

Article

Distribution of Diatom Resting Stages in Sediment near Artificial Reefs Deployed in the Dysphotic Zone: Exploration of New Artificial Reef Function

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Featured Application: Our findings provide a crucial perspective on a novel accumulation effect of diatom resting stages by the deployment of artificial reefs, which may enhance the primary production of the overlying water column, subsequently contributing to the augmentation of fishery productivity through food chains.

Abstract: Artificial reefs (ARs) have multiple beneficial effects involved in increased fishery production. The present study investigated diatom resting stages in the sediments near ARs at a depth of about 90 m by the combined use of direct microscopic observation and the MPN method, seeking a novel function of AR deployment that potentially increases biological production. Although the concentration of diatom resting stages at A2 (north end station of the AR area), composed mainly of *Chaetoceros*, was ten times the concentration at A5 (non-reef station), the concentration at A1 (south end station of the AR area) was the same level as at A5. These results suggest that the AR contributes to the accumulation of diatom resting stages while the degree of accumulation is attributed to the arrangement of ARs and their physical environment such as flow directions. Interestingly, live pennate diatoms possessing photosynthetic pigments were constantly observed in all samples despite the low light intensity ($\leq 1.2 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$), which provides new ecological insight into their biological and physiological characteristics. In this study, the distribution of viable diatom resting stages and live pennate diatoms in the sediment around ARs in a dysphotic zone was revealed for the first time, casting a light on the novel function of ARs potentially enhancing primary production around them.

Keywords: artificial reef; diatom resting stages; pennate diatoms; artificial reef arrangement; wake region; primary production



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1. Introduction

Artificial reefs (ARs), which are already used worldwide for multiple purposes, mainly involving fishery enhancement [1–3], are defined as “submerged structures deliberately constructed or placed on the seabed to emulate some functions of a natural reef such as protecting, regenerating, concentrating, and/or enhancing populations of living marine resources” [4], and the deployment of ARs has been proposed as an effective tool to enhance fishery resources in the ocean [5]. Although it is a well-known fact that fish aggregate densely around ARs, it is still controversial whether the fish aggregation is attributed to attraction (ARs merely attract fish from surrounding areas) or production (ARs contribute to fish reproduction), and Simon et al. [6] concluded that both attraction and production may occur around ARs by comparing ARs with natural reefs. An ecosystem perspective on the function of ARs, such as enhanced primary productivity, will surely provide new insight into the unresolved “attraction versus production” debate [7,8]. Actually, an increase in

phytoplankton in a water column was pointed out by previous studies after the deployment of ARs [9,10]. In this way, AR deployment may play a role in enhancing the primary production of the overlying water column, subsequently contributing to the augmentation of fishery productivity through food chains.

Among the primary producers in the ocean, diatoms (Bacillariophyceae) are the main organisms and are estimated to contribute as much as 20% to the total primary production [11,12]. Many diatom species have been shown to form dormant cells called resting stages (resting spores and/or resting cells) to survive under unfavorable environmental conditions [13,14]. The formation of resting stages is induced when they are exposed to adverse conditions, such as nutrient depletion [15]. Resting stages settle down on the seafloor and will germinate/rejuvenate when the environmental conditions are favorable for their growth by being resuspended in a water column by a disturbing event such as vertical mixing and/or upwelling [16,17]. Therefore, diatom resting stages can serve as a seed population for a future diatom bloom [18]. If AR deployment increases pelagic diatom populations in the water column, the subsequent formation of diatom resting stages around AR is likely to be enhanced simultaneously.

One of the beneficial physical characteristics of ARs is a wake region generated behind an AR on the downstream side of the prevailing flow, where counterflows and eddies occur [19]. The flows and eddies allow phytoplankton and biodeposits in the water column to remain there and accumulate around the structure [19,20]. It is thus likely that an artificial reef also traps settling diatom resting stages and accumulates them in the vicinity. Through the above-mentioned processes, deployment of an AR may not only enhance primary production in the sympatric water column but also potentially boost primary production by supplying diatom resting stages. To our best knowledge, however, no studies have ever investigated the distribution of diatom resting stages around ARs.

