Studies on the nematode community structure and diversity during the seasonal hypoxia in Omura Bay, Nagasaki, Japan

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長崎県・大村湾の季節性貧酸素形成期における線虫群集の構造と多様性に関する研究

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Abstract

Hypoxia in bottom environments of coastal marine ecosystems is one of the major threats adversely affecting numerous benthic organisms and thereby local fisheries. Under the ongoing global trend of the seawater temperature rise, it is very much likely that incidents of hypoxia would become more frequent and the severity of that would become more intensified around coastal seas. Considering the growing concerns on the impacts of ocean deoxygenation on the coastal fisheries, it is urgently needed to monitor precisely and consistently how benthic organisms respond to the decline of dissolved oxygen (DO) in the bottom environments. Meiobenthic organisms (meiobenthos) represent a major part of biodiversity of the bottom environments and play roles in carbon cycles and energy transfer through the ecosystems. As meiobenthos are much more numerous and considered to respond more sensitively to environmental perturbations than are macrobenthic organisms (macrobenthos), meiobenthos can serve as suitable target organisms for environmental monitoring to gain insights into the ecosystem response to hypoxia.

In this dissertation study, I particularly focused on nematodes as they predominate among meiobenthic fauna, and monitored nematode abundance, composition and feeding types under pre-, mid-, and post-hypoxic conditions during the period from June 2013 through October 2018 in Omura Bay, Nagasaki, Japan. The bay is almost completely enclosed, and consistently experiences seasonal hypoxia at the bottom every summer. The results of the present study are presented in three chapters, namely Chapter 2, 3 and 4.

In Chapter 2, I monitored abundance, genus level composition and feeding types of the nematode community in the uppermost layer of the sediment at a fixed sampling site locating in a central part of Omura Bay and found a positive correlation between DO concentration and nematode abundance over the entire sampling period of 2013 through 2015 ($r=0.61$, $p<0.05$). 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 (selective and non-selective deposit feeders, referred as 1A and 1B type, respectively) in hypoxic periods, relative to normoxic periods, further suggested that the transfer of organic matter from bacteria through nematodes became more predominant in the sediment 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}).

In Chapter 3, I assessed horizontal distribution of the nematode community in the bay by examining their abundance, community structure and diversity in the uppermost sediment of 4 selected sites representing gradients of some environmental variables across the bay during the study period of 2017. The severity of hypoxia varied typically along north-south axis of the bay, which was intensified southwardly. Nematode abundance and diversity were highest in the northern site than the other sites, and nematode communities were clustered into three groups by the sampling

site. There were significant differences in composition (Two-way ANOSIM, Rho = 0.726, $p<0.05$) and in feeding types (Two-way ANOSIM, Rho = 0.589, $p < 0.05$) among the groups. Organic matter content alone was the best predictor for the shift in nematode compositions (Spearman's Rho = 0.666, $p < 0.05$), whereas the combination of salinity and DO correlated well with the shift in nematode feeding types (Spearman's Rho = 0.568, $p < 0.05$). These findings strongly suggest that the diversity and the structures of nematode assemblages were strongly affected by the gradients in terms of seasonal DO availability, salinity change and persistent food availability (organic carbon accumulation) over the surface sediment of the bay.

In Chapter 4, I examined vertical distribution of nematode community in the sediment down to 4 cm depth in 2018. Nematode abundance and the amount of chlorophyll a in the sediment below 2 cm depth were apparently less than those above the depth, suggesting vertical distribution of nematodes is strongly affected by the availability of phytodetritus as a food source in the sediment column. Genus level composition of the nematode population revealed that the uppermost sediment (0-1 cm depth) was predominated by a single nematode genus at each sampling time. However, the predominance declined with depth and the genus level diversity tended to increase at subsurface (1-4 cm). There was also a clear difference in the nematode feeding-type composition between the uppermost and the subsurface sediment layers. Relative percentage of epistrate feeding nematode (referred as 2A type) was highest in the uppermost sediment, whereas that of both 1A and 1B type nematodes increased at subsurface regardless of the time of sampling. This suggests that the

importance of bacteria as a food source for nematode changes not only seasonally but also vertically in the sediment.

All of these results highlighted that free-living nematode abundance, genus level diversity and feeding type composition in the surface sediment of Omura Bay are highly responsive to DO concentration of the bottom water. One of the most important findings was that the proportion of nematode without teeth (1A and 1B types) to that with teeth (2A and 2B) could serve as a robust and sensitive measure to gauge the community response to the development and reduction of hypoxia in the bottom. Although DO availability inside the sediment was not determined, it is very much likely that DO availability for the infauna within the depth range of the present study closely reflect the extent of DO concentration in the bottom water. Therefore, monitoring the nematode community response to varying DO levels in the bottom water should give us ecologically relevant information on how nematode community would be affected by and respond to hypoxia.

Chapter 1

General introduction

1. Coastal seasonal hypoxia

Availability of dissolved oxygen (DO) is critical for most of the macroscopic living creatures to breathe in aquatic habitats. It is known that negative effects would appear in the majority of marine fish and shellfish below 3 mg O_2/L (Wu 2002). In enclosed coastal sea, such as inner bay, concentration of DO near the bottom sediment typically declines in summer to develop a dense, cold water mass containing less amount of DO compared with that near the sea surface. This is often called “summer hypoxia” or “seasonal hypoxia” at the bottom. Development of such hypoxic water mass threatens directly the communities of both macro- and meiobenthic organisms (Diaz and Rosenberg 2008, Middelburg and Levin 2009). In some areas, such as the northern Gulf of Mexico and Baltic Sea, that receive large amount of river runoff mixed with discharge from agricultural/industrial activities, the extent and severity of the negative impacts of hypoxic water mass could become enormous (Breitburg et al. 2018). Excessive amount of organic and inorganic nutrient inputs to the sea often leads to persistent eutrophication and hence sustained explosive growth of phytoplankton which eventually reaches to the bottom environment as massive load of labile organic matter. Microbial degradation of a large amount of labile organic matter deposited on the sediment consumes DO rapidly and entirely from its surroundings, thereby creating a huge water

mass devoid of oxygen over the sediment, known as “dead zone” (Diaz and Rosenberg 2008). Many of those areas have suffered not only from mass mortality of benthic animals, but also from habitat compression or loss of pelagic organisms (fish and zooplanktons), threatening local fisheries and ecosystem functioning in the affected areas (Zhang et al. 2009). To make things worse, there is a global trend of increasing dead zones over the last couple of decades (Diaz and Rosenberg 2008). In order to find ways to cope with the challenges and difficulties due to deoxygenation in coastal areas, it is fundamental to assess the status of benthic ecosystem by examining abundance, community composition (taxonomic diversity) and functional traits of the bottom fauna.

2. Free-living marine nematode

Meiobenthic organisms, also known as “meiofauna” are defined as animals that pass through a 500 μm or 1 mm sieve but are retained on a 32 to 40 μm sieve (Higgins and Thiel 1988; Giere 2009). Meiofauna are represented by diverse invertebrate taxa, such as 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 play important roles in marine ecosystem functioning, such as organic matter degradation, mineralization, solute transport and trophic link between bacteria and macroscopic organisms (Higgins and Thiel 1988; Schratzberger and Ingels 2018). Free-living nematodes often dominate among other taxa in marine meiofaunal communities (Gingold et al. 2013, Sergeeva and Zaika 2013). As the nematode

biodiversity is considered to be extremely large with an estimate of 50,000 species, only less than 15 % of which are currently known (Moens et al. 2013). In ecological studies of marine nematodes, specimens are often identified at species level, but also classified into four feeding types based on morphology of the buccal cavity (Giere 2009). The four feeding types consist of two primary groups of nematodes that are divided by the presence or absence of "buccal armature" (a tooth or teeth), which are then subdivided into two (Wieser 1953; Wieser 1960; Moens et al. 2013). 1A and 1B nematodes have no buccal armature. They are both called deposit feeders, but are distinguished from each other based on the size of the buccal cavity. 1A are referred to as selective deposit feeders, characterized by small to minute mouth openings, which only allow ingestion of very small, bacterial sized food particles. 1B are, on the other hand, non-selective deposit feeders that have more spacious buccal cavities, enabling them to exploit particles with a wider size range. 2A and 2B nematodes have buccal armature. 2A are epistratum (diatom) feeders that are characterized by the presence of a tooth or other mouthpart structures, enabling them to scrape off bacteria or microalgae from a substratum. 2B are predators or omnivores that are often large nematodes, characterized by the presence of spacious mouth openings, equipped with sclerotized tooth. Besides, 2B nematodes may have additional feeding strategies such as herbivory and bacterivory. They may even show some deposit-feeding behaviors as well (Moens et al. 2013). Thus, classification by feeding type alone does not automatically ensure the trophic link between the nematodes and prey material.

Moreover, nematode communities are often comprised of physiologically and

phylogenetically diverse species that have varying degrees of sensitivity to environmental disturbances including hypoxia (Tsujino 1998, Setoguchi et al. 2014). Therefore, shifts in nematode community composition and abundance have been used as a sensitive indicator to assess impacts of hypoxia on coastal ecosystems (Sergeeva and Zaika 2013, Taheri et al. 2015; Giere 2009, Wilson and Kakouli-Duarte 2009).

3. Omura Bay

Omura Bay is located in the center of Nagasaki Prefecture, in western Kyushu, Japan (Fig. 1). The bay covers an area of 320 km^2, with an average depth of 14.7 m (Iizuka and Min 1989, Takahashi et al. 2009). As the bay is connected to the Sasebo Bay through two narrow channels (Hario strait and Haiki strait), the water exchange between Omura Bay and the open ocean is very restricted. The tidal range in the bay is therefore significantly reduced with a mean value of 0.74 m at spring tides (Iizuka and Min, 1989). Incoming water from Sasebo Bay through the two straits is mixed well at the bay mouth and holds relatively high DO concentration with more than 5 mg/L all year round (Takahashi et al. 2008). The incoming water has usually higher density compared with Omura Bay, therefore flows into the bottom layer of the bay. In summer, however, the mixed water enters into the intermediate layer of the bay (usually at around 10 m depth) as the density becomes lighter due to water temperature rise at the bay mouth. A thermocline is thus formed near the bottom (Nogami and Matsuno 2001). Water stratification in Omura Bay during summer is hence reinforced

by the presence of two thermoclines: one near the surface (around 5 m) and the other near the bottom in the central region of the bay (beneath 17 m) which is known as “the second thermocline” (Nogami et al. 2000). The water below the second thermocline becomes extremely stagnant to form a basin-wide hypoxic water mass every summer.

The development of hypoxic water mass near the bottom prevails in the central region of the bay and often cause a mass mortality of fish and other marine organisms (Yokoyama 1995, Fukumoto and Kobayashi 2005, Takahashi et al. 2009). Although the hypoxic water mass at the bottom would expand to any directions in the bay, the northern area is less affected by hypoxia due to its proximity to the bay mouth compared with the center and south-east areas. As the south-east area is farthest from the bay mouth and close to the closed-off section of the bay, hypoxic condition can remain for longer period than other areas (Yokoyama 1995, Fukumoto and Kobayashi 2005, Takahashi et al. 2009). Therefore, it is expected that benthic organisms in different areas of the bay would respond differently along with gradients of oxygen and other environmental variables (such as salinity, organic matter content).

4. Research problem and objectives

4-1. Response of nematode community structure to hypoxia in Omura Bay

As the hypoxic water mass (<3 mg O_2 l^{-1}) develops at the bottom on a basin-wide scale every summer, it has long been considered to be largely responsible for the decline in the local fisheries

catch. The severity of the negative impacts of hypoxia varies year to year, yet could become so intensified that it would lead to mass mortality of macrobenthos and demersal fish near the shallow coast (Takahashi et al. 2008). Despite the large concern, however, little attempts have been made to address quantitatively how benthic life forms are impacted by the hypoxic water mass, and to predict how the benthic ecosystem would behave if the duration and the severity of the seasonal hypoxia become more intensified in the near future. The scarcity of such studies is due in large part to the difficulties of monitoring the biodiversity and the functioning of benthic ecosystem in the bay in ways which are ecologically relevant, yet feasible enough to perform consistently for long time. Monitoring nematode communities as environmental indicators is therefore worthy of attention in that it gives effective and affordable means to put such monitoring efforts in practice (Danovaro et al. 2009).

Before initiating my study, there was a preliminary result in our laboratory which indicated (1) nematodes were the most abundant and dominant component of the meiobenthic community of Omura Bay throughout May through October 2012, 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 were yet to be done.

To address the questions raised by the preliminary study, and to obtain more insight into the interactions between DO and the nematode community in Omura Bay, I 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).

4-2. Spatial (horizontal and vertical) distribution of nematode communities in Omura Bay

In addition to DO conditions, other environmental gradients also plays important roles in shaping macro- and meiofauna community diversity and structures at a regional (basin-wide) scale (Levin et al. 2010, Kovalenko et al. 2012, Stein et al. 2014). Therefore, dynamics of nematode community composition in deoxygenated coastal areas should be addressed in conjunction with the environmental gradient of bottom environments in order to make more accurate understanding and realistic predictions for the ecosystem impacts of deoxygenation (Giere 2009, Traunspurger and Majdi 2017).

In the case of Omura Bay, Yokoyama (1995) found that the DO availability and sediment characteristics of the bay controlled the macrofaunal communities. Nguyen et al. (2018) reported that nematode community structures temporally shifted between normoxic and hypoxic conditions. However, analyses on spatial (horizontal and vertical) distribution of nematode communities, and how the nematode assemblages respond to the development and the reduction of hypoxia within the bay have yet to be done. Taking environmental characteristics of the bay into consideration, I have examined horizontal and vertical distribution patterns of the nematode community at the surface sediment and address how deoxygenation and environmental gradients control the diversity and

functions of nematode community in Omura Bay.

Chapter 2

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

Introduction

There is a growing need to quantitatively monitor how benthic life forms are impacted by the hypoxic water mass, and to predict how the benthic ecosystem would behave if the duration and the severity of the seasonal hypoxia become more intensified in the near future. Monitoring nematode communities as environmental indicators can provide effective and affordable means to address the above-mentioned needs (Danovaro et al. 2009). In this chapter, my objective was to address the following questions: (1) To what extent do nematode population persist during summer hypoxia?, (2) How would it recover after hypoxia disappeared?, and (3) Are there any notable patterns of shifts in the genus-level community composition and the feeding type composition of nematodes in response to decline and recovery of DO conditions in Omura Bay? In order to address these questions, I monitored the nematode community for three consecutive years (2013–2015).

Materials and Methods

1. 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: 21m 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 25ºC, and carefully transported to the laboratory within 3 h after sampling to avoid direct exposure to sunlight and other physical disturbances. 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.

2. 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 a preliminary study has indicated that nematodes in the top layer (0-10 mm depth) of the center site seemed to be most strongly influenced by low DO condition compared to the subsurface (Fig. 2), 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 a 1 mm and 32 μm mesh screens, sediment specimens retained on the 32 μm mesh were re-suspended in tap water based on the method of Setoguchi et al. (2014) and centrifuged three times with colloidal silica (Ludox HS40; Sigma-Aldrich, St. Louis, Missouri, USA). The supernatants were transferred to Petri dishes with grids, and nematodes were collected using a 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. Those plates were put into an incubator (60ºC) 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, randomly-picked nematodes were identified up to 50. 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 (1A), non-selective deposit feeders (1B), epistrate (diatom) feeders (2A), and predators/omnivores (2B) (Lambshead 1986).

3. Statistical analysis

To plot the data of DO, water temperature, and nematode abundance, R-3.4.1 software was used (https://cran.r-project.org/bin/windows/base/). Multivariate analyses were performed using PRIMER6 software (PRIMER-E Ltd., Plymouth, UK). For multivariate analyses, nematode abundance was square-rooted transformed to down-weight the effects of abundant genera. To visualize similarities in nematode community composition under pre-, mid-, and post- hypoxia, both cluster analysis and non-metric multidimensional scaling (nMDS) were used (Clarke and Warwick

2001). A one-way analysis of similarities (ANOSIM) among pre-, mid-, and post-hypoxia groups was performed with the hypothesis that hypoxia would have a significant effect on the nematode community structure. In this study, similarity percentage analyses (SIMPER) were conducted to identify the group of nematode taxa or morphotypes that was contributing most to the similarity or dissimilarity.

Results

1. Effects of DO concentration on nematode abundance

In the present study, hypoxia was defined as DO concentrations less than 3 mg L^{-1}. As shown in Fig.3, clear patterns of seasonal hypoxia were obvious. DO started to decline below 3 mg L^{-1} after mid-June each year. Hypoxic and anoxic conditions developed during July through September with a couple of episodes of transient recovery to normoxic conditions. DO increased and remained over 3 mg L^{-1} for more than 10 days after mid-September. Water temperatures at the bottom also shown in Fig. 3 was described as follows: (1) temperatures began to increase after mid-June or mid-July; and (2) the temperatures declined gradually from mid-September (2013 and 2015) or end-September (2014). Nematode abundance at the time of sampling was summarized in Table 1. There is 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 the DO changes (R= 0.57, $p < 0.05$), while the

changes in water temperature did not affect nematode abundance ($R= 0.30$, $p > 0.05$).

2. 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. I examined the structural changes of nematode communities in June, August, and late September/October, which corresponds 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%) remained unidentified because they were too small or seriously damaged (Table 2).

As shown in Fig. 4, 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 was referred as post-hypoxia, were clustered with the samples from August 2013 through 2015 (mid-hypoxia). The SIMPER analysis indicated within group similarity was largest in pre-hypoxia (61.9%), followed by mid-hypoxia (58.5%), and post-hypoxia (37.9%) (Table 3). The genus that showed the greatest contribution to the group similarity under pre-hypoxic conditions (contrib% = 64.0%) was *Atrochromadora*. *Axonolaimus* contributed most of the average similarities in the mid-hypoxia group (contrib% = 50.0%). In the average similarities in post-hypoxia, *Chromadora* was

the most strongly contributing genus (contrib% = 40.2%). It was noted that *Halalaimus* increased their contribution ranks only in mid-hypoxia, while *Oncholaimus* enlarged their contributions only in post-hypoxia.

3. Effects of DO concentration on nematode trophic diversity

Although a one-way ANOSIM did not detect any significant differences in nematode feeding types among hypoxia-related periods or sampling months (Global R = 0.218, $p > 0.05$), a recurring pattern of the nematode trophic diversity in the center of Omura Bay is likely. As shown in Fig. 5, the relative percentage of toothless nematodes (types 1A and 1B) increased in mid-hypoxia in 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 nematode, while type 2A dominated over 2B in the nematode with teeth. In the nematode assemblages of September 2015, which was considered as post-hypoxia samples in the present study, type 1B dominated over other feeding type nematodes.

Discussion

The present study clearly demonstrated a decline in nematode abundance during hypoxic conditions, which is consistent with previous reports (Sergeeva and 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. However, some nematode genera, such as *Sabatieria*,

Linhomoeus, *Metalinhomoeus*, and *Monhystera,* can switch between aerobic and anaerobic metabolism, and therefore, can survive in long-term hypoxic conditions (Sergeeva and 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 to withstand low oxygen stress (Taheri et al. 2015).

I also found significant shifts in the nematode community structures among pre-, mid-, and post-hypoxia in the center of Omura Bay for three conservative years (2013-2015). The fact that both *Axonolaimus* (1B, non- selective deposit feeders) and *Halalaimus* (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, 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 for the shift, not only in taxonomic composition but in trophic diversity of free-living nematodes. According to the feeding type of nematode (Moens et al. 2013), the present data showed epistrate feeders (type 2A) were dominant in oxic conditions, whereas non-selective deposit feeders (type 1B) became dominant in hypoxic conditions (Table 3, Fig.4). Dominance of these two feeding-types is consistent with other reports on the northern Adriatic Sea where nematodes were subjected to experimentally induced anoxia (Taheri et al. 2015). It was also found that most dominate genus of 2A type in pre-

hypoxia (*Atrochromadora*) was different from that in post-hypoxia (*Chromadora*). As *Chromadora* became the second most contributor for the average similarity in mid-hypoxia, it is likely that *Chromadora* 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 as less dominant in post-hypoxia. Considering *Atrochromadora* would recover its dominance by next June, these 2A type nematodes may be the most effective colonizer 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 should be responsible for the observed difference between the two genera.

As suggested by Taheri et al. (2015), interactions between DO conditions and food availability might have impacts on the responses of the nematode community to low-oxygen stress. Because diatoms serve as a main food source for epistrate feeder nematodes (2A type), both *Atrochromadora* (dominant pre-hypoxia) and *Chromadora* (dominant post-hypoxia) may have suffered from the lower availability of diatoms during hypoxic conditions. Increase of predators/omnivores (*Oncholaimus*, 2B) feeding on protozoa, meiofauna, and other nematodes in their contribution ranks under normoxic samples (Table 3, Fig.5) may be partly explained by the re-appearance of their food sources after deoxygenation. As non-selective feeders (1B) are supposed to have a wider range of food sources, including bacteria and detritus, than selective deposit feeders

(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 29th, 2015) were clustered with a group of nematode communities under hypoxic conditions (Fig. 2). Although the DO level of the sample on Sep 2015 is more than 3 mg L^{-1}, it did not induce the community recovery from hypoxia to normoxia. 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 nematode community structure from hypoxia. Therefore, the placement of nematode community structure in the September 2015 sample within nematode samples of the hypoxia period in other years could be explained by the discontinuous period of re-oxygenation in September 2015. As for the source of the nematode population for recovery after the hypoxia, we think there are two possibilities: (1) nematodes in the bottom would migrate up to the surface in post-hypoxia, and (2) nematodes from surroundings would migrate horizontally (or be brought) into the sampling site in post-hypoxia. To better understand the recovery process in Omura Bay, it is clearly needed to investigate horizontal and vertical distribution patterns of nematode communities in the future study.

Chapter 3

Horizontal distribution of nematode communities in a seasonally hypoxic bay (Omura Bay, Japan)

Introduction

It has been known that hypoxic water mass develops at the central region of Omura Bay (Nogami et al. 2000; Takahashi et al. 2008). Although the hypoxic water mass can expand to any directions in the bay, extent of the impact by hypoxia may vary along with the gradient of environmental variables, such as DO concentration, salinity and organic matter content, along with the north-south axis of the bay. In order to address the possibility I examine the horizontal distribution of nematode community in the bay.

Materials and Methods

1. Sample collection, and environmental parameters monitoring

Sediment core samples of Omura Bay were obtained with a multiple core sampler, Ashura (RIGOSHA Co., Ltd.) on a training ship, Kakuyo Maru, Nagasaki University at four sampling sites: (1) The north (St. N), (2) the center (St. C), (3) the south west (St. SW), and (4) the south east of the bay (St. SE) between June and October 2017 (Fig. 6) . Detailed locations and dates of sampling are

summarized in Table 4. At each site, triplicate sediment cores (82-mm inner diameter) were collected. Besides those cores, extra triplicate core sample were taken in June, August, and October for the analysis of sediment parameters (gain size, organic matter, and sediment chlorophyll a). Sediment cores were extruded and horizontally sliced into layers with a pair of clean stainless-steel straight blades on board a ship. Each sediment layer 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}). All formalin-fixed samples were maintained at approximately 25 °C, and carefully transported to the laboratory within 1-2 days after sampling to avoid direct exposure to sunlight and other physical disturbances (Nguyen et al. 2018). Water column parameters (DO concentration, temperature, salinity, and water chlorophyll a) in overlying water 1 m above the sediment surface were monitored with a multi-parameter monitoring device (AAQ, JFE-Alec) at each sampling site.

2. Sediment gain size, organic matter (OM), and chlorophyll a (Chl a) analysis

As suggested by Nguyen et al. (2018), hypoxic conditions in the bay fully developed in August, while normoxic conditions occurred in June and October. Therefore, a portion of the sediment core samples from 3 months (June, August and October) were examined for grain size, organic matter and chlorophyll a content. Small amounts of sediment (approximately 3 ml) were taken from each sample to determine median grain sizes, which were measured in triplicate using a laser diffraction particle size analyzer (SALD-3100, Shimadzu Corp., Kyoto, Japan). The samples for organic matter analysis were placed into plastic bags and kept frozen at −20 °C. Sediment

samples in glass cupboard were dried at 105^{o}C for 1h, cooled down to room temperature and determined their dry weights. Dried sediments were gradually heated until they reached temperatures of 500^{o}C, and kept for 4 h. The samples were then put into a desiccator and weighed at room temperatures. The amount of organic matter (Loss on ignition; LOI) is the weight difference between the dry matter and the 500^{o}C ash (Vereș and Daniel Ș, 2002). For extracting sediment Chl a., a small amount (approximately 1 ml) of each sediment sample was put into 5 ml N, N-dimethylformamide (DMF). Concentrations of Chl a were then measured fluorometrically in triplicate using a 10-AU Field Fluorometer (Turner Designs, USA).

3. Sample processing and nematode identification

As nematodes in the top layer (0−10 mm depth) seemed to be most strongly influenced by low DO conditions compared to the subsurface (Nguyen et al. 2018), the top layer was examined across all the sediment cores. Samples were divided into quarters using a Folsom plankton splitter prior to the laboratory sieving processes, and either a 1/8 or 1/4 of the sediment was treated until nematode numbers exceeded 50 (Setoguchi et al., 2014). Sieving and sorting of specimens were done as described in a previous paper (Nguyen et al. 2018).

Up to 50 randomly-picked nematodes were identified to the genus level by referring to Warwick et al. (1998), Schmidt-Rhaesa (2013), and the keys on the NeMys online identification system (http://nemys.ugent.be/). 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) (Moens et al. 2013).

4. Statistical analysis

To test whether there were any differences in means of the environmental parameters (sediment and water column parameters), the pooled data of nematode abundance or diversity between sampling sites or months, Kruskal–Wallis test was performed (Kruskal and Wallis, 1952). Multiple pairwise comparisons were made to further compare all pairs of medians for each factor by using the Steel-Dwass-Critchlow-Fligner test (Spurrier, 2006). Those tests were done by using XLSTAT software (XLSTAT 2018: Data Analysis and Statistical Solution for Microsoft Excel. Addinsoft, Paris, France).

Multivariate analyses, calculations of diversity, and BIO-ENV procedure were performed using the PRIMER6 software (PRIMER-E Ltd., Plymouth, UK). Abundance data were square-rooted transformed to weigh down the effects of the abundant genera. To visualize similarities in the nematode community compositions and feeding types in four stations (St. N, C, SW and SE) or 5 months (June-October), non-metric multidimensional scaling (nMDS) was used. A two-way analysis of similarities (ANOSIM) without replication were made to make comparisons between the means of groups of data, where sampling sites and months were considered. Furthermore, one-

way ANOSIM to indicate pairwise differences between the means of each group like sampling sites or months. Similarity percentage analyses (SIMPER) were conducted to identify the group of nematode taxa or morphotypes that was contributing most to any similarity or dissimilarity. DIVERSE analyses were performed to calculate a set of univariate biodiversity measures (Shannon-Weiner index, H' and Simpson diversity index, 1-lambda) among station groups. To find the best variable combinations between nematode community structure and environmental parameters among sampling sites, the BIOENV procedure was conducted using weighted Spearman rank correlation (p_w) (Clarke and Warwick 2001).

Results

1. Environmental parameters

1.1 Bottom water parameters

Figure 7 illustrates changes in four bottom water parameters at four stations of Omura Bay between Jun and Oct 2017. Temperature of the bottom water began to increase from June to August (from around 19^{o}C to 26^{o}C), and then declined from September to October (from around 26^{o}C to 23^{o}C) across the sampling sites. There was no significant difference in the water temperature among the sampling sites (Kruskal–Wallis test, $p > 0.05$). Salinity of the bottom water at St. N, C and SW remained rather constant ranging from 30 to 32, while at St. SE it fluctuated notably with a peak

(32) in July followed by a decline to 24 in October. However, statistical tests failed to show significant difference among the four sites (Kruskal–Wallis test, $p > 0.05$) in salinity. In contrast, there was significant difference among sampling months (Kruskal–Wallis test, $p < 0.05$), although the pairwise test did not detect any significant difference in salinity between months (Steel-Dwass-Critchlow-Fligner test, $p > 0.05$). In the present study, hypoxia was defined as DO concentrations of less than 3 mg L^{-1} (Wada et al., 2012). DO concentration at St. N mostly remained normoxic, while the St. SE suffered from persistent hypoxia. The difference in the overall mean of DO between St. N and St. SE was significant (Steel-Dwass-Critchlow-Fligner test, $p < 0.05$). DO concentration at St. C and St. SW ranged widely; DO started to fall below 3 mg L^{-1} after July and hypoxic conditions developed from August until September, while it recovered over 3 mg L^{-1} by October. The Chl a concentration of the bottom water ranged from 0.73 through 5.26 μg L^{-1} at St. N, C and SW. The St. SE showed the large fluctuation of Chl a concentration with the highest in July (11.44 μg L^{-1}) and the lowest in October (0.18 μg L^{-1}). However, there was no significant difference in the mean Chl a concentration of the bottom water Chl a in the five sampling occasions between June and October among the sampling sites (Kruskal–Wallis test, $p > 0.05$).

1.2 Sediment parameters

Sediment grain size (the mean values of the median diameter of the sediment particles) tended to increase from 8.77 μm to 18.64 μm across the sampling sites between June and October, but difference among the sites was not significant (Kruskal–Wallis test, $p > 0.05$) (Fig. 8). The mean

values of LOI at the St. N (from 13.4 to 14.8%) were lower and significantly different from other stations (Steel-Dwass-Critchlow-Fligner test, $p < 0.05$). There was no significant difference in LOI among the stations (Kruskal–Wallis test, $p > 0.05$). The Chl a content of the sediment ranged from 640 mg kg $^{-1}$ to 2000 mg kg $^{-1}$ at the three sampling sites (St. C, SW, and SE) between June and October. The highest value of the Chl a content of the sediment (6212 mg kg $^{-1}$) was found at St. N in June. There was no significant difference in sediment Chl a among the stations (Kruskal–Wallis test, $p > 0.05$).

2. Abundance, genus composition, and genus diversity of nematode community

2.1 Nematode abundance

The variations of the mean abundance among the different sampling sites at five months (June-October) were statistically significant (Kruskal-Wallis test, $p < 0.05$). The Steel-Dwass-Critchlow-Fligner test showed that the overall mean of nematode abundance throughout the study period at the St. N (626 ind. 10 cm^{-2}) was significantly larger than those observed at St. C (215 ind. 10 cm^{-2}, $p < 0.05$) and SW (226 ind. 10 cm^{-2}, $p < 0.05$) and SE (310 ind. 10 cm^{-2}, $p < 0.05$). There was a decreasing trend for the nematode mean abundance from 480 to 266 ind. 10 cm^{-2} across the sampling sites between July and September (Fig. 9). The variations of the mean abundance among the different sampling months at four stations were statistically significant (Kruskal-Wallis test, $p < 0.05$); however, the pairwise test did not detect any significant difference in abundance between months (Steel-Dwass-Critchlow-Fligner test, $p > 0.05$).

2.2 Genus composition of the nematode community

As shown in Table 5 and Table 6, *Neotonchus* (176 out of 733 individuals, 24.0%) and *Axonolaimus* (338 out of 749 individuals, 45.1%) were found to be the most abundant genera at St. N and St. SE, respectively. *Chromadorina* dominated at St. C (195 out of 743 individuals, 26.2%) and SW (215 out of 737 individuals, 29.2%). A clear distinction between St. N ad SE was largely attributed to the predominance of *Neotonchus*, one of the epistrate feeding nematodes (2A), at St. N and that of *Axonolaimus*, one of the non-selective deposit feeding nematodes (1B), at St. SE. Three nematode genera, *Chromadonina*, *Axonolaimus* and *Pseudolella* remained and accounted for more than 50% of the nematode population during hypoxic conditions.

There was a significant difference in the genus level composition among the sampling sites (two-way ANOSIM, Rho = 0.726, $p < 0.05$). The pairwise test showed that genus level composition in St. N was significantly different from other three sites ($p < 0.05$), and that of St. SE was significantly different from St. C ($p < 0.05$). Further analyses showed that the nematode communities noted for the five sampling occasions at the four sampling sites were clustered into three different groups with more than 60% similarity except one at St. N in October (Fig. 10). Group I consisted of most plots at St. N (except October), in which *Neotonchus* was the most abundant. Group II included all of the plots at St. SE (dominated by *Axonolaimus*) and August at St. SW (dominated by *Chromadorina*). Group III consists of the rest of plots at St. C and SW (dominated

by *Chromadorina*) at the similarity level of 60%. However, there were no significant differences in the composition among sampling months (Rho = 0.176, $p > 0.05$).

The SIMPER analysis indicated that the within group similarity was the largest at St. SE (67.9%), followed by St. C (65.1%), St. SW (62.5%) and St. N (62.3%) (Table 6). The genus that showed the greatest contribution to the group similarity at the St. SE (contrib%= 24.2%) was *Axonolaimus*. *Chromadorina* contributed most to the average similarities at the St. C and St. SW (contrib%= 18.3% and contrib%= 22.9%, respectively), while *Neotonchus* contributed most to the average similarities at the St. N (contrib%= 17.8%).

2.3 Genus diversity of the nematode

At St. N, the genus-level species richness (H') and the evenness (1- Lambda) were on average 2.51 and 0.88 respectively and highest among the four sampling sites. On the other hand, lowest values for the diversity indices were found at St. SE (on average 1.87 for H' and 0.71 for 1-Lambda) (Fig. 11, Fig. 12). It was also noted that variations of the diversity were smallest at St. N, whereas they were largest at St. SE. The diversity indices at St. C and SW were intermediate between N and SE. The Steel-Dwass-Critchlow-Fligner test indicated that H' at St. N was significantly different from that at SW ($p < 0.05$). These results demonstrated that St. N had higher species richness (genus-level) and evenness, while the nematode community at St. SE was less rich and the genus was not equally abundant. These findings were also consistent with the rank abundance of nematode genera (Table 7). Seven genera are needed to achieve 70% cumulative

percentage of the community abundance in St. N, whereas only four genera occupied more than 70% in St. SE. Neither of the two diversity indices were statistically different among sampling months (Kruskal-Wallis test, $p > 0.05$).

2.4. Nematode feeding types

There was a significant difference in the feeding type compositions among the four sampling sites (two-way ANOSIM, Rho = 0.589, $p < 0.05$). The pairwise test detected that the feeding type compositions in St. SE were significantly different from other three sites (one-way ANOSIM, $p < 0.05$). Further analyses revealed that most of the nematode communities based on the feeding type were clustered into three different groups, except October at St. N, which was not included in any of the groups (Fig.13). Group I consisted of all the communities at St. SE and one from August at St. SW, which were dominated by type 1B nematodes. Group II included most of the communities at St. N, the one in July at St. SW, and the two other communities at St. C (July and October). They were dominated by type 2A nematodes. Group III consists of the rest of the communities at St. C and SW. The level of dominance for type 2A and 1B was comparable in this group. The SIMPER analysis indicated that the within group similarity was the largest at St. N (90.7%), followed by St. SW (87.7%), St. C (86.8%) and St. SE (86.6%). The feeding type that showed the greatest contribution to the group similarity at the St. N (39.3%) was 2A. 2A group also contributed most to the average similarities at St. SW (30%) and C (33.1%). The 1B type nematodes contributed most to the average similarities at St. SE (46.1%) (Table 8).

As shown in Fig. 14, the relative percentage of the nematodes with teeth (types 2A and 2B, 63.9%) was higher than that of toothless nematodes (types 1A and 1B, 36.1%) at St. N. On the other hand, the relative percentage of toothless nematodes (78.3%) was higher than that of the nematodes with teeth (21.7%) at St. SE. Type 2A (50.3%) was more dominant than 2B (13.6%) among the nematodes with teeth at St. N, while type 1B (61.3%) dominated over 1A (17.0%) within the toothless nematodes at St. SE through the study period. Relative abundance of toothless nematodes reached a maximum in August at St. C (59.7%) and SW (72.3%). Among the toothless nematodes, 1B type predominated at all sites but the relative abundance of 1A type tended to increase in August through September, except at St. C.

2.5. Correlation between nematode community structure and environmental variables

To correlate the patterns of the nematode community structure (nematode composition and feeding types) and the environmental variables, BioEnv analysis was conducted. As shown in Table 9, loss on ignition (LOI) showed the best match (p_w = 0.666) with the MDS ordination of the nematode community composition. A combination of LOI and sediment chlorophyll a content yielded the second highest correlation (p_w = 0.41). On the other hand, a combination of all the variables but water temperature gave rise to the highest correlation (p_w = 0.598) with the ordination plot of the nematode feeding type. The best 2-variable combination involved salinity and DO (p_w = 0.568).

Discussion

Basin wide gradient of environmental conditions in Omura Bay was clearly demonstrated in relation to the bottom-water DO and sediment organic matter (LOI) between bay mouth (St. N) and closed section of the bay (St. SE). In contrast to N, hypoxia and enrichment of organic matter was apparent in SE. This is consistent with a notion that sediment organic matter is well-preserved under low DO conditions in the bottom-water (Jessen et al., 2017; Mori et al. 2018). On the other hand, a greater fluctuation of DO concentration at St. C and SW during the study period demonstrates the seasonal transition between normoxia and hypoxia in the bay. Such seasonality of DO condition was less pronounced at St. N and SE. Highly fluctuating, lower values of salinity at St. SE also indicated a kind of gradient of the bottom environment in Omura Bay. This is attributable to the influence of freshwater discharge from the nearby coast. The peak of Chl a concentration of the bottom water coinciding with that of salinity at St. SE in July may therefore reflect an algal growth promoted by extra input of nutrients such as nitrogen and phosphorous from terrestrial origin to the inner bay in early summer (during June to July). The Chl a content of the sediment can be an indicator for the input of easily degradable organic matter from upper layer of the water column (Boon and Duineveld, 1998). A greater amount of sediment Chl a found in June at St. N than other sites may therefore indicate downward flux of potential food resource to benthic fauna was transient, but larger at St. N. A report on the aerosol particles (> 0.3 microns) transported from Eurasia to Nagasaki atmosphere (Takatsuji et al. 2017) suggests that amount of aerosols increased during

summer in 2017. This may help explain the apparent increase in sediment grain size found from June through October in Omura Bay. The overall mean of nematode abundance throughout the study period at the St. N (626 ind. 10 cm^{-2}) was significantly larger than those observed at St. C (215 ind. 10 cm^{-2}, $p < 0.05$) and SW (226 ind. 10 cm^{-2}, $p < 0.05$) and SE (310 ind. 10 cm^{-2}, $p < 0.05$) (Fig. 9), which suggests that hypoxic condition restricts population growth of nematodes in Omura Bay.

Significant differences in the nematode community structure based on genus level composition among the sampling sites (Fig. 10, Table 6) further suggest that the variations of DO and food availability play important roles in shaping the community compositions and the trophic diversity. A clear distinction between St. N ad SE was largely attributed to the predominance of *Neotonchus,* one of the epistrate feeding nematodes (2A), at St. N and that of *Axonolaimus*, one of the non-selective deposit feeding nematodes (1B), at St. SE (Table 5, Table 6). However, it should be noted that three nematode genera, *Chromadonina*, *Axonolaimus* and *Pseudolella* persisted and accounted for more than 50% of the nematode population during hypoxic conditions (Table 5, Table 6). In the previous report (Nguyen et al. 2018), neither *Chromadorina*, nor *Pseudolella* was shown as a major group of nematodes at St. C regardless of the sampling months during the 3 consecutive years (2013-2015), except that *Axonolaimus* remained in the higher rank making up of more than 22% of the nematode population (Nguyen et al 2018). The differences in the dominant nematode composition between 2017 and 2013-2015 at St. C therefore suggests that the faunal replacement at

genus level had stochastically occurred after 2015. The results also supported the previous notion that *Axonolaimus* are well adapted to low oxygen stress in Omura Bay.

In contrast to the significant differences between the north and southeast communities, there were no significant differences between the center (St. C) and southwest (St. SW) communities. At these sites, toothless nematodes (types 1A and 1B) predominated in hypoxic conditions, while the nematodes with teeth (types 2A and 2B) prevailed in normoxic conditions (Fig. 13). The very similar patterns on trophic diversity between St. C and SW suggests a gradient of environmental parameters were negligible between the two sites. Even though a two-way ANOSIM failed to detect significant differences in nematode feeding types between sampling months, a horizontal gradient of nematode trophic diversity was obvious across the north and south axis of the bay (Figure 13).

According to the results of BIOENV analysis (Table 9), it was clearly demonstrated that the gradient of organic matter content across the basin has profound impacts on the horizontal pattern of genus-level community composition. As the LOI varied more significantly among sampling sites than sampling months (Figure 8), it should represent the bulk of organic matter that is built up by long-term processes of sedimentation and degradation throughout the year. In contrast, a single abiotic variable did not provide a very successful match with the ordination pattern of the feeding type. However, the combination of salinity and DO yielded much better correlation than any other 2-variable subset. In the previous study, it was noted that seasonal decline in DO availability would select for toothless nematodes (1A, 1B types) during the stratified period of Omura Bay (Nguyen et

al. 2018). However, the present results strongly suggest that horizontal gradient of DO conditions also play important roles in determining the extent of relative contribution of the feeding types in nematode community. Salinity may impact the nematode community structure through tolerance or preference to fluctuating salinity (Platt 1977). Limitation to dispersion of nematode (Broman et al. 2018) may also be taken into consideration in our study sites.

The present results support the previous finding that gradients of organic matter influenced nematode community structure (Adão et al. 2009, Warwick 1971). Furthermore, the findings provided the first insight into how strongly the environmental gradient in terms of seasonal DO availability, changes in salinity and persistent food availability, which varies along with the unique geomorphological characteristics of enclosed bay, affects the diversity and the structures of nematode assemblages.

Chapter 4

Vertical distribution of nematode communities in a seasonally hypoxic bay (Omura Bay, Japan)

Introduction

Nematode abundance at the upper-most layer of the sediment is generally higher than that at deeper sediment, due to greater availability of DO and food sources near the surface (Moens et al. 2013). Under hypoxic conditions, the nematode abundance would decline due not only to lower availability of DO, but also to that of food source for nematode (Taheri et al. 2015). However, nematode abundance at subsurface may behave differently, as the extent of physical, chemical and biological disturbances are often different between the uppermost and subsurface sediments. In the central region of Omura Bay, vertical profile of microorganisms has hardly been examined except for bacteria (Wada et al. 2012; Mori et al. 2018).

In order to gain basic information on the vertical distribution of nematode community in Omura Bay, I examined the nematode abundance, community composition and feeding type in the sediment samples of 0-4 cm depth from the central region in the bay.

Material and methods

1. Sampling

Due to limited availability of the sediment samples in Omura Bay in 2018, we used selected sediment core samples (three replicates for each sampling) that had been collected at the center site (Fig. 1 in chapter 2) from June through October 2018 on board of Kakuyo-Maru, Nagasaki University. Sediment cores were cut into three depth fractions (D01= 0 - 1 cm depth, D12 = 1 - 2 cm depth, and D24 = 2 - 4 cm depth). At each sampling, the three depth fractions of undisturbed sediment cores were pooled and fixed immediately in DESS solution. Detailed locations, dates of sampling, and depth fractions are summarized in Table 10. A portion of these core samples were used for the analysis of sediment parameters (gain size, organic matter, and sediment chlorophyll a). Water column parameters (DO concentration, temperature, salinity, and water chlorophyll a) in overlying water 1 m above the sediment surface were monitored with a multi-parameter monitoring device (AAQ, JFE-Alec, Japan) at the sampling site. DO was also monitored with a DO logger (AROW2-USB, JFE Advantec, Japan)

2. Sample processing

Samples of the three depth fractions were divided into quarters using a Folsom plankton splitter prior to the laboratory sieving processes, and either a 1/8 or 1/16 of the sediment was treated until nematode numbers exceeded 50 (Setoguchi et al., 2014). Sieving and sorting of specimens, nematode identification and chlorophyll a (Chl a) analysis were done as described in the chapter 3.

3. Statistical analysis

To test whether there were any differences in means of the sediment parameters, the pooled data

of nematode abundance between depth fractions or sampling months, Kruskal–Wallis test was performed (Kruskal and Wallis, 1952). Multiple pairwise comparisons were made to further compare all pairs of medians for each factor by using the Steel-Dwass-Critchlow-Fligner test (Spurrier, 2006). Those tests were done by using XLSTAT software (XLSTAT 2018: Data Analysis and Statistical Solution for Microsoft Excel. Addinsoft, Paris, France).

