, E., Gebhardt, K., Langer, M. 2011. Coastal pollution monitoring: Foraminifera as tracers of environmental perturbation in the port of Boulogne-sur-Mer (Northern France). Neues Jahrbuch für Geologie und Paläontologie. 262. 91–116.

Aylagas, E., Borja, Å., Tangherlini, M., Dell'Anno, A., Corinaldesi, C., Michell, C.T., Irigoien, X., Danovaro, R. and Rodriguez-Ezpeleta, N., 2017. A bacterial community-based index to assess the ecological status of estuarine and coastal environments. Marine pollution bulletin, 114(2), 679-688.

Bernhard, J. M., Ostermann, D. R., Williams, D. S., Blanks, J. K. 2006. Comparison of two methods to identify live benthic foraminifera: A test between Rose Bengal and CellTracker Green with implications for stable isotope paleoreconstructions. Paleoceanography, 21(4).

Bindiu, R., & Filipescu, S. 2011. Agglutinated foraminifera from the Northern Tarcău Nappe (Eastern Carpathians, Romania). Studia UBB Geologia, 56(2), 31-41.

Borja, A., Elliott, M., Andersen, J. H., Berg, T., Carstensen, J., Halpern, B. S., Rodriguez-Ezpeleta, N. 2016. Overview of integrative assessment of marine systems: the ecosystem approach in practice. Frontiers in Marine Science, 3, 20.

Borja, A., Franco, J., Pérez, V. 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. Marine pollution bulletin, 40(12), 1100-1114.

Borja, A., Muxika, I., Franco, J. 2003. The application of a Marine Biotic Index to different impact sources affecting soft-bottom benthic communities along European coasts. Marine Pollution Bulletin, 46(7), 835-845.

Borja, A. 2018. Testing the efficiency of a bacterial community-based index (microgAMBI) to assess distinct impact sources in six locations around the world. Ecological Indicators, 85, 594-602.

Bouchet, V.M.P., Alve, E., Rygg, B., Telford, J. 2012. Benthic foraminifera provide a promising tool for ecological quality assessment of marine waters. Ecological Indicators. 23, 66-75.

Bouchet, V.M.P., Goberville, E., Frontalini, F. 2018. Benthic foraminifera to assess Ecological Quality Statuses in Italian transitional waters. Ecological Indicators. 84, 130-139.

Brady, H. B. 1878. XLIX.—On the reticularian and Radiolarian Rhizopoda (Foraminifera and Polycystina) of the North-Polar Expedition of 1875–76. Journal of Natural History, 1(6), 425-440.

Brady, H. B. 1879. Notes on some of the reticularian Rhizopoda of the'Challenger'expedition. 1. On new or little known arenaceous types. Quarterly Journal of Microscopial Science. New Series, 19, 20-63.

Brady, H. B. 1884. Report on the foraminifera dredged by HMS Challenger during the years 1873-1876. Report on the scientific results of the voyage of the HMS Challenger during the years 1873-1876, Zoology, 9, 1-814.

Burke, S.K., Berger, W.H., Coulbourn, W.T., Vincent, E.. 1993. Benthic foraminifera in box core ERDC 112, Ontong Java Plateau. J. Foraminiferal Res., 23 (1), 19–39.

Carvalho, S., Gaspar, M. B., Moura, A., Vale, C., Antunes, P., Gil, O., Falcao, M. 2006. The use of the marine biotic index AMBI in the assessment of the ecological status of the Óbidos lagoon (Portugal). Marine Pollution Bulletin, 52(11), 1414-1424.

Cordes, E. E., Jones, D. O., Schlacher, T. A., Amon, D. J., Bernardino, A. F., Brooke, S., Gates, A. R. 2016. Environmental impacts of the deep-water oil and gas industry: a review to guide management strategies. Frontiers in Environmental Science, 4, 58.

Cushman, J. 1927. New and interesting Foraminifera from Mexico and Texas. Contr. Cushman Lab. Foram. Res., 3, 22.

Cushman, J. A. 1922. Shallow-water foraminifera of the Tortugas region (No. 311). Carnegie Institution.

Cushman, J. A. 1922. The Foraminifera of the Atlantic Ocean. 104, Government print: Office.

d'Orbigny, A. D. 1826. Tableau méthodique de la classe des Céphalopodes. In Annales des Sciences Naturelles. Series, 1, 96-314).

d’Orbigny, A.D. 1839. Voyage dans l'Amerique Meridionale. Paris and Strasbourg, 5 (5), 1–86.

Dean, W. E., 1974. Determination of carbonate and organic matter in calcareous sediments and sedimentary rocks by loss on ignition: Comparison with other methods. J. Sed. Petrol. 44, 242–248.

Donnici, S., Serandrei-Barbero, R., Bonardi, M., & Sperle, M. 2012. Benthic foraminifera as proxies of pollution: the case of Guanabara Bay (Brazil). Marine Pollution Bulletin, 64(10), 2015-2028.

Eichler, P. P., Rodrigues, A. R., Pereira, E. D. R. M., Eichler, B. B., Kahn, A., & Vital, H. 2015. Foraminifera as environmental condition indicators in Todos os Santos Bay (Bahia, Brazil). Open Journal of Ecology, 5(07), 326.

Ernst, S.R., Bours, I., Djuinsteem Y., & Van der Zwaan, G.J. 2005. Experimental effects of an organic matter pulse and oxygen depletion on a benthic foraminiferal shelf community. Journal of Foraminiferal Research, 35, 177-197.

European Commission. 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. J. Eur. Communities, 2000.

Frontalini, F., Buosi, C., Da Pelo, S., Coccioni, R., Cherchi, A., & Bucci, C. 2009. Benthic foraminifera as bio-indicators of trace element pollution in the heavily contaminated Santa Gilla lagoon (Cagliari, Italy). Marine Pollution Bulletin, 58(6), 858-877.

Glemarec, M., Hily, C. 1981. Perturbations apportées à la macrofaune benthique de la baie de Concarneau par les effluents urbains et portuaires. Oecologica Applicata, 2, 139-150.

Heiri, O., Lotter, A. F., & Lemcke, G. 2001. Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. Journal of paleolimnology, 25(1), 101-110.

Jeffreys, R. M., Fisher, E. H., Gooday, A. J., Larkin, K. E., Billett, D. S. M., & Wolff, G. A. 2015. The trophic and metabolic pathways of foraminifera in the Arabian Sea: evidence from cellular stable isotopes. Biogeosciences, 12(6), 1781-1797.

Jones, T. R., & Parker, W. K. 1860. On the Rhizopodal fauna of the Mediterranean, compared with that of the Italian and some other Tertiary deposits. Quarterly Journal of the Geological Society, 16(1-2), 292-307.

Kennedy, M. J., Pevear, D. R., Hill, R. J. 2002. Mineral surface control of organic carbon in black shale. Science, 295(5555), 657-660.

Lei, Y. L., Li, T. G., Bi, H., Cui, W. L., Song, W. P., Li, J. Y., & Li, C. C. 2015. Responses of benthic foraminifera to the 2011 oil spill in the Bohai Sea, PR China. Marine pollution bulletin, 96(1-2), 245-260.

Lindroth, A., 1971. On biocoenoses; coenotypes and coenoclines. Thalassia jugosl, 7, 185–194.

Martínez-Colón, M., Hallock, P., & Green-Ruíz, C. 2009. Strategies for using shallow-water benthic foraminifers as bioindicators of potentially toxic elements: a review. The Journal of Foraminiferal Research, 39(4), 278-299.

McCrone, A.W., Schafer, C. 1966. Geochemical and sedimentary environments of foraminifera in the Hudson River estuary, New York. Micropaleontology, 12, 505-509.

Mills, E.I. 1969. The community concept in marine zoology, with comments on continua and instability in some marine communities: a review. J. Fish. Res. Bd. Can. 26, 1415-1428.

Mitsch, W.J., Day, J.W., Gilliam, J.W., Groffman, P.M., Hey, D.L., Randall, G.W., Wang, N. 2011.Reducing Nitrogen Loading to the Gulf of Mexico from the Mississippi River Basin: Strategies to Counter a Persistent Ecological Problem: Ecotechnology—the use of natural ecosystems to solve environmental problems—should be a part of efforts to shrink the zone of hypoxia in the Gulf of Mexico, BioScience, 51(5), 373–388.

Munoz-Salinas, E., Castillo, M. 2015. Streamflow and sediment load assessment from 1950 to 2006 in the Usumacinta and Grijalva Rivers (Southern Mexico) and the influence of ENSO. Catena, 127, 270-278.

Murawski, S. A., Ainsworth, C. H., Gilbert, S., Hollander, D. J., Paris, C. B., Schlüter, M., & Wetzel, D. L. 2020a. Summary of Progress on Major Research Issues: Deep-Sea Oil Spills. In Deep Oil Spills, 584-594. Springer, Cham.

Murawski, S. A., Hollander, D. J., Gilbert, S., Gracia, A. 2020b. Deepwater Oil and Gas Production in the Gulf of Mexico and Related Global Trends. In Scenarios and Responses to Future Deep Oil Spills, 16-32. Springer, Cham.

Muxika, I., Borja, A., Bonne, W. 2005. The suitability of the marine biotic index (AMBI) to new impact sources along European coasts. Ecological indicators, 5(1), 19-31.

Muxika, I., Borja, A., Franco, J. 2003. The use of a biotic index (AMBI) to identify spatial and temporal impact gradients on benthic communities in an estuarine area. ICES CM2003/Session J-01, Tallinn (Estonia), 24-28.

National Research Council. 2012. Approaches for Ecosystem Services Valuation for the Gulf of Mexico After the Deepwater Horizon Oil Spill: Interim Report. Washington, DC: The National Academies Press, https://doi.org/10.17226/13141.

Nelson, J. R., & Grubesic, T. H. 2018. Oil spill modeling: Risk, spatial vulnerability, and impact assessment. Progress in Physical Geography: Earth and Environment, 42(1), 112–127, https://doi.org/10.1177/0309133317744737.

Osterman, L. E. 2003. Benthic foraminifers from the continental shelf and slope of the Gulf of Mexico: an indicator of shelf hypoxia. Estuarine, Coastal and Shelf Science, 58(1), 17-35.

Parker, F. L. 1954. Distribution of the foraminifera in the northeastern Gulf of Mexico. The Museum, 111, 10.

Parker, K. R., & Wiens, J. A. 2005. Assessing recovery following environmental accidents: environmental variation, ecological assumptions, and strategies. Ecological Applications, 15(6), 2037-2051.

Pearson, T.H., Rosenberg, R. 1978. Macrobenthic Succession in Relation to Organic Enrichment and Pollution of the Marine Environment. Oceanography and Marine Biology—An Annual Review, 16, 229-311.

Pearson, T.H., Stanley, S.O. 1979. Comparative measurement of the redox potential of marine sediments as a rapid means of assessing the effect of organic pollution. Mar. Biol., 53, 371-379.

Phleger, F. B. 1953. North Atlantic core foraminifera. Reports on the Swedish Deep-Sea Expedition, 1-122.

Phleger, F. B., & Parker, F. L. 1954. Gulf of Mexico foraminifera.

Prazeres, M., Martinez-Colon, M., and Hallock, P., 2019. Foraminifera as bioindicators of water quality: The FoRAM Index revisited. Environmental Pollution. https://doi.org/10.1016/j.envpol.2019.113612

Rabalais, N. N. 1999. Characterization of Hypoxia: Topic 1, Report for the Integrated Assessment on Hypoxia in the Gulf of Mexico (No. 15). US Department of Commerce, National Oceanic and Atmospheric Administration, Coastal Ocean Program.

Rabalais, N. N., Turner, R. E., Díaz, R. J., Justić, D. 2009. Global change and eutrophication of coastal waters. ICES Journal of Marine Science, 66(7), 1528-1537.

Rees, H.L., Hyland, J.L., Hylland, K., Mercer Clarke, C.S.L., Roff, J.C., Ware, S. 2006. Environmental indicators: utility in meeting regulatory needs. An overview. ICES Journal of Marine Science, 65: 1381-1386.

Romano, E., Bergamin, L., Finoia, M. G., Celia Magno, M., Ausili, A., Gabellini, M. 2009. The effects of human impact on benthic foraminifera in the Augusta harbour (Sicily, Italy).Integrated Coastal Zone Management. Blackwell Publishing Ltd., Chichester, UK, 97-115.

Rotstigen, A. B. 2009. Benthic foraminifera as a proxy for natural versus anthropogenic environmental change in the German Bight, North Sea: a record over the last 1000 years (Master's thesis).

Ryan, W. B. F., S.M. Carbotte, J. Coplan, S. O'Hara, A. Melkonian, R. Arko, R.A. Weissel, V. Ferrini, A. Goodwillie, F. Nitsche, J. Bonczkowski, and R. Zemsky 2009. Global Multi-Resolution Topography (GMRT) synthesis data set, Geochem. Geophys. Geosyst., 10, Q03014, doi:10.1029/2008GC002332.

Salas, F., Neto, J. M., Borja, A., & Marques, J. C. 2004. Evaluation of the applicability of a marine biotic index to characterize the status of estuarine ecosystems: the case of Mondego estuary (Portugal). Ecological indicators, 4(3), 215-225.

Schlitzer, Reiner, Ocean Data View, odv.awi.de, 2020.

Schmiedl, G., Hemleben, C., Keller, J., & Segl, M. 1998. Impact of climatic changes on the benthic foraminiferal fauna in the Ionian Sea during the last 330, 0000 years. Paleoceanography, 13(5), 447-458.

Schönfeld, J., Alve, E., Geslin, E., Jorissen, F., Korsun, S., Spezzaferri, S. Abramovich, S., Almogi-Labin, A., Châtelet, E., Barras, C., Bergamin, L., Bicchi, E., Bouchet, V., Cearreta, A., & Bella, L., & Dijkstra, N., Disaró, S., Ferraro, L., Frontalini, F., Tsujimoto, A. 2012. The FOBIMO (FOraminiferal BIo-MOnitoring) initiative—Towards a standardised protocol for soft-bottom benthic foraminiferal monitoring studies. Marine Micropaleontology. 94-95. 1-13. 10.1016/j.marmicro.2012.06.001.

Schwing P.T., Romero I.C., Larson R.A., et al. 2016. Sediment Core Extrusion Method at Millimeter Resolution Using a Calibrated, Threaded-rod. J Vis Exp. 2016;(114):54363.

Schwing, P. T., O'Malley, B. J., & Hollander, D. J. 2018. Resilience of benthic foraminifera in the northern Gulf of Mexico following the Deepwater Horizon event (2011–2015). Ecological indicators, 84, 753-764.

Scott, D., Medioli, F.S., Schafer, C.T. 2001. Monitoring in Coastal Environments Using Foraminifera and Thecamoebian Indicators. 10.1017/CBO9780511546020.

Sen Gupta, B.K., Platon, E., Bernhard, J.M., & Aharon, P. 1997. Foraminiferal colonization of hydrocarbon-seep bacterial mats and underlying sediment, Gulf of Mexico Slope. Journal of Foraminiferal Research, 27, 292-300.

Shannon, C.E. & Weaver, W.W. 1963. The mathematical theory of communications. University of Illinois Press, Urbana, 117.

Shepard, A.N., Valentine, J.F., D'Elia, C.F., Yoskowitz, D.W., Dismukes, D.E. 2013.

Economic Impact of Gulf of Mexico Ecosystem Goods and Services and Integration Into Restoration Decision-Making. Gulf of Mexico Science, 10-27.