In the present study, we investigated the concentration and composition of resting stages and vegetative cells of diatoms by direct microscopic observation and a culturing method (MPN method) of the sediment around ARs and a non-reef station at a depth of about 90 m in the dysphotic zone to reveal the accumulation effect of ARs.

2. Materials and Methods

2.1. Study Area

The study area was around an artificial reef system established by the Hokkaido prefectural government in 2017, located 10 km off the south of Rishiri Island (45°10' N, 141°13' N), Hokkaido, in the northernmost part of Japan (Figure 1a). The AR system comprised 3 artificial tower reefs made of steel (10 m by 10 m, 20 m tall) in the center and 156 cube reefs made of concrete (3 × 3 × 3 m) sporadically deployed in the outer circular zone (Figure 1b,c). Samplings were conducted at three stations (A1, A2, and A5) in the study area (Figure 1d). A1 was located at the innermost side of the circular zone; in contrast, A2 was located at the outermost side, while A5 was a non-reef station 150 m off the outer edge of the circular zone. Detailed information on the position and bottom depth of each station is summarized in Table 1.

Table 1. The position and bottom depth of the sampling stations (A1, A2, and A5).

Station	Latitude (N)	Longitude (E)	Water Depth (m)
A1	45°02'32.600''	141°07'54.368''	90.9
A2	45°02'34.220''	141°07'55.028''	91.7
A5	45°02'39.079''	141°07'54.848''	93.7