Multivariate analyses were performed using the PRIMER6 software (PRIMER-E Ltd., Plymouth, UK). Abundance data were square-rooted transformed to weigh down the effects of the abundant genera. To visualize similarities in the nematode community compositions and feeding types in three depth fractions (D01, D12, and D24) or 3 months (June, August, October), non-metric multidimensional scaling (nMDS) was used. A two-way analysis of similarities (ANOSIM) without replication were made to make comparisons between the means of groups of data, where depth fractions and sampling months were considered. Furthermore, one-way ANOSIM to indicate pairwise differences between the means of each group like depth fractions or sampling months. DIVERSE analyses were performed to calculate a set of univariate biodiversity measures (Shannon-Weiner index, H' and Simpson diversity index, 1-Lambda) among depth fraction groups. Similarity percentage analyses (SIMPER) were conducted to identify the group of nematode taxa or morphotypes that was contributing most to any similarity or dissimilarity (Clarke and Warwick 2001).

Results

1. Environmental parameters

Temperature of the bottom water increased from June to August (from around 18.5ºC to 26.5ºC) and remained more than 24ºC until October. Salinity of the bottom water was above 32 throughout the study period. As the hypoxia was defined as less than 3 mg O_2 L^{-1} in the present study (Wada et al., 2012), the bottom hypoxia of Omura Bay fully developed in August, while normoxia prevailed in June and October (Table 11, Fig.15). The concentration of Chl.a in the bottom water in August was lowest (1.4 µg L^{-1}) in August, while it was above 1.6 µg/L in June and October.

The mean concentration of Chl.a was highest (2882 mg Kg -1) at the surface D01 (Fig 16). The D12 showed higher and wider range of sediment Chl a, while the D24 showed lower value of sediment Chl a (1363 mg Kg -1) compared to the other depth fractions. The mean concentration of Chl.a in the sediment was lowest (1414 mg Kg $^{-1}$) in October, but it was above 2446 mg Kg $^{-1}$ in June and August (Fig.17). The variations of the sediment Chl a value among depth fractions were statistically significant (Kruskal-Wallis test, K=8.667, $p < 0.05$). In addition, the pairwise test showed that the variations of the sediment Chl a value between D01 and D24 was statistically significant (Steel-Dwass-Critchlow-Fligner test, $p < 0.05$). There was no significant difference in the sediment Chl a value among sampling months ($p > 0.05$).

2. Nematode abundance

There was a decreasing trend for the nematode abundance from 172 to 163 ind. 10 cm^{-3} across the depth fractions from June to August (Fig. 18). The nematode abundance increased from 163 to

192 ind. 10 cm^{-3} from August to October. The average abundance at the surface D01 (210 ind. 10 cm^{-3}) was higher than that of D12 (205 ind. 10 cm^{-3}) and D24 (114 ind. 10 cm^{-3}) across the three sampling months. There was no significant difference in the nematode abundance among sampling months ($p > 0.05$) and among depth fraction ($p > 0.05$).

3. Genus composition of the nematode community

As shown in Table 12 and Table 13, among the 450 nematode specimens examined, *Chromadorina* was found to be the most abundant genus (113 out of 450 individuals, 25%), followed by *Actinonema* (88 individuals, 20%) and *Microlaimus* (46 individuals, 10%). However, 40 nematode specimens (9%) remain unidentified because they were too small or seriously damaged (Table 12). *Chromadorina* was predominant at all depth fractions; it contributed to 56.6, 25.1 and 29.9 % of the total nematode for D01, D12, and D24, respectively

There was a significant difference in the genus level composition among the depth fractions (two-way ANOSIM, Rho = 1, $p < 0.05$); however, the pairwise test did not detect any significant difference in the composition between depth fractions ($p > 0.05$). The same analyses did not detect any significant difference in the composition among sampling month groups (Rho = 0.167, $p > 0.05$). Further analyses showed that the nematode communities noted for the three sampling occasions in the three depth fractions were clustered into two different groups at 40% similarity (Fig. 19). Group 1 consisted of all the depth fractions in June and the D01 in October. All of these fractions were from normoxic conditions. In contrast, all the depth fractions in August and the subsurface fractions

(D12 and D24) in October clustered as Group II.

4. Genus diversity of the nematode

The genus-level species richness (H′) and the evenness (1 − Lambda) were lowest at the upper most sediment surface (on average 1.07 for H′ and 0.50 for 1 − Lambda). On the other hand,-those at subsurface and deeper sediment fractions were on average 1.89 for H′ and 0.79 for 1-Lambda, respectively (Fig. 20, Fig. 21). It was also noted that variations of the diversity were largest in the top layer, whereas they were smallest at the deepest section. The average diversity indices in D12 were almost equal to those of D24. However, neither of the two diversity indices were statistically different among depth fractions or sampling months ($p > 0.05$).

5. Nematode feeding types

2A type dominated in the D01 and D12 (74.9% and 48.7%, respectively), while 1B type was the most abundant feeding types in the D24 (33.5%) (Fig. 22). The relative percentage of toothless nematodes (types 1A and 1B) increase from D01 (2%; 37%;11%) to D24 (63%; 59%; 36%) across three months (June-October), and that of the nematodes with teeth (types 2A and 2B) dominated in pre- (70%) and post-hypoxia (74%) (June and October, respectively). Type 1B (22%) was more dominant than 1A (12%) within the toothless nematodes, while type 2A (60%) dominated over 2B (5%) among the nematodes with teeth.

Although a one-way ANOSIM did not detect any significant differences in nematode feeding types among depth fraction or sampling months (two-way ANOSIM, $p > 0.05$), the separation of

nematode communities into different groups of depth fraction is considerable (Fig. 23, Fig. 24). Nematode communities based on feeding type analysis were clustered into three different groups. Group I consisted of two plots in October and June in D01. Group II included all of the plots in D12, August in D01, and October in D24. Group III consists of the rest of the plots in D24 at the similarity level of 80%.

Discussion

Sediment Chl a can be an indicator for the input of easily degradable organic matter from upper layer of the water column (Boon and Duineveld, 1998). A clear decline of Chl a from the surface (D01) to the deeper sections (D12, D24) is likely to reflect active biodegradation of this molecule had happened in the subsurface sediment (Fig. 17).

As for the nematode abundance across the depth fractions, there was a decreasing trend from June to August and an increasing trend from August to October (Fig. 18). Nematode abundance in D24 section followed the overall trend indicating a decline in nematode abundance in August. It seemed that the overall nematode population in the deepest section was negatively impacted by the severer hypoxic conditions in Omura Bay, which is consistent with our previous report (Nguyen et al. 2018). On the other hand, nematode abundance in D01 and D12 did not appear to do so. Although the nematode abundance was higher at the D01 compared to other depth layers (D12 and D24), there was no significant difference among depth fractions. As Wada et al. (2012) demonstrated the amount

of organic matter (measured as TOC) and bacteria density in the surface sediment of the same site remained rather constant within the depth range 0-5 cm, it was suggested that food availability was not a limiting factor for deposit feeding and/or bacterivore nematodes.

Significant differences in the nematode community structure based on genus level composition among the depth fractions (Fig. 19, Table 13) further suggest that the variations of DO and food availability play important roles in shaping the community compositions and the trophic diversity. It was noted that the nematode communities of D01 in October were placed in normoxic group, while that of D12 and D24 in the same month belonged to hypoxic group. The separation of October samples in DO1 (normoxic group) and D12 and D24 (hypoxic group) suggests that nematode communities in top layer responded differently compared with those in sub-layers after hypoxia. One possibility is that nematodes in D01 was exchanged to a large part with those originated from oxygenated, upper most layer of surrounding sediment, whereas those in D12 and D24 were hardly exchanged with nearby nematode populations.

It was found that variations of the diversity were largest in the top layer, whereas they were smallest at the deepest section. The average diversity indices in D12 were almost equal to those of D24 (Fig. 20 & 21). These results demonstrated that the nematode community in D01 was less rich and the genus was not equally distributed, while the subsurface and deeper sediment had higher species richness (genus-level) and evenness. The higher diversity indices in D12 and D24 compared to D01 might indicate that the nematode communities in sub-layers had more stable environment

with lesser extent of grazing pressure or other disturbances. This is also consistent with the notion raised for a vertical profile of the bacterial richness in the same region of the bay (Wada et al. 2012).

Regarding the feeding type of nematodes, our data showed that the relative percentage of toothless nematodes (types 1A and 1B) increased from D01 to D24 across three sampling months, and that of the nematodes with teeth (types 2A and 2B) dominated in pre- and post-hypoxia. Type 1B was more dominant than 1A within the toothless nematodes, while type 2A dominated over 2B among the nematodes with teeth (Fig. 22). This trend was consistent with the results in the previous chapters. 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), they may have suffered from the lower availability of diatoms as well as DO. 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) (Moens et al., 2013), type 1B nematodes may be able to cope with hypoxic conditions better than type 1A nematodes.

As it is not feasible at present to infer the relative contribution of the two factors (availability of DO and food) to the decline of 2A+2B and the increase of 1A+1B along with depth, it is safe to interpret the results as a reflection of the combined effects of scarcity of DO and food source.

Chapter 5

General Discussion

1. Summary

The primary objective of the present study was to gain ecological insights into how nematode community may be affected by and respond to the transition of seasonal hypoxia in Omura Bay, a typical enclosed bay in Japan. As the hypoxic water mass (<3 mg O_2 l^{-1}) develops at the bottom on a basin-wide scale every summer, it has long been considered to be largely responsible for the decline in the local fisheries catch. The severity of the negative impacts of hypoxia varies year to year, but could become so intensified that it would lead to mass mortality of macrobenthos and demersal fish near the shallow coast (Takahashi et al. 2008). Despite the large concern, however, little attempts have been made to address quantitatively how benthic life forms are impacted by the hypoxic water mass, and to predict how the benthic ecosystem would behave if the duration and the severity of the seasonal hypoxia become more intensified in the near future. The scarcity of such studies is due in large part to the difficulties of monitoring the biodiversity and the functioning of benthic ecosystem in the bay in ways which are ecologically relevant, yet feasible enough to perform consistently for long time. Monitoring nematode communities as environmental indicators is therefore worthy of attention in that it gives effective and affordable means to put such monitoring efforts in practice (Danovaro et al. 2009). As shown in the present study, it was clearly demonstrated

that nematode abundance, genus-level composition and feeding-type composition in the surface sediment were all noticeably responsive to the environmental changes under pre-, mid-, and post-hypoxic conditions in the bay (Chapter 2, 3 and 4). It was also demonstrated that there were differences in the extent of variations of the nematode community parameters along horizontal and vertical environmental gradients in the bay bottom (Chapter 3 and 4). Furthermore, as the nematode community responses to the development and reduction of seasonal hypoxic water mass in Omura Bay were found to be robust and predictable, intensification of irregularity in any of the community responses over the course of the monitoring might be used to issue an early warning for a regime shift in the benthic ecosystem of the bay in the near future. One possible application of the present findings could be to watch a disproportionate increase in the abundance of 1A and 1B type nematodes relative to those of 2A and 2B types among multiple samples of the surface sediment, as it is likely to happen during the course of an intensified, basin-wide deoxygenation. In the following sections, I discuss further the findings and the interpretations of the present study.

2. How did DO variation in the bottom water exert impacts on the nematode community in the surface sediment?

Throughout the present study, I focused on the nematode community at the upper-most layer of the sediment except in Chapter 4. The reason is that nematode abundances are generally higher in the upper centimeters of the sediment (Moens et al. 2013) due to greater availability of DO and

food in the upper most surface sediment than in the subsurface. Apparently, DO availability is one of the environmental factors that exert direct physiological impacts on nematodes. Although it is generally considered that nematodes are more tolerant to the low oxygen conditions than macrofauna and other meiofaunal taxa (Levin et al. 2009), nematode survival to hypoxia is highly species-specific, and most species are negatively affected due to failure of aerobic respiration (Moen et al. 2013). Anoxic conditions that emerge after extended period of hypoxia can be even more lethal to most nematode due to buildup of highly toxic hydrogen sulfide in the sediment.

DO availability in the sediment is primarily determined by the balance between supply and consumption of DO within the sediment. It is well known that many sands and muds are only oxygenated in the uppermost millimeter-thin layer (Revsbech and Jørgensen 1986) due to high demand of DO by the respiratory activities of sediment microorganisms. However, muddy sediment that is inhabited by burrowing fauna would show completely different patterns of oxygen distribution (Pischedda et al 2008). In the presence of dense population of active burrowing fauna including polychaete and crustacea, oxygen can penetrate into sediment as deep as the burrow structures are found. This should apply to the case in Omura Bay, even though oxygen penetration depth (OPD) was not precisely determined in the study site. According to Mori et al. (2018), it is possible to infer OPD under oxic conditions based on the side views of undisturbed sediment cores that were retrieved from the study site at the time of sampling. During normoxic period, protruding tubes of polychaetes were abundant on the sediment surface and their burrows were well developed

within 0–5 mm depth from the surface. Some of the burrow structures were seen even at several centimeters below the surface. When these burrows were found, it is very much likely that OPD reached at least 5 mm depth. On the other hand, such burrowing fauna and their burrow structures were not seen for some time during August through September, and therefore OPD was considered to be virtually zero. Under these circumstances, the uppermost sediment turned dark gray to black in color and was often associated with microbial mats consisting of sulfur oxidizing bacteria (Wada et al. 2012; Mori et al. 2018). Collectively, the variations of OPD were closely associated with those of DO in the bottom of Omura Bay (Mori et al. 2018) and DO availability for the nematode community within the depth range (0-1 cm) should be tightly coupled to the extent of DO concentration in the bottom water.

With regard to the availability of food for nematode, it is generally considered that fresh phytodetritus derived from algal bloom in the photic zone of the water column, and microphytobenthos (MPB) thriving over the sediment surface serve as the primary food sources for the vast majority of marine nematode (Montagna et al. 1983). As the supply of organic matter from the light-dependent autotrophs to the benthic environments should be highest at the uppermost sediment, the nematode community near the sediment-water interfaces has greater access to the food sources compared with that residing in the subsurface. The level of food availability to the nematode community is, however, subjected to change possibly due to variations in the extent of settlement of phytoplankton aggregates from photic zone of the water column and/or the availability of light

and inorganic nutrients to MPB on the sediment surface. In the case of Omura Bay, the latter source seemed to be less important to the nematodes, as the transparency of the water at any of the sampling locations were usually less than 5 m (Wada, personal communication) and light conditions for photosynthesizing algae seemed far too weak to achieve net production. Therefore, settlement of fresh, photosynthetically fixed organic matter is likely to be critical as food supply to the nematode community in the study sites. Water column stratification during the hypoxic period is reinforced by two layers of thermocline, one near the surface (around 5 m) and the other near the bottom (beneath 17 m) known as "the second thermocline" (Nogami et al. 2000). The second thermocline acts as a stable barrier to transfer of DO between upper and lower depths of water, and consequently exacerbate hypoxia. It seems possible that the thermocline also restricts the settlement of phytoplankton aggregates derived from above and hence lowers the availability of algal food for nematodes.

Taking the above-mentioned notions into account, not only the availability of DO, but also that of food for the nematode community could be seriously reduced during hypoxia in Omura Bay. This has implications for the interpretation of the present results. As discussed briefly in chapter 2, some members of the epistrate feeding (diatom feeding) nematode (2A) seemed to be suffered from a shortage of food sources in the middle of hypoxia. At genus level, it was *Atrochromadora* and *Chromadora* that lowered the abundance in the mid hypoxia. Similarly, *Actinonema*, another 2A type nematode, was predominantly distributed all across the sediment column (0-4 cm depth) in

June, but disappeared thoroughly in August, and somewhat recovered in October (see chapter 4). The observed patterns of variation in the nematode groups are consistent with the report by Bongers et al. (1991) in that they assigned the two genera, *Atrochromadora* and *Actinonema,* to a group of nematodes sensitive to environmental disturbance compared with other genera. However, as it is not feasible at present to infer the relative contribution of the two factors (availability of DO and food) to the decline of nematode abundance, it is safe to interpret the results as a reflection of the combined effects of scarcity of DO and food source.

In contrast to what is suggested for the availability of fresh microalgal material to the nematode community under the hypoxic period, low DO conditions generally enhance preservation of organic matter (OM) in the sediment by slowing down aerobic bacterial degradation of OM (Jessen et al. 2017). However, vast majority of sediment bacteria are capable of thriving through either facultative or strict anaerobic metabolism and pursuing OM degradation in the absence of DO. In fact, when hypoxic conditions become intensified, there emerges “dead zones” in the bottom environments where most macro- and meiofauna can no longer tolerate (Diaz and Rosenberg 2008), yet a variety of anaerobic bacteria prevail. Therefore, the availability of OM and bacteria increases under hypoxia and anoxia, and members of nematodes that can survive under sever hypoxia should have an advantage over less tolerant species in gaining access to the food sources (Moens and Vincx 1997). Increase in the proportion of 1A (bacteria feeder) and 1B (deposit feeder), relative to 2A and 2B types in the nematode community found in the present study (Chapter 2, 3 and 4) are fully

consistent with this notion.

As has been noted above, summer hypoxia of Omura Bay consistently resulted in a community shift from epistrate feeder (2A) dominance to more deposit feeders (1A and 1B types), which eventually returned to a 2A dominated state by next spring. This whole process should have important implications in the energy transfer through benthic food web of the bay as suggested by Diaz and Rosenberg (2008). Under a normoxic period, nematode community dominated by 2A type would facilitate transfer of organic matter from fresh phytodetritus to 2B type and/or other meiobenthic predators, which could ultimately nourish macrobenthic organisms. On the other hand, seasonal hypoxic and anoxic conditions would favor nematode community dominated by deposit feeders (1A+1B types) and increased utilization of bacterial organic matter in the benthic food web. As biological activities of macrobenthic communities are severely hampered under the low DO conditions, it is generally expected to cause lowered efficiency of energy transfer from meio- to macrobentos. However, carbon flow from deposit feeding nematodes to other benthic organisms at higher trophic levels might increase during a transition from hypoxic (anoxic) to normoxic period in Omura Bay.

3. Notes on valuable information from the nematode community composition that was examined based on genus level identification

As stated above, survival of free-living marine nematode under low DO conditions is highly

species-specific (Moens et al. 2013) and shifts in the nematode community composition due to hypoxia have often been revealed at species level (for example, Van Colen et al. 2009; Arroyo et al. 2012; Taheri et al. 2014). Despite the limitation of taxonomic resolution inherent in the genus level identification, the present study successfully demonstrated that not only the abundance, but also nematode community composition and diversity in the surface sediment of Omura Bay were highly responsive to the variation of DO conditions in the bottom water, in ways that are compared with the existing knowledge of the ecological response of nematodes to environmental disturbances.

Some of the nematode genera, such as *Atrochromadora* and *Actinonema* (see chapter 2 and 4), that showed notable decrease in number during hypoxic period matched up with those that were sensitive to environmental disturbance (Bongers et al. 1991). In contrast, other nematode genera, such as *Axonolaimus*, *Halalaimus* (see chapter 2) and *Chromadorina* (chapter 3 and 4), that appeared to be highly tolerant to hypoxia in Omura Bay have not been represented as abundant in other hypoxic areas. Considering the high degree of physiological plasticity and short generation time of nematodes, varying from a few days to weeks, in general (Moens et al. 2013), some groups in the nematode community might have locally adapted to a repeated cycle of seasonal hypoxia in Omura Bay (Nguyen et al. 2019 & 2020). Unfortunately, a sever lack of information on the eco-physiological traits that are shared among nematode taxa makes it hard to construct a plausible hypothesis that could account for the possibility any further. In the case of *Chromadorina,* however, the knowledge on the feeding strategy of the nematode genus may give us a clue on how they

dominated in the center of the bay throughout the study period from 2017 (see chapter 3) through 2018 (chapter 4). *Chromadorina* nematodes belong to 2A type and feed on diatoms by puncturing or cracking diatom frustules and to suck inner cellular contents (Moens and Vinx, 1997), but they also have the ability to agglutinate surrounding detritus using mucus secreted from their caudal glands, thereby fostering bacterial colonization on the detrital agglutinations and eventually ingesting them as a food source (Riemann and Schrage, 1978; Majdi et al., 2012). The presence of such feeding modes could have increased the efficiency of nutrient intake for the nematodes in otherwise starving condition.

In light of the need for sustainable environmental monitoring, genus-level identification of nematodes can be sufficient to detect the effects of ecosystem disturbance (Kandratavicius et al., 2018), and yet to raise questions on eco-physiological traits of nematodes that could be solved by experiments with nematode specimens that are identified at species and even strain-level resolution.

4. The significance of the present study

In addition to the genus level identification, grouping of nematodes based on the feeding type was also proved to be effective in detecting responses to hypoxia in Omura Bay. In fact, it was the proportion of nematodes without teeth (1A and 1B types) to those with teeth (2A and 2B) that responded sensitively and consistently to the variation of DO level in the bay. The ratio of "1A+1B type nematodes" to "2A+2B type nematodes", referred as "1AB/2AB" ratio in the following

sentences, increased under hypoxia and decreased under normoxia (chapter 2, 3 and 4).

There are a number of reports on the nematode community response to low DO conditions (hypoxia) (see Levin et al. 2009 for review). Most of them have focused on intertidal flats or estuaries that developed hypoxia due to high organic load from human perturbations such as, sewage disposal (Lambshead, 1986), intensive fish farming (Mirto et al., 2002), and oil spillage (Danovaro et al. 1995). Similar to what was found in Omura Bay, those studies often reported conspicuous increase in abundance of 1A and/or 1B type nematodes under the hypoxic sites compared with other reference sites. However, there have been no reports on the "1AB/2AB" ratio that is consistently responsive to seasonal hypoxia. As the results is based on the consistent monitoring in Omura Bay for a total of 5 years, I would propose that the "1AB/2AB" ratio can be used as a sensitive ecological index to gauge whether the extent of deoxygenation is intensified or relaxed in the bay.

There are ecological indices that have been developed specifically for analysis of nematode assemblages (Ferris and Bongers 2009). For example, the Nematoda/Copepoda ratio (Ne/Co ratio; Raffaelli and Mason 1981) is calculated as Ne/Co = the total number of nematode individuals divided by that of copepod individuals. The Index of Trophic Diversity (ITD; Heip et al., 1985) is another index that is calculated as ITD = $(g1A)^2+(g1B)^2+(g2A)^2+(g2B)^2$, where g is the relative contribution (percentage) of each trophic group (ie, g1A for 1A type, g1B for 1B type, g2A for 2A type and g2B for 2B type nematodes) to the total number of nematode populations. The Maturity Index (MI) is based on ecological characteristics and reproductive strategies of nematodes. MI is

computed as the weighted average of the individual colonizer-persistent (c-p) values in a sample (Bongers et al., 1991). There is another feeding-type based index, “1B/2A” (Lambshead 1986) that is calculated as the number of 1B type nematodes divided by that of 2A type nematodes. This index is quite similar to the “1AB/2AB”, although it has not been used as often as other indices. Therefore, it is clear that, along with all the above-mentioned indices, the “1AB/2AB” ratio needs to be tested further as to whether it would hold the reproducibility, consistency and sensitivity as an index for environmental changes not only in Omura Bay but also in other seasonally hypoxic areas.

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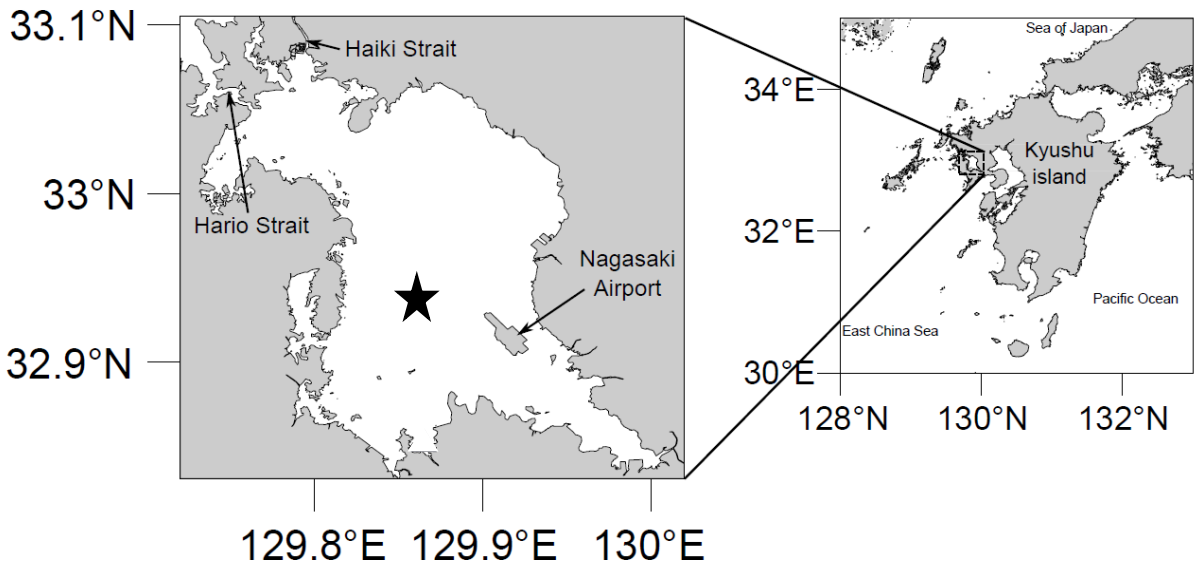

Fig. 1. Omura Bay and the center site (★)

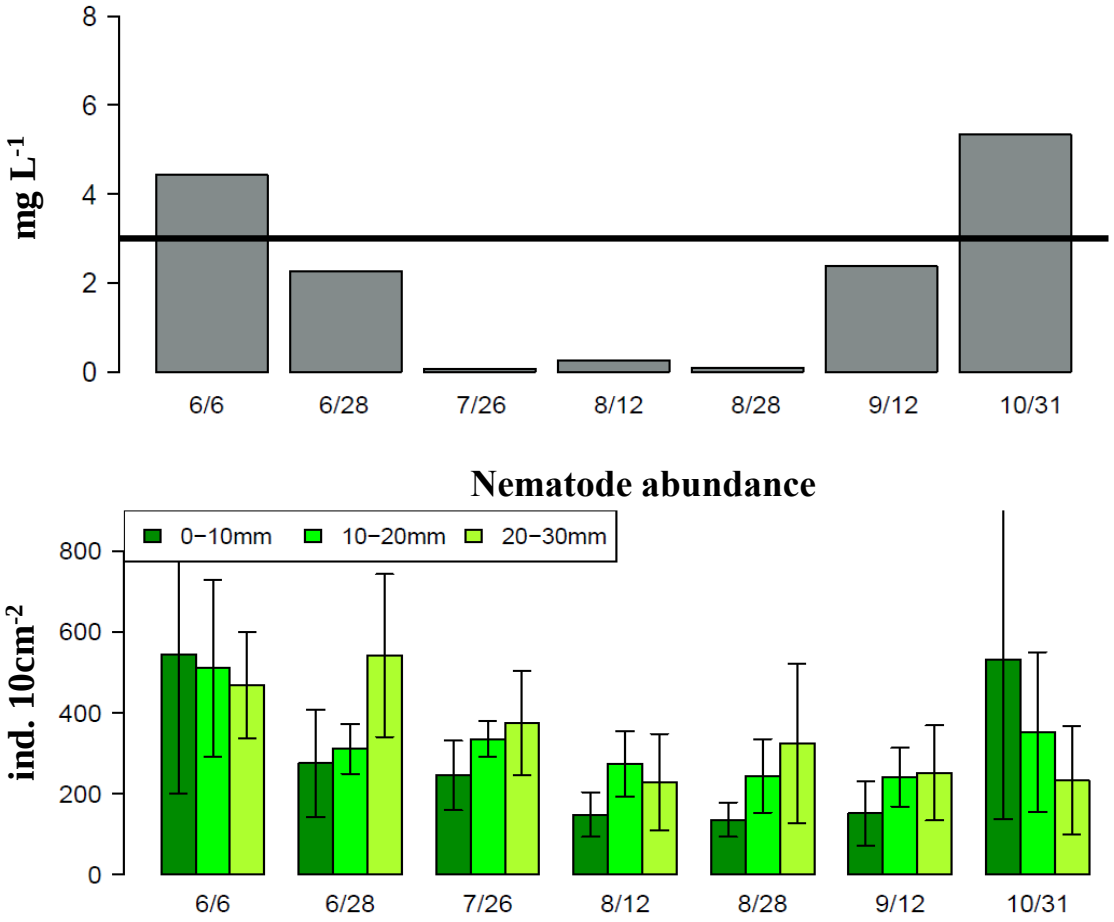

Fig. 2. DO concentration and vertical distribution of nematode communities in 2013 in the center site of Omura Bay

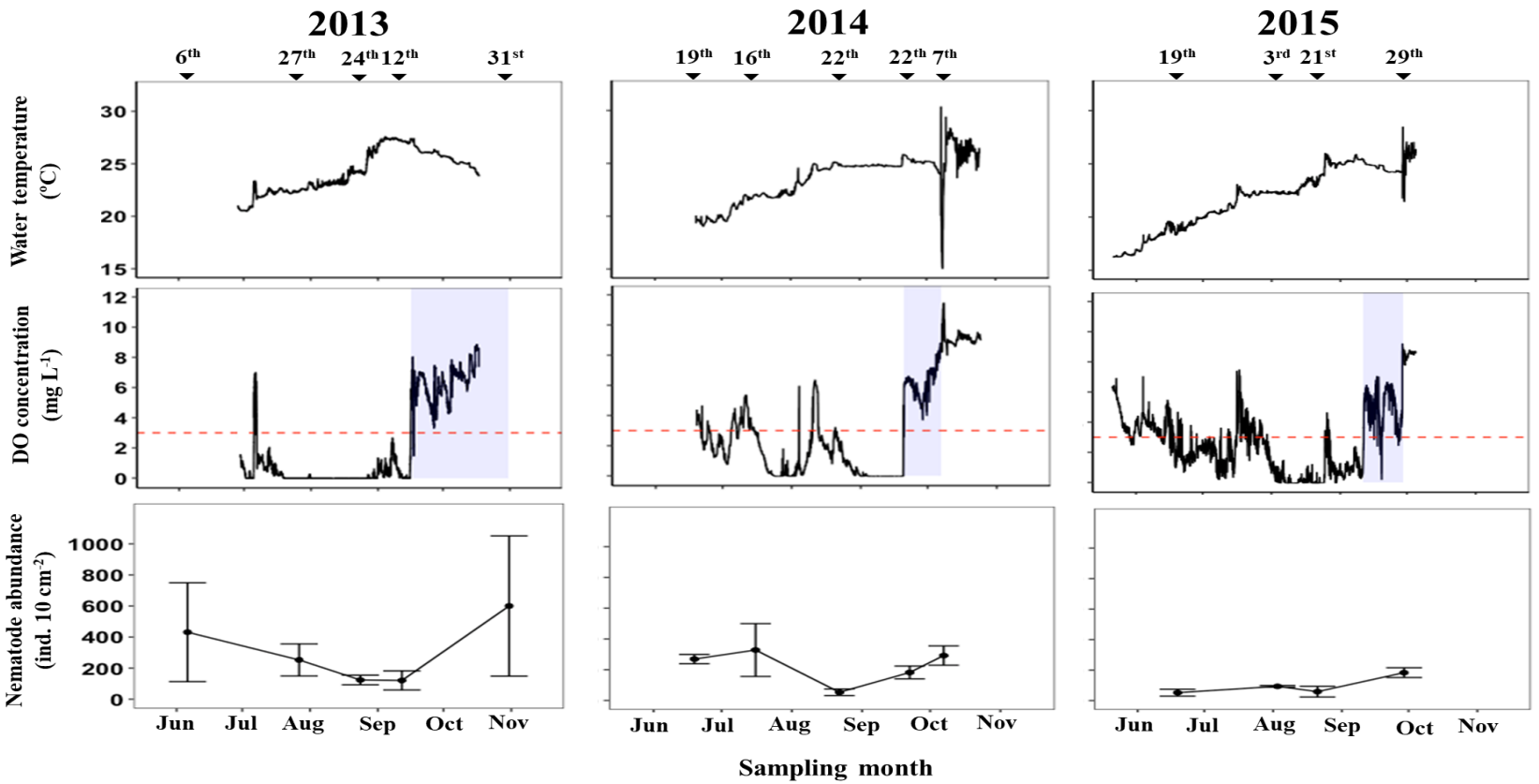

Fig 3. Environmental variables (DO concentration and water temperature at 1 m above the bottom) and nematode abundance (mean ± standard deviation, n = 3) in the center of Omura Bay for three consecutive years (2013-2015). The inverted triangles indicate the sampling dates for each year. The dashed lines denote the transition point of DO concentration (3 mg L^{-1}) between hypoxic and normoxic conditions. The shaded rectangles represent the period of re-oxygenation for each sampling year.

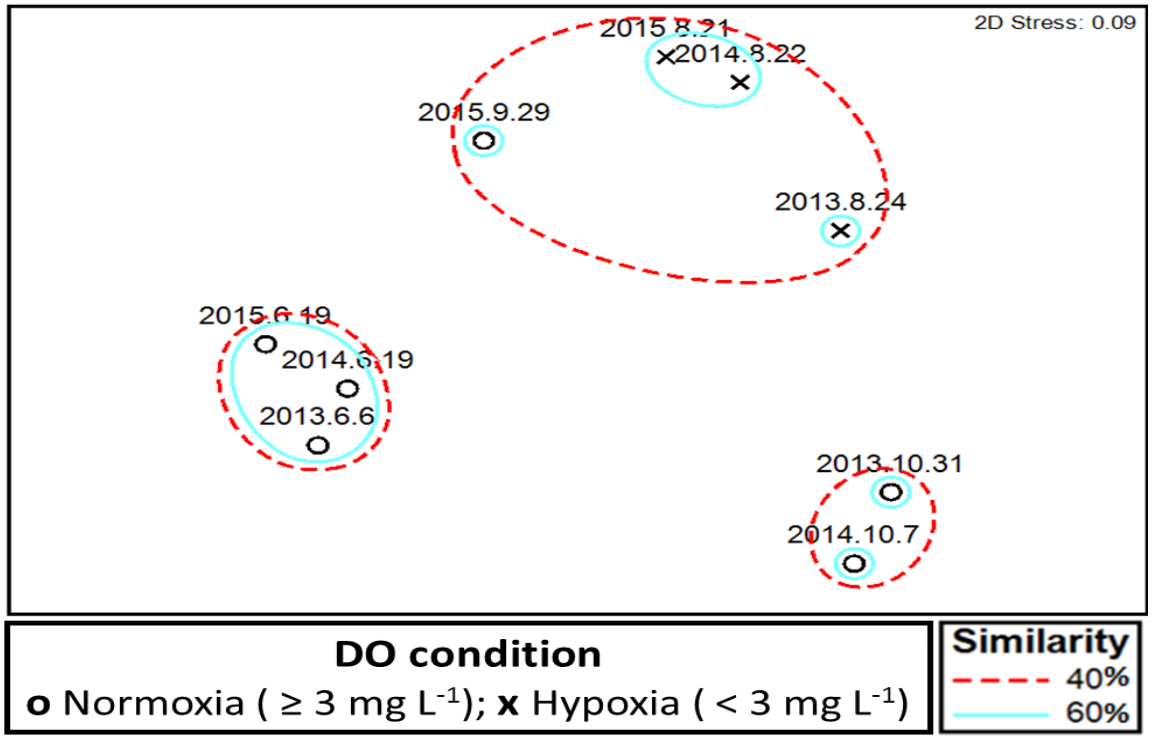

Fig. 4. Non-metric multidimensional scaling (nMDS) plot of nematode community structure in the center of Omura Bay for three years (2013-2015) with superimposed clusters based on the Bray–Curtis similarity (Standardized sample by total; Transform: Square root). Dashed and continuous lines refer to similarity levels of 40% and 60%, respectively.

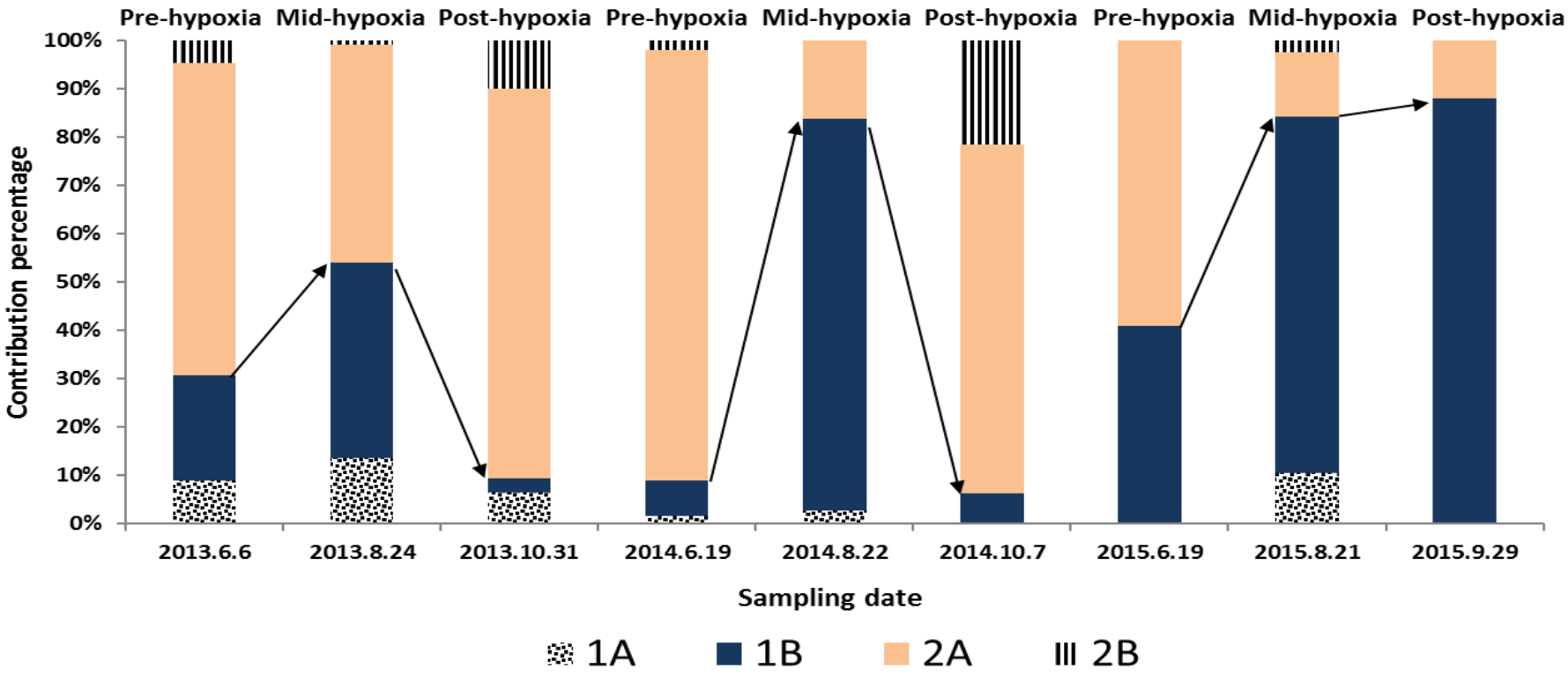

Fig. 5. Percent contribution of nematode feeding types (1A, 1B, 2A, and 2B) in the center of Omura Bay for three years (2013-2015). The arrows indicate changes in the relative percentage of toothless nematodes (types 1A and 1B) and nematodes with teeth (types 2A and 2B) over three sampling years. Selective deposit feeders (1A), non-selective deposit feeders (1B), epistrate (diatom) feeders (2A), and predators/omnivores (2B).

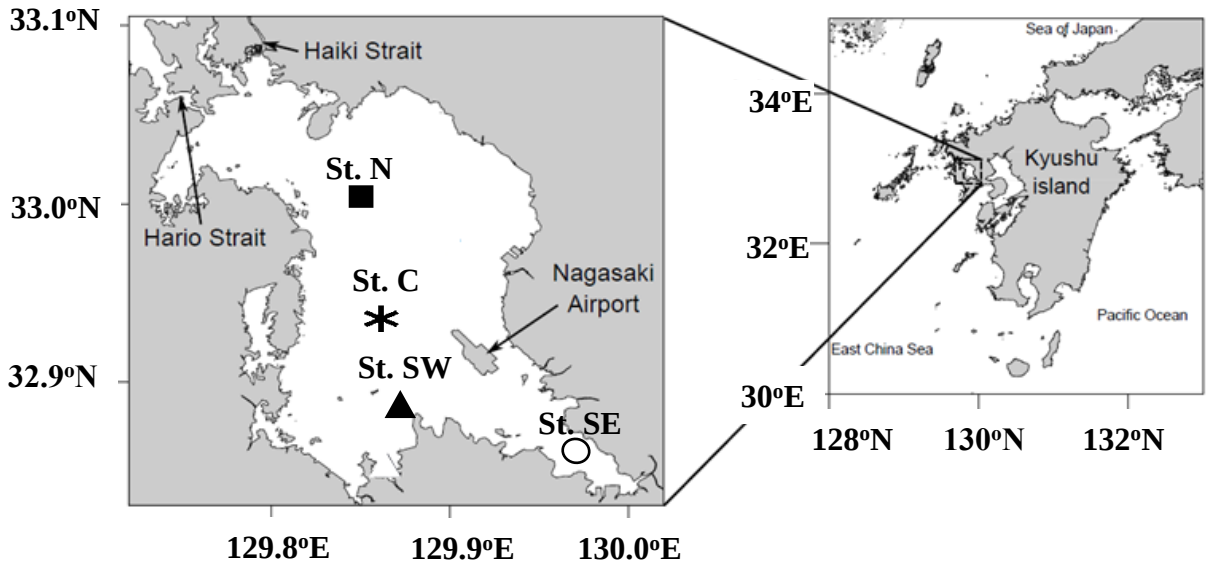

Fig. 6 Omura Bay and four sampling sites

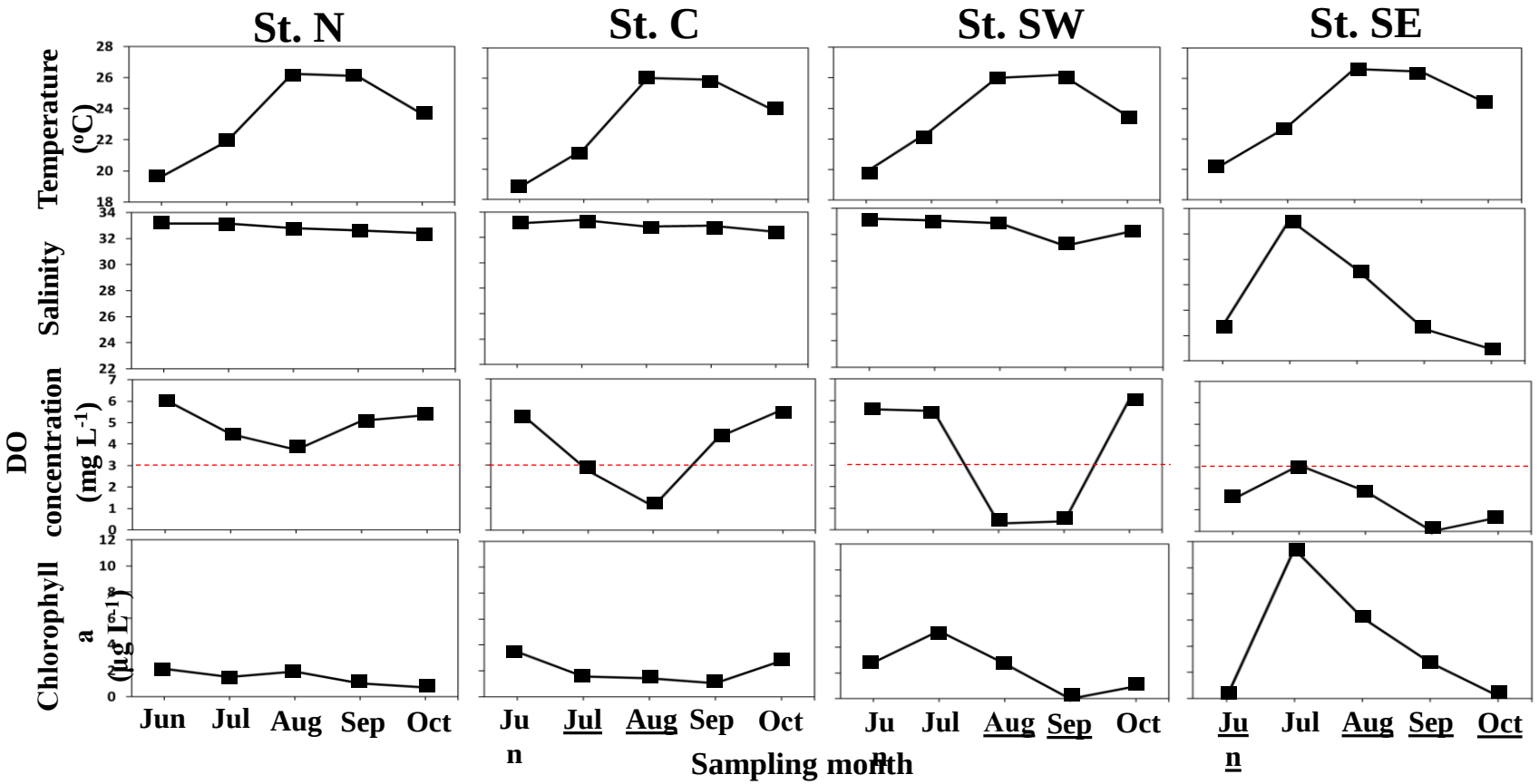

Fig. 7 Four parameters (DO concentration, water temperature, salinity, and water chlorophyll a) of the bottom water at the four sampling sites in Omura Bay between June and October 2017. The dashed lines show the transition point of DO concentration (3 mg L-1) between hypoxia and normoxia. Hypoxic months were underlined in order to separate from normoxic ones

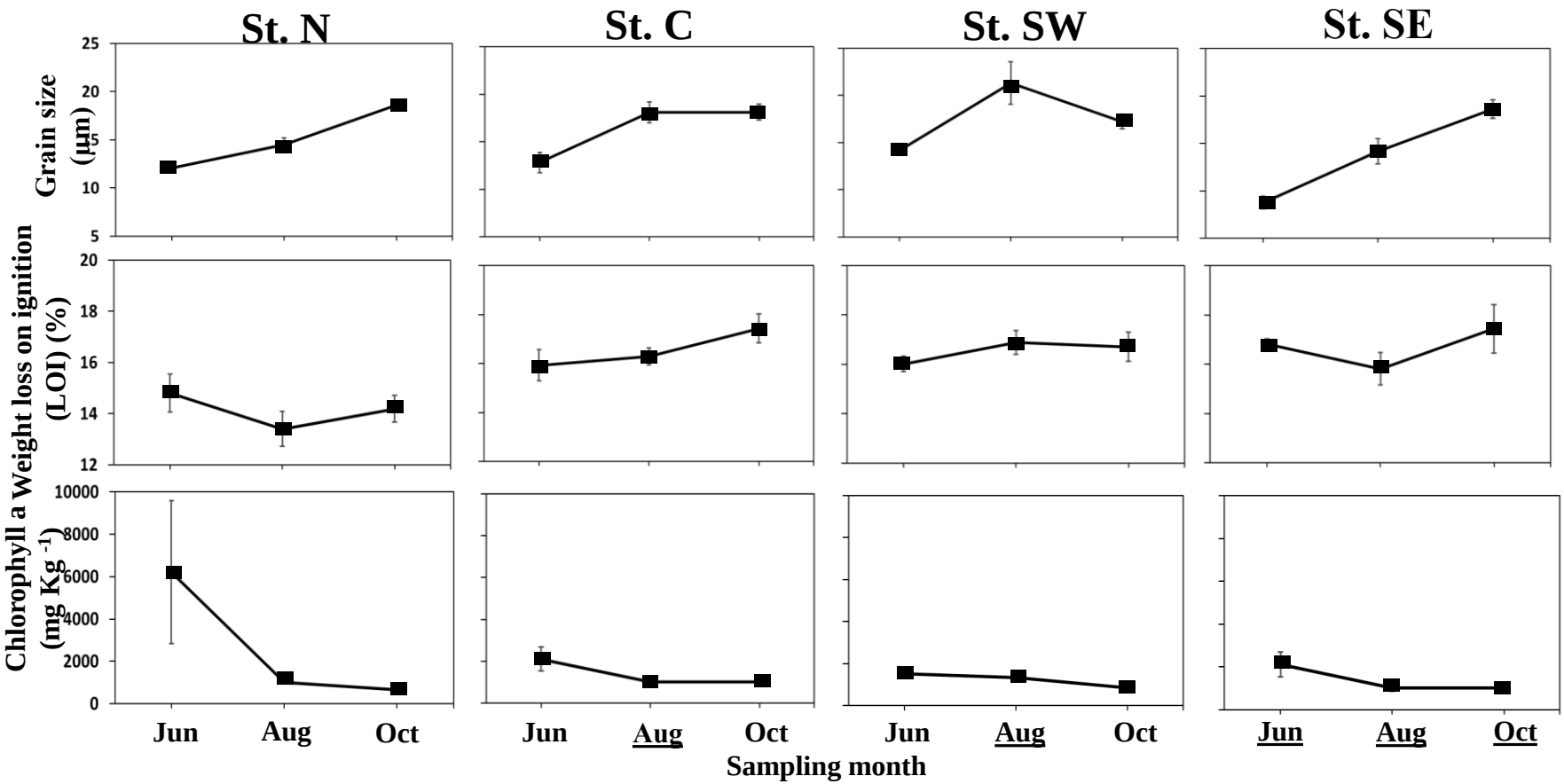

Fig. 8 Three parameters (grain size, weight loss on ignition, and sediment chlorophyll a) (mean± standard deviation, n = 3) of the sediment at the four sampling sites in Omura Bay between June and October in 2017. Hypoxic months were underlined in order to separate from normoxic ones.

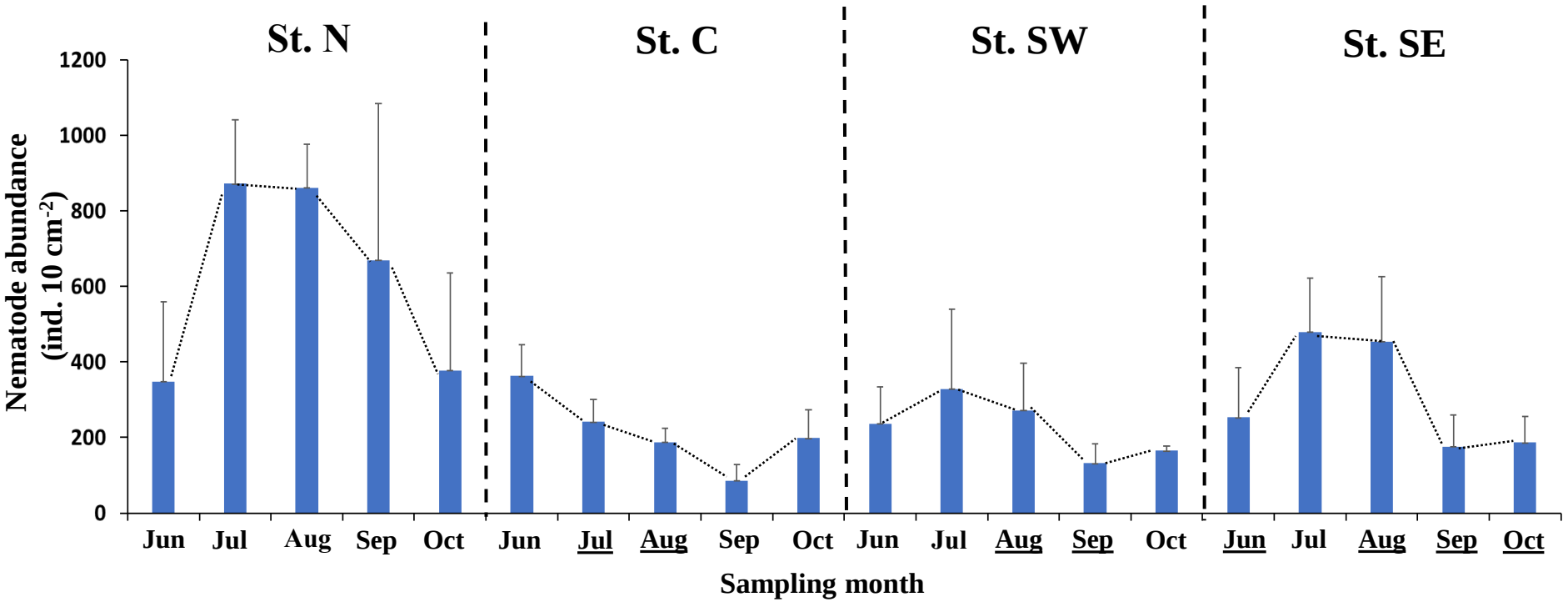

Fig. 9 Nematode abundance at the four sampling sites in Omura Bay between June and October in 2017. The error bars in the lower panel represent the standard deviation of nematode abundance. The round-dot lines indicate changes in the nematode abundance over the four sampling sites. Hypoxic months were underlined in order to separate from normoxic ones

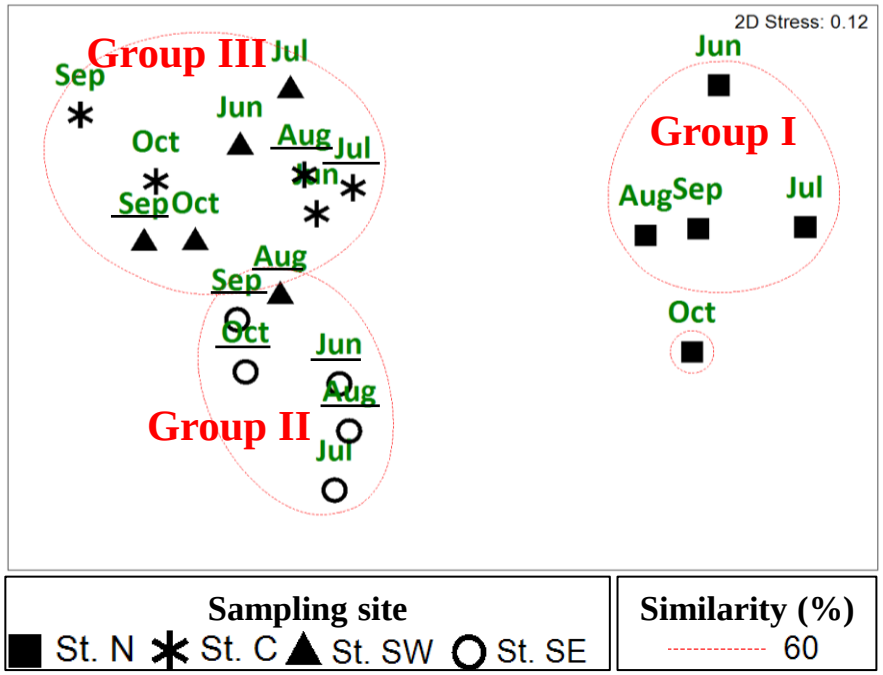

Fig. 10 Non-metric MDS plot of nematode community structure at the four sampling sites in Omura Bay between June and October in 2017 with superimposed clusters based on the Bray–Curtis similarity (standardize sample by total; transformation: square root). The round-dot lines refer to similarity levels of 60%. Hypoxic months were underlined in order to separate from normoxic ones.

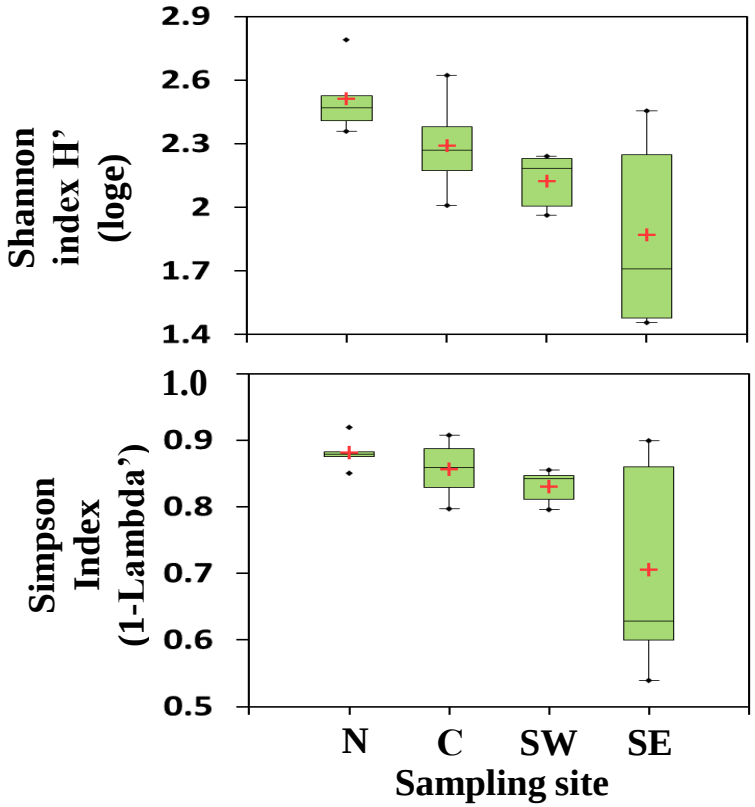

Fig. 11 Shannon and Simpson indexes of nematode diversity at the four sampling sites of Omura Bay between June and October in 2017. The crosses indicate the means. The central horizontal bars denote the medians. The lower and upper limits of the box plot are the first and third quartiles, respectively. Points above or below the whiskers' upper and lower bounds are considered as outliers**.**

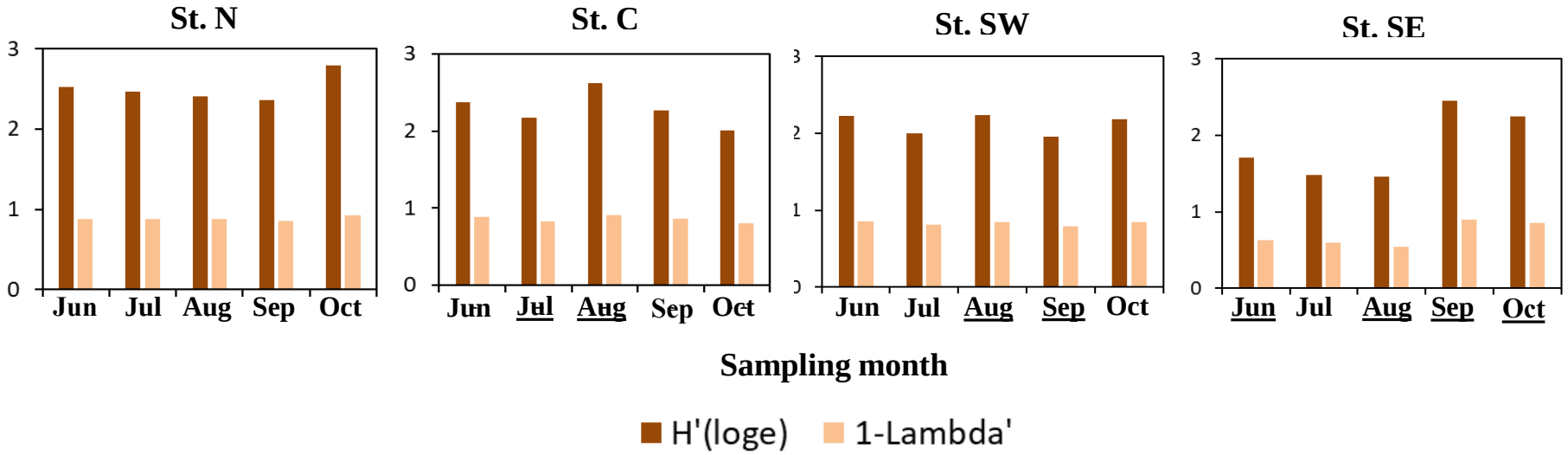

Fig. 12 Shannon and Simpson indexes of nematode diversity at the four sampling sites of Omura Bay between June and October in 2017. Hypoxic months were underlined in order to separate from normoxic ones.

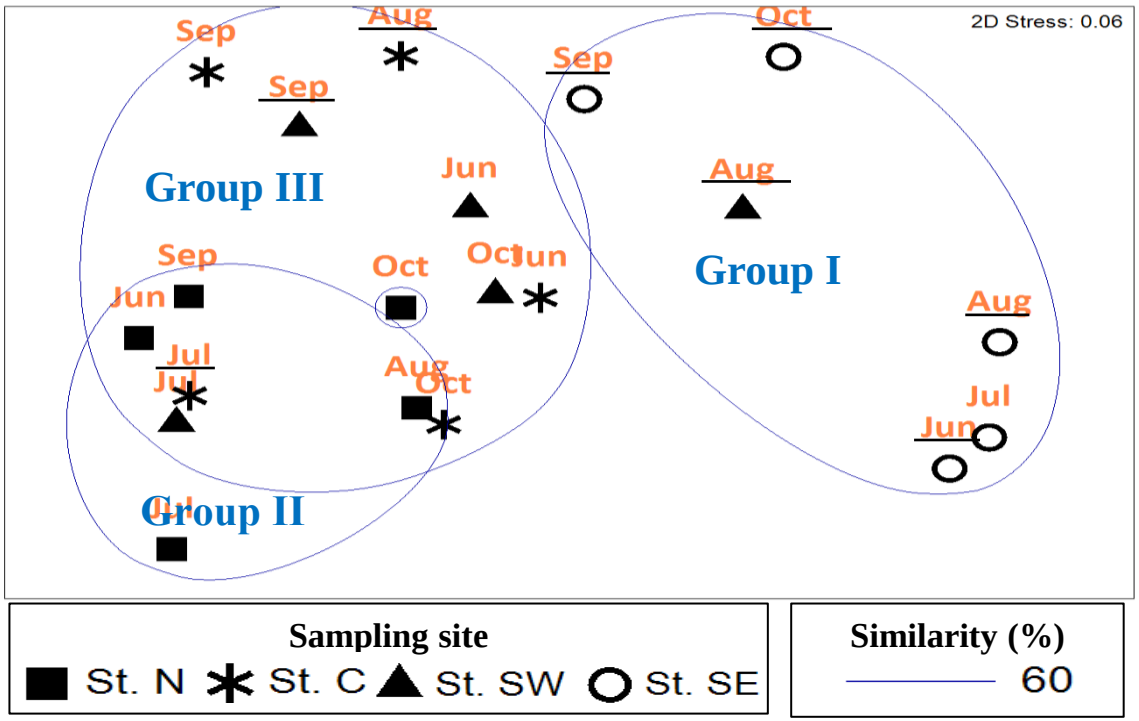

Fig. 13 Non-metric MDS plot of nematode feeding types at the four sampling sites in Omura Bay between June and October in 2017 with superimposed clusters based on the Bray–Curtis similarity (standardize sample by total; transformation: square root). Continuous lines refer to similarity levels of 60%. Hypoxic months were underlined in order to separate from normoxic ones.

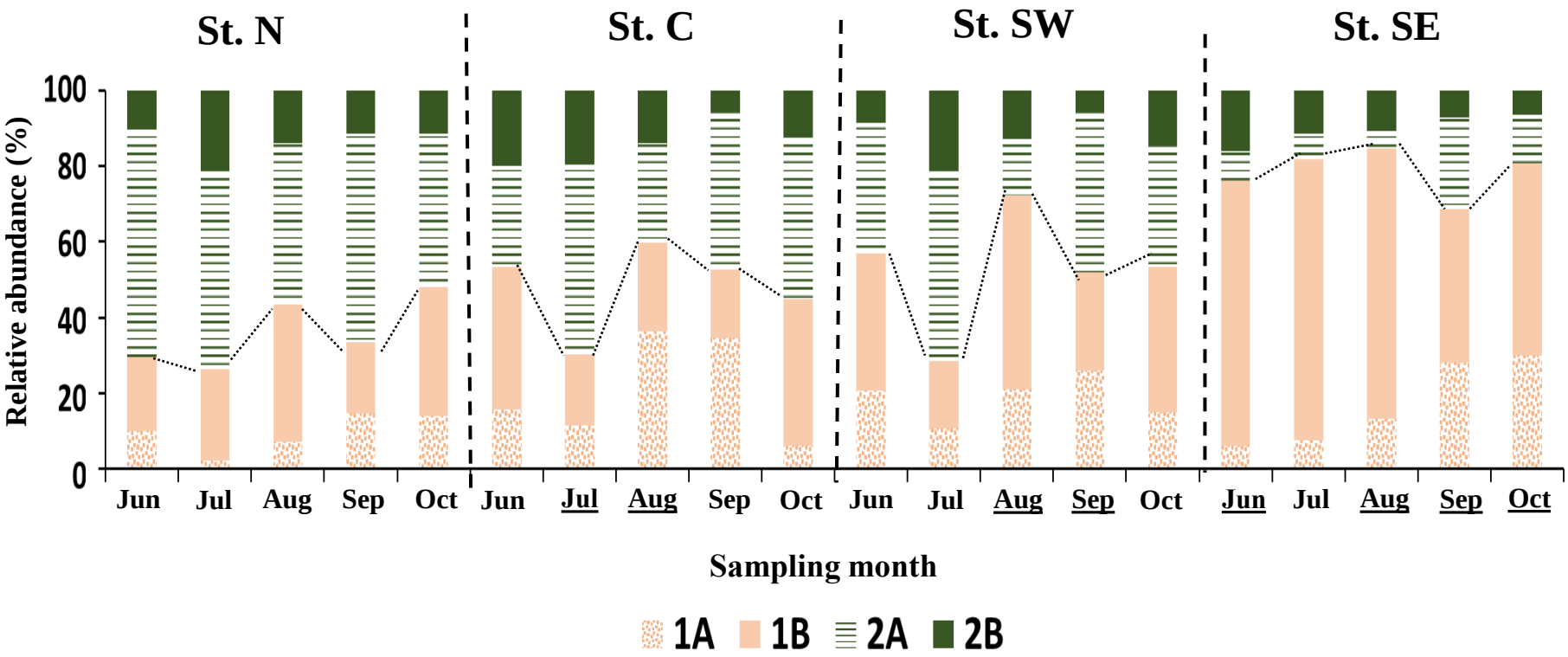

Fig. 14 Relative abundance of nematode feeding types (1A, 1B, 2A, and 2B) at the four sampling sites of Omura Bay between June and October 2017. The round-dot lines indicate changes in the relative percentage of toothless nematodes (types 1A and 1B) and nematodes with teeth (types 2A and 2B) over the four sampling sites. Selective deposit feeders (1A), non-selective deposit feeders (1B), epistrate (diatom) feeders (2A), and predators/omnivores (2B). Hypoxic months were underlined in order to separate from normoxic ones **.**

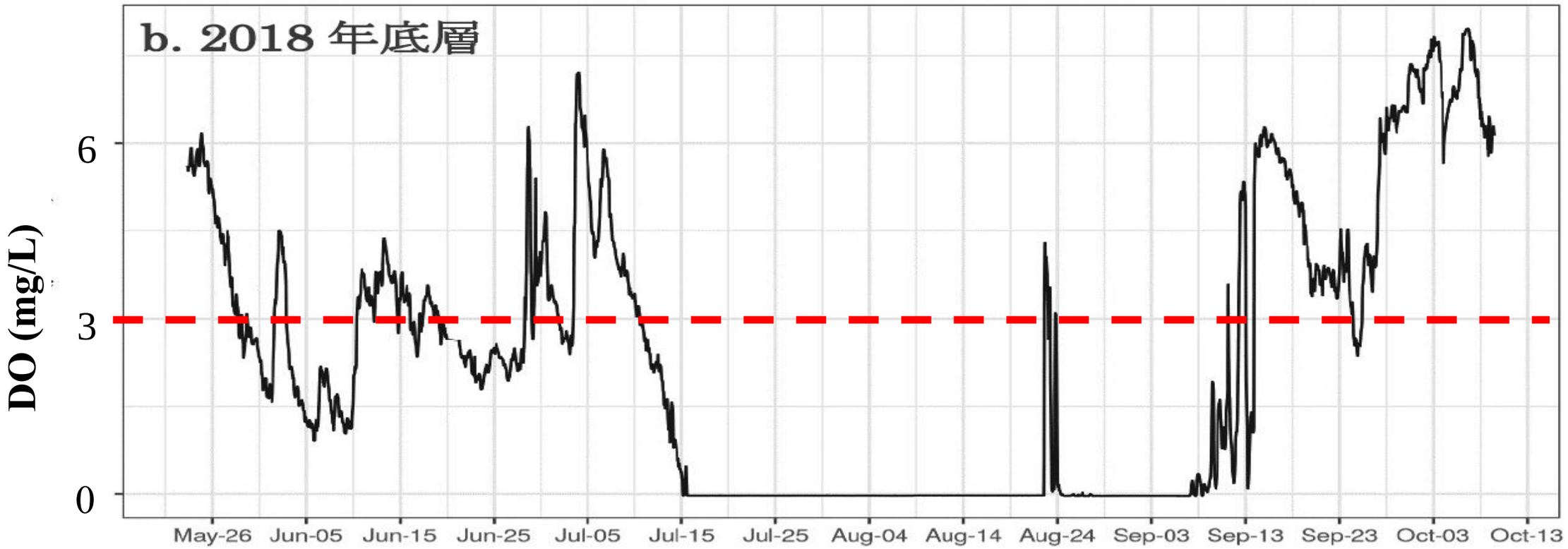

Fig. 15. DO concentration at 1 m above the bottom in the center of Omura Bay in 2018. The dashed lines denote the transition point of DO concentration between hypoxic and normoxic conditions.

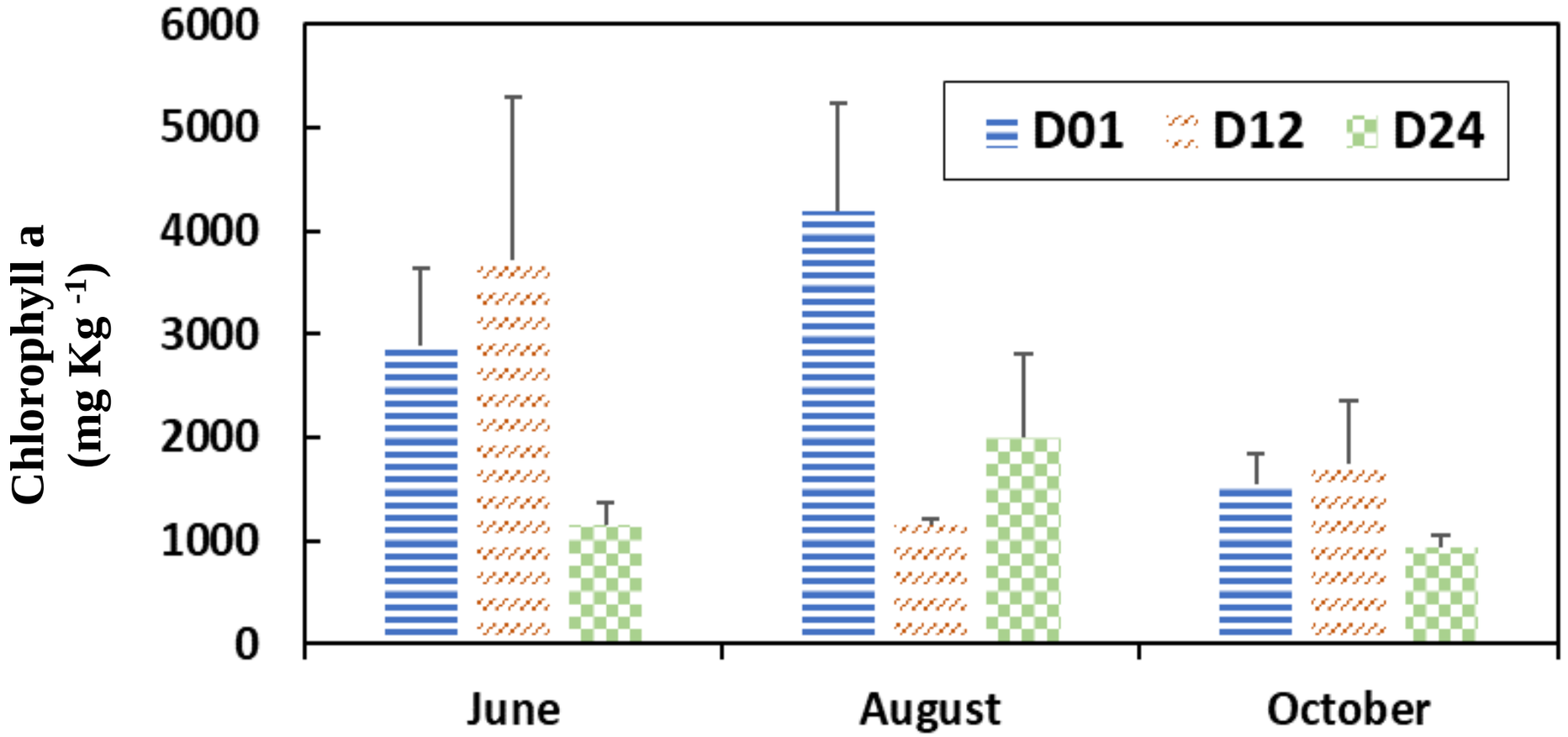

Fig. 16. Sediment chlorophyll a (mean ± standard deviation, n=3) in each depth fraction in the center of Omura Bay between June and October 2018. Hypoxic months were underlined in order to separate with normoxic ones.

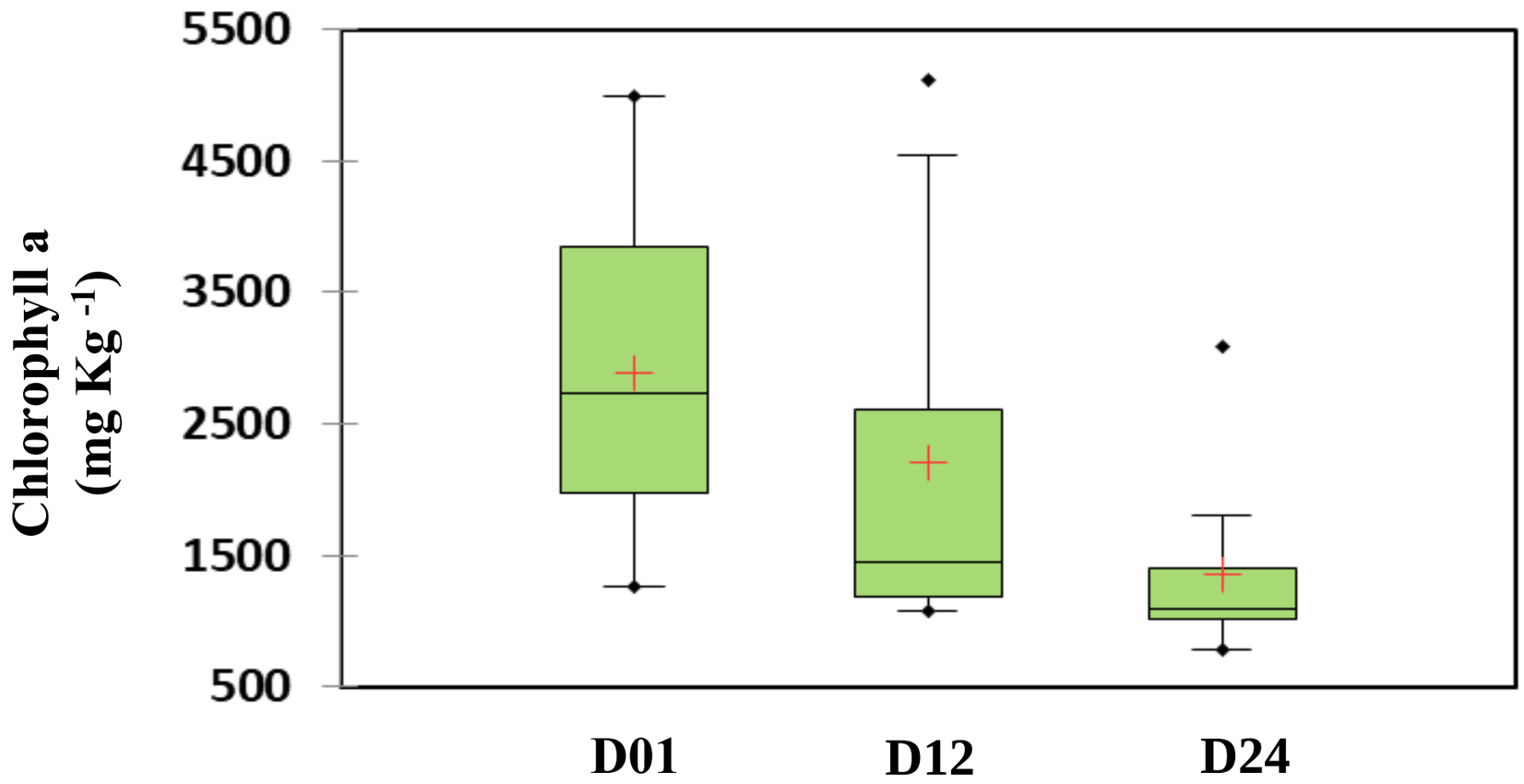

Fig. 17. Sediment chlorophyll a in each depth fraction in the center of Omura Bay between June and October 2018. The crosses indicate the means. The central horizontal bars denote the medians. The lower and upper limits of the box plot are the first and third quartiles, respectively. Points above or below the whiskers' upper and lower bounds are considered as outliers.

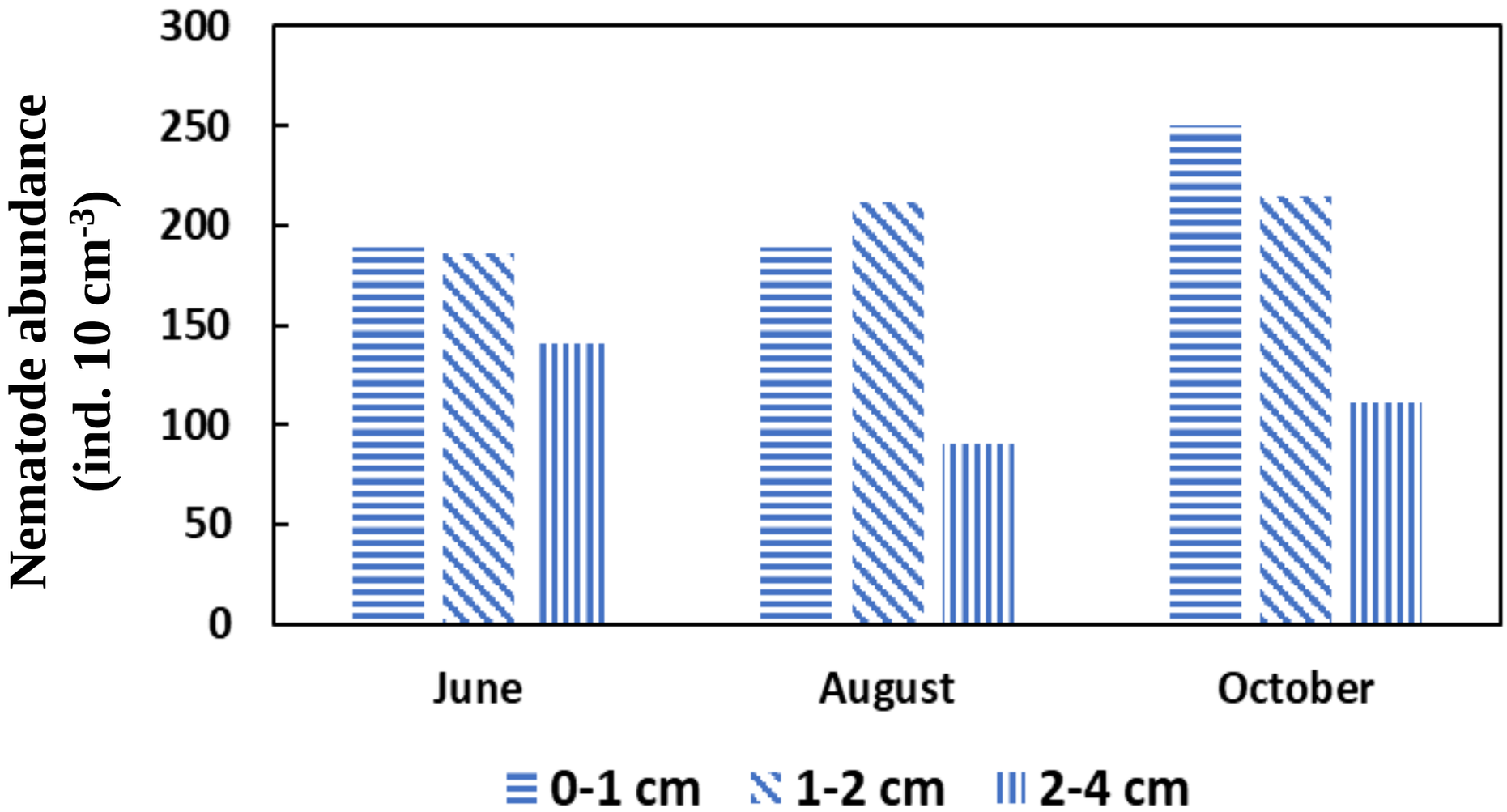

Fig. 18. Nematode abundance in each depth in the center of Omura Bay between Jun and Oct 2018. Hypoxic months were underlined in order to separate with normoxic ones.

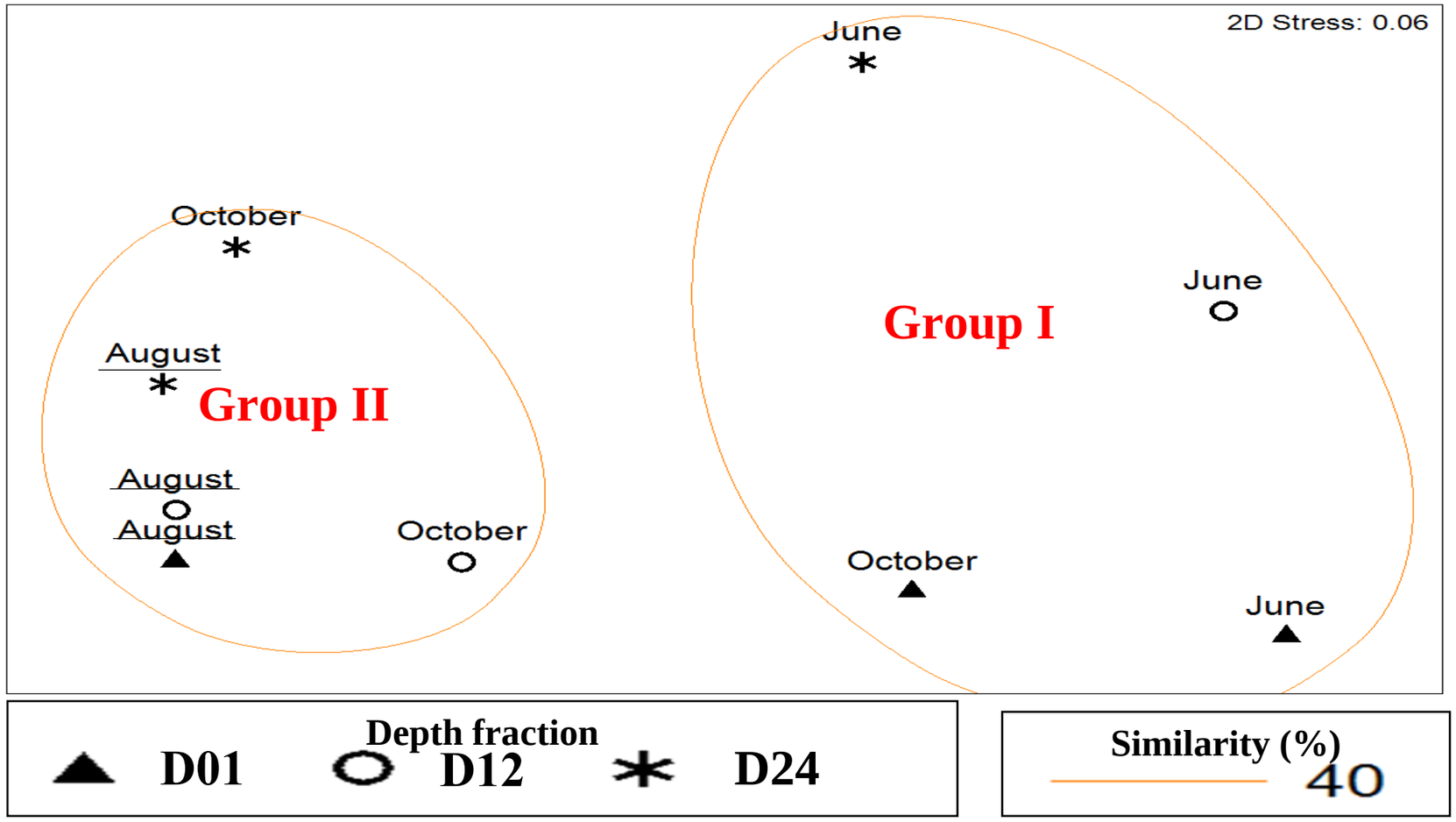

Fig. 19 Non-metric MDS plot of nematode genus composition in each depth fraction in the center of Omura Bay between June and October 2018 with superimposed clusters based on the Bray–Curtis similarity (standardize sample by total; transformation: square root). Continuous lines refer to similarity levels of 40%. Hypoxic months were underlined in order to separate from normoxic ones.

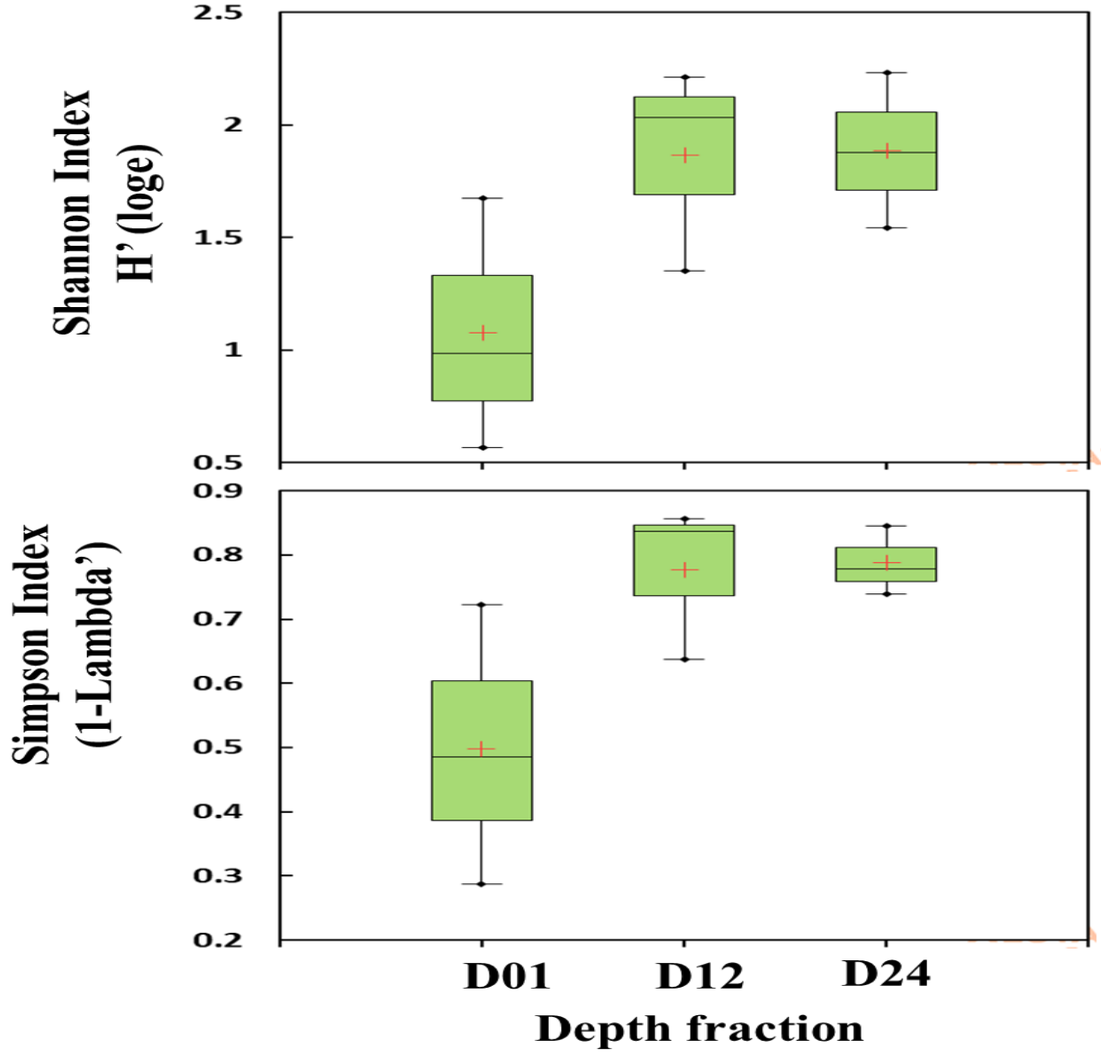

Fig. 20 Shannon and Simpson indexes of nematode diversity at the three depth fractions of Omura Bay between June and October in 2018. The crosses indicate the means. The central horizontal bars denote the medians. The lower and upper limits of the box plot are the first and third quartiles, respectively. Points above or below the whiskers' upper and lower bounds are considered as outliers

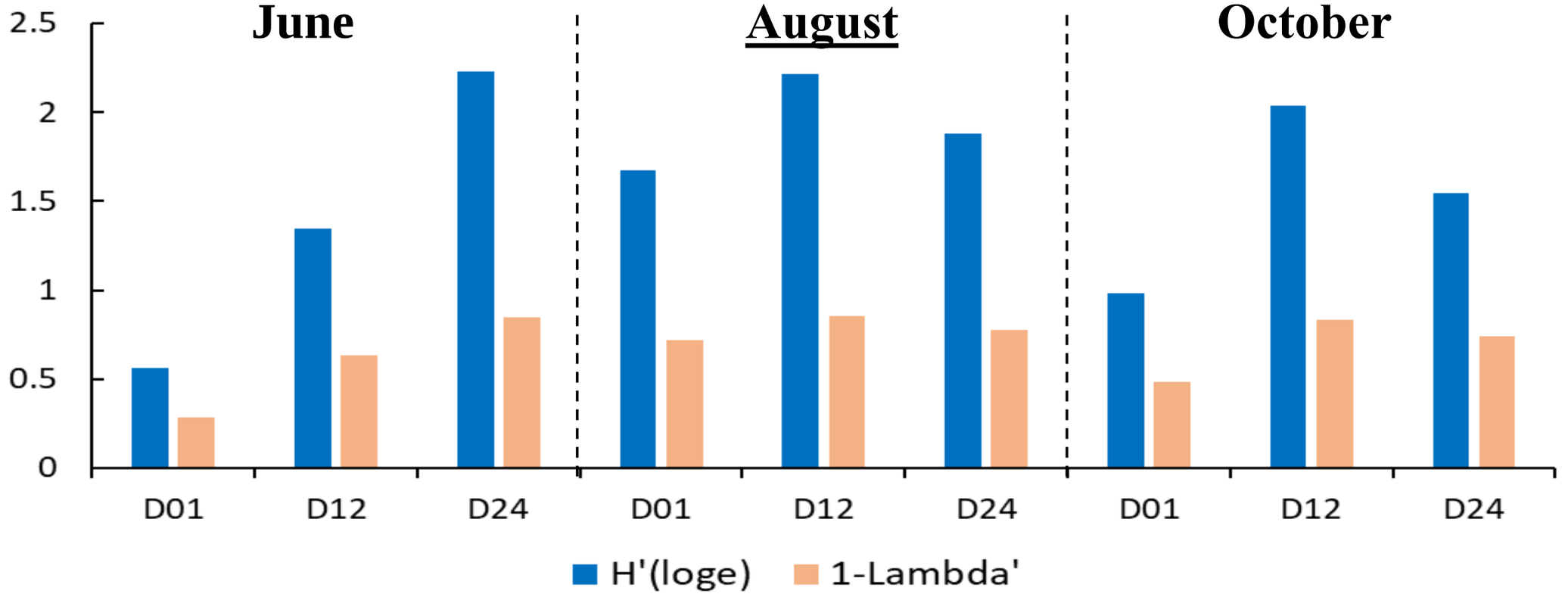

Fig. 21 Shannon (H') and Simpson indexes (1- Lambda') of nematode diversity at the three depth fractions of Omura Bay between June and October in 2018. Hypoxic months were underlined in order to separate from normoxic ones.

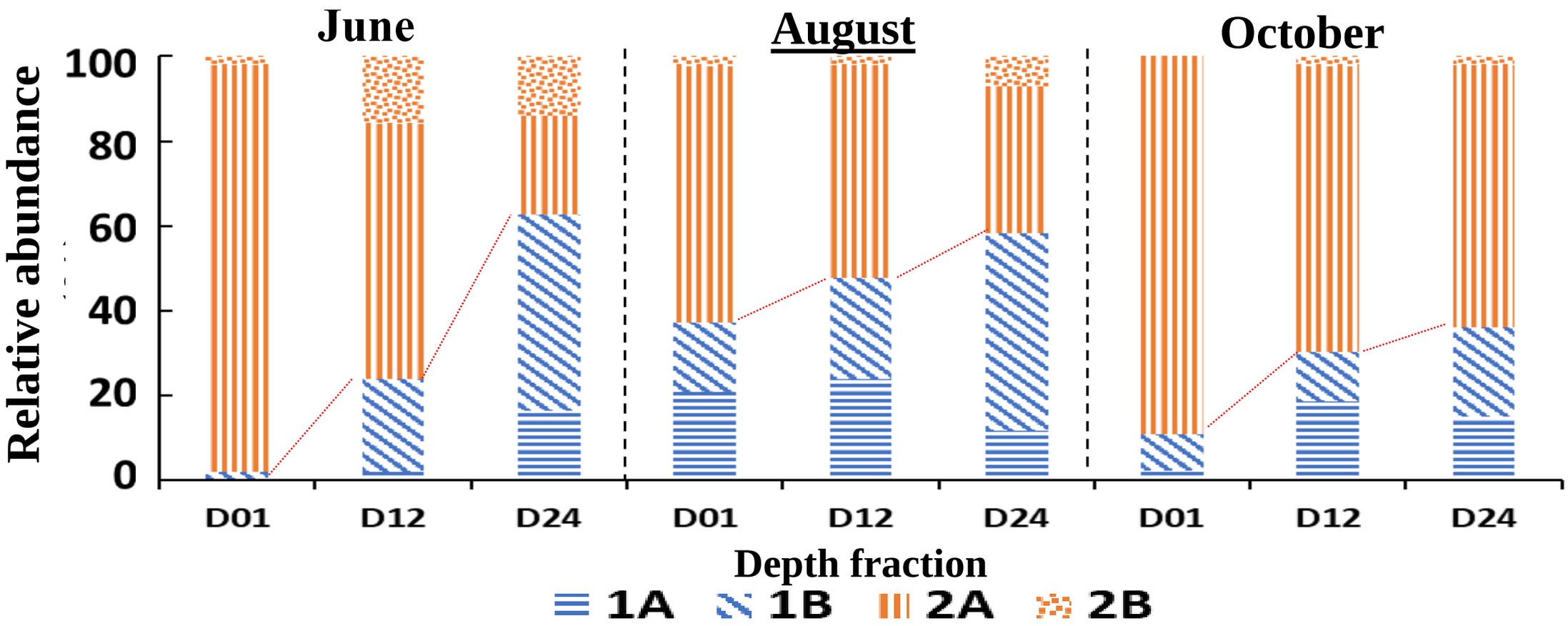

Fig. 22 Relative abundance of nematode feeding types (1A, 1B, 2A, and 2B) at the three depth fractions of Omura Bay between June and October 2017. The red round-dot lines indicate changes in the relative percentage of toothless nematodes (types 1A and 1B) and nematodes with teeth (types 2A and 2B) over the three depth fractions. Selective deposit feeders (1A), non-selective deposit feeders (1B), epistrate (diatom) feeders (2A), and predators/omnivores (2B). Hypoxic months were underlined in order to separate from normoxic ones.

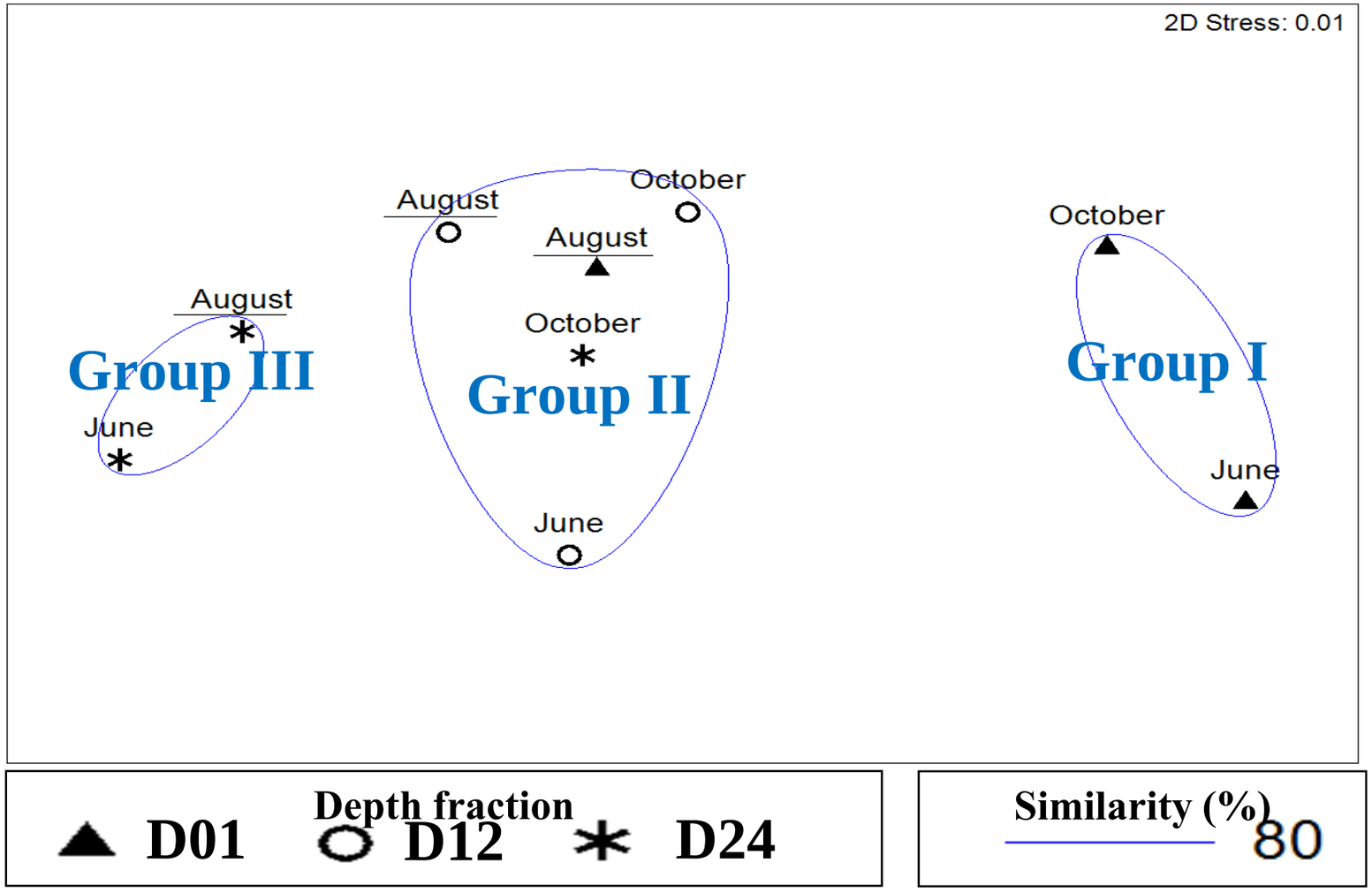

Fig. 23 Non-metric MDS plot of nematode feeding types in each depth fraction in the center of Omura Bay between June and October 2018 with superimposed clusters based on the Bray–Curtis similarity (standardize sample by total; transformation: square root). Continuous lines refer to similarity levels of 80%. Hypoxic months were underlined in order to separate from normoxic ones.

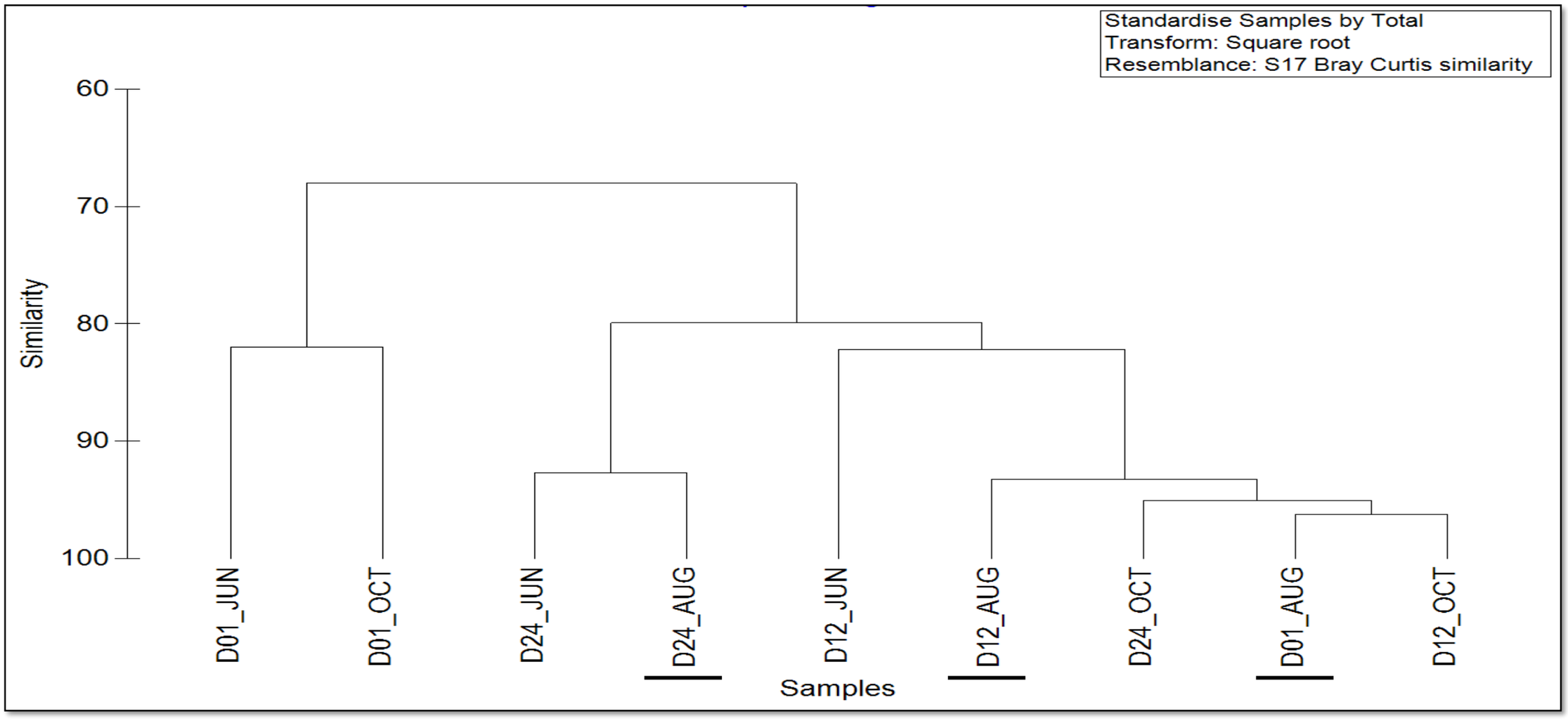

Fig. 24 Hierarchical Cluster analysis of nematode feeding types in each depth fraction in the center of Omura Bay between June and October 2018 with superimposed clusters based on the Bray–Curtis similarity (standardize sample by total; transformation: square root). Hypoxic months were underlined in order to separate from normoxic ones.

Table 1. Summary of DO and water temperature determined with a multi-parameter monitoring device and mean of nematode abundance in the core samples

No.	Sampling date	DO ($mg\ L^{-1}$)	Water temperature (^{o}C)	Mean abundance ($ind.cm^{-2}$)	SD of Mean abundance	SE of Mean abundance
1	2013.06.06	4.45	17.6	431.3	318.2	225.0
2	2013.07.27	0.12	22.7	252.7	103.0	72.8
3	2013.08.24	0.13	26.6	123.7	31.1	22.0
4	2013.09.12	0.10	27.1	120.7	61.4	43.4
5	2013.10.31	6.27	22.1	600.0	451.4	319.2
6	2014.06.19	2.85	19.3	269.3	30.1	21.3
7	2014.07.16	2.24	22.0	328.0	171.6	121.4
8	2014.08.22	1.74	24.9	53.3	21.4	15.1
9	2014.09.22	5.25	25.5	182.0	41.6	29.4
10	2014.10.07	6.50	23.9	292.0	62.9	44.5
11	2015.06.19	4.07	17.0	52.0	22.7	16.1
12	2015.08.03	1.30	22.5	93.3	5.8	4.1
13	2015.08.21	0.66	23.2	58.7	35.2	24.9
14	2015.09.29	3.97	24.3	183.3	32.1	22.7

Table 2. Number of identified nematodes (ind.5 cm^{-2}) in the center of Omura Bay for three years (2013-2015).

Genus	Feeding type	2013.6.6.		
		Core. 1	Core. 2	Core. 3
Atrochromadora	2A	39	44	23
Araeolaimus	1A	6	4	1
Diplopeltula	1A	1		
Chromadorita	2A	1	2	
Halalaimus	1A	1	1	
Axonolaimus	1B		11	23
Daptonema	1B		1	1
Oncholaimus	2B		3	3
Pareurystomina	2B			1
Molgolaimus	2A		1	
Dolicholaimus	1B		1	
Enoplus	2B		1	
Oxystomina	1A		1	
Campylaimus	1B			
Chromadora	2A			
Setoplectus	1A			
Dorylaimus	1A			
Neotonchus	2A			
Siphonolaimus	2B			
Comesoma	1B			
Viscosia	2B			
Camacolaimus	2A			
Symplocostoma	2B			
Metalinhomoeus	1B			
Identified specimens		**48**	**70**	**52**
Unidentified specimens		**2**	**0**	**0**

Feeding type
1A: selective deposit feeders
1B: non-selective deposit feeders
2A: epistrate (diatom) feeders
2B: predators/omnivores

Table 2 (continued)

Genus	2013.8.24			2013.10.31			2014.6.19		
	Core. 1	Core. 2	Core. 3	Core. 1	Core. 2	Core. 3	Core. 1	Core. 2	Core. 3
Atrochromadora	3			2	1	6	40	33	37
Araeolaimus					1				
Diplopeltula									
Chromadorita		1	1	1		6			3
Halalaimus	2	1		1			1	1	
Axonolaimus	5	16	12		1		1	3	4
Daptonema	2	2	1					1	1
Oncholaimus				7	3	1		1	
Pareurystomina									
Molgolaimus	8	13	3	14	3	1			
Dolicholaimus	1								
Enoplus									
Oxystomina									
Campylaimus	2	1	3	1					
Chromadora	10	7	1	12	23	18	1	5	1
Setoplectus	3	2	3		1	4			
Dorylaimus	2		2						
Neotonchus		2	1						
Siphonolaimus		1							
Comesoma					1				
Viscosia								2	
Camacolaimus									1
Symplocostoma									
Metalinhomoeus									
Identified specim	**38**	**46**	**27**	**38**	**34**	**36**	**43**	**46**	**47**
Unidentified spec	**0**	**0**	**0**	**12**	**16**	**14**	**7**	**4**	**3**

Table 2 (continued)

Genus	2014.8.22			2014.10.7			2015.6.19		
	Core. 1	Core. 2	Core. 3	Core. 1	Core. 2	Core. 3	Core. 1	Core. 2	Core. 3
Atrochromadora							3	10	3
Araeolaimus									
Diplopeltula									
Chromadorita		1		3					
Halalaimus			1						
Axonolaimus	14	6	10	3		3	6	5	
Daptonema									
Oncholaimus				5	6	10			
Pareurystomina									
Molgolaimus									
Dolicholaimus									
Enoplus									
Oxystomina									
Campylaimus									
Chromadora	1	1	3	24	23	19			
Setoplectus									
Dorylaimus									
Neotonchus				1					
Siphonolaimus									
Comesoma									
Viscosia									
Camacolaimus									
Symplocostoma									
Metalinhomoeus									
Identified specime	**15**	**8**	**14**	**36**	**29**	**32**	**9**	**15**	**3**
Unidentified speci	**3**	**0**	**0**	**14**	**21**	**18**	**6**	**2**	**3**

Table 2 (continued)

Genus	2015.8.21			2015.9.29			Total	Percentage
	Core. 1	Core. 2	Core. 3	Core. 1	Core. 2	Core. 3		
Atrochromadora				3	1	1	249	31%
Araeolaimus							12	1%
Diplopeltula							1	0%
Chromadorita		1				1	21	3%
Halalaimus		3					12	1%
Axonolaimus	4	10	13	14	9	5	178	22%
Daptonema		1		14	12	15	51	6%
Oncholaimus							39	5%
Pareurystomina							1	0%
Molgolaimus				1			44	5%
Dolicholaimus							2	0%
Enoplus							1	0%
Oxystomina			1				2	0%
Campylaimus							7	1%
Chromadora			3	1		1	154	19%
Setoplectus							13	2%
Dorylaimus							4	0%
Neotonchus							4	0%
Siphonolaimus							1	0%
Comesoma							1	0%
Viscosia							2	0%
Camacolaimus		1			1		3	0%
Symplocostoma			1				1	0%
Metalinhomoeus				1			1	0%
Identified specin	**4**	**16**	**18**	**34**	**23**	**23**	**804**	**82%**
Unidentified spe	**0**	**3**	**2**	**16**	**17**	**19**	**182**	**18%**

Table 3. 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 SIMPER procedure.

Pre-hypoxia			**Mid-hypoxia**			**Post-hypoxia**		
Average similarity: 61.9%			Average similarity: 58.5%			Average similarity: 37.9%		
Genera_Feeding type	Contrib%	Cum%	Genera_Feeding type	Contrib%	Cum%	Genera_Feeding type	Contrib%	Cum%
Atrochromadora _2A	64.0	64.0	*Axonolaimus* _1B	50.0	50.0	*Chromadora* _2A	40.2	40.2
Axonolaimus _1B	25.7	89.7	*Chromadora* _2A	23.3	73.2	*Axonolaimus* _1B	18.3	58.4
Chromadorita _2A	3.15	92.8	*Halalaimus* _1A	12.4	85.6	*Chromadorita* _2A	15.9	74.3
			Chromadorita _2A	11.0	96.5	*Oncholaimus* _2B	12.5	86.8
						Atrochromadora _2A	9.09	95.9

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.

Table 4 Summary of sampling positions, stations, locations, depths, and dates around Omura Bay in 2017

Samling area	Station	Location	Depth (m)	Samling date
North	St. N	33°00′00″ N, 129°51′13.5″ E	18	22-Jun
				14-Jul
				21-Aug
				8-Sep
				17-Oct
Center	St. C	32°56′1.3″ N, 129°51′50.4″ E	20	21-Jun
				13-Jul
				21-Aug
				8-Sep
				16-Oct
South west	St. SW	32°53′11.1″ N, 129°52′19.9″ E	19	21-Jun
				14-Jul
				22-Aug
				9-Sep
				17-Oct
South east	St. SE	32°51′36″ N, 129°58′24″ E	13	22-Jun
				13-Jul
				22-Aug
				9-Sep
				17-Oct

Table 5 List of nematode specimens identified for each station of Omura Bay beween June and October 2017. Figures in parentheses denote proportions of amount of sediment from which investigated specimens were extracted per total amount of sampled sediment. Selective deposit feeders (1A), non-selective deposit feeders (1B), epistrate (diatom) feeders (2A), and predators/omnivores (2B).

Genus	Feeding type	N6 (1/8)	N7 (1/8)	N8 (1/8)	N9 (1/8)	N10 (1/8)
Actinonema	2A	9	5	0	0	3
Amphimonhystera	1A	0	0	0	0	1
Anticoma	1A	2	0	0	0	0
Antomicron	1A	0	0	0	0	0
Axonolaimus	1B	1	11	26	5	14
Bathyeurystomina	2B	1	0	1	0	1
Calligyrus	1A	0	1	0	0	1
Calyptronema	1A	0	0	0	0	0
Campylaimus	1A	1	0	0	1	2
Cervonema	1A	1	0	0	1	6
Chromadorella	2A	15	16	30	38	17
Chromadorina	2A	3	1	1	2	4
Comesoma	1B	0	8	0	0	1
Cyartonema	1A	0	1	1	2	0
Diplolaimella	1B	1	0	0	1	0
Diplopeltula	1B	0	0	0	0	3
Eleutherolaimus	1B	0	0	0	1	1
Enoplolaimus	2B	0	3	3	1	1
Enoplus	2B	0	1	0	0	0
Eurystomina	2B	2	13	9	1	0
Gnomoxyala	1B	0	0	0	0	0
Gomphionchus	2A	0	1	0	0	0
Graphonema	2A	0	0	1	0	0
Halalaimus	1A	1	1	6	5	0
Leptolaimus	1B	0	0	0	0	0
Linhomoeus	1B	0	0	0	0	0
Metalinhomoeus	1B	0	0	0	0	0
Microlaimus	2A	0	0	1	1	0
Molgolaimus	2A	1	0	0	0	1
Nemanema	1A	0	0	0	1	0
Neotonchus	2A	40	41	26	40	29
Oxystomina	1A	2	0	0	0	1
Paramonhystera	1B	0	0	0	0	12
Pareurystomina	2B	4	2	2	5	12
Paroxystomina	1A	4	0	0	1	0
Phanodermopsis	1A	0	0	0	0	0
Procamacolaimus	2A	0	0	0	0	2
Prooncholaimus	2B	1	1	1	3	0
Pseudolella	1B	19	3	10	8	6
Ptycholaimellus	2A	8	11	5	1	3
Richtersia	1B	6	13	15	12	9
Sabatieria	1B	0	0	3	1	1
Southerniella	1B	0	0	0	0	4
Sphaerolaimus	2B	3	2	3	0	0
Spilophorella	2A	7	0	0	0	0
Symplocostoma	2B	2	0	0	0	0
Synonchiella	2B	1	5	1	4	2
Terschellingia	1A	3	0	4	11	10
Vasostoma	2A	0	1	0	1	2
Viscosia	2B	0	4	1	3	1
Total		**138**	**145**	**150**	**150**	**150**
Unidentified		12	5	0	0	0

Table 5
(continued)

Genus	Feeding type	C6 (1/8)	C7 (1/8)	C8 (1/8)	C9 (1/4)	C10 (1/8)
Actinonema	2A	22	20	4	0	1
Amphimonhystera	1A	2	2	4	3	0
Anticoma	1A	0	0	0	0	0
Antomicron	1A	0	0	0	1	1
Axonolaimus	1B	28	16	15	0	38
Bathyeurystomina	2B	0	1	1	0	0
Calligyrus	1A	4	1	3	1	1
Calyptronema	1A	0	0	0	0	0
Campylaimus	1A	2	0	16	12	3
Cervonema	1A	0	0	0	0	0
Chromadorella	2A	0	0	0	0	0
Chromadorina	2A	15	51	32	44	53
Comesoma	1B	0	1	0	0	0
Cyartonema	1A	0	1	2	2	0
Diplolaimella	1B	0	0	0	0	0
Diplopeltula	1B	0	0	0	0	0
Eleutherolaimus	1B	0	0	0	0	0
Enoplolaimus	2B	0	0	0	0	0
Enoplus	2B	0	0	0	0	0
Eurystomina	2B	0	0	0	0	0
Gnomoxyala	1B	3	0	0	0	1
Gomphionchus	2A	0	0	0	0	0
Graphonema	2A	0	0	0	0	0
Halalaimus	1A	8	8	14	11	2
Leptolaimus	1B	0	0	1	0	2
Linhomoeus	1B	0	0	0	0	3
Metalinhomoeus	1B	0	0	0	0	0
Microlaimus	2A	2	1	2	18	10
Molgolaimus	2A	0	0	0	0	0
Nemanema	1A	0	0	0	1	0
Neotonchus	2A	0	1	1	0	0
Oxystomina	1A	3	1	7	9	1
Paramonhystera	1B	0	0	0	0	2
Pareurystomina	2B	21	20	6	0	7
Paroxystomina	1A	0	0	0	0	0
Phanodermopsis	1A	0	0	1	2	0
Procamacolaimus	2A	0	0	0	0	0
Prooncholaimus	2B	3	1	8	6	10
Pseudolella	1B	21	10	14	23	12
Ptycholaimellus	2A	0	1	0	0	0
Richtersia	1B	3	1	5	3	0
Sabatieria	1B	0	0	0	1	0
Southerniella	1B	0	0	0	0	0
Sphaerolaimus	2B	1	2	4	2	2
Spilophorella	2A	0	0	0	0	0
Symplocostoma	2B	0	0	0	1	0
Synonchiella	2B	0	1	1	0	0
Terschellingia	1A	4	4	7	10	1
Vasostoma	2A	0	0	0	0	0
Viscosia	2B	4	4	1	0	0
Total		**146**	**148**	**149**	**150**	**150**
Unidentified		4	2	1	0	0

Table 5
(continued)

Genus	Feeding type	SW6 (1/8)	SW7 (1/8)	SW8 (1/8)	SW9 (1/4)	SW10 (1/8)
Actinonema	2A	7	19	0	0	0
Amphimonhystera	1A	0	0	2	0	3
Anticoma	1A	0	0	0	0	0
Antomicron	1A	0	0	0	1	0
Axonolaimus	1B	26	11	49	19	35
Bathyeurystomina	2B	5	2	2	0	0
Calligyrus	1A	6	2	2	0	0
Calyptronema	1A	0	0	0	0	4
Campylaimus	1A	0	0	1	1	1
Cervonema	1A	0	0	0	0	0
Chromadorella	2A	0	0	0	0	0
Chromadorina	2A	37	56	22	59	41
Comesoma	1B	0	0	2	0	0
Cyartonema	1A	2	0	0	0	0
Diplolaimella	1B	0	0	0	0	0
Diplopeltula	1B	0	0	0	0	0
Eleutherolaimus	1B	0	0	0	0	0
Enoplolaimus	2B	0	0	0	0	0
Enoplus	2B	0	0	0	0	0
Eurystomina	2B	0	0	0	0	0
Gnomoxyala	1B	0	0	0	0	0
Gomphionchus	2A	0	0	0	0	0
Graphonema	2A	0	0	0	0	0
Halalaimus	1A	11	5	5	5	8
Leptolaimus	1B	0	0	0	2	0
Linhomoeus	1B	0	0	0	0	5
Metalinhomoeus	1B	0	3	0	0	0
Microlaimus	2A	3	0	0	4	7
Molgolaimus	2A	2	0	0	0	0
Nemanema	1A	7	0	0	0	0
Neotonchus	2A	0	0	0	0	0
Oxystomina	1A	2	0	6	19	0
Paramonhystera	1B	0	0	7	1	8
Pareurystomina	2B	1	20	6	0	15
Paroxystomina	1A	0	0	0	0	0
Phanodermopsis	1A	0	0	0	0	0
Procamacolaimus	2A	0	0	0	0	0
Prooncholaimus	2B	3	5	2	6	3
Pseudolella	1B	25	13	16	16	9
Ptycholaimellus	2A	0	0	0	0	0
Richtersia	1B	0	0	1	0	0
Sabatieria	1B	0	0	0	1	0
Southerniella	1B	0	0	1	0	0
Sphaerolaimus	2B	1	0	6	2	1
Spilophorella	2A	0	0	0	0	0
Symplocostoma	2B	0	0	0	0	0
Synonchiella	2B	0	0	0	1	0
Terschellingia	1A	1	9	15	13	6
Vasostoma	2A	0	0	0	0	0
Viscosia	2B	2	5	3	0	2
Total		**141**	**150**	**148**	**150**	**148**
Unidentified		9	0	2	0	2

Table 5
(continued)

Genus	Feeding type	SE6 (1/8)	SE7 (1/8)	SE8 (1/8)	SE9 (1/8)	SE10 (1/8)
Actinonema	2A	0	0	0	0	0
Amphimonhystera	1A	1	1	1	2	0
Anticoma	1A	0	0	0	0	0
Antomicron	1A	0	0	0	0	0
Axonolaimus	1B	90	92	101	20	35
Bathyeurystomina	2B	5	0	4	0	0
Calligyrus	1A	2	1	2	2	1
Calyptronema	1A	0	0	0	0	0
Campylaimus	1A	0	1	0	0	1
Cervonema	1A	0	0	0	0	0
Chromadorella	2A	0	1	0	0	0
Chromadorina	2A	12	9	7	27	13
Comesoma	1B	2	0	1	1	2
Cyartonema	1A	0	0	0	0	1
Diplolaimella	1B	0	0	0	0	0
Diplopeltula	1B	0	0	0	0	0
Eleutherolaimus	1B	0	0	0	1	0
Enoplolaimus	2B	1	1	1	2	2
Enoplus	2B	0	1	0	0	0
Eurystomina	2B	0	0	0	0	0
Gnomoxyala	1B	0	0	0	0	0
Gomphionchus	2A	0	0	0	0	0
Graphonema	2A	0	0	0	0	0
Halalaimus	1A	3	1	6	13	8
Leptolaimus	1B	0	0	0	0	0
Linhomoeus	1B	0	0	0	0	0
Metalinhomoeus	1B	1	0	0	0	1
Microlaimus	2A	0	0	0	9	6
Molgolaimus	2A	0	0	0	0	0
Nemanema	1A	0	0	0	0	0
Neotonchus	2A	0	0	0	0	0
Oxystomina	1A	1	0	2	5	6
Paramonhystera	1B	0	0	0	13	3
Pareurystomina	2B	3	0	2	2	0
Paroxystomina	1A	0	0	0	0	0
Phanodermopsis	1A	0	0	0	2	0
Procamacolaimus	2A	0	0	0	0	0
Prooncholaimus	2B	6	0	2	0	0
Pseudolella	1B	8	15	2	21	30
Ptycholaimellus	2A	0	0	0	0	0
Richtersia	1B	1	0	0	0	0
Sabatieria	1B	3	4	3	5	5
Southerniella	1B	0	0	0	0	0
Sphaerolaimus	2B	1	1	1	3	3
Spilophorella	2A	0	0	0	0	0
Symplocostoma	2B	1	0	0	0	0
Synonchiella	2B	1	3	4	0	1
Terschellingia	1A	2	7	9	18	28
Vasostoma	2A	0	0	0	0	0
Viscosia	2B	6	11	2	4	4
Total		**150**	**149**	**150**	**150**	**150**
Unidentified		0	1	0	0	0

Table 6 Contributions of nematode genera to average similarity between all pairs of samples within each sampling site in Omura Bay between June and October 2017 were examined by the SIMPER procedure. Selective deposit feeders (1A), non-selective deposit feeders (1B), epistrate (diatom) feeders (2A), and predators/omnivores (2B).

North (St. N)			**Center (St. C)**			**South west (St. SW)**			**South east (St. SE)**		
Average similarity: 62.3%			Average similarity: 65.1%			Average similarity: 62.5%			Average similarity: 67.9%		
Genera Feeding type	Contrib%	Cum%	Genera Feeding type	Contrib%	Cum%	Genera Feeding type	Contrib%	Cum%	Genera Feeding type	Contrib%	Cum%
Neotonchus 2A	17.8	17.8	*Chromadorina* 2A	18.3	18.3	*Chromadorina* 2A	22.9	22.9	*Axonolaimus* 1B	24.2	24.2
Chromadorella 2A	13.4	31.2	*Pseudolella* 1B	12.1	30.4	*Axonolaimus* 1B	16.7	39.6	*Chromadorina* 2A	11.1	35.2
Richtersia 1B	9.5	40.7	*Axonolaimus* 1B	8.6	39.0	*Pseudolella* 1B	13.7	53.3	*Pseudolella* 1B	9.6	44.9
Pseudolella 1B	7.4	48.1	*Halalaimus* 1A	7.9	46.9	*Halalaimus* 1A	9.0	62.3	*Terschellingia* 1A	8.7	53.6
Axonolaimus 1B	6.8	54.9	*Pareurystomina* 2B	5.9	52.8				*Sabatieria* 1B	6.8	60.4
Ptycholaimellus 2A	5.3	60.2	*Prooncholaimus* 2B	5.8	58.6						
			Terschellingia 1A	5.7	64.3						

Contrib% = contribution of each genus to total average similarity within a sampling site. Cum% = cumulative contribution. Each list was truncated when 60% was reached.

Table 7 Rank abundance (%) of nematode genera for each sampling site in Omura Bay based on the total specimens through the study period. "n" indicates the total number of the nematode specimens identified to genus for each sampling site. Selective deposit feeders (1A), non-selective deposit feeders (1B), epistrate (diatom) feeders (2A), and predators/omnivores (2B).

St. N (n = 733)				St. C (n = 743)			
Genera	Feeding type	Rank abundance	Cumulative percentage	Genera	Feeding type	Relative abundance	Cumulative percentage
Neotonchus	2A	24.0%	24.0%	*Chromadorina*	2A	26.2%	26.2%
Chromadorella	2A	15.8%	39.8%	*Axonolaimus*	1B	13.1%	39.3%
Axonolaimus	1B	7.8%	47.6%	*Pseudolella*	1B	10.8%	50.1%
Richtersia	1B	7.5%	56.0%	*Pareurystomina*	2B	7.3%	57.3%
Pseudolella	1B	6.3%	62.0%	*Actinonema*	2A	6.3%	63.0%
Ptycholaimellus	2A	3.8%	66.0%	*Halalaimus*	1A	5.8%	69.0%
Terschellingia	1A	3.8%	70.0%				

St. SW (n = 737)				St. SE (n = 749)			
Genera	Feeding type	Relative abundance	Cumulative percentage	Genera	Feeding type	Relative abundance	Cumulative percentage
Chromadorina	2A	29.2%	29.2%	*Axonolaimus*	1B	45.1%	45.1%
Axonolaimus	1B	19.0%	48.2%	*Pseudolella*	1B	10.1%	55.3%
Pseudolella	1B	10.7%	58.9%	*Chromadorina*	2A	9.1%	64.4%
Terschellingia	1A	6.0%	64.9%	*Terschellingia*	1A	8.5%	72.9%
Pareurystomina	2B	5.7%	70.6%				

Each list was truncated when 70% was reached.

Table 8 Contributions of nematode feeding types to average similarity between all pairs of samples within each sampling site in Omura Bay beween June and October 2017 were examined by the SIMPER procedure. Selective deposit feeders (1A), non-selective deposit feeders (1B), epistrate (diatom) feeders (2A), and predators/omnivores (2B).

North (St. N)			**Center (St. C)**			**South west (St. SW)**			**South east (St. SE)**		
Average similarity: 90.7%			Average similarity: 86.8%			Average similarity: 87.7%			Average similarity: 86.6%		
Feeding type	Contrib%	Cum%	Feeding type	Contrib%	Cum%	Feeding type	Contrib%	Cum%	Feeding type	Contrib%	Cum%
2A	39.3	39.3	2A	33.1	33.1	2A	30.0	30	1B	46.1	46.1
1B	27.1	66.4	1B	27.4	60.5	1B	29.9	59.9	1A	19.4	65.5
2B	19.5	86.0	1A	20.2	80.7	1A	22.7	82.6	2B	18.2	83.7
1A	14.0	100	2B	19.3	100	2B	17.4	100	2A	16.3	100

Contrib% = contribution of each genus to total average similarity within a sampling site. Cum% = cumulative contribution.

Table 9 The best combinations of seven enviromental variables and nematode commmunity structure measured by weighted Spearman rank correlation (p_w). Temperature (^oC) (1), Salinity (‰) (2), Water chlorophyl a ($\mu g\ L^{-1}$) (3), DO ($mg\ L^{-1}$) (4), Grain size (μm) (5), Weight loss on ignition (%) (6), Sediment chlorophyl a ($mg\ Kg^{-1}$) (7)

Nematode genus compositions		**Nematode feeding types**	
Rho: 0.666	$p < 0.05$	Rho = 0.598	$p < 0.05$
Correlation (p_w)	Selections	Correlation (p_w)	Selections
0.666	6	0.598	2,3,4,5,6
0.41	6,7	0.597	2,4,5
0.402	2,4,6,7	0.595	2,4,5,6
0.4	3,4,6,7	0.586	2,3,4,5
0.399	2,6,7	0.576	2,4,6
0.398	3,6,7	0.574	2,3,4,6
0.398	2,3,4,6,7	0.573	2,3,4
0.397	4,6,7	0.568	2,4
0.396	1,2,4,6,7	0.567	3,4,5
0.393	7	0.561	1,2,3,4,6

Table 10. Summary of sampling positions, dates, locations, depths from sea surface, depth of sampled sediment, and sample name around Omura Bay in 2018

Samling date	Depth of sampled sediment (cm)	Sample name
6-Jun	0 - 1	D01_JUN
6-Jun	1 - 2	D12_JUN
6-Jun	2 - 4	D24_JUN
23-Aug	0 - 1	D01_AUG
23-Aug	1 - 2	D12_AUG
23-Aug	2 - 4	D24_AUG
9-Oct	0 - 1	D01_OCT
9-Oct	1 - 2	D12_OCT
9-Oct	2 - 4	D24_OCT

Table 11. Summary of bottom water parameters (DO concentration, water temperature, salinity, and water chlorophyll a) in the center of Omura Bay between June and October 2018.

Sampling Date	Water temperature (^{o}C)	Salinity (‰)	Chlorophyl a in water ($\mu g\ L^{-1}$)	DO ($mg\ L^{-1}$)
6-Jun	18.5	32.9	1.68	3.34
23-Aug	26.5	32.6	1.41	2.46
9-Oct	24.3	32.7	1.66	5.28

Table 12 List of nematode specimens identified for each depth fraction of Omura Bay beween June and October 2018. Figures in parentheses denote proportions of amount of sediment from which investigated specimens were extracted per total amount of sampled sediment. Selective deposit feeders (1A), non-selective deposit feeders (1B), epistrate (diatom) feeders (2A), and predators/omnivores (2B). The major genera were wirtten in red and bold font.

Genus	Feeding type	D01_JUN (1/8)	D12_JUN (1/8)	D24_JUN (1/16)	D01_AUG (1/8)	D12_AUG (1/8)	D24_AUG (1/16)	D01_OCT (1/8)	D12_OCT (1/8)	D24_OCT (1/16)	**Total**
Actinonema	**2A**	**41**	**28**	**2**	**0**	**0**	**0**	**8**	**8**	**1**	88
Amphimonhystera	1A	0	0	1	0	0	0	0	0	0	1
Araeolaimus	1B	0	0	0	2	4	0	0	3	0	9
Axonolaimus	1B	1	9	14	0	1	2	3	1	0	31
Camacolaimus	1A	0	0	0	0	0	0	0	1	0	1
Campylaimus	1A	0	0	1	1	1	1	0	3	4	11
Chromadorina	**2A**	**6**	**2**	**8**	**21**	**14**	**10**	**32**	**12**	**8**	113
Cyartonema	1A	0	0	0	1	1	2	0	1	3	8
Diplolaimella	1B	0	0	0	1	1	1	0	1	0	4
Enoplus	2B	0	0	1	0	1	1	0	0	0	3
Halalaimus	1A	0	1	2	1	3	1	1	1	0	10
Microlaimus	2A	0	0	0	5	7	4	1	9	20	46
Nemanema	1A	0	0	0	6	4	0	0	2	0	12
Oxystomina	1A	0	0	2	0	1	1	0	0	0	4
Paracyatholaimus	2A	0	0	0	0	2	0	0	0	0	2
Pareurystomina	2B	0	1	1	0	0	0	0	0	0	2
Pareurystomina	2B	0	0	1	0	0	0	0	0	0	1
Prooncholaimus	2B	1	0	0	0	0	0	0	1	0	2
Pseudolella	1B	0	1	3	4	5	17	1	0	10	41
Retrotheristus	1B	0	1	3	0	0	0	0	0	0	4
Sphaerolaimus	2B	0	0	0	0	0	2	0	0	0	2
Spilophorella	2A	0	0	0	0	0	1	0	0	0	1
Symplocostoma	2B	0	0	2	0	0	0	0	0	0	2
Synonchiella	2B	0	0	0	1	0	0	0	0	0	1
Terschellingia	1A	0	0	1	0	1	0	0	0	0	2
Thoonchus	2B	0	7	1	0	0	0	0	0	1	9
Unidentified	Unidentified	1	0	7	7	4	7	4	7	3	40
Total		50	50	50	50	50	50	50	50	50	450

Table 13. Contributions of nematode genera to average similarity between all pairs of samples within each depth fraction in Omura Bay between June and October 2018 were examined by the SIMPER procedure. "n" indicates the total number of the nematode specimens identified to genus for each depth fraction. Selective deposit feeders (1A), non-selective deposit feeders (1B), epistrate (diatom) feeders (2A), and predators/omnivores (2B).

D01 (n = 138)			**D12 (n = 139)**			**D24 (n = 133)**		
Average similarity: 39.2%			Average similarity: 41.04%			Average similarity: 47.9%		
Genera Feeding type	Contrib%	Cum%	Genera Feeding type	Contrib%	Cum%	Genera Feeding type	Contrib%	Cum%
Chromadorina 2A	56.6	56.6	*Chromadorina* 2A	25.1	25.1	*Chromadorina* 2A	29.94	29.94
Actinonema 2A	20.3	76.9	*Actinonema* 2A	13.0	38.1	*Pseudolella* 1B	24.05	53.99
Axonolaimus 1B	6.9	83.8	*Axonolaimus* 1B	12.5	50.7	*Campylaimus* 1A	10.92	64.9
Halalaimus 1A	5.4	89.2	*Halalaimus* 1A	12.2	62.8	*Microlaimus* 2A	7.95	72.85
Microlaimus 2A	5.4	94.6	*Microlaimus* 2A	9.9	72.7	*Cyartonema* 1A	5.62	78.47
			Araeolaimus 1B	6.7	79.3	*Axonolaimus* 1B	4.66	83.14
			Nemanema 1A	5.5	84.8	*Actinonema* 2A	3.49	86.62
			Pseudolella 1B	4.05	88.83	*Thoonchus* 2B	3.49	90.11
			Campylaimus 1A	3.72	92.55			

Contrib% = contribution of each genus to total average similarity within a sampling site. Cum% = cumulative contribution. Each list was truncated when 90% was reached.