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Key Points:
- The microhabitat of *Cribrononion subincertum* was found to be epifaunal in the Changjiang Estuary
- *Cribrononion subincertum* Mn/Ca ratios were responsive to variations of bottom water dissolved oxygen
- Epifaunal benthic foraminifera can be a robust monitor for coastal hypoxia; more species need to be similarly studied

Supporting Information:

Supporting Information may be found in the online version of this article.

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A Benthic Monitor for Coastal Water Dissolved Oxygen Variation: Mn/Ca Ratios in Tests of an Epifaunal Foraminifer

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Abstract An appropriate proxy could help to better understand dissolved oxygen variations in the past, helping to predict potential outcomes of future environmental changes. In the Changjiang Estuary (China), the foraminifer *Cribrononion subincertum* (*C. subincertum*) shows a distinct population maximum in the topmost sediment, an indication of an epifaunal species. Therefore, the geochemical composition of *C. subincertum* tests could record changes in the region's bottom water chemistry. Our results showed that Mn/Ca ratios in tests of living (Rose-Bengal stained) *C. subincertum* analyzed by LA-ICP-MS were responsive to variations of bottom water dissolved oxygen concentrations, with average foraminiferal Mn/Ca ratios three times higher during low-oxygen period than in winter. In the uppermost centimeters of sediment, wider ranges of foraminiferal Mn/Ca occurred in summer compared to winter ranges. Our results imply that this epifaunal benthic foraminiferal species could serve as a useful benthic monitor with the Mn/Ca ratios representing a reliable proxy of hypoxia in the past.

Plain Language Summary We explored how different dissolved oxygen contents impacts the geochemical composition of benthic foraminiferal shells (tests) in a dynamic coastal setting. We collected sediment samples and identified living (Rose Bengal stained) foraminifera from the Changjiang (Yangtze) Estuary, one of the largest seasonally hypoxic (low oxygen) zones in the world. Sampling at 1-cm intervals revealed *Cribrononion subincertum* predominantly inhabits the surface sediments in this area. Mn/Ca ratios in living tests of this benthic foraminifer were measured via laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). As expected, Mn/Ca ratios responded to changing Mn in the bottom waters which itself is correlated to DO levels. Our results imply that this epifaunal benthic foraminiferal species could serve as a useful benthic monitor with the Mn/Ca ratios representing a reliable proxy of hypoxia in the past.

1. Introduction

The coastal oceans are undergoing changes influenced by anthropogenic activities. Ocean acidification, eutrophication and hypoxia are all becoming major concerns (e.g., Cai et al., 2011; Wallace et al., 2014; J. Zhang et al., 2010). When dissolved oxygen (DO) decreases below 2 mg/L (or 63 μM), a condition deemed as hypoxia, most metazoans are negatively impacted, some to the point of suffocation (Diaz, 2001; Fennel & Testa, 2019). Coastal seasonal hypoxia has been observed in the ocean off the Changjiang (Yangtze) Estuary since the 1950s (D. Li et al., 2002). Hypoxia there typically begins in late spring or early summer, reaching a maximum in August, then it subsides in autumn and is absent during winter (B. Wang et al., 2012). Recently, the magnitude and lateral extent of hypoxia has become more severe in response to increasing nutrient inputs and rising temperatures (H. Li et al., 2014; Q. S. Wei et al., 2017). In order to better evaluate the development of seasonal hypoxia and predict its effects on estuarine ecosystems in the future, it is necessary to fully understand the history of hypoxia development, including its duration and magnitude. In the ocean, direct measurement of DO over large areas and long periods is logistically challenging and unrealistic due to resource limitations and technical challenges. Therefore, an appropriate proxy of hypoxia is highly desired to enable dissolved oxygen conditions in the recent and distant past. Reconstruction of past hypoxia could help us better understand the potential factors that influence hypoxia formation and variation mechanisms (natural or anthropogenic activity), and it could allow us to predict future patterns of hypoxia.

There are a variety of benthic foraminiferal geochemical proxies proven to be reliable (Boyle & Keigwin, 1985; Keul et al., 2017; Z. Lu et al., 2016; Marchitto & Broecker, 2006), though "vital effects" (biological processes) may impact foraminiferal test geochemical records (Bentov & Erez, 2005; de Nooijer et al., 2009), complicating interpretations of these records. Redox sensitive elemental compositions of benthic foraminiferal tests have recently been investigated as a potential proxy for bottom water DO variations (e.g., Glock et al., 2012; Guo et al., 2019; Reichart et al., 2003). Iodine has long been known as a redox-sensitive and biophilic element, mainly present as iodate and iodide in seawater, with iodate converting to iodide under anoxic conditions (Z. Lu et al., 2010). Iodine is incorporated as iodate within biogenic carbonates (Feng & Redfern, 2018), and thus studies have applied foraminiferal I/Ca ratios to monitor changes in oxygenation in open ocean environments (Glock et al., 2014; Z. Lu et al., 2016; W. Lu et al., 2020). Manganese (Mn) is sensitive to oxygen depletion in the oceans (Algeo & Li, 2020; Froelich et al., 1979). As oxygen concentration in near bottom waters decrease, Mn oxides and/or Mn hydroxides in sediments are reduced to soluble Mn^{2+} in pore waters (Slomp et al., 1997). As a consequence, Mn^{2+} ions diffuse upwards into overlying bottom waters, leading to relatively high Mn^{2+} concentrations during hypoxic conditions (Middelburg et al., 1987; Scholz et al., 2011). Benthic foraminifera are known to incorporate Mn proportionally with ambient seawater Mn concentration into $CaCO_3$ during biomineralization (Barras et al., 2018; Munsel et al., 2010). During calcification foraminifera can incorporate Mn into their calcium carbonate crystal lattice (Koho et al., 2015; Reichart et al., 2003). The most important characteristic is that once precipitated, the Mn concentration should remain fixed in the foraminiferal shells and should not be subject to diagenetic reduction or oxidation (Koho et al., 2015). Mn/Ca in tests of some benthic foraminiferal species have already been successfully used as a proxy for variable DO concentrations from shallow-water (Groeneveld & Filipsson, 2013; Guo et al., 2019; Petersen et al., 2019) to deep-sea areas (Fhlaithearta et al., 2018; Koho et al., 2015). However, the influence of microhabitat zonation on benthic foraminiferal geochemical proxy records of seawater oxygenation remains to be investigated.