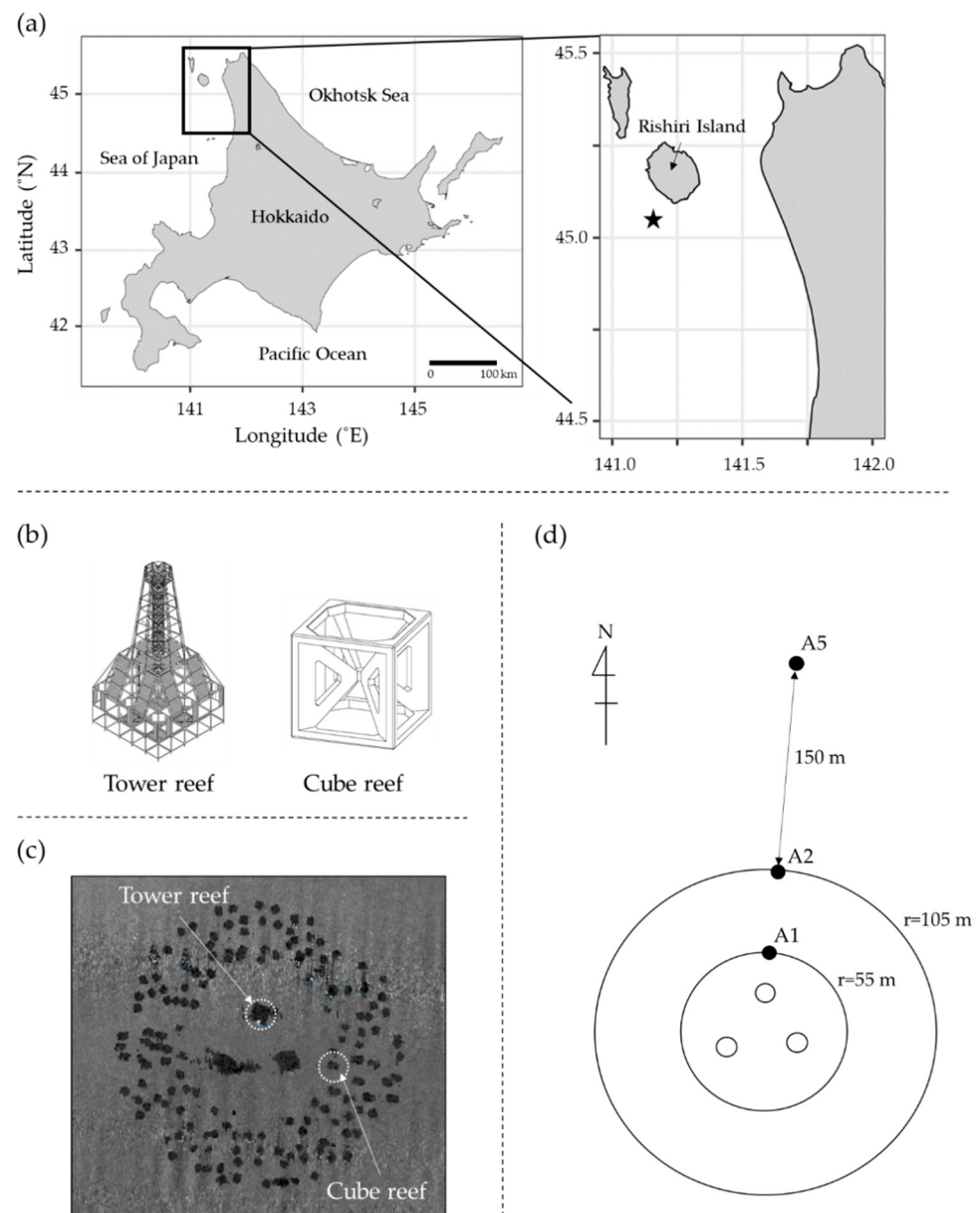


Figure 1. Location of the study area and details of the artificial reef (AR) system: (a) location of the AR system (black star), (b) detailed structure of a tower reef (20 m high), and a cube reef (3 m high) deployed in the AR system, (c) the distribution of tower reefs and cube reefs in the AR system detected by side-scan sonar, and (d) location of the sampling stations (A1, A2, and A5); an open circle represents a tower reef.

2.2. Hydrographic Observation

The water temperature and light intensity at A2 and A5 were continuously observed with a water temperature logger (HOBO TidbiT v2 UTBI-001, Onset, Bourne, MA, USA) installed right above the bottom and a light quantum meter (COMPACT-LW, JFE Advantech, Hyogo, Japan) deployed 3.0 m above the seafloor. To observe the flow speed and direction near the seafloor, an electromagnetic flowmeter (INFINITY-EM, JFE Advantech, Hyogo, Japan) was also installed at 3.0 m above the seafloor of A5. All the observation devices were moored to an anchored rope at each station. The observation period was from 25 July 2018 to 7 August 2018. Vertical profiles of environmental variables (temperature, salinity, density, and chlorophyll fluorescence) were also measured with CTD (RINKO-Profilier ASTD102, JFE Advantech, Hyogo, Japan) at all the stations on 29 July 2018.

2.3. Particle Size Distribution and Organic Matter Measurements

Bottom sediments were collected with a Smith–McIntyre grab (0.05 m²) (RIGO, Tokyo, Japan) at each station. The particle size distribution in part of each sample was determined according to the Japanese Industrial Standards A 1204 (JIS A 1204). Sediment samples for loss on ignition (LOI) measurement were weighed and then heated to 600 °C for 2 h in a muffle furnace (FO510, Yamato Scientific, Tokyo, Japan) after drying at 105 °C until a constant weight was obtained. The total organic carbon (TOC) and total organic nitrogen (TON) concentrations of each sediment were also determined by dry combustion using a Sumigraph NC-220F (Sumika Chemical Analysis Service, Osaka, Japan).

2.4. Diatom Community and Population in Sediment

2.4.1. Sampling

Bottom sediments for diatom analysis were collected with the Smith–McIntyre grab at A5, while the samples at A1 and A2 were collected with an Ekman–Birge grab (0.02 m²) (RIGO, Tokyo, Japan) attached to a remotely operated vehicle (ROV) (Mitsui RTV. N-Hyper300EXY, Mitsui E&S Holdings, Tokyo, Japan) from the vicinity of a cube reef after visual confirmation through the ROV. The sediment samples were rapidly transported under cool-dark conditions to a laboratory and stored for two months at 4 °C under dark conditions. In the present study, compositions and concentrations of diatom resting stages were determined using the two methods below.

2.4.2. Direct Microscopic Observation

Resting stages of diatoms in the sediment samples were counted directly as follows, according to the method described by Ishii et al. [21]. One gram in wet weight of the sediment sample was suspended in filtered autoclaved seawater and sieved with 100, 20, and 10 µm nylon mesh in succession. The respective fractions captured on the 20 and 10 µm nylon mesh were each suspended in 10 mL of filtered autoclaved seawater. An aliquot of the suspensions was then placed on a glass slide and examined with inverted fluorescence microscope (IX-70, OLYMPUS, Tokyo, Japan) to identify and enumerate vegetative cells and resting stages of diatoms. The presence or absence of chloroplasts was also determined using the inverted fluorescence microscope. Identification of diatom resting stages was conducted in reference to Rines and Hargraves [22], Hasle and Syvertsen [23], Jensen and Moestrup [24], and Ishii et al. [25].

2.4.3. MPN Method

The number of viable diatom resting stages in the sediments was estimated by the most probable number (MPN) method [26,27]. The sediment sample (1.0 g wet weight) was suspended in autoclaved culture medium (Daigo's IMK medium (Shiotani MS, Hyogo, Japan) + silicate) at a concentration of 0.1 g mL⁻¹. The suspension was successively diluted with the medium (10⁻², 10⁻³, 10⁻⁴, and 10⁻⁵), and 1 mL aliquots of each dilution were inoculated into five wells each on a microplate (48 wells). Incubation was carried out at 20 °C under a light intensity of about 100 µmol photons m⁻² s⁻¹ with a 14:10 h light–dark photocycle for 7 days, and the appearance of diatom vegetative cells in the five wells of each dilution series was examined with the inverted fluorescence microscope to calculate MPN, according to the statistical table of Throndsen [28].

3. Results

3.1. Environmental Conditions

The vertical profiles of the environmental variables closely agreed between the stations (Figure 2). Thermal and salinity stratification was observed at depths greater than about 55 m and chlorophyll fluorescence also peaked at around 55 m depth at all the stations. The vertical profile of water temperature was consistent among the stations, ranging from 7.5 to 20 °C. In particular, the influence of artificial reefs on hydrography was not seen. The temporal changes in water temperature near the seabed at A2 and A5 also

exhibited a similar trend, ranging from 7.0 to 7.9 °C and from 7.1 to 8.0 °C, respectively. The light intensity near the seafloor was generally consistent at the two stations, ranging between 0.10 and 1.2 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at A2, and between 0.10 and 0.89 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at A5, respectively. The measured average flow speed near the seafloor at A5 was 0.06 m s^{-1} , and the maximum flow speed was 0.16 m s^{-1} during the study period, and north-northwestward flows were dominant (31.4%) (Figure 3). The particle size in the bottom sediment was the coarsest at A1 among the stations; the fraction of 4.75–19 mm contributed 44.4% and the median diameter (D_{50}) was 3.70 mm (Table 2). The particle size distribution at A2 showed a higher proportion of particles smaller than 0.85 mm than at A1 (A1: 35.8%, A2: 49.7%), although the fraction of particles of 4.75–19 mm was also relatively high (29.5%), while the D_{50} showed a middle value (0.89 mm) among the sites. The particle size distribution of A5 was dominated by the fraction of <0.005 mm (28.3%) and the D_{50} was the smallest among the three stations (0.12 mm). Overall, the particle sizes of the sediment at the AR stations (A1, A2) were larger than at the non-reef station (A5). The LOI of the sediment sample was 4.1% at A2 and A5 and was relatively low at A1 (3.5%). The TOC concentration in the sediment of A1, A2, and A5 was 5.2, 5.9, and 5.4 mg g^{-1} , respectively, while the content of TON was under the limit of quantification (<1.0 mg g^{-1}) at all the stations. The LOI and TOC concentrations at A2 showed the highest values among the three stations. In contrast, those at A1 located in the AR area also showed the lowest values among the stations.

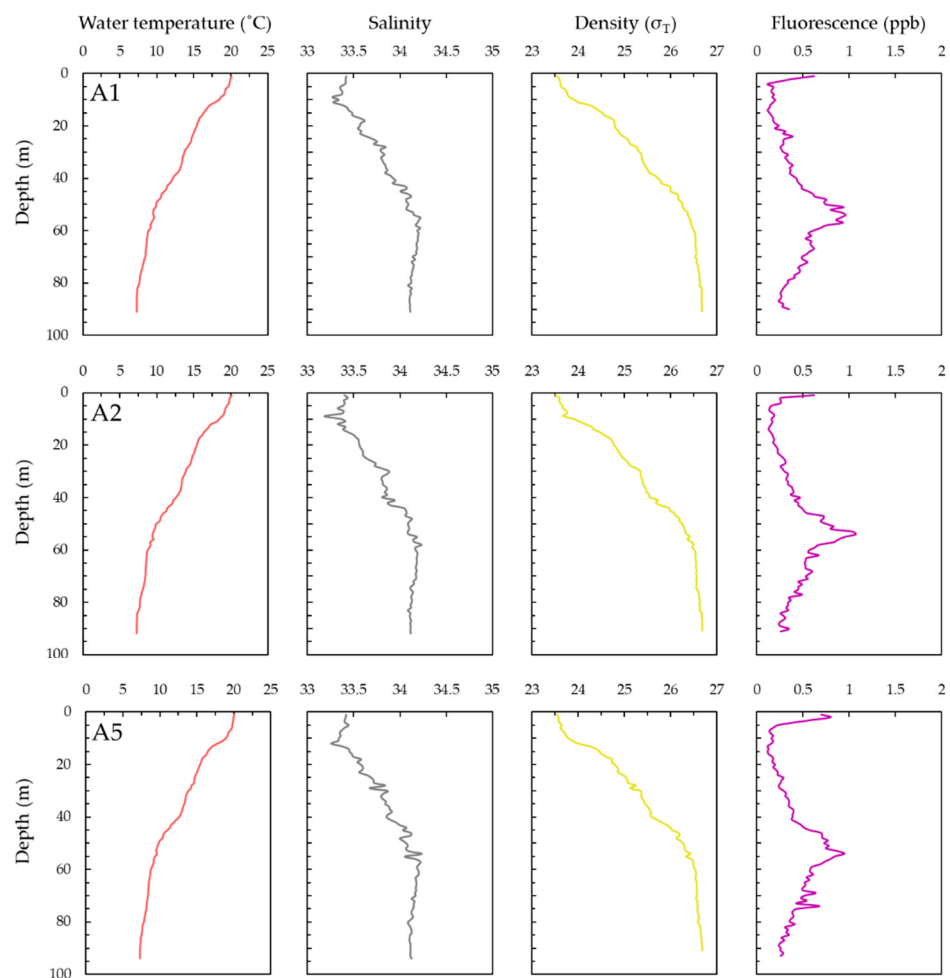


Figure 2. Vertical profiles of the environmental variables (water temperature, salinity, density (σ_T), and chlorophyll fluorescence) at A1, A2, and A5.

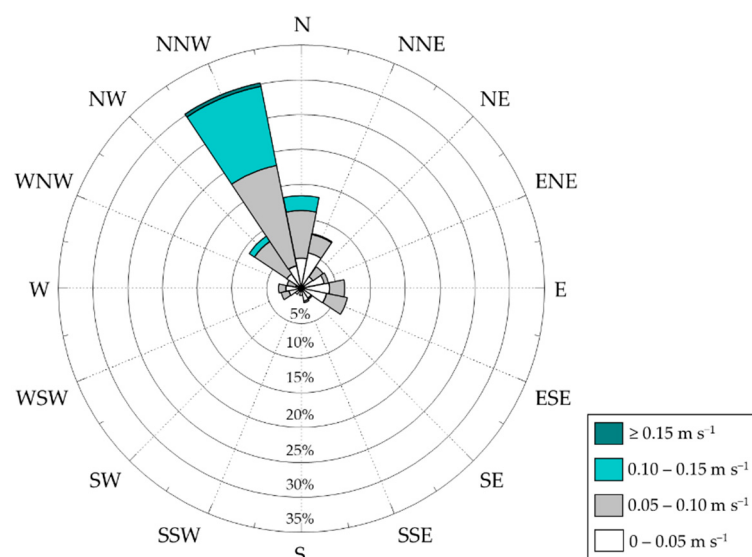


Figure 3. Frequency distribution of flow speed and direction at 3 m above the bottom of A5 (non-reef station) from 25 July to 7 August 2018. Flow speeds and directions were measured every one second for two minutes at an interval of twenty minutes through the period.

Table 2. The particle size distribution and D_{50} (median diameter) of the bottom sediment at A1, A2, and A5.

Station	Particle Size Distribution (%)								D ₅₀ (mm)
	Clay	Silt	Sand			Gravel			
			Fine	Medium	Coarse	Fine	Medium	Coarse	
			<0.005 mm	0.005–0.075 mm	0.075–0.25 mm	0.25–0.85 mm	0.85–2.0 mm	2.0–4.75 mm	
A1	10.5	9.9	7.1	8.3	5.8	14.0	44.4	0.0	3.70
A2	16.6	11.9	8.8	12.4	5.9	14.9	29.5	0.0	0.89
A5	28.3	18.1	13.8	20.9	3.8	5.7	9.4	0.0	0.12

3.2. Diatom Resting Stages

3.2.1. Abundances and Composition Based on Direct Microscopic Observation

The concentration of viable cells at A1, A2, and A5 was 560, 8820, and 840 cells g^{-1} wet sediment, respectively, and the proportion of *Chaetoceros* was more than 50% at all the stations, in particular, accounting for 93.4% at A2 (Figure 4a). The concentration and composition of the dominant *Chaetoceros* resting stages are displayed in Figure 4b, and seven different representative resting stage types morphologically identified as *Chaetoceros* in the present study are also shown in Figure 4c. However, the resting stages of *C. affinis* and *C. vanheurnkii* were counted as “*Chaetoceros affinis* or *vanheurnkii*”, owing to inadequate information on morphological characteristics to distinguish between the two species. There were also multiple resting stages of *Chaetoceros* that could not be identified at the species level in this study. The species composition of *Chaetoceros* exhibited a general resemblance among the stations, in which *C. contortus* and *C. radicans* were consistently dominant. Putative resting stages of *Actinocyclus* sp., *Actinopterychus senarius*, and *Odontella aurita*, and *Thalassiosira* sp., together with resting spores of *Leptocylinndrus danicus*, were also observed (Figure 5). It is noteworthy that several motile pennate diatoms with chloroplasts were constantly observed in all the sediment samples, reaching the highest concentration (260 cells g^{-1} wet sediment) at A2 (Figure 6 and Table 3). The concentration of diatom resting stages at A2 located on the northernmost side of the AR area was about 10-fold that

at A5 (non-reef station), although the concentration at A1, the south end station of the AR area, was lower than at A5.

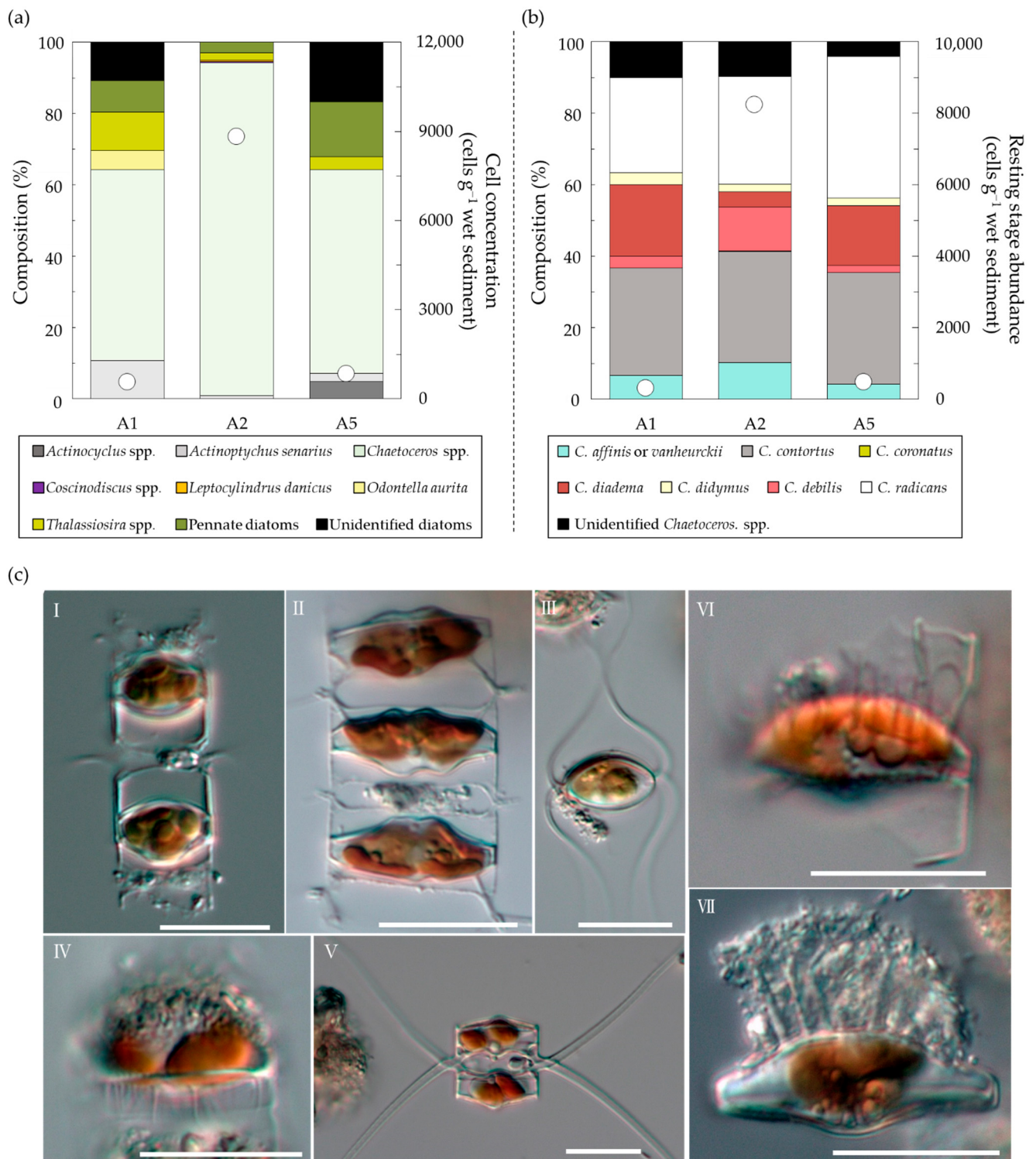


Figure 4. Cell concentrations and species compositions of (a) diatoms and (b) *Chaetoceros* species in the bottom sediment at A1, A2, and A5, determined by direct microscopic observation (an open circle represents cell concentration), and (c) photomicrographs of the resting spores/cells of *Chaetoceros* spp.; (I) *C. contortus*, (II) *C. debilis*, (III) *C. radicans*, (IV) *C. affinis* or *vanheurnckii*, (V) *C. didymus*, (VI) *C. coronatus*, (VII) *C. diadema*. Scale bars are 20 µm.

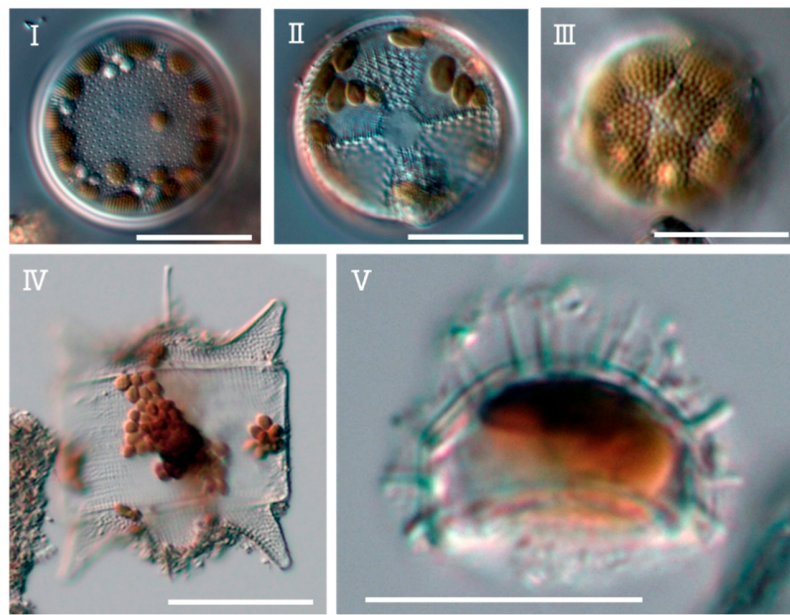


Figure 5. Photomicrographs of the resting stages of diatoms other than *Chaetoceros* spp. by direct microscopic observation; (I) *Actinocyclus* sp., