Response of nematode community structure to hypoxia in an enclosed coastal sea, Omura Bay, for three consecutive years

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Abstract: Hypoxia in bottom environments of coastal marine ecosystems is a serious problem adversely affecting both benthic life and local fisheries. In this study, we monitored abundance, composition, and feeding types of nematode communities under pre-, mid-, and post-hypoxic conditions in Omura Bay, Nagasaki, Japan, for three consecutive years (2013–2015). The bay is almost completely enclosed, and experiences hypoxia at the bottom every summer. A positive correlation was found between dissolved oxygen (DO) concentration and nematode abundance over the entire sampling period (p<0.05, r=0.61). The nematode community compositions among the pre-, mid-, and post-hypoxic conditions were significantly different (one-way analysis of similarities (ANOSIM), p<0.05), which suggests that DO in the bottom water acts as a major driver for the community shift. The increases in abundance of nematodes with toothless feeding apparatus in hypoxic periods, relative to normoxic periods, further suggested that the transfer of organic matter from bacteria through nematodes became more important in the bay under hypoxia than normoxia. It was also demonstrated that full recovery of nematode populations from hypoxic to normoxic conditions would require more than two weeks of continuous normoxic DO levels (>3 mg L⁻¹). These findings will help us to understand how global trends of ocean deoxygenation could shape the meiofaunal community and alter benthic ecosystem functioning in coastal areas.

Key words: hypoxia, nematode, Omura Bay

Introduction

Hypoxia is a serious problem in bottom environments of coastal marine ecosystems that has a great impact on the benthic communities of both macro- and meiofauna (Diaz & Rosenberg 2008, Middelburg & Levin 2009). Low oxygen conditions select for meiofaunal organisms capable of tolerating hypoxia, which could affect their community structure and function (Braeckman et al. 2013, Taheri et al. 2014). The community of meiofauna, represented by polychaetes, bivalves, copepods, ostracods, cumaceans, nematodes, turbellarians, and foraminiferans, forms a major part of the marine benthic biodiversity (Giere 2009, Grego et al. 2013), and plays important roles in marine ecosystem functioning (Higgins & Thiel 1988). Therefore, the effects of hypoxia on each faunal component need to be fully understood. Free-living marine nematodes often dominate meiofaunal communities (Gingold et al. 2013, Sergeeva & Zaika 2013), and can generally be classified into four feeding types, such as selective deposit feeders, non-selective deposit feeders, epistrate (diatom) feeders, and predators/omnivores (Lambshead 1986). They also play fundamental roles as recyclers of various organic substances, and/or as trophic mediators between the micro- and macro-benthic organisms. Moreover, nematode communities are often comprised of physiologically and phylogenetically diverse species that have varying degrees of sensitivity to
environmental stresses including hypoxia (Tsujino 1998, Setoguchi et al. 2014); therefore, shifts in their community composition can be viewed as a sensitive indicator of oxygen depletion in the environment (Sergeeva & Zaika 2013, Taheri et al. 2015). This is of particular importance because it would help in the assessment of the resilience of coastal ecosystems under low dissolved oxygen (DO) conditions imposed by recent climate change (Giere 2009, Wilson & Kakouli-Duarte 2009).

Omura Bay is located in the center of Nagasaki Prefecture, in western Kyushu, Japan (Fig. 1). The bay covers an area of 320 km², with an average depth of 14.7 m (Iizuka & Min 1989, Takahashi et al. 2009). As the bay is connected to the East China Sea through extremely narrow channels, the water exchange between Omura Bay and the open ocean is very restricted. As a result, hypoxia of the bottom-water (henceforth, “bottom hypoxia”) occurs every summer in the center of the bay, and is often accompanied by the mortality of fish and other marine organisms (Yokoyama 1995, Fukumoto & Kobayashi 2005, Takahashi et al. 2009). From these documents and oceanographic observations that have been conducted so far, it is reasonable to conclude that the bay’s center should be regarded as a representative site for examining the development of hypoxic water mass and its effects on the benthic organisms in Omura Bay.