Previous distributional and ecological studies have shown that benthic foraminifera exhibit in-sediment depth microhabitat zonation (Corliss, 1985). Foraminiferal species have been categorized into one of four main groups: epifaunal, shallow-infaunal, intermediate-infaunal and deep-infaunal (Corliss, 1991; Jorissen, 1999; Koho & Piña-Ochoa, 2012), with some species ("epifaunal") living in the upper centimeter, and other species ("infaunal") having clear subsurface maxima in their population.

Because each foraminiferal species inhabits, and presumably calcifies in, a particular range of sediment depth, it is important to identify that depth range as accurately as possible before relying on foram Mn/Ca ratios as proxies for seawater oxygenation. Globally, only a few studies have thus far explored benthic foraminiferal Mn/Ca ratios in relation to their preferred micro-habitats (Groeneveld & Filipsson, 2013; Koho et al., 2015, 2017). Theoretically, epifaunal foraminifera would serve as a more appropriate monitor recording variations in the benthic boundary layer. The "benthic boundary layer" refers to the surface sediment layer that connects to the bottom water. Since epifaunal benthic foraminifera live in the uppermost sediment, the geochemical composition of epifaunal benthic foraminiferal shells could reflect chemical variations within the benthic boundary layer. Unfortunately, this category has been understudied thus far.

The foraminifer *Cribrononion subincertum* has been previously reported from sediments in the East China Sea and the South Yellow Sea (Lei & Li, 2016; Wang et al., 1988; Wang, Gua, et al., 2016). Here, we determined, for the first time, the vertical distributions of living *C. subincertum* specimens in two sediment cores from the seafloor off the Changjiang Estuary. We also measured Mn/Ca ratios in the carbonate tests of these living foraminifera, which were collected from both surface sediments and sediment cores. We collected cores during different seasons and compared Mn/Ca ratios to measured bottom water DO concentrations. Our objective in analyzing the Mn/Ca of calcite from living individuals of an epifaunal foraminiferal species was to assess the potential use of that species' fossil and relict remains for determining past periods of coastal hypoxia.

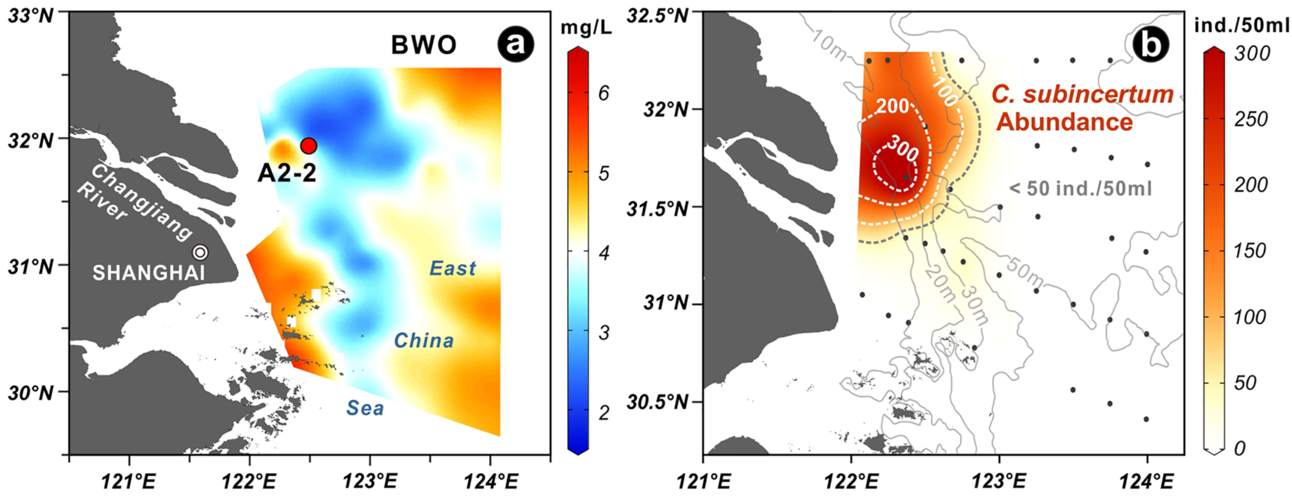

Figure 1. (a) Distributions of bottom water dissolved oxygen concentrations during summer seasons (July 2016, July 2017 July 2018, and August 2019) in the Changjiang Estuary. (b) Distributions of living (Rose-Bengal stained) *C. subincertum* from the surface sediment in summer (July 2016).

