Impact of seasonal hypoxia on benthic copepod communities in Omura Bay, a highly enclosed coastal sea in southwestern Japan

KAZUKI KAWANO¹, MOTOHIRO SHIMANAGA²*, RYO UEDA³, QUyen T. D. NGUYEN³ & MINORU WADA³

¹Graduate School of Science and Technology, Kumamoto University, 39–1, Kurokami 2-chome, Chuo-ku, Kumamoto 860–8555, Japan
²Center for Water Cycle, Marine Environment and Disaster Management, Kumamoto University, 39–1, Kurokami 2-chome, Chuo-ku, Kumamoto 860–8555, Japan
³Graduate School of Fisheries and Environmental Sciences, Nagasaki University, 1–14 Bunkyo-machi, Nagasaki-shi, Nagasaki 852–8521, Japan

Received 28 August 2020; Accepted 7 October 2020 Responsible Editor: Dongsung Kim
doi: 10.3800/pbr.16.93

Abstract: Declining oxygen concentrations in aquatic habitats represent extreme conditions that threaten benthic life. Hypoxia has recently become an important research topic, as areas affected by these phenomena are spreading globally. Omura Bay is one of the most highly enclosed seas in Japan, and severely hypoxic conditions occur at the bottom every summer. We conducted a preliminary study in the center of the bay to evaluate how seasonal hypoxia affects the abundance and community composition of benthic meiofauna, with particular reference to copepods. The copepod densities and their nauplii differed significantly among seasonal categories (before, during, and after hypoxia). Furthermore, the degree of the seasonal decline in copepods during hypoxia seemed much more severe than that in nematodes, the most abundant meiofauna. The assemblages of adult copepods had the simplest composition during hypoxia, when harpacticoid copepods in the family Cletodidae, which have smaller and slender bodies, occurred at significantly higher frequencies (a contribution of 84% to the mean similarity among seasons). After hypoxia, the relative abundance of copepods in the families Ectinosomatidae and Longipediidae increased, which may likely be attributed to their higher swimming abilities and rapid recruitment via specific planktonic nauplius stages, respectively. High frequencies of copepods in the Cletodidae family have also been observed under hypoxic conditions in the Mediterranean Sea, suggesting that similar processes affect benthic copepod communities, which work to the advantage of cletodid species with small and slender forms in the subtidal sediment bottom under severe hypoxia in Omura Bay and other regions.

Key words: Cletodidae, Harpacticoida, hypoxic condition, meiofauna

Introduction

A reduced oxygen supply, which is often associated with toxic hydrogen sulfide, represents an extreme condition that threatens most benthic metazoans in sediment (Giere 2019). Recently, oxygen has declined in both the open ocean and coastal waters, and global warming is likely an ultimate cause of deoxygenation (Breitburg et al. 2018).

Omura Bay is located in the center of Nagasaki, western Kyushu, Japan (Fig. 1). The bay covers 321 km² and has the highest geographical enclosed index (54.29) among the semi-enclosed seas larger than 100 km² in Japan (International Center for Environmental Management of Enclosed Coastal Seas, https://www.emecs.or.jp/encsea/japan, accessed on September 25, 2020). The bay is connected to the East China Sea by extremely narrow channels, and the water exchange between Omura Bay and the open ocean is minimal. Due to these geological characteristics, severe hypoxia occurs every summer at the sea bottom in the bay's center (Nagasaki Prefecture Government, https://www.pref.nagasaki.jp/bunrui/kurashi-kankyo/kankyohozen-
ondankataisaku/omura/hinsanso-omura/, accessed on September 25, 2020), which has been reported to be associated with marine fish mortality and decreases and/or changes in benthic macrofauna’s community composition (Yokoyama 1995a, b, Takahashi et al. 2009).

For smaller organisms, previous studies have shown that the surface sediment on the sea bottom in the center of Omura Bay is characterized by higher microbial respiration and greater diversity of bacterial components compared with the non-hypoxic sediment at the fringe of the bay (Wada et al. 2012). Furthermore, shifts in bacterial communities were also evident in response to the availability of dissolved oxygen (DO). While the relative abundance of Gammaproteobacteria was found to correlate positively with DO, the abundance of Deltaproteobacteria was inversely correlated with DO (Mori et al. 2018, Mori & Wada 2019). These results strongly suggest that DO availability on the sea bottom plays a fundamental role in shaping the bacterial community in sediment surfaces in the area.

In contrast, little is known about seasonal hypoxia impacts on metazoan meiofauna in the bay. Meiofaunas are regarded as ideal indicators of aquatic ecosystem health because of their intimate association with sedimentary substrates, high abundance, and short generation intervals (Giere 2009). Among the metazoan meiofauna, nematodes, the most abundant metazoan meiofaunal taxa, usually prevail in suboxic or even sulfidic environments (Giere 2009). Recently, Nguyen et al. (2018, 2020) reported that the community composition of nematodes temporally shifted between normoxic and hypoxic conditions in Omura Bay. However, these temporal patterns were also affected by spatial heterogeneities in the degree of seasonal hypoxia and other environmental factors in the surface sediment of the bay. Benthic copepods, on the other hand, the second most abundant meiofaunal taxa, are mainly composed of harpacticoids, which are generally sensitive to oxygen depletion and thus restrict their occurrence to the upper sediment layer and favor an epibenthic life (Giere 2009). Although a few taxonomic groups among benthic copepods have been found experimentally to tolerate low oxygen concentrations (De Troch et al. 2013, Grego et al. 2014), information on the response of benthic copepod communities to seasonal hypoxic conditions in a natural setting is still rare.

In this study, we focused on benthic copepods to provide the first insight into the impact of seasonal hypoxia on the temporal changes in their abundance and community composition in the center of Omura Bay.

**Materials and Methods**

**Benthic copepod sampling and sample processing**

We set up a study site in the center of Omura Bay (32°55′39″N, 129°51′35″E, and water depth: 21 m, Fig. 1). We collected sediment samples at the study site from June through October 2013–2015, using an acrylic corer (inner diameter: 2.6 cm) by scuba diving. We collected four cores at each sampling date in 2013 and three cores in 2014 and 2015. The dissolved oxygen (DO) concentration was measured using a multi-parameter monitoring device (AAR, JFE-Alec) placed 1 m above the sediment surface. Details regarding these processes and measurements of DO values have been provided in our previous study (Nguyen et al. 2018). The sampling dates of this study are shown in Table S1.

In the laboratory, sediment cores were sectioned into several layers and fixed and dyed in 5% buffered seawater formalin containing borax and Rose Bengal (final conc. = 1 gL⁻¹). As most benthic copepods are known to be distributed in the sediment’s top layer (0–1 cm depth, Shimanaga
et al. 2004), we only examined the top layer in this study. Copepods (adults and juvenile copepodites) and nauplii, most of which were considered as those of copepods, were extracted from sediment by centrifugation with a 32 µm mesh and colloidal silica (Ludox HS40, Sigma-Aldrich). The details of these processes are also provided by Nguyen et al. (2018), who focused on the composition of nematode communities extracted from the same samples. Extracted copepods and nauplii in each supernatant were sorted and counted under a binocular stereo microscope.

We investigated only adult specimens because of the difficulty associated with morphological identification of immature copepodites. Little is known about the taxonomy of benthic copepod fauna on the sea bottom around Japan. Therefore, we did not identify individuals at the species level but identified the specimens to the order level. Subsequently, specimens in the order Harpacticoida were identified to the family level using a key based on Huys et al. (1996), Boxshall and Halsey (2004), and Wells (2007) under a compound microscope equipped with differential interference optics. Recently, copepods belonging to the families Canuellidae and Longipediidae were subdivided into order Canuellida from Harpacticoida; thus, they were categorized in this study as a new order (Khodami et al. 2017). We investigated all available adult specimens for each sampling period when the number was <100, although some specimens collected on June 28, July 26, and October 31, 2013, were lost during the 2016 Kumamoto earthquakes. For the periods in which we obtained sufficient numbers of specimens, we randomly selected specimens from replicates until 100 adults had been investigated. The list of specimens identified in each sampling period is shown in Table S1.

Statistical analyses

Two-way permutational multivariate analysis of variance (PERMANOVAs) was conducted to test the null hypothesis that densities of copepods and nauplii did not differ among studied years or seasons (before, during, and after hypoxia), after log-transformation of values (log[x+1], Anderson et al. 2008). In this study, hypoxia was defined as a DO concentration in the bottom water of less than 2.8 mg L$^{-1}$ (2 mL L$^{-1}$), based on Diaz & Rosenberg (1995).

We also analyzed the dataset of higher taxonomic level community composition (order-family) of adult copepods using non-metric multidimensional scaling (nMDS) and similarity percentages (SIMPER) based on standardized values of copepod abundance for each sample (%) and using the Bray-Curtis index as a similarity index. nMDS and SIMPER were used to visualize similarities between samples and to examine the individual contributions of the different taxa to the mean similarity among samples in each seasonal category, respectively (Clarke & Warwick 2001). However, for most sampling dates, the available specimen numbers were <50 (Table 1). Thus, we considered the pooled data on copepod composition for each sampling date as a sample unit for these analyses of copepod composition. The sampling dates for which the total number of specimens was ≤2 were not considered in these analyses. PERMANOVA, nMDS, and SIMPER were performed using the PRIMER 7 software with the add-on package PERMANOVA+ (PRIMER-E).

Because of the lack of samples for some seasons in each year, as well as a small specimen number in some sampling periods (Table S1), it was not possible to conduct two-way PERMANOVAs to evaluate the difference in composition between years and seasons. Instead, a chi-square test for the independence of frequencies of copepod taxa and seasons was performed (Zar 2010), based on the pooled data of all identified specimens in each season category, without considering differences in sampling dates. In this analysis, the minor taxa were summarized as “other” to prevent each category’s expected frequency from being too small (Zar 2010).

### Results

DO concentrations in the water at the bottom of Omura Bay started to decrease from late June, reached hypoxic levels in July and August, and recovered to normoxic levels after mid-September in each year (Fig. 2A). The degree of hypoxia was the highest in 2013 when DO values were

| Family            | Before Hypoxia | Hypoxia | After Hypoxia |
|-------------------|----------------|---------|--------------|
| Mean              | 36.5           | 23.3    | 59.3         |
| Cont%             | 42.9           | 33.2    | 84.0         |
| Cum%              | 42.9           | 76.0    | 84.0         |

### Table 1. Absolute (Cont) and and cumulative (Cum) contributions of main families to average similarity in family composition in each seasonal category.

| Family            | Before Hypoxia | Hypoxia | After Hypoxia |
|-------------------|----------------|---------|--------------|
| Mean              | 48.1           | 23.4    | 49.8         |
| Cont%             | 58.0           | 25.3    | 58.0         |
| Cum%              | 58.0           | 83.3    | 58.0         |

Samples with specimen no. <9 were excluded from analyses (cf. Fig. 3). Mean values represent mean relative abundance of families among samples from each seasonal category (%). Each list is truncated at 70%.

Impact of hypoxia on copepods in Omura Bay
close to 0 mg L\(^{-1}\) during hypoxia. Densities of copepods (adults and juvenile copepodites) and nauplii showed seasonal fluctuations in 2013; both decreased to the level at which no individuals could be detected in whole samples during the hypoxic season, and recovered to approximately 10 individuals cm\(^{-2}\) after hypoxia (Fig. 2B, C). In 2014, copepods and nauplii densities decreased again during hypoxia but never recovered, and remained low (around 1 ind. cm\(^{-2}\)) until the last sampling period in 2015. Two-way PERMANOVA detected significant differences in copepods and nauplii densities among the survey years and among the season categories (all tests detected \(p<0.01\)).

The taxonomic composition of adult copepods on each sampling date is shown in Fig. 3. Although only no or few specimens were obtained on most sampling dates during hypoxia, the copepod compositions tended to become simpler during hypoxia than those observed before or after hypoxia when the consideration was restricted to the dates when \(\geq 9\) specimens were obtained. nMDS based on \(\geq 9\) specimens showed that the samples collected in the same seasonal categories tended to be plotted close to each other (Fig. 4A), although strict PERMANOVAs to test the significance could not be done because of the lack of samples with adequate numbers of specimens in some seasons in 2013 (during hypoxia) and 2015 (after hypoxia).

SIMPER showed that the two harpacticoid families contributed \(\geq 70\%\) of the mean similarity among samples taken before hypoxia (Cletodidae: 43%, Ectinosomatidae: 33%, Table 1, see also Fig. 4B). Among the samples collected during hypoxia, the relative abundance of Cletodidae increased (Fig. 4B), which was selected by SIMPER with a contribution of 84% to the mean similarity among those samples (Table 1). On the other hand, after hypoxia, the most abundant family was Ectinosomatidae, contribut-
ing 58% of the mean similarity among the samples in the category. Longipediidae (Canuelloida) was also chosen as the second contributor (25%, Table 1, and Fig. 4B).

The chi-square test based on the pooled data ignoring sampling dates suggested that the frequencies of the top three most abundant taxa chosen by SIMPER (+sum of the others) were significantly different among seasons ($\chi^2=70.9, p<0.01$, Fig. 5).

**Discussion**

The temporal change in the total density of benthic copepods at the Omura Bay study site was within the range of values observed in muddy sand and mud bottoms in shallow subtidal zones globally ($0.2–25$ ind. cm$^{-2}$, Higgins & Thiel 1988). Seasonal hypoxia in the bay, however, affected copepods more drastically than nematodes in the same habitat. The present study indicates that the densities of benthic copepods were significantly lower during hypoxia than during normoxia. In contrast, there was no significant difference in nematode (the most abundant meiobenthic taxon) abundance during the same period at the same study site (Nguyen et al. 2018). We are unsure why the copepod densities at the study site did not recover after the decrease in copepod densities during hypoxia in 2014, even though they showed some recovery trend after hypoxia in 2013. A possibility is that the impacts of hypoxia in 2013, which was the most hypoxic year in the 3-year study, may have resulted in a certain time lag in the recovery of copepod densities, and the effects of these conditions may have continued until 2015.

Although there were no drastic changes in nematode abundance during hypoxia, the nematode community composition differed significantly between seasonal categories at the study site, and the frequency of nematodes with toothless feeding apparatus increased during hypoxic periods (Nguyen et al. 2018). These results suggest that the DO concentration in bottom water is one of the main factors affecting the nematode community composition shift, and the transfer of organic matter from bacteria to nematodes is greater under hypoxic conditions at the center of the bay.

One of the present study’s limitations was the small survey area covered by the sampling devices used (5.3 cm$^2$ per core sampler). Although the survey area was sufficient for the analyses of the nematode community composition (the most abundant taxa), the survey area was not sufficient for the less abundant copepods, and it was often the case that we were not able to collect an adequate number of copepods for systematic statistical analyses at the study site, especially during hypoxia. Despite the limited data available in this study, we found evidence for temporal changes in copepod community composition, suggesting that the DO concentration in bottom water has substantial effects on copepod communities. Furthermore, our results indicate that copepods in the family Cletodidae dominate the benthic copepod community in the center of Omura Bay during hypoxia.

In the northern Adriatic Sea (Mediterranean), Grego et al. (2014) deployed in situ plexiglass chambers on the sea bottom, at a depth of 24 m, to simulate anoxic events lasting nine days, one month, two months, and ten months. With increasing anoxia duration, the relative abundance of copepods in the family Cletodidae increased in the benthic copepod composition, and these copepods survived the anoxic conditions for 1 month and even 2 months, although only a few specimens were obtained after 2 months of anoxia. Another in situ experiment found that copepods in the family Ectinosomatidae were also dominant in anoxic conditions that lasted for a short period (5 days), with Ectinosomatidae recorded at the second-highest relative abundance (37%), following Cletodidae (47%, De Troch et al. 2013). These results are similar to those of our study (Fig. 5).

Morphologically, Cletodidae harpacticoids are characterized by smaller and slender body shapes (Boxshall & Halsey 2004), which would be adaptive for oxygen and carbon dioxide exchange between ambient pore water and internal bodies of copepods (Grego et al. 2014). Furthermore, recent carbon isotope analyses have suggested that Cletodidae are specialists feeding almost exclusively on chemosynthetic bacteria flourishing under low oxygen levels, in contrast with the majority of harpacticoids, which typically depend on phototrophic primary production (Vafeiadou et al. 2014, Cnudde et al. 2015). As such, Grego et al. (2014) regarded Cletodidae as a “winner” of coastal hypoxia among benthic harpacticoid copepods.

Considering reports from the coasts of the Mediterranean Sea or the Atlantic Ocean, our results suggest that the benthic copepod communities’ response to hypoxia is universal. During hypoxia, the abundance of Cletodidae harpacticoids decreases (in this study, their overall mean density during hypoxia was 0.4 ind. ml$^{-1}$ sediment, 16% of that observed before hypoxia), but their survival rates are higher than those of the other copepods (in this study, the overall mean densities of Ectinosomatidae and Longipediidae during hypoxia were 0.1 ind. ml$^{-1}$ [3% before
hypoaxia], and 0.01 ind. ml⁻¹ [0.6%], respectively), resulting in their highest relative abundance in the small-sized copepod communities. Under hypoxia, chemosynthetic bacteria, which are consumed by not only toothless nematodes but also Cletididae harpacticoids, would increase their importance as food items.

Ectinosomatidae seemed to tolerate hypoxia to a certain degree in both previous and our studies. Many species of this family are good swimmers and demonstrate emergence behavior, that is, temporary excursions into the water column (Thistle et al. 2007). Their ability to swim to the water column above the seafloor may help the rapid recovery of Ectinosomatidae at the beginning of the elimination of hypoxic conditions in bottom water (their overall mean density after hypoxia was about seven times as large as that in hypoxia in this study).

While Harpacticoida copepods usually have direct benthic developments, species in the family Longipediidae (Canuellelloidea), which was classified as a "Polyarthra" harpacticoid family until recently, have planktonic nauplii larval stages (Huys et al. 1996) and larger clutch sizes than other benthic copepods (Itô 1985). These seemingly "r-selected" characteristics in the family's reproductive system would have at least partly promoted the increases in their frequency in copepod composition after hypoxia at our study site. However, since the present study was based on small-volume sediment samples taken at a single site, we are not sure whether the increases in Longipediidae after hypoxia (1.0 ind. ml⁻¹ on average) were caused by (1) the recruitment of planktonic larvae from other areas in the same bay where the seasonal hypoxia impacts were not as severe, or (2) the recovery from the decreased densities that were lower than the limit of detection by the sampling efforts adopted in this study.

Further studies based on adequate specimens from several sites in the bay, using samplers covering larger sampling areas, are needed in the near future to confirm the patterns of seasonal impacts of hypoxia on different benthic copepod taxa found in this study.

Acknowledgements

This study was partially supported by JSPS KAKENHI (Grant numbers: JP25292114, JPI7H03854, JP18K19236), MEXT, Japan. We thank Dr. F. Mori for the assistance provided with field sampling. Comments by anonymous reviewers greatly improved our manuscript.

References

Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth, 214 pp.
Boxshall GA, Halsey SH (2004) An Introduction to Copepod Diversity. Ray Society, London, 966 pp.
Breitburg D, Levin LA, Oschlies A, et al. (2018) Declining oxygen in the global ocean and coastal waters. Science 359: eaam7240.
Clarke KR, Warwick RM (2001) Change in Marine Communities: An Approach to Statistical Analysis and Interpretation, 2nd ed. PRIMER-E, Plymouth, 176 pp.
Courde C, Moens T, Werbrouck E, Lepoint G, Van Gansbeke D, De Troch M (2015) Trophodynamics of estuarine intertidal harpacticoid copepods based on stable isotope composition and fatty acid profiles. Mar Ecol Prog Ser 524: 225–239.
De Troch M, Roelofs M, Riedel B, Grego M (2013) Structural and functional responses of harpacticoid copepods to anoxia in the Northern Adriatic: an experimental approach. Biogeosciences 10: 4259–4272.
Diaz RJ, Rosenberg R (1995) Marine benthic hypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna. Oceanogr Mar Biol Ann Rev 33: 245–303.
Grego M, Riedel B, Stachowitsch M, De Troch M (2014) Meiobrana winners and losers of coastal hypoxia: case study harpacticoid copepods. Biogeosciences 11: 281–292.
Giere O (2009) Meiobenthology. The Microscopic Motile Fauna of Aquatic Sediments, 2nd edition. Springer, Berlin, 527 pp.
Giere O (2019) Perspectives in Meiobenthology: Reviews, Reflections, and Conclusions. Springer, Cham, 80 pp.
Higgins RP, Thiel H (1988) Introduction to the Study of Meiobrana. Smithsonian Institution Press, London, 488 pp.
Huys R, Gee, JM, Moore, CG, Hamond R (1996) Marine and Brackish Water Harpacticoid Copepods, Part 1. Field Studies Council, Shrewsbury, 352 pp.
Itô T (1985) Suna no Sukima no Ikimono tachi (Fauna in interstices of sand particles). Kaimei-sha, Tokyo, 241 pp. (in Japanese)
Khodami S, McArthur JV, Blanco-Bercial L, Martinez Arbizu P (2017) Molecular phylogeny and revision of copepod Orders (Crustacea: Copepoda). Sci Rep 7: 1–11.
Mori F, Umezawa Y, Kondo R, Wada M (2018) Effects of bottom-water hypoxia on sediment bacterial community composition in a seasonally hypoxic enclosed bay (Omura Bay, West Kyushu, Japan). FEMS Microbiol Ecol 94: fiy053.
Mori F, Wada M (2019) Evaluation of sulfate-reducing bacteria community composition in surface sediment of a seasonally hypoxic enclosed bay as assessed using dsrA and 16S rRNA genes. Proceedings of the 2019 International Conference on Climate Change, Disaster Management and Environmental Sustainability, Kumamoto University: 702–708. ISBN 978-4-600-00316-6.
Nguyen QTD, Ueda R, Mori F, Kang T, Kim D, Shimanaga M, Wada M (2018) Response of nematode community structure to hypoxia in an enclosed coastal sea, Omura Bay, for three consecutive years. Plankton Benthos Res 13: 59–65.
Nguyen QTD, Kim D, Shimanaga M, Uchida J, Aisguna T, Wada M (2020) Horizontal distribution of nematode communities in a seasonally-hypoxic enclosed sea (Omura Bay, Japan). J Oceanogr: https://doi.org/10.1007/s10872-020-00558-2.
Shimanaga M, Kitazato H, Shirayama Y (2004) Temporal patterns in diversity and species composition of deep-sea benthic copepods in bathyal Sagami Bay, central Japan. Mar Biol 144: 1097–1110.
Takahashi T, Nakata H, Hirano K, Matsuoka K, Iwataki M, Ya-
Impact of hypoxia on copepods in Omura Bay

maguchi H, Kasuya T (2009) Upwelling of oxygen-depleted water (Sumishio) in Omura Bay, Japan. J Oceanogr 65: 113–120.
Thistle D, Sedlacek L, Carman KR, Fleeger JW, Barry JP (2007) Emergence in the deep sea: evidence from harpacticoid copepods. Deep Sea Res I 54: 1008–1014.
Vafeiadou AM, Materatski P, Adão H, De Troch M, Moens T (2014). Resource utilization and trophic position of nematodes and harpacticoid copepods in and adjacent to Zostera noltii beds. Biogeosciences 11: 4001–4014.
Wada M, Suzuki S, Nara T, Umezawa Y, Shimanaga M, Matsuoka K, Nakata H (2012) Microbial community respiration and structure of dead zone sediments of Omura Bay, Japan. J Oceanogr 68: 857–867.

Wells JBJ (2007) An Annotated Checklist and Keys to the Species of Copepoda Harpacticoida (Crustacea). Magnolia Press, Auckland, 872 pp.
Yokoyama H (1995) Macrobenthic assemblages in Omura Bay-I: Community parameters versus bottom environmental factors (in Japanese). Bull Natl Res Inst Aquaculture 24: 43–53. (in Japanese with English abstract)
Yokoyama H (1995) Macrobenthic assemblages in Omura Bay-II: species composition and faunal zones (in Japanese). Bull Natl Res Inst Aquaculture 24: 55–72. (in Japanese with English abstract)
Zar JH (2010) Biostatistical Analysis, 5th ed. Prentice-Hall, Englewood Cliffs, 944 pp.