Despite progress in our understanding of the physical processes driving bottom hypoxia in Omura Bay (Nogami et al. 2000, Takahashi et al. 2009, Suzuki et al. 2013), relatively little is known about the ecological consequences of seasonal oxygen deficiency at the bottom of the bay. A preliminary study in our laboratory revealed that: (1) nematodes were the most abundant and dominant component of the meiofaunal community of Omura Bay throughout the year, and (2) the nematode population persisted even in summer, when DO was nearly zero (Ueda et al. 2014). However, detailed analyses on the community compositions of the nematode assemblages, and how the nematode assemblages respond to hypoxia are yet to be done.

To address the questions raised by the preliminary study, and to obtain more comprehensive insight into the interactions between DO and the nematode community in Omura Bay, we have examined changes in the abundance, community composition, and trophic diversity of nematodes in response to DO conditions in Omura Bay for three consecutive years (2013–2015). We have also discussed the consequences of hypoxia on the benthic ecosystem in the bay.

**Materials and Methods**

**Sample collection, and DO and temperature monitoring**

At a site located in the center of Omura Bay (32°55′39″N, 129°51′35″E, water depth: 21 m; Fig. 1), sediment was collected with an acrylic tube (31-cm long with 26-mm inner diameter), by scuba diving in June through October for three consecutive years (2013–2015), unless otherwise specified. For each sampling date, triplicate sediment cores were collected. All core samples were maintained at approximately 25°C, and carefully transported to the laboratory within 3 h after sampling, to avoid direct exposure to sunlight and other physical disturbances (Wada et al. 2012). DO concentration and water temperature were both monitored with a DO logger (U26-001, HOBO, Onset) and a multi-parameter monitoring device (AAQ, JFE-Alec), that were placed 1-m above the sediment surface.

**Sample processing and sorting nematode specimens**

In the laboratory, sediment cores were extruded and sectioned into layers, using a pair of clean plastic blades. As nematodes in the top layer (0–10 mm depth) of the center site seemed to be most strongly influenced by low DO conditions compared to the subsurface (Fig. S1), the top layer was examined across all the sediment cores. Each sediment section was fixed immediately and preserved in 5% buffered seawater formalin containing borax (final conc.=30–40 g L⁻¹) and rose bengal (final conc.=1 g L⁻¹). After sieving through 1-mm and 32-µm mesh screens, sediment specimens retained on the 32-µm mesh were resus-
Nematode community under hypoxia in Omura Bay

Suspended in tap water according to the method of Setoguchi et al. (2014), and centrifuged three times with colloidal silica (Ludox HS40, Sigma-Aldrich). The supernatants were transferred to Petri dishes with grids, and nematodes were collected using small splinter forceps and an Irwin loop under a binocular microscope (SZ-PT, Olympus). All specimens were counted and transferred to a cell culture plate (TR5 001, TrueLine) containing 1 mL of 80% glycerol in each cell. Thymol (final conc. = 1 mg mL\(^{-1}\)) was added to the solution to avoid fungal growth. These plates were put into an incubator (60°C) to allow the water to evaporate slowly, leaving the nematodes in pure glycerol.

If the number of nematodes in each core sample was less than 100, half of them were randomly chosen for identification. If nematode numbers were larger than 100, up to 50 randomly-picked nematodes were identified. Each nematode specimen was mounted on a glass slide for identification using a compound microscope (BX51, Olympus) with differential interference contrast optics. Nematodes were identified to the genus level by referring to Warwick et al. (1998), Schmidt-Rhaesa (2013), and the keys on the WoRMS online identification system (http://www.marinespecies.org). Finally, based on the morphology of the buccal cavity, the identified nematodes were categorized into four feeding types: selective deposit feeders (type 1A), non-selective deposit feeders (type 1B), epistrate (diatom) feeders (type 2A), and predators/omnivores (type 2B) (Lambshead 1986).

Statistical analysis

To plot the data of DO, water temperature, and nematode abundance, the R-3.4.1 software was used (https://cran.r-project.org/bin/windows/base/). Multivariate analyses were performed using the PRIMER6 software (PRIMER-E Ltd., Plymouth, UK). For multivariate analyses, nematode abundance was square-root transformed to weigh down the effects of the abundant genera. To visualize similarities in the nematode community compositions under pre-, mid-, and post-hypoxia, both cluster analysis and non-metric multidimensional scaling (nMDS) were used (Clarke & Warwick 2001). A one-way analysis of similarities (ANOSIM) among the pre-, mid-, and post-hypoxia groups was performed, using the hypothesis that hypoxia would have a significant effect on nematode community structure. In this study, similarity percentage analyses (SIMPER) were conducted to identify the group of nematode taxa or morphotypes that was contributing the most to any similarity or dissimilarity.

Results

Effects of DO concentration on nematode abundance

In the present study, hypoxia was defined as DO concentrations of less than 3 mg L\(^{-1}\) (Wada et al. 2012). Our data indicated clear patterns of seasonal hypoxia as follows: (1) the DO started to fall below 3 mg L\(^{-1}\) after mid-
June in each year; (2) hypoxic and anoxic conditions developed during July through September, with a couple of episodes of transient recovery to normoxic conditions; and (3) the DO increased and remained over 3 mg L\(^{-1}\) for more than 10 days after mid-September. Water temperatures at the bottom were also observed as follows: (1) temperatures began to increase after mid-June or mid-July; and (2) the temperatures declined gradually from mid-September in 2013 and 2015, and late September in 2014 (Fig. 2). There was no statistically significant difference in nematode abundance between hypoxia (DO<3 mg L\(^{-1}\)) and normoxia (DO≥3 mg L\(^{-1}\)) (Mann–Whitney U test, \(U=147, p>0.05\)). However, the correlation test showed that nematode abundance responded to DO changes (\(r=0.57\), \(p<0.05\)), whereas, changes in water temperature did not affect nematode abundance (\(r=0.30, p>0.05\)).

**Effects of DO concentration on nematode community composition**

As shown above, bottom hypoxia of Omura Bay fully developed in August, while normoxia prevailed in June and late September/October. We examined the structural changes in nematode communities in June, August, and late September/October, which correspond to pre-, mid-, and post-hypoxia, respectively. Among the 804 nematode specimens examined, *Atrochromadora* was found to be the most abundant genus (249 out of 804 individuals, 31%), followed by *Axonolaimus* (178 individuals, 22%) and *Chromadora* (154 individuals, 19%). However, 182 nematode specimens (18%) remain unidentified because they were too small or seriously damaged (Table S1).

As shown in Fig. 3, nematode communities were clustered into three groups by sampling period, and there was a significant difference in composition among the groups (one-way ANOSIM, Global \(R=0.572, p<0.05\)). However, it was noted that the samples of September 2015, which constituted the post-hypoxia group, were clustered with the samples from August (mid-hypoxia) from all three sampling years. The SIMPER analysis indicated that the within-group similarity was the largest in pre-hypoxia (61.9%), followed by mid-hypoxia (58.5%), and post-hypoxia (37.9%) (Table 1). The genus that showed the greatest contribution to the group similarity under pre-hypoxic conditions (contrib\%=64.0%) was *Atrochromadora*. *Axonolaimus* contributed the most to the average similarities in the mid-hypoxia group (contrib\%=50.0%). Of the average similarities in the post-hypoxia group, *Chromadora* was the most strongly contributing genus (contrib\%=40.2%). It was noted that *Halalaimus* increased their contribution only under mid-hypoxia, while *Oncholaimus* increased their contribution only under post-hypoxia.