2. Materials and Methods

The Changjiang is the third longest river in the world and is the largest river in China in terms of river discharge (Chen et al., 2017). It flows into the East China Sea at a rate of approximately 10^{12} m^3/year, forming an enormous plume of freshwater and delivering a sediment load of 0.5×10^9 ton/year (Zhang et al., 2007). Outside the Changjiang river mouth, a fan-shaped submerged delta extends more than 100 km offshore, which covers an area of more than 10,000 km^2 (Chen et al., 1985). The Changjiang estuary is characterized by a semidiurnal tide, and is identified as a seasonal hypoxia area since 1950s (Li et al., 2002).

2.1. Field Sampling

Bottom water temperature and salinity were obtained by a SeaBird CTD (SBE 25, SeaBird Inc., USA) and chlorophyll *a* (Chl *a*) was analyzed fluorometrically (Strickland & Parsons, 1972) with a F-4500 fluorometer (Hitachi Inc.). Bottom waters were collected via a Rosette sampler and DO concentrations were measured via Winkler titrations (Bryan et al., 1976).

Surface sediment samples were collected using a box corer from 33 stations in the Changjiang Estuary in July 2016 (Table S1 in Supporting Information S1 and Figure 1b). Surface sediment samples were collected from station A2-2 (Figure 1a) using a box corer in February 2017, May 2017, July 2017, March 2018, and October 2020. These sediments were recovered with approximate top 1-cm interval and were stained in a 1 g/L solution of Rose Bengal in 95% ethanol immediately after collection (Bernhard, 2000; Walton, 1952). Protein stain Rose Bengal is the most common and rapid method used to distinguish cytoplasm-containing foraminiferal tests (alive or recently alive) from empty tests (Bernhard, 1992; Duros et al., 2011; W. Lu et al., 2020), but this method may overestimate abundance (Bernhard et al., 2006). Foraminiferal specimens with a brilliant rose color were identified as living (Figure 2).

Two sediment cores at station A2-2 were collected using a box corer with PVC tubes in February 2017 and July 2017. The February and July winter and summer sampling, correspond to non-hypoxic and hypoxic periods respectively. The bottom water DO concentrations during these two seasons should represent quite opposite values based on many previous studies (B. Wang et al., 2012; Q. S. Wei et al., 2017). It should be noted that A2-2 is the only station in the northern hypoxic zone in the Changjiang Estuary where we were able to collect sediment cores. All other stations in this hypoxic area were covered with coarser grain-sized sediments (Lin et al., 2017). Upon arrival on board, those two sediment cores were subsequently sliced (sub-sampled) down to 5 cm depth, at 1-cm intervals. As for box-core samples, these samples were similarly preserved in a Rose Bengal-ethanol solution, as noted above.

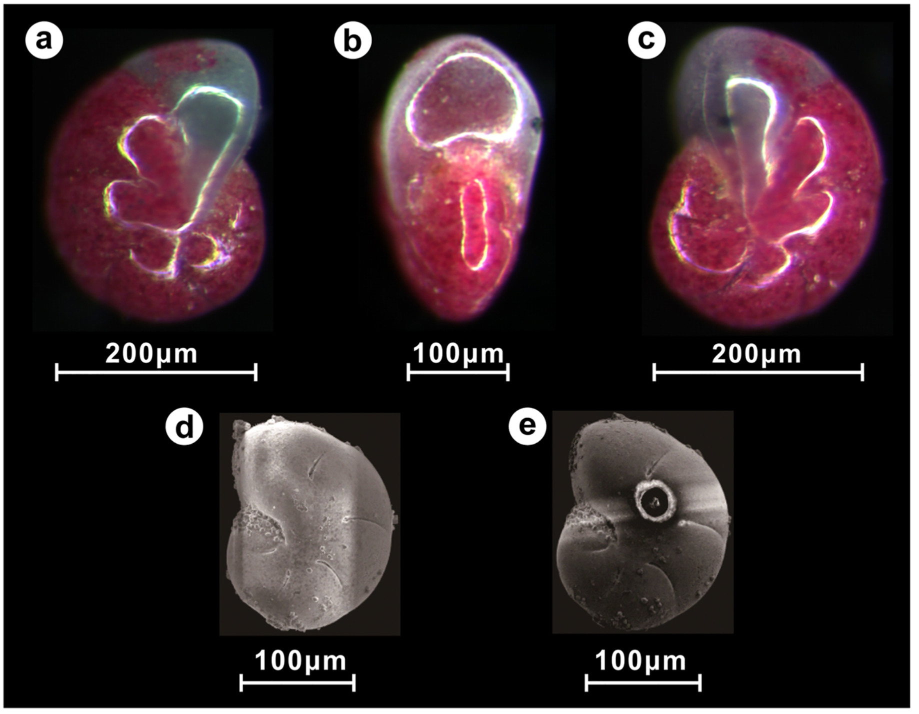

Figure 2. Reflected light micrographs of one living (Rose-Bengal stained) *Cribrononion subincertum* (Asano, 1950) specimen in our study area. (a and c) Side view; (b) Apertural view. SEM photo of *C. subincertum* before (d) and after (e) LA-ICP-MS analysis.

In the laboratory at Ocean University of China, sediment samples were wet-sieved through a 63-μm mesh. Stained *C. subincertum* specimens were handpicked from the >63-μm fraction of both surface sediments and sediment cores using a dissecting microscope (Figures 2a–2c). The abundance of *C.subincertum* at each sediment depth was counted under the microscope and presented as numbers of individuals per 50 mL of wet sediment. The diameters of *C. subincertum* individuals ranged from 160 to 470 μm.

2.2. Foraminiferal Cleaning and Elemental Composition Analyses

Prior to inductively coupled plasma mass spectrometry (LA-ICP-MS) analyses, living (Rose-Bengal stained) *C. subincertum* specimens were air dried and then cleaned according to established physical cleaning procedures. The physical cleaning protocol used was mainly adapted from Barker et al. (2003) and Koho et al. (2015). Physical cleaning aims to remove clay adhering to foraminiferal shell surfaces. Foraminiferal specimens were transferred to microcentrifuge tubes and ultrasonically cleaned and rinsed three times with ultrapure water, followed by two rinses in methanol in an ultrasonic bath and finally three rinses with ultrapure water. All specimens were dried and stored until geochemical analysis. Mn/Ca ratios from individual foraminiferal tests were analyzed using an ArF excimer 193 nm nano-second laser (Coherent Co.) coupled to an Agilent 7700e quadrupole-LA-ICP-MS. The laser spot size was 24 μm in this study. Isotopes selected for analyses were ^{27}Al, ^{43}Ca and ^{55}Mn that were used to calculate Mn/Ca and Al/Ca ratios. The Al/Ca serves as an indicator of possible contamination. The Al signals in contaminated sections were usually orders of magnitude higher than in the non-contamination section. Only data unaffected by contaminations (integration window shown by red rectangle in Figure S1 in Supporting Information S1) were selected and averaged as the best estimate of the elemental content. The integration window of the ablation profiles depends on the size and thickness of the foraminiferal specimens. All integration windows were longer than 10 s in our study. In order to be consistent between samples, we ablated the penultimate chamber of each individual specimen. The penultimate chamber of stained C. *subincertum* individuals were ablated once, from the exterior to the interior (Figures 2d and 2e). While the elemental contents of the last-formed (final) chamber of living foraminifera should reflect the ambient water environment nearest the last sampling date, the last chamber of *C. subincertum* tends to be relatively thin and therefore difficult to ablate with the laser. Following

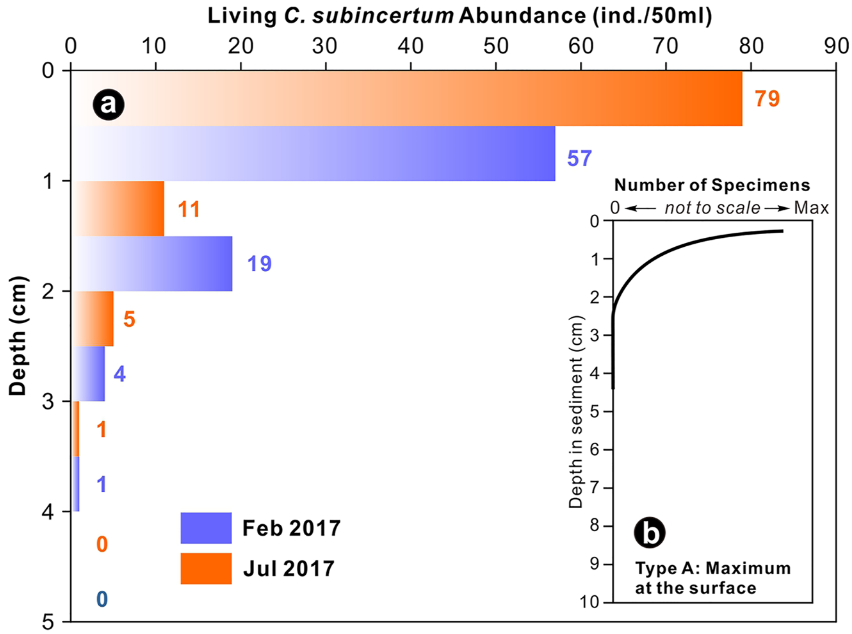

Figure 3. (a) Living *C. subincertum* abundance in A2-2 sediment cores from February and July 2017; (b) Generalized Type A vertical distribution pattern of a benthic foraminiferal species (Jorissen, 1999).

Koho et al. (2015) and Guo et al. (2019), penultimate chambers of *C. subincertum* specimens were ablated instead. Raw intensity ratios were converted to molar ratios by calibration against a NIST 610 glass standard, which was ablated once every 5 samples, with an internal precision of 3.6% for Mn/Ca. More details of operational settings, standards, data calibration and processing are provided in Guo et al. (2019).

3. Results and Discussion

3.1. Microhabitat Zonation for *C. subincertum*

Living foraminifers were observed from 32 of all 33 stations (Figure 1b) with water depths ranging from 8 to 57 m; 31 living species were identified in total in July 2016. Among all identified species, *C. subincertum* showed a unique distribution characteristic. First, stained *C. subincertum* were found in 10 of the stations, which were located in the relatively nearshore area of the estuary (with water depths between 18 to 57 m; Figure 1b). Second, *C. subincertum* occurred over a wide range of physicochemical characteristics, with bottom water temperatures, salinities and DO ranging from 19.7° to 22.6°C, 29–34‰, and 3.14–5.57 mg/L, respectively (Table S2 in Supporting Information S1). At the site A2-2, over our sampling period, bottom water salinity was relatively consistent, but both temperature and DO showed significant seasonal variations. These characteristics make *C. subincertum* a potential candidate for a possible geochemical proxy of marine environmental conditions in shallow-water areas.

Our results of the vertical distributions for living *C. subincertum* in sediment cores showed a markedly higher abundance in the uppermost sediment layers (depth < 1 cm) at station A2-2 (Figure 3a). Our results correspond well with the "type A" profile defined by Jorissen (1999) (Figure 3b), meaning that the maximum population appears in the surface/uppermost interval (0–1 cm) with only a few individuals in deeper sediment. We also calculated the "average living depth" (ALD) (defined by Jorissen et al., 1995):

$$ALD_X = \sum_{i=1,x} (n_i * D_i)/N$$

where x is the lower boundary of the deepest sample included in the calculation; n_i is the number of specimens in interval; D_i is the midpoint of sample interval i; N is the total number of individuals for all levels. The ALD of *C. subincertum* occurred at 0.87 and 0.71 cm in February 2017 and July 2017, respectively. Though the results were based on low sample size, the similar ALD between summer and winter strongly suggested *C. subincertum* to be a typical epifaunal species in the Changjiang Estuary.

C. subincertum abundance in summer was 79 individuals per 50 mL wet sediment in the surface 1 cm of sediment, about 30% more than that in winter (Figure 3). The distribution suggested that relatively smaller specimens (i.e., median value of diameters) were mainly found in the summer months (Figure S2 in Supporting Information S1). Oxygen concentration and food availability are two important ecological factors for benthic foraminifera (Jorissen et al., 1995; Koho & Piña-Ochoa, 2012). On the one hand, the bottom water DO is about 50% lower in summer than that in winter (Table S2 in Supporting Information S1). Low-oxygen condition usually have a negative impact on benthic foraminiferal survival and growth (Gross, 2000; Jorissen, 1999) though there are species that tolerate low-oxygen conditions including anoxia and the potential mechanisms involved in this relationship have been discussed (Bernhard et al., 1997; Glock et al., 2019; Risgaard-Petersen et al., 2006). Relatively smaller specimens in summer in this study may be due to the oxygen deficiency. On the other hand, we observed high Chl-*a* in May and July (Table S2 in Supporting Information S1). Studies have shown that quasi-annual diatom blooms occur in the Changjiang Estuary in spring and summer (Jiang et al., 2015; Q. S. Wei et al., 2017). A more abundant food supply might cause reproduction of foraminifera (e.g., Alve, 1999; Filipsson et al., 2004), thus explaining why more individuals with smaller size (juveniles) typically occur in summer (median diameter in Figure S2 in Supporting Information S1). However, since microhabitat preferences of benthic foraminifera is dynamically adapted to a seasonally variable environment (Barmawidjaja et al., 1992; Linke & Lutze, 1993), more data is required to reveal how does variable food supply interact with variable oxygen content in influencing the reproduction and/or growth of *C. subincertum*.

3.2. Influence of Bottom Water Oxygenation on Mn Incorporation

Previous study suggested that foraminiferal calcification begins with sea water uptake into vacuoles by endocytosis, while the trace elements in sea water are transported into a biomineralization space along with the foraminiferal test calcification (Erez, 2003). Trace elements are expected to be incorporated into the calcite in the same proportion as in the original seawater (Munsel et al., 2010). Once $CaCO_3$ has precipitated, the elemental concentration is assumed to remain fixed into the foraminiferal shell (test) and is not prone to reduction or oxidation (Koho et al., 2015). Thus, the geochemical composition of these tests is a promising tool for recording ambient seawater conditions at the time of calcification, including foraminiferal Mn/Ca as a proxy for seawater oxygenation (Reichart et al., 2003).

Mn/Ca ratios in tests of living *C. subincertum* from surface sediments had an inverse relationship with respect to bottom water DO (Figure 4). Bottom water DO was 4.89 mg/L at A2-2 in summer 2017, with the average foraminiferal Mn/Ca of 0.55 ± 0.06 mmol/mol (ranging from 0.03 ± 0.02 to 7 ± 1 mmol/mol, $n = 31$), which was more than 3 times higher than that observed in winter (average of 0.16 ± 0.02 mmol/mol, ranging from 0.03 ± 0.01 to 0.8 ± 0.3 mmol/mol, $n = 39$) when bottom water DO was 10.09 mg/L. This seasonal variation corresponds with Mn^{2+} concentrations in bottom waters of the same study area (Wang, Ren, et al., 2016), which also showed an inverse relationship of Mn^{2+} to bottom water DO (Figure 4). It is not surprising to have higher Mn^{2+} concentrations in summer compared to than winter due to the extensive consumption of dissolved oxygen during summer. While the bottom water DO was not technically hypoxic when measured in summer of 2017, the historic data indicate that hypoxia is typical in summers since 1950s (Figure 4 and reference therein). In order to supplement the observed data, we applied a high-resolution ecosystem model by W. Zhang et al. (2018) to simulate bottom water DO from January to December in 2011 in the Changjiang Estuary, with a resolution of month. On the other hand, water mixing processes induced by strong winds can eliminate water stratification in the Changjiang Estuary in winter (B. Wang et al., 2012), thus dissolved oxygen can penetrate into the sediment, cause soluble Mn^{2+} oxidized into particulate Mn oxides and/or Mn hydroxides, leading to low Mn^{2+} concentration in the bottom water in winter.

