

An exploratory surface-subsurface trace metal analysis of benthic foraminifera from San Salvador, Bahamas

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Abstract

Foraminifera, protozoans which form preservable siliciclastic or calcareous tests, are invaluable tools for proxy-based oceanographic and paleoenvironmental studies. However, anthropogenic contamination of marine ecosystems can lead to the over-accumulation of trace metals in foraminiferal tests, such that can induce test morphological abnormalities. Species-specific differences in foraminiferal ontogenesis result in differential responses to anthropogenically-elevated trace metal concentrations, causing foraminiferal community disruption. Consequently, high concentrations of trace metals are purported to deleteriously affect the fidelity with which tests are preserved in the microfossil record, such that nullify their utility as proxies for paleoecological reconstruction. Herein, we predict that surface foraminiferal assemblages contain higher concentrations of trace metals than subsurface assemblages and accordingly feature a higher incidence of morphological abnormalities. We obtained surface sediment (top ~ 1 cm of seabed) and subsurface sediment (~ 65 cm below seabed) samples from Graham's Harbor at San Salvador Island, Bahamas. ~ 65 mg of benthic foraminifera were isolated from surface and subsurface sediment and dissolved in HNO₃. Inductively coupled plasma optical emission spectroscopy (ICP-OES) analysis revealed that concentrations of Al, Fe, Mn and Zn were higher in surface foraminiferal assemblages than in subsurface assemblages; the inverse trend was reported for Cu and Cr. Scanning electron microscope (SEM) analysis of representative tests from surface and subsurface assemblages revealed a greater incidence of morphological abnormalities

in surface tests, such that accorded with the ICP-OES results and corroborated our prediction. Our research supports the postulate that anthropogenic impact on benthos ecosystems deleteriously affects foraminiferal test integrity. Further research investigating the relationship between trace-metal derived morphological abnormalities and live-dead foraminiferal assemblage discordance is thus highly warranted.

Introduction

Ecological indices for species abundance and evenness provide insight into community dynamics that is difficult to obtain when solely performing paleo-environmental analyses.^{1,2} Paleo-ecological reconstruction is largely restricted by the amount of information which can be obtained from geologic facies, an issue which manifests itself notably when considering soft-bodied organisms due to their low propensity for sedimentation resulting in their underrepresentation in the geological record.¹ Fortunately, various proxies have been established as reliable means whereby to study environmental factors over the span of geological time and use them to create a context by which to gauge current environmental change.²

Foraminifera, unicellular eukaryotes (protozoan supergroup Rhizaria³) known for their formation of siliciclastic and calcareous tests, have been widely used for biostratigraphical and paleo-environmental analyses.¹ Foraminifera are commonly classified based on the mechanism of test formation.⁴⁻⁶ Although foraminiferal ontogenesis is still largely unexplained, test chamber addition to a primary organic matrix encapsulating the protozoan cytoplasm follows one of two pathways: agglutination or calcification.⁴⁻⁶ In the former mechanism, the cementing of readily available bioclastic and inorganic grains to a tectinous membrane characterizes foraminiferal growth.⁵ Importantly, most agglutinating foraminiferal species (Allogromiina) are indifferent to the materials from which they construct their tests and thus represent the environment wherein

they developed.⁵ Among calcifying foraminifera, species utilize both intracellular (miliolids) and extracellular (hyaline) mechanisms for the precipitation of mineral ions integral to test formation.⁴ Nevertheless, vacuolization (pinocytosis) of seawater is fundamental to calcite precipitation in both miliolid and hyaline foraminifera.⁴ As in the Allogromiina, test formation in calcifying foraminifera is essentially dependent on the constitution of their seawater medium, making the microfossils invaluable tools for proxy-based oceanographic and paleo-oceanographic studies.^{4,7,8}

The utility of benthic foraminifera for proxy-based studies is associated with their role as symbionts with many organisms, especially soft-bodied, their diversity and ubiquity among global benthos ecosystems, and the fidelity with which they are represented in the microfossil record.^{1,9,10} Foraminifera can be used as proxies for environmental parameters via analysis of their chemical constitution or the association between species-specific organism abundance and evenness and a given environmental factor.² Many studies have exploited the utility of microfossils as proxies to assess the anthropogenic impact on climate change⁵ and ecosystem contamination.¹¹⁻¹⁵ Furthermore, scientific literature on the subject reveals considerable research published on the use of foraminiferal assemblage data to perform paleoecological community reconstructions.¹⁰ However, foraminiferal tests are subject to deformities leaving them unrecognizable as a result of natural environmental and anthropogenic stresses.^{11-12,16} These include reduction of nutritive resources, fluctuations in environmental conditions like salinity and pH, and high concentrations of trace metals.¹⁶

Most studies which assess the effects of anthropogenic environmental change on foraminiferal ontogenesis and test integrity either exclusively consider the discordance in live – dead and surface – subsurface assemblages or measure test trace metal concentrations without evaluating community disruption.^{9,11,12,17} A dearth of research exists on the relationship between

the ecological and chemical metrics of foraminiferal viability. Herein, we postulate that **anthropogenically-derived metal ion contaminants may accumulate in surface sediment and induce morphological abnormalities in foraminiferal tests, such that would impair their utility for proxy-based studies.**

Studied Area

San Salvador (Fig. 1) is a small island in the eastern part of the Bahamian archipelago with an area of approximately 160 km.¹⁸ The island is surrounded by the North Atlantic Ocean with shallow coastal regions and calm waters that allows for easy study of the benthic biota.^{1,18,19} A single site was chosen on the northwestern coast of the island to minimize effects of directional current: North Point at Graham's Harbor (24°7.217'N, 74°27.739'W). North Point, an inner lagoon environment, represents one of the regions most affected by anthropogenic activity due to the establishment of the Gerace Research Centre in 1971.^{1,18} The locality represents a region of low hydrodynamic energy as determined by low water depth and the abundance of *Thalassia* seagrass patches such that buffer the extent of bioturbation.¹

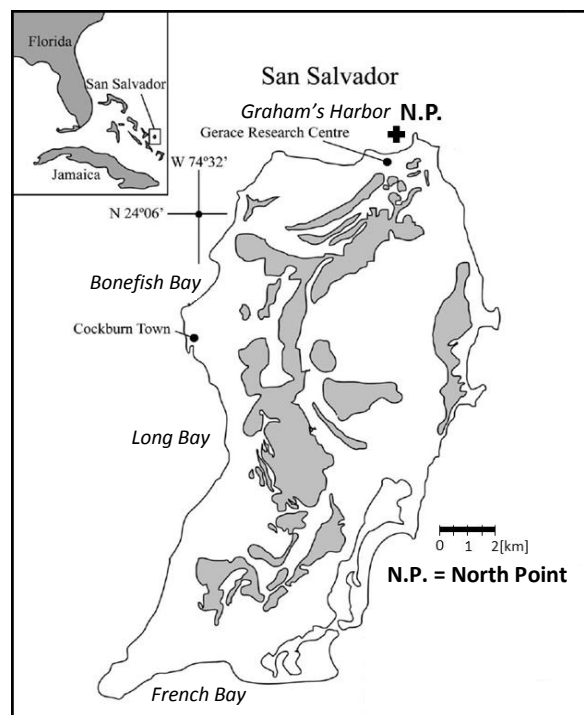


Figure 1. Map of San Salvador, Bahamas with studied locations marked; adapted from map presented by Mylroie and Carew (2010)¹⁸

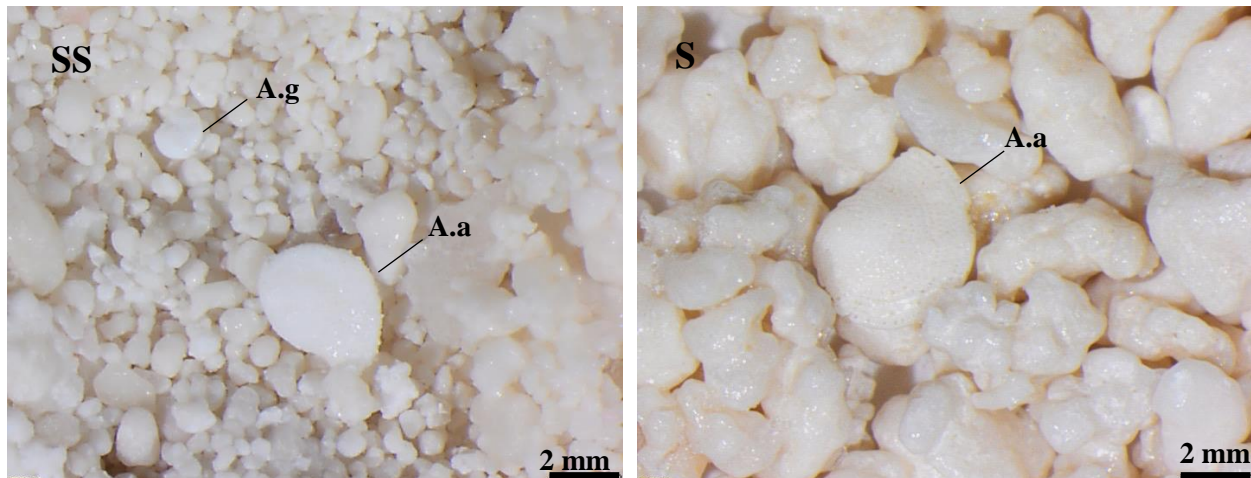


Figure 2. Subsurface (SS) sediment and surface (S) sediment from Graham's Harbor at North Point. The calcareous sediment both at the surface and subsurface was composed largely of bioclastic grains. The S sediment was composed of large grains relative to the SS. Benthic foraminifera including *Archaias angulatus* (A.a) and *Amphistegina gibbosa* (A.g.) were readily abundant in both S and SS sediment.

Materials and Methods

A total of two samples were collected from Graham's Harbor at North Point; one surface and one subsurface. Sampling locations were chosen away from sea grass, large rocks, and corals while remaining at a water-depth of ~1m to mitigate the effects of bioturbation. 12-15 cm³ of surface sediment was collected from the first 1 cm layer of the seabed

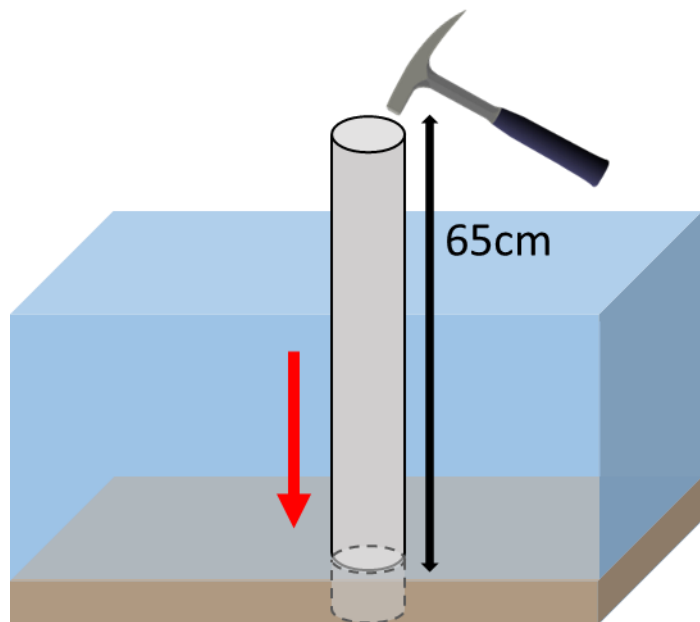


Figure 3. Setup of core extraction protocol. Cores were extracted using a 65 cm PVC tube with a diameter of 2.5 cm

directly into a test tube. 12-15 cm³ of subsurface sample was collected from the bottom of a 65 cm core that was manually extracted using a PVC corer (diameter of 2.5 cm). Sediment from the very bottom of the core was discarded to ensure contamination from the surface sediment did not occur. The core collection procedure was adapted from Darroch, 2012.¹ All samples were desiccated in

an oven at 120 °C for 10 minutes. 150-200 foraminifera of various unspecified genera were isolated from surface (60 mg) and subsurface (65 mg) sediment using fine tip brushes under an Olympus SZ61 stereo microscope. Select foraminifera were photographed using a JEOL JSM 6610LV scanning electron microscope at the University of Toronto, St. George, allowing for superior identification of morphological traits.

The samples were digested in 0.500 mL of 70% HNO₃ in a borosilicate glass beaker and left overnight. 9.5 mL of Milli-Q water was added to the digest to dilute and prevent damage to the inductively coupled plasma-optical emission spectrometer (ICP-OES) (Thermo Scientific © iCAP 6500 Duo) used for elemental analysis. Serial dilutions of a factor of 10 and 100 were made to the diluted digest resulting in a total of six samples (3 surface and 3 subsurface) with concentrations of 1/20, 1/200, 1/2000 relative to the initially digested foraminifera. Samples were run in triplicates with a 10 mL of Fluka multielement standard solution 4 for ICP as a standard and 10 mL of 2% HNO₃-Milli-Q water as a blank. All values obtained were within detection limits as provided by Thermo Scientific ©.²⁰

Results

Elemental analysis was performed on 10 trace metals: Cu, Ni, Al, As, Cd, Cr, Fe, Mn, Pb and Zn, however, only the results for those concentrations whose relative standard deviation was below 6 % as determined by the signal to noise ratio of the ICP-OES. Aluminium constituted the highest concentration of the six trace metals assessed in both the surface and subsurface tests (Table 1, Fig. 3). In contrast, chromium was found to have the lowest concentration in both regions (Table 1, Fig. 3). Aluminium and Iron were both present at high concentration (>30 ppb) relative to the other four metals (<11 ppb). Surface-subsurface concentration disparities for all six metals yielded a significant effect ($p < 0.001$). Four of the six metals (Al, Fe, Mn, and Zn) were present at

higher concentrations in foraminiferal tests on the surface than in the subsurface. The remaining two metals (Cu and Cr) displayed the opposite trend wherein metal concentration was higher in subsurface tests. SEM analysis revealed that surface foraminiferal tests featured greater morphological abnormalities and less structural integrity than those from the subsurface.

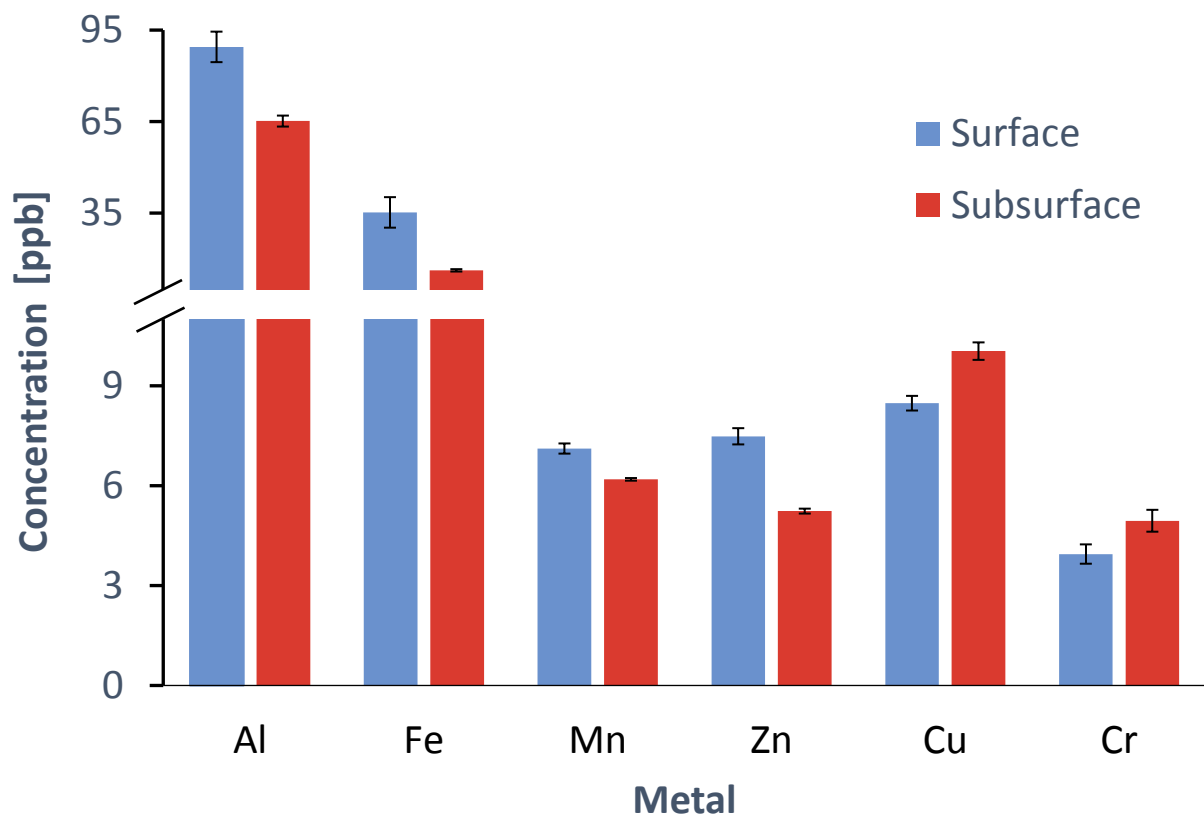


Figure 4. Concentrations of trace metals [ppb] in benthic foraminifera from surface and subsurface sediment collected at North Point. Error bars represent 95 % C.I

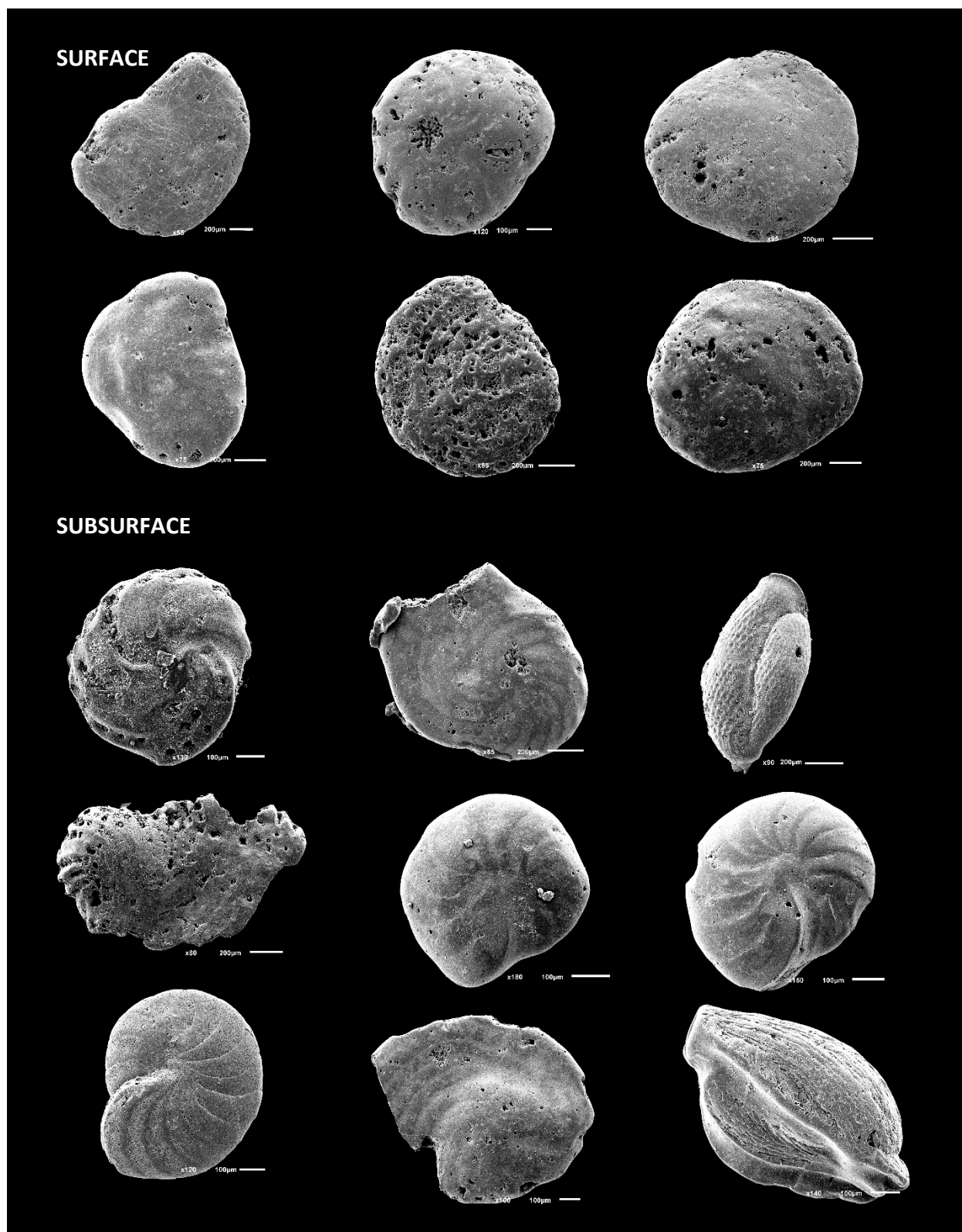


Figure 5. Benthic foraminifera from surface and subsurface sediment of Graham's Harbor at North Point

Discussion

Although foraminiferal calcite has been extensively analyzed for the presence of most trace elements and both radiogenic and stable isotopes, the incorporation of metals into foraminiferal tests remains a subject of active research.⁶ Many studies have measured Mg/Ca ratios in foraminiferal calcite to perform paleoclimatic reconstructions, seeing that Mg^{2+} substitutes for calcium more readily with increasing temperature of seawater.^{21,22} However, temperature, salinity, pH, and genetically-determined foraminiferal growth rate all modulate the rate of calcite formation, confounding the association between any two variables considered in a proxy-based study.^{4,7,8,23} Moreover, hyaline foraminifera can actively increase the pH of their cytoplasm via proton pumps to promote calcification in marine environments with relatively high magnesium levels.^{4,8} This circumventive mechanism likewise influences the extent of incorporation of other metals into the foraminiferal test, representing a major limitation of foraminiferal sclerochronology.^{24,25} Given that polluted environments often feature hypoxic or anoxic sediments due to sulfate reduction bacteria, trace metals typically exist as sparingly soluble sulphides with low bioavailability.^{7,25} Consequently, foraminiferal tests provide largely conservative estimates of actual trace metal concentrations in marine ecosystems. Despite these limitations, however, foraminiferal test analysis, both qualitative and quantitative, can reveal a considerable amount of information regarding the health of these environments.

The concentrations of trace metals in foraminifera extracted from the surface sediment (Fig. 4; Table A1) were considerably lower than those reported in related studies.^{11,12} We suspect that our data indicate that the marine environment around San Salvador island is less polluted than those in similar studies investigating lagoon ecosystems. Further research investigating both intra-site surface-subsurface and inter-site surface foraminiferal test trace metal concentrations at

different locations on San Salvador island (e.g. Bonefish Bay, Long Bay and French Bay; Fig.1) would help to elucidate the association between proximity to a source of pollution and the concentration of trace metals in foraminiferal tests.

Excepting Al and Cr, the trace metals detectable in the foraminiferal tests whose concentrations were reported with $RSD < 6\%$ play essential roles in organismal ontogenesis. Thus it is important to consider the role they play in natural foraminiferal test development.

Iron

A vital component of marine ecosystems, iron forms oxides such that strengthen the tests of agglutinating foraminifera.⁵ Foraminifera likely obtain iron through phagocytosing organic complexes chelated to iron and releasing it as colloidal hydrated Fe_2O_3 .⁵ Studies have shown that high concentrations of iron in marine environments promote the blackening of tests¹². Interestingly, oceanic surface waters are becoming increasingly depleted of the trace metal.²⁶ Insofar as the surface Fe concentration was 35 ppb (Fig. 4; Fig. A1), representing a value up to five magnitudes smaller than that reported in related studies^{11,12}, and all of the foraminifera examined had white or cream colored porcelaneous tests, we do not suspect that iron is a significant contaminant at Graham's Harbor.

Manganese

Like Fe in its conditions of dissolution and accumulation¹¹, Mn forms carbonates which may coat the external surface of foraminiferal tests.²⁷ However, recent studies using electron microprobe mapping (EMP) and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) have demonstrated that foraminifera contain a inner kutnohorite ($Ca(Mn, Mg)(CO_3)_2$) – rich phase, suggesting that Mn incorporation into foraminiferal tests goes beyond

adsorption at the test exterior.²⁸ The concentrations of Mn in both surface and subsurface foraminifera from Graham's Harbor (Fig. 4; Fig. A1) were lower than those reported in similar studies^{11,12}, suggesting that Mn is not a significant contaminant at the research site.

Copper

Copper is essential for the health of the marine biota, most notably phytoplankton.²⁹ Nonetheless, it is toxic at concentrations even slightly higher than those required for organismal ontogenesis.¹¹ Accordingly, oceanic copper levels are regulated through the production of various copper-binding ligands secreted by photosynthesizing prokaryotes.²⁹ Whether these ligands modify the bioavailability of copper for foraminifera remains to be determined, however, we suspect their relative abundance in oceanic waters might explain why a higher Cu concentration was reported for subsurface foraminifera.²⁹ Alternately, surface foraminifera may have been exposed to excessively high levels of Cu leading to the precocious death of certain individuals and creating a misrepresentation in the surface foraminiferal facie. This postulate is supported through experiments showing that high Cu concentrations inhibited the development of certain foraminiferal species²⁵ and the fact that zinc concentrations reported for both surface and subsurface foraminiferal samples were low relative to values reported by similar studies.¹²

Interestingly, studies have shown that despite the potential for high Cu concentrations to impede calcification, likely because copper carbonates do not exist in crystalline structures but form morphed octahedrons or tetragonal pyramids, the partition coefficient for copper in foraminiferal tests remains the same irrespective of Cu concentration.²⁵ This is intelligible given that the foraminifer-driven removal of Cu at the location of CaCO₃ precipitation requires a lot of energy.²⁵ Furthermore, certain species of benthic foraminifera contain proteins rich in tryptophan such that can bind Cu²⁺ and mitigate cytosolic Cu concentrations.²⁵

Zinc

The concentration of Zn in marine ecosystems is minimally influenced by human impact, deriving predominantly from such contaminants like batteries, galvanized steel and automobile tires.¹¹ Most of the Zn in oceanic waters is chelated to organic ligands which have a lower bioavailability for incorporation into foraminiferal tests.²⁹ When integrated into foraminiferal tests, zinc exists as a carbonate isomorphous with CaCO_3 .¹¹

Aluminum

Aluminum represented the most prevalent trace metal in both surface and subsurface foraminifera. Although Al is not excessively toxic, it has been shown to induce test deformation in foraminifera at high concentrations. The Al at Graham's Harbor may have derived from alum or any other Al-based adsorbent utilized for the removal of contaminants in the treatment of water.³⁰

Chromium

Like copper, the concentration of chromium was higher in the subsurface foraminiferal tests than in those from the surface. However, unlike Cu it is unlikely that photosynthesizing prokaryotes regulate oceanic Cr levels, given the high toxicity of the trace element. Rather, high oceanic levels of Cr may have impeded the development of certain species of foraminifera leading to an underrepresentation of Cr-containing foraminiferal tests at the surface.

Our deduction that trace metal concentrations accumulate more readily in surface foraminifera due to anthropogenic impact on the environment is wholly contingent on the assumption that our subsurface sample was beyond the zone of sediment susceptible to hydrodynamic and organismal disturbance. Although we made our assumption based on previous

literature reports¹, future investigations should date sediment at the subsurface to ensure that this assumption is justifiable. Furthermore, we isolated foraminifera from both surface and subsurface sediment indiscriminately. Current standards for foraminiferal research encourage the use of the 63 – 125 μm and $> 125 \mu\text{m}$ fractions of sediment to exclude juvenile individuals from morphological and elemental analysis.³¹ In addition, our elemental analysis was performed for a group of foraminifera likely comprised of various genera; insofar as it is known that environmental factors affect foraminiferal ontogenesis on a species-specific basis, future research considering a species-specific analysis of trace metals in surface and subsurface assemblages would be highly warranted. Such an investigation would elucidate the differential effects of anthropogenically-elevated trace metal concentrations on foraminiferal species, providing greater insight into anthropogenic community disruption.

Conclusion

The present study provides a basis for the postulate that anthropogenic influence on benthos ecosystems deleteriously affects foraminiferal test integrity. ICP-OES analysis of surface and subsurface foraminiferal assemblages from North Point revealed that concentrations of Al, Fe, Mn and Zn were significantly higher in surface than in subsurface assemblages. The inverse trend was exhibited for Cu and Cr. Preliminary SEM analysis of surface and subsurface tests substantiated our trace metal analysis in that, a greater prevalence of morphological abnormalities in surface tests were apparent, affirming our initial prediction. Moreover, our findings perpetuate the negation of foraminiferal tests as proxies for paleoecological reconstruction as high concentrations of trace metals appear to invoke deteriorating effects on test integrity. Our data suggest that the marine environment surrounding San Salvador island is less polluted than in similar studies investigating lagoon ecosystems.¹¹⁻¹² To address species-

specific differences in foraminiferal ontogenesis in response to anthropogenically-induced trace metal accumulation, further research must be conducted to investigate live-dead assemblages and assess test preservation fidelity in the microfossil record. Additionally, an inter-site surface assemblage analysis at different locations on San Salvador island would help elucidate any association between proximity to a source of pollution and the accumulation of trace metals in tests. Despite this, our preliminary work demonstrates a obstacle in the use of foraminiferal tests as proxies that should be recognized and corrected for when utilizing tests for paleoecological reconstruction.

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Appendix

Table A1: Trace metal concentrations in foraminifera from North Point (standardized to 1 mg of sample)

North Point	Mean trace metal concentration [ppb]					
	Al	Fe	Mn	Zn	Cu	Cr
Surface	89	35	7	7	8	4
Subsurface	65	16	6	5	10	5
t-test unequal var.	t ₍₃₎ = 30.57***	t ₍₂₎ = 89.42***	t ₍₃₎ = 8.97 ***	t ₍₃₎ = -61.05***	t ₍₄₎ = -23.15***	t ₍₄₎ = -10.66***
F _(2,2)	0.23	3.63	12.60	9.28	0.60	0.67

* p < 0.05 ** p < 0.01 *** p < 0.005