**Effects of DO concentration on nematode trophic diversity**

Although a one-way ANOSIM did not detect any significant differences in nematode feeding types between hypoxia-related periods or sampling months (Global \(R=0.218, p>0.05\), a recurring pattern of nematode trophic diversity in the center of Omura Bay is likely. As shown in Fig. 4, the relative percentage of toothless nematodes (types 1A and 1B) increased in mid-hypoxia across the

### Table 1. Contributions of genera to average similarity between all pairs of samples within each hypoxia-related period in the center of Omura Bay for three years (2013–2015) were examined by the SIMPER procedure.

<table>
<thead>
<tr>
<th>Genera Feeding type</th>
<th>Pre-hypoxia</th>
<th>Mid-hypoxia</th>
<th>Post-hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average similarity: 61.9%</td>
<td>Genera Feeding type</td>
<td>Contrib%</td>
<td>Cum%</td>
</tr>
<tr>
<td><em>Atrochromadora</em> 2A</td>
<td>64.0</td>
<td>64.0</td>
<td><em>Axonolaimus</em> 1B</td>
</tr>
<tr>
<td><em>Axonolaimus</em> 1B</td>
<td>25.7</td>
<td>89.7</td>
<td><em>Chromadora</em> 2A</td>
</tr>
<tr>
<td><em>Chromadora</em> 2A</td>
<td>3.15</td>
<td>92.8</td>
<td><em>Halalaimus</em> 1A</td>
</tr>
<tr>
<td><em>Chromadora</em> 2A</td>
<td>11.0</td>
<td>96.5</td>
<td><em>Chromadora</em> 2A</td>
</tr>
<tr>
<td><em>Chromadora</em> 2A</td>
<td>9.09</td>
<td>95.9</td>
<td><em>Atrochromadora</em> 2A</td>
</tr>
</tbody>
</table>

Contrib\%=contribution of each genus to total average similarity within a hypoxia-related period. Cum\%=cumulative contribution. Each list was truncated when 90% was reached.
three years, while that of the nematodes with teeth (types 2A and 2B) dominated in pre- and post-hypoxia (2013 and 2014, respectively). Type 1B was more dominant than 1A within the toothless nematodes, while type 2A dominated over 2B among the nematodes with teeth. In the nematode assemblages of September 2015 (post-hypoxia samples) in the present study, type 1B nematodes dominated over other feeding types.

Discussion

The present study clearly demonstrated a decline in nematode abundance during hypoxic conditions, which is consistent with the findings from previous reports (Sergeeva & Zaika 2013, Taheri et al. 2014, Taheri et al. 2015). Since most marine nematodes depend on aerobic respiration, they might not survive under long-term low oxygen stress (Giere 2009). However, some nematode genera, such as Sabatieria, Linhomoeus, Metalinhomoeus, and Monhystera, can switch between aerobic and anaerobic metabolisms, and therefore, can survive in long-term hypoxic conditions (Sergeeva & Zaika 2013). In other nematode genera, such as Camacolaimus, Daptonema, Terschellingia, and Viscosia, a higher length/width ratio and low respiration rates may increase the chances of withstanding low oxygen stress (Taheri et al. 2015).

We also found significant shifts in the nematode community structures among pre-, mid-, and post-hypoxia condition in the center of Omura Bay for the three consecutive years (2013–2015). The fact that both Axonolaimus (type 1B, non-selective deposit feeders) and Halalaimus (type 1A, selective deposit feeders) increased their contribution ranks only in mid-hypoxia suggests they are well adapted to hypoxia. As these two nematode genera have not been reported to be abundant in other low DO environments (Modig & Ölafsson 1998, Urban-Malinga et al. 2006), a kind of local adaptation to hypoxic conditions may have occurred in these nematodes.

The present results further demonstrated that DO acted as a major driver of the shift, not only in taxonomic composition, but in trophic diversity of free-living nematodes. Regarding the feeding type of nematodes (Lambshead 1986), our data showed that epistrate feeders (type 2A) were dominant in oxic conditions, whereas non-selective deposit feeders (type 1B) became dominant in hypoxic conditions (Table 1, Fig. 4). Dominance of these two feeding types is consistent with that reported by studies from the northern Adriatic Sea, where nematodes were subjected to experimentally induced anoxia (Taheri et al. 2015). It was also found that the most dominant genus of type 2A in pre-hypoxia (Atrochromadora) was different from that in post-hypoxia (Chromadora). As Chromadora became the second greatest contributor to the average similarity in mid-hypoxia, it is likely that Chromadora species were more resistant to hypoxia than Atrochromadora, and thus increased their abundance more rapidly than Atrochromadora in post-hypoxia. In contrast, Atrochromadora was dominant in pre-hypoxia, but became less dominant in post-hypoxia. Considering that Atrochromadora would recover its dominance by the following June, these type 2A nematodes may be the most effective colonizers in the bottom environment of Omura Bay from autumn to spring.

According to Warwick et al. (1998), there are few substantial morphological distinctions between Atrochromadora and Chromadora. Therefore, physiological traits are likely to be responsible for the observed differences between the two genera.

As suggested by Taheri et al. (2015), interactions between DO conditions and food availability might affect nematode community’s responses to low-oxygen stress. Because diatoms serve as a main food source for epistrate feeder nematodes (type 2A), both Atrochromadora (domi-
nant pre-hypoxia) and Chromadora (dominant post-hypoxia) may have suffered from the lower availability of diatoms during hypoxic conditions. Increase in predators/omnivores (Oncholaimus, type 2B) feeding on protozoa, meiofauna, and other nematodes, and the increase in their contribution ranks in normoxic samples (Table 1, Fig. 4) may be partly explained by the reappearance of their food sources after deoxygenation. As non-selective feeders (type 1B) are supposed to have a wider range of food sources, including bacteria and detritus, than selective deposit feeders (type 1A), type 1B nematodes may be able to cope with hypoxic conditions better than type 1A nematodes. To test the above-mentioned hypothesis with regard to the nematode community shift and food availability in Omura Bay, information on organic matter content and abundance of diatoms and bacteria in the sediment should be examined in the future.

One of the important findings in the present study is that the nematode communities from seemingly post-hypoxic conditions (September 29, 2015) were clustered with a group of nematode communities under hypoxic conditions (Fig. 2). Although the DO level of the sample from September 2015 is more than 3 mg L$^{-1}$, a community recovery from hypoxia to normoxia was not observed. This may reflect the non-linear nature of the recovery processes of benthic fauna as suggested by Diaz and Rosenberg (2008), and demonstrate that prolonged and continuous normoxic conditions, presumably for at least 2–3 weeks, are essential for the full recovery of the nematode community from hypoxia. Therefore, the placement of the nematode community structure of the September 2015 sample within the nematode samples of the hypoxia period in other years may be explained by the discontinuous period of re-oxygenation in September 2015.

As for the recovery of nematode populations after hypoxia, we think there are two possible sources: (1) nematodes in the subsurface would migrate up to the surface in post-hypoxia, or (2) nematodes from surrounding areas would migrate horizontally (or be brought) into the sampling site in post-hypoxia. To better understand the recovery process in Omura Bay, we need to investigate horizontal and vertical distribution patterns of nematode communities in future studies.

In conclusion, our results confirmed that shifts in nematode community structures (abundance, community composition, and feeding type distribution) depended on the availability of DO in the center of Omura Bay, during three consecutive years (2013–2015). The increase in the abundance of nematodes with toothless feeding apparatus (types 1A and 1B) in the hypoxic period, compared with that in the normoxic period further suggests that the transfer of organic matter from bacteria through nematodes would be of greater importance in the bay under hypoxia than normoxia. Our data clearly demonstrated that the re-oxygenation duration influenced the recovery process of marine nematode communities. These findings should help to clarify how global trends in ocean deoxygenation can shape meio-benthic communities and alter benthic ecosystem functioning in coastal areas.

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