Histograms of living *C. subincertum* Mn/Ca ratios at station A2-2 are shown in Figure S3 in Supporting Information S1. In February, all Mn/Ca data points were lower than 1.0 mmol/mol. Specifically, 95% of the data were within 0–0.6 mmol/mol. In March, 18 out of 19 data points (95%) of Mn/Ca ratios were below 1.0 mmol/mol, and 89% of the data were within 0–0.6 mmol/mol. In May, around 94% of the data points of Mn/Ca ratios were lower than 1 mmol/mol. The percentage of data points with Mn/Ca ratios between 0-0.6 mmol/mol decreased to 78%, and around 22% of data showed Mn/Ca ratios higher than 0.6 mmol/mol. This may imply that Mn in ambient

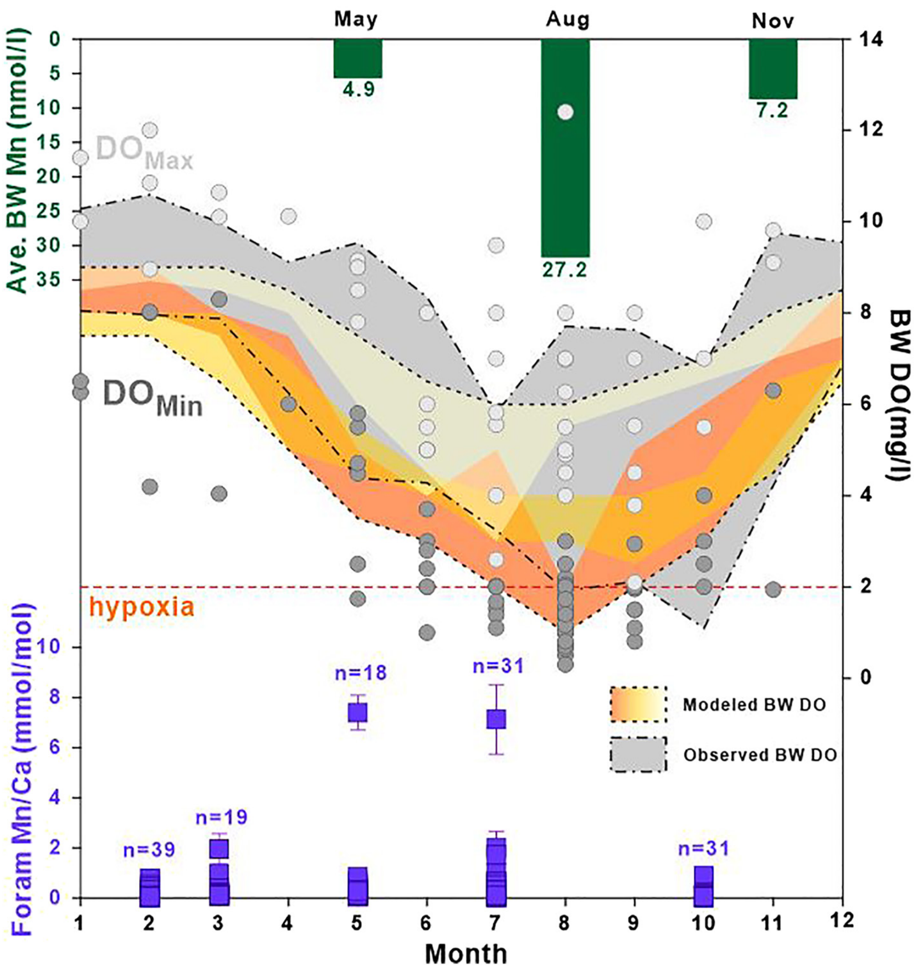

Figure 4. Mn/Ca ratios (blue bars) in tests of living *C. subincertum* at station A2-2 from five time points (February 2017, May 2017, July 2017, March 2018, and October 2020). Average bottom water Mn concentrations in the Changjiang Estuary in 2011 (data from Wang, Ren, et al., 2016) shown as green bars. Monthly variations of average modeled bottom water DO and average observed bottom water DO from 1959 to 2019 in the Changjiang Estuary shown in orange and gray colors, respectively. Minimum and maximum DO values in each study are shown in light and dark gray solid circles, respectively. Error bars represent the standard deviation (1 σ) based on counting, which corresponds to the variability within the LA profile of each spot. (Bottom water DO data were collected from previous studies and related references are shown in the reference list. See Table S3 in Supporting Information S1).

seawater increased and the foraminifera began to incorporate more Mn into their shells. In July, there are 10% of data points with Mn/Ca ratio higher than 1 mmol/mol which suggested that foraminifera could uptake more Mn into their calcite shells during summer seasons. In October, all Mn/Ca ratios were lower than 1 mmol/mol, and 94% of data concentrated in 0–0.6 mmol/mol. Variations of foraminiferal Mn/Ca ratios during different seasons reflects well with seasonal bottom water Mn variations which was induced by dissolved oxygen concentrations in the Changjiang Estuary (Wang, Ren, et al., 2016). Thus, our results suggest that Mn/Ca ratios in shells of *C. subincertum* could be a potential proxy for coastal seasonal hypoxia in the Changjiang Estuary.

By measuring the Mn/Ca ratios in tests of living (Rose Bengal stained) *Florilus decorus* (P. Wang et al., 1988), another benthic foraminifer, in the Changjiang Estuary, Guo et al. (2019) suggested that at stations with lower bottom water DO (<3 mg/L), Mn/Ca displayed relatively higher values and larger ranges (0.02–6.42 mmol/mol, averaging 0.75 mmol/mol) than those with higher bottom water DO (>3 mg/L with Mn/Ca ranges from 0.04 to 4.32 mmol/mol, averaging 0.33 mmol/mol). Their results were within the range of Mn/Ca ratios in tests of *C. subincertum* in our study. In another foraminifer, *Ammonia tepida*, from the saline and sometimes hypoxic to anoxic artificial Lake Grevelingen (Netherlands), geochemical compositions of tests of live showed high Mn/Ca ratios in the most recently precipitated chambers (0.18–0.36 mmol/mol), which reflect increased Mn refluxing in

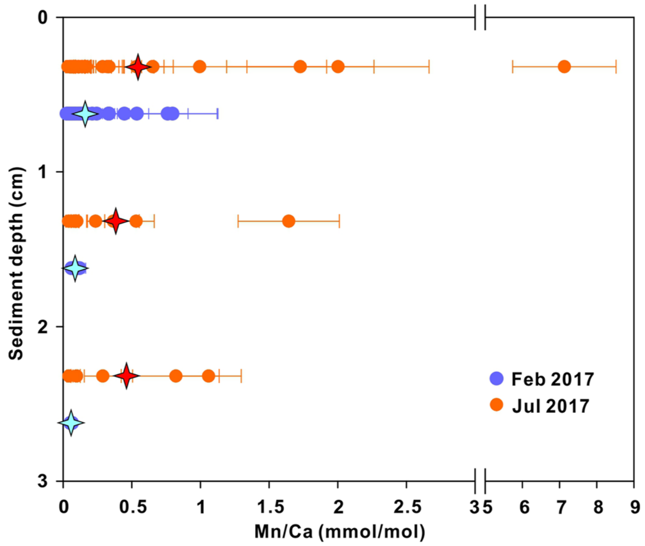

Figure 5. Mn/Ca ratios of living *C. subincertum* tests in A2-2 sediment cores from February and July 2017. Cyan and red stars are average Mn/Ca at each sediment depth. No available data for Mn/Ca in deeper intervals because of lack of foraminiferal specimens.

the surface sediment due to summer hypoxia (Petersen et al., 2019). Though the Mn/Ca ratios were equivalent to our results, but their high Mn/Ca ratios were not nearly as high as our values. The inter-species variability may due to biologically mediated (vital) effects (Hintz et al., 2006). Culture experiments showed that Mn/Ca ratios in tests of benthic foraminifers increased under hypoxic conditions with magnitudes depending upon species-specific biomineralization (Barras et al., 2018), and the authors suggested that the species-specific effect on Mn incorporation is probably due to different biological controls during the biomineralization processes. Those previous studies agree with our results that Mn/Ca in benthic foraminiferal tests can be used to indicate bottom water DO variations. However, the earlier studies did not simultaneously determine the vertical distributions of the foraminiferal species (e.g., sediment depth of maximum "living" abundance), which could influence the interpretations of the foraminiferal Mn/Ca ratios as an indicator for DO variations in bottom waters.

In this study, Mn/Ca ratios in living *C. subincertum* tests at three sediment depths (0–1, 1–2, and 2–3 cm) showed higher values (4–7 fold higher) during the summer low-oxygen period than those in winter (Figure 5). Specifically, our results showed that the average Mn/Ca ratios were 0.55 ± 0.06 ($n = 31$), 0.38 ± 0.05 ($n = 8$), and 0.46 ± 0.08 mmol/mol ($n = 5$) in layers 0–1 cm, 1–2 cm and 2–3 cm, respectively, in summer. These ratios decreased in winter to 0.16 ± 0.02 mmol/mol ($n = 39$), 0.09 ± 0.02 mmol/mol ($n = 6$) and 0.06 ± 0.02 mmol/mol ($n = 2$) at those same sediment depths, respectively. During both winter and summer, the Mn/Ca ratios showed a decreasing trend with sediment depth. Though the 2–3 cm average is slightly higher than the 1–2 cm average in summer, noting that the 1–2 cm value might increase more with analyses of additional specimens. Zhao et al. (2017) reported that higher pore water Mn^{2+} were found in the upper sediment layers in both winter and summer in the Changjiang Estuary. This agrees with our observed foraminiferal Mn/Ca ratios. We found that higher pore-water Mn^{2+} in summer, especially in the uppermost layer, would contribute to higher foraminiferal Mn/Ca ratios.

Different geochemical signatures such as Mn/Ca ratios from foraminiferal tests are expected from specimens from different microhabitats. This might be the case as foraminifera live and/or calcify at different sediment depths with varying bottom and pore water Mn^{2+} concentrations. However, very few studies have focused on this topic, especially in coastal ocean settings with complex terrestrial material sources and strong physical driving forces. Here, we summarized results of previous studies on benthic foraminiferal Mn/Ca ratios among coastal hypoxia areas and oxygen minimum zones (OMZs) (Table S4 in Supporting Information S1). Relatively higher benthic foraminiferal Mn/Ca ratios mainly occurred in shallow-water coastal hypoxia areas, where benthic taxa mainly live in the uppermost sediment. In deep-sea hypoxic areas, the range of benthic foraminiferal Mn/Ca ratios was lower than those of foraminifera that live in shallow-water areas. While inter-species variability due to "vital" effect might be one of the factors that influences Mn^{2+} uptake into foraminiferal tests, microhabitat zonation of benthic foraminiferal distributions might also play a critical role in controlling Mn/Ca ratios. For example, Koho et al. (2017) studied Mn/Ca ratios in shallow, intermediate, and deep infaunal foraminiferal tests along a bottom water oxygen gradient (1.06–3.58 mg/L) on the continental slope of NE Japan (western Pacific Ocean), with water depths between 500 and 2,000 m, and compared the foraminiferal Mn/Ca ratios with pore water Mn^{2+} concentrations. They classified *Elphidium batialis* as a shallow-infaunal species due to its ALD in the upper 1–1.2 cm. *Uvigerina* spp. and *Bolivina spissa* were classified as intermediate infaunal taxa because their ALDs range from 1.0 to 2.1 cm and close to 2 cm, respectively (Koho et al., 2017). Their results suggested that Mn/Ca ratios in those two intermediate-infaunal foraminiferal species are a promising proxy for redox reconstruction because their test chemistry would reflect variable ambient pore water Mn^{2+} content. As for shallow-infaunal taxa, they argued that since shallow-infaunal taxa are expected to mainly inhabit a surficial microhabitat, they are not exposed to pore water Mn, leading to a hampering of any redox signal.

In deep-sea hypoxic scenarios such as oxygen minimum zones (OMZ), when bottom water DO has been depleted for extended periods (Olson et al., 1993), aqueous Mn^{2+} might be thoroughly depleted from the uppermost sediment. In such a case, little reducible Mn would remain in the sediment and negligible Mn^{2+} would be present in the pore water, so foraminiferal Mn/Ca ratios are predicted to remain very low. On the other hand, under anoxia, the maximum dissolved manganese concentration in interstitial solutions would typically be limited by precipitation of manganese carbonates (Pedersen & Price, 1982). In extremely reducing sediments, manganese sulfide may form (Lenz et al., 2015), leaving Mn^{2+} absent from the pore water. However, in our coastal shallow-water study area that periodically undergoes low-oxygen episodes, the benthic foraminifer *C. subincertum* mainly inhabited the surface sediments, and the bottom-water geochemical variations were likely recorded within the actively calcifying foraminiferal test. The seasonal DO cycle in the Changjiang Estuary corresponded well with higher Mn/Ca ratios in the *C. subincertum* tests due to increased Mn refluxing in the surface sediment during summer low-oxygen period and relatively lower foraminiferal Mn/Ca ratios in aerated period. Thus, Mn/Ca ratios of *C. subincertum* acts as a recorder of bottom water redox conditions on a seasonal timescale. One issue might need to be considered when using foraminiferal Mn/Ca ratios for historical seasonal hypoxia reconstruction, which is the possible presence of Fe-Mn oxides and/or $MnCO_3$ overgrowths. Previous studies have documented the occurrence of Fe-Mn oxides and Mn coatings on foraminiferal shells that could present contamination difficulties for hypoxia reconstruction (Boyle, 1983; Pena et al., 2005).

4. Conclusions

We demonstrated that *Cribrononion subincertum* is an epifaunal benthic foraminiferal species which mainly lives in the top 1 cm of sediment in the Changjiang Estuary. Mn/Ca ratios in these foraminiferal tests from surface sediment displayed a large variability when bottom water DO concentrations were relatively low. Mn/Ca ratios in *C. subincertum* tests from sediment cores were compared for both winter and summer seasons. The Mn/Ca ratios in the carbonate of *C. subincertum* living in the upper 3 cm sediment showed higher values in the summer lower oxygen period than that in the more aerated winter, reflecting seasonal changes in pore water Mn^{2+} concentrations. Our results highlight that Mn/Ca ratios in tests of at least one epifaunal benthic foraminiferal species can be a robust proxy for coastal seasonal hypoxia, given that *C. subincertum* Mn/Ca was linked to bottom water DO variations.

Data Availability Statement

All of the raw data was published in Harvard Dataverse (https://dataverse.harvard.edu/dataset.xhtml?persistentId=doi:10.7910/DVN/1PDFFE), data set persistent DOI/ID is https://doi.org/10.7910/DVN/1PDFFE.

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