Stewart, R. E., & Stewart, K. C. 1930. Post-Miocene Foraminifera from the Ventura Quadrangle, Ventura County, California: Twelve New Species and Varieties from the Pliocene. Journal of Paleontology, 4(1), 60-72.

Stubbles, S.J. 1993. Recent benthic Foraminiferida as indicators of pollution in Restronguet Creek, Cornwall. Proc. Ussher Soc., 8, 200-204.

Teixeira, H., Weisberg, S. B., Borja, A., Ranasinghe, J. A., Cadien, D. B., Velarde, R. G., Ritter, K. J. 2012. Calibration and validation of the AZTI's Marine Biotic Index (AMBI) for Southern California marine bays. Ecological Indicators, 12(1), 84-95.

Waśkowska, A. 2014. Distribution of the agglutinated foraminifer Ammolagena clavata (Jones and Parker) in Western Tethyan Upper Cretaceous and Paleogene deep-water deposits (Outer Carpathians, Poland). Micropaleontology, 77-88.

Waśkowska, A., & Kaminski, M. A. 2019. Pleistocene epilithic foraminifera from the Arctic Ocean. PeerJ, 7, e7207.

Waterson, E. J., & Canuel, E. A. (2008). Sources of sedimentary organic matter in the Mississippi River and adjacent Gulf of Mexico as revealed by lipid biomarker and δ13CTOC analyses. Organic Geochemistry, 39(4), 422-439.

Williamson, W. C. 1858. On the recent foraminifera of Great Britain.

Woodruff, F. 1992. Deep-sea benthic foraminifera as indicator of Miocene oceanography Studies in Benthic Foraminifera: Proceedings of the Fourth International Symposium on Benthic Foraminifera, Sendai, 1990: Tokai University Press, Tokyo, 55–66.

Chapter 3

Trophic Comparability and Ecological Quality in the Gulf of Mexico, Contributions, and Future Work

3.1 Comparisons

The application of a standardized index like the Foram-AMBI is valuable due to its ability to be directly compared to Ecological Quality Statuses (EcoQS) defined by other benthic organisms like macroinvertebrates. This paints a picture of trophic connectivity in the Gulf of Mexico as benthic foraminifera are at an intermediate level of the food web that connect the bacterial community to macroinvertebrates (Lipps & Valentine, 1971; Nomaki et al., 2008). Santibañez-Aguascalientes et al. (2018) published a multivariate-AMBI (M-AMBI) study in the Southern Gulf of Mexico (SGoM) and used macroinvertebrates to define the benthic EcoQS across the Campeche Bay and Yucatán Peninsula. There were two Foram-AMBI sites (IXTOC1A; 4.42, LT3; 4.57) located in the oil production area and both had "poor" or "transitional to heavy polluted" EcoQS ($4.3 < \text{AMBI} \leq 5.5$). Eleven out of the twenty-seven sites in the M-AMBI study also had a "poor" EcoQS (Santibañez-Aguascalientes et al., 2018). These studies, using completely different benthic organisms, which reflected the same environmental conditions, demonstrates the utility of the AMBI across trophic levels and geospatial scales (Gulf-wide, regional, and local) (Fig. 10).

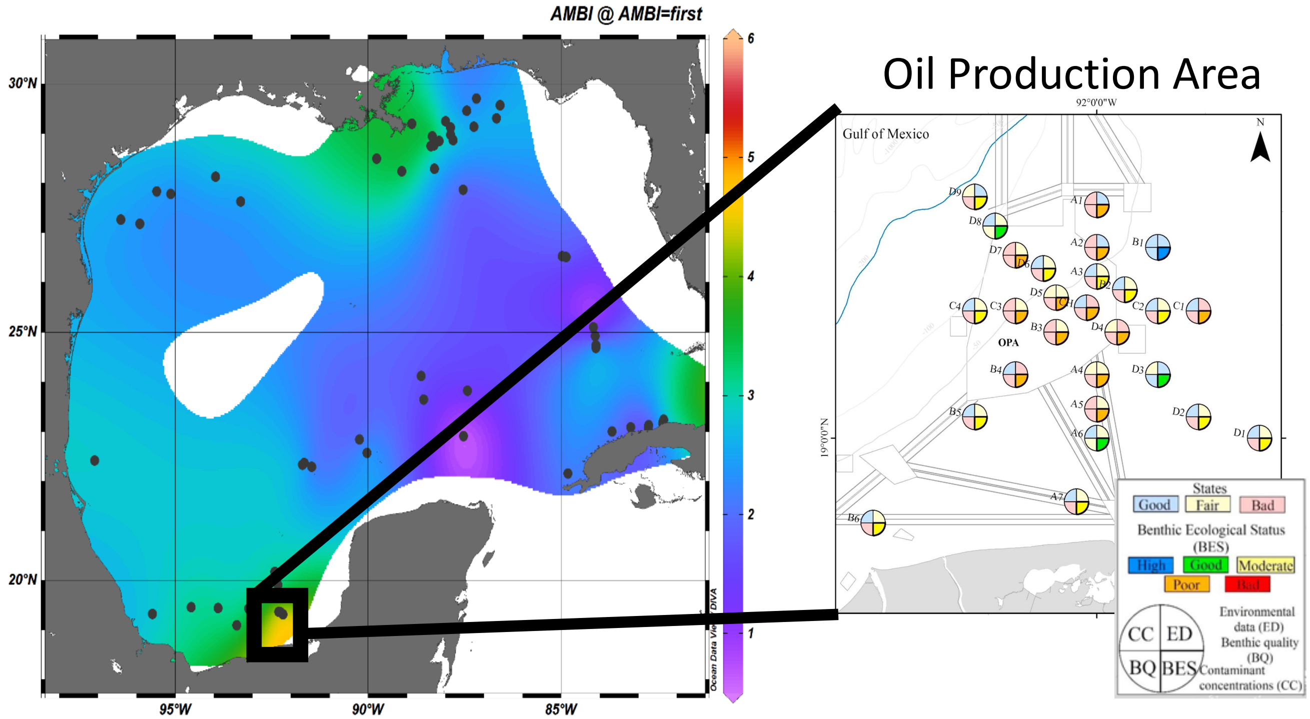


Figure 10. EcoQS derived from Foram-AMBI values compared to EcoQS (bottom-right quadrant of circle) from the M-AMBI (Santibañez-Aguascalientes et al., 2018) in the oil production area (OPA) of the Campeche Bay in the SGoM.

As a post-Deepwater Horizon map of reference conditions, there are good agreements with other post-Deepwater Horizon "baseline" maps from the GoM. In regard to trophic connectivity, the high Foram-AMBI values ("poor" to "moderate") spatially line up with areas of low macroinvertebrate diversity (H') representing regions of environmental stress around the Mississippi River Basin and the Bay of Campeche (Fig. 11) (Montagna et al., 2017).

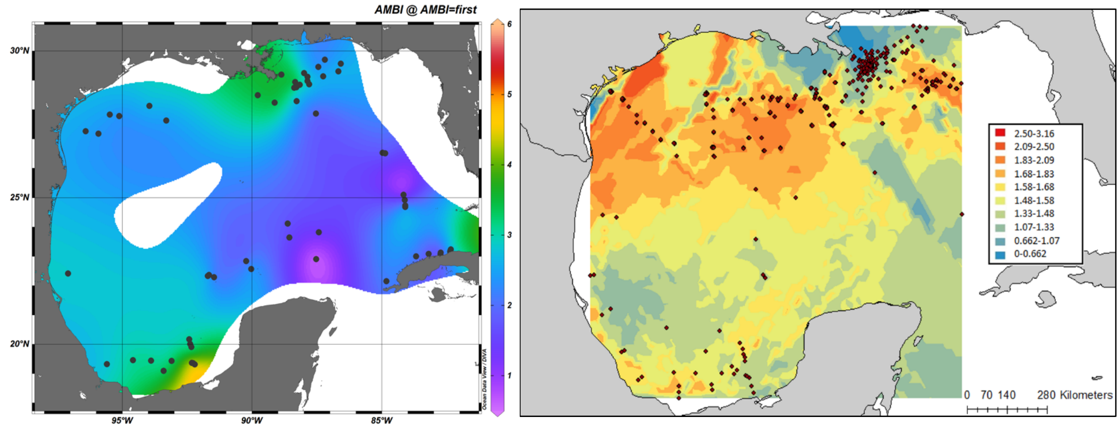


Figure 11. Foram-AMBI values (left) compared to post-Deepwater Horizon Shannon-Weaver diversity values for macroinvertebrates (right) across the GoM (Montagna et al., 2017).

Another possibility for the higher than average Foram-AMBI values (3.27-4.57) in the Campeche Bay and the Mississippi River Basin could be fluvial influence through freshwater input and salinity variations as the Mississippi River and the Usumacinta-Grijalva River are responsible for large water discharges in the GoM (Fig. 12). These waters bring nutrients and high levels of eutrophication have been observed from both river basins (Rabalais et al., 1999; Mitsch et al., 2001; Machain-Castillo et al., 2016).

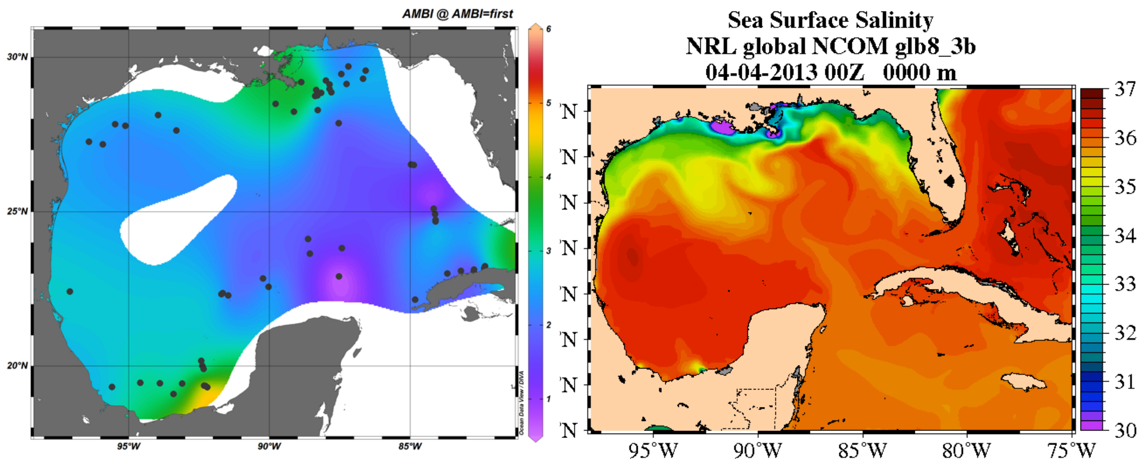


Figure 12. Comparison of Foram-AMBI values to sea surface salinity (Navy Coastal Ocean Model, 2020).

The GoM is a hotspot for oil production and is home to a multitude of oil rigs and drilling platforms (Murawski. 2020). Oil rigs are a source of benthic contamination as discovered by Mojtahid et al. (2006) in a study that quantified benthic foraminiferal communities with proximity to the drilling platform. It was found that oiled drill muds introduce hydrocarbons into the immediate surrounding environment and benthic faunal health decreases with increasing proximity to this (Mojtahid et al., 2006). Oil platforms exist in high densities off of Louisiana and the Mississippi River outflow in the northern GoM (NGoM) as well as off of the Usumacinta-Grijalva river system in the Campeche Bay (Murawski et al., 2020). These areas of high oil platform density show some agreement with our higher Foram-AMBI values found in the NGoM and the SGoM (Fig. 13).

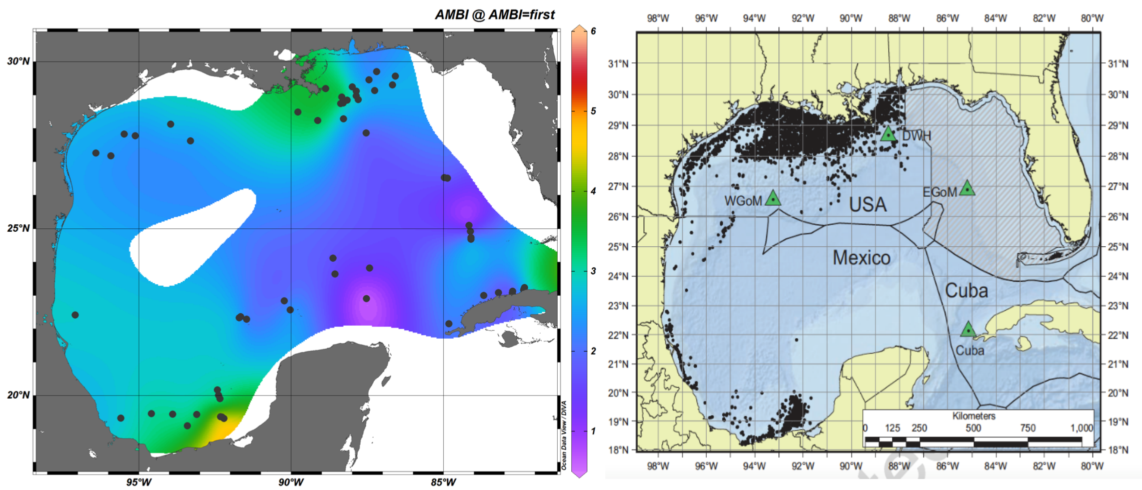


Figure 13. Comparison of Foram-AMBI values to oil rig density in the GoM (Murawski et al., 2020).

Through the comparison of the Foram-AMBI to these various metrics, certain local, regional, and basin wide trends across trophic levels become apparent. It is clear that the combined stressors of fluvial influence (nutrient loading, freshwater input) and oil production (hydrocarbon contamination) are demonstrated through benthic foraminifera and their predators, macroinvertebrates, like shrimp, polychaetes, and gastropods. The oil production area in the SGoM, is particularly stressed with “poor” EcoQS determined separately through the M-AMBI (Fig. 10) (Santibañez-Aguascalientes et al., 2018) and the Foram-AMBI in this study. This is an area of high oil rig density and significant riverine influence from the Usumacinta-Grijalva River (Fig. 12, Fig. 13) demonstrating the Foram-AMBI’s sensitivity to multiple stressors.

3.2 Contributions

This study developed the Foram-AMBI for use as a decision support tool in the Gulf of Mexico and defined reference conditions in case of future impact events or perturbations. The Gulf of Mexico is economically vital to the Mexico, Cuba, and the United States providing invaluable ecosystem services and supporting millions of livelihoods (Adams et al., 2004). To ensure the sustainability of the Gulf of Mexico's resources, there is need for long-term monitoring programs that are capable of quantitatively defining Ecological Quality Status. The European Union has found success with the European Water Framework Directive (WFD, 2000/60/EC), which puts ecological integrity at the basis of all management decisions. The AMBI is used as a standardized metric to define Ecological Quality Statuses in European bodies of water and introducing it in the Gulf of Mexico allows for inter-regional comparisons of Ecological Quality. Foraminifera may have different ecological preferences as well as species endemism that may vary in different regions, therefore it is important to conduct regional studies (Alve, 1995). This study fits into the global effort of the Foraminiferal Biomonitoring Group (FOBIMO) to standardize and construct foraminifera-based indices for regulatory and managerial purposes (Schönfeld et al., 2012). Additionally, the introduction of the Foram-AMBI adds the unique ability to compare how different benthic organisms deal with environmental stress (Alve et al., 2016). As a result of this study, a master list of 155 benthic foraminifera species assignments was made publicly available for immediate use in developing Ecological Quality Statuses for the Gulf of Mexico. Another useful takeaway from this study is an SEM identification plate of 44 of the species assigned to ensure that foraminifera are being correctly identified across studies.

The Foram-AMBI meets the requirements of useful Ecological Quality monitoring tools proposed by Rees et al. (2006; 2008). From a managerial point of view, decision making tools must be easily and accurately measured, sensitive to anthropogenic influence, scientifically valid, easily communicable, and cost-effective (Rees et al. 2006; 2008). The use of organic matter content as the proxy for anthropogenic influence in the Foram-AMBI make it cost-effective and easily quantified through the loss on ignition method (Dean et al., 1974; Heiri et al. 2001). Also the collection of foraminifera through coring is a relatively cheap method to collect statistically robust sample sizes with small sampling volumes. The Gulf of Mexico Foram-AMBI has demonstrated sensitivity as this study singled out the Mississippi River basin, the Usumacinta-Grijalva River basin, and Havana Bay as areas of environmental stress. Possible reasons for the higher Foram-AMBI values in the Usumacinta-Grijalva River basin and the Mississippi River basin could include the water discharge and subsequent nutrient loading from anthropogenic sources causing eutrophication and hypoxia or the high oil-rig density present in both regions (Munoz-Salinas and Castillo, 2015; Rabalais et al., 1999; 2009; Murawski et al., 2020). It is also possibly taking into account lingering effects from the two of the largest oil spills in history that occurred in both of these regions, the Deepwater Horizon oil spill in the Northern Gulf and the IXTOC-1 oil spill in the Southern Gulf. Likely, the Foram-AMBI is responding to a combination of these things and the Foram-AMBI values are elevated due to a multiple stressors response.

3.3 Broader Implications

The timing of the development of this index is appropriate as the BLUE GLOBE act, or the Bolstering Long-Term Understanding and Exploration of the Great Lakes, Oceans, Bays, and Estuaries Act (H.R.3548/S.933) is currently stalled in the United States congress but has promising bipartisan support in the ensuing 117th United States Congress. The BLUE GLOBE Act promotes the growth of U.S. ocean industries through the monitoring, observation, and exploration of the United States' oceans, bays, estuaries, and coasts. It focuses on the collection, synthesis, and database of standardized ecological data crucial to these ocean industries. This bill would increase federal investments in ocean data and monitoring as well as reauthorize NOAA's Ocean Exploration program, Integrated Ocean and Coastal Mapping programs, and Hydrographic Services Improvement programs. The Foram-AMBI is an appropriate suitor to provide benthic ecological health data across U.S. bodies of water. This is important in the establishment of environmental baseline studies for environmental impact assessments as well as the ability to properly evaluate natural resource damage from events like oil spills. If the BLUE GLOBE Act gets passed, then it would be appropriate to pitch the Foram-AMBI to NOAA monitoring programs, as this is a standardized index that can determine benthic habitat suitability for commercially important fish species and can be directly compared to other water bodies in the United States. It can also be compared to commercially important water bodies in Europe that are under the management of the European Water Framework Directive (WFD, 2000/60/EC). This is in line with the primary directives of the UNESCO ocean decade, which support global scientific partnerships in an effort to provide science-based management to United Nations members and a common goal of sustainable development in the world's oceans (Ryabinin et al., 2019). The Foram-AMBI's ability to be inter-regionally compared would aid the UNESCO ocean decade's ultimate goals of maintaining a healthy, resilient, safe, predicted, sustainably harvested, and productive ocean (Ryabinin et al., 2019).

3.4 Future Work

The Foram-AMBI is immensely useful due to its capability to cost-effectively identify regions of anthropogenic influence. Future studies need to be done to determine the true potential of the tool. The Foram-AMBI is multifaceted and has potential to recreate past Ecological Quality Statuses due to the high preservation potential of foraminifera. Using thanatocoenoses, or total assemblages downcore, paired with Pb-210 sediment dating, benthic foraminifera can be used to retroactively calculate Foram-AMBI values to determine ecological health of past environments. This makes it possible to establish true pre-human environmental baselines.

The Foram-AMBI has the potential be refined and tailored to specific environmental stressors. In order to determine distinct impacts of specific stressors the assignment calibration graphs can be customized to any environmental pressure that can be quantified. This method compares the trend of how relative abundance changes to a gradient of that specific stressor. This study used total organic matter because it was the most logical cost-effective option for monitoring programs. If the quantification tools are available (Gas Chromatography-Mass Spectrometer, Liquid Chromatography-Mass Spectrometer, Inductively Coupled Plasma-Mass Spectrometer) then specific Foram-AMBI calibrations can be generated for Polycyclic Aromatic Hydrocarbons for the identification of petroleum contamination, Polychlorinated Biphenyls and pesticides for the identification of anthropogenic pollution, and heavy metals for the identification of industrial pollution (Tchounwou et al., 2012; Olsson et al., 1986; Romero et al., 2015).

A project collaborating with Eckerd College is currently in development that would create an automatic foraminifera identification software using a microscope camera and a neural network of SEM micrographs and light micrographs of benthic foraminifera species throughout the Gulf of Mexico. This would greatly expedite the Foram-AMBI process for managers and standardize the taxonomic process.

3.5 References

Adams, C. M., Hernandez, E., and Cato, J. C., 2004. The economic significance of the Gulf of Mexico related to population, income, employment, minerals, fisheries and shipping. Ocean & Coastal Management, 47(11-12), 565-580.

Alve, E., 1995. Benthic foraminiferal responses to estuarine pollution: a review. Journal of Foraminiferal Research, 25(3), 190-203.

Alve, E., Korsun, S., Schönfeld, J., Dijkstra, N., Golikova, E., Hess, S., Panieri, G., 2016. Foram-AMBI: A sensitivity index based on benthic foraminiferal faunas from North-East Atlantic and Arctic fjords, continental shelves and slopes. Marine Micropaleontology, 122, 1-12.

Dean, W. E., 1974. Determination of carbonate and organic matter in calcareous sediments and sedimentary rocks by loss on ignition: Comparison with other methods. J. Sed. Petrol. 44, 242–248.

European Commission, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. J. Eur. Communities, 2000.

GovTrack.us, 2020. S. 933 — 116th Congress: BLUE GLOBE Act. Retrieved from https://www.govtrack.us/congress/bills/116/s933.

Heiri, O., Lotter, A. F., & Lemcke, G. 2001. Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. Journal of paleolimnology, 25(1), 101-110.

Montagna, P.A., Baguley, J.G., Cooksey, C. and Hyland, J.L., 2017. Persistent impacts to the deep soft-bottom benthos one year after the Deepwater Horizon event. Integrated environmental assessment and management, 13(2), pp.342-351.

Munoz-Salinas, E. and Castillo, M., 2015. Streamflow and sediment load assessment from 1950 to 2006 in the Usumacinta and Grijalva Rivers (Southern Mexico) and the influence of ENSO. Catena, 127, pp.270-278.

Murawski, S. A., Ainsworth, C. H., Gilbert, S., Hollander, D. J., Paris, C. B., Schlüter, M., & Wetzel, D. L., 2020. Summary of Progress on Major Research Issues: Deep-Sea Oil Spills. In Deep Oil Spills, 584-594. Springer, Cham.

Nomaki, H., Ogawa, N. O., Ohkouchi, N., Suga, H., Toyofuku, T., Shimanaga, M., ... & Kitazato, H., 2008. Benthic foraminifera as trophic links between phytodetritus and benthic metazoans: carbon and nitrogen isotopic evidence. Marine Ecology Progress Series, 357, 153-164.

Olsson, M., Reutergårdh, L., 1986. DDT and PCB pollution trends in the Swedish aquatic environment. Ambio, 103-109.

Rabalais, N. N., 1999. Characterization of Hypoxia: Topic 1, Report for the Integrated Assessment on Hypoxia in the Gulf of Mexico (No. 15). US Department of Commerce, National Oceanic and Atmospheric Administration, Coastal Ocean Program.

Rabalais, N. N., Turner, R. E., Díaz, R. J., Justić, D. 2009. Global change and eutrophication of coastal waters. ICES Journal of Marine Science, 66(7), 1528-1537.

Rees, H. L., Boyd, S. E., Schratzberger, M., & Murray, L. A., 2006. Role of benthic indicators in regulating human activities at sea. Environmental Science & Policy, 9(5), 496-508.

Rees, H.L., Hyland, J.L., Hylland, K., Mercer Clarke, C.S.L., Roff, J.C., Ware, S., 2008. Environmental indicators: utility in meeting regulatory needs. An overview. ICES Journal of Marine Science, 65: 1381-1386.

Romero, I.C., Schwing, P.T., Brooks, G.R., Larson, R.A., Hastings, D.W., Ellis, G., Goddard, E.A. and Hollander, D.J., 2015. Hydrocarbons in deep-sea sediments following the 2010 Deepwater Horizon blowout in the northeast Gulf of Mexico. PLoS One, 10(5), p.e0128371.

Ryabinin, V., Barbière, J., Haugan, P., Kullenberg, G., Smith, N., McLean, C., Troisi, A., Fischer, A.S., Aricò, S., Aarup, T. and Pissierssens, P., 2019. The UN decade of ocean science for sustainable development. Frontiers in Marine Science, 6, p.470.

Santibañez-Aguascalientes, N.A., Borja, Á., Kuk-Dzul, J.G., Montero-Muñoz, J.L. and Ardisson, P.L., 2018. Assessing benthic ecological status under impoverished faunal situations: A case study from the southern Gulf of Mexico. Ecological indicators, 91, pp.679-688.

Schönfeld, J., Alve, E., Geslin, E., Jorissen, F., & Korsun, S., Spezzaferri, S. Abramovich, S., Almogi-Labin, A., Châtelet, E., Barras, C., Bergamin, L., Bicchi, E., Bouchet, V., Cearreta, A., & Bella, L., & Dijkstra, N., Disaró, S., Ferraro, L., Frontalini, F., Tsujimoto, A., 2012. The FOBIMO (FOraminiferal BIo-MOnitoring) initiative—Towards a standardised protocol for soft-bottom benthic foraminiferal monitoring studies. Marine Micropaleontology. 94-95. 1-13. 10.1016/j.marmicro.2012.06.001.

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., Sutton, D. J., 2012. Heavy metal toxicity and the environment. In Molecular, clinical and environmental toxicology (pp. 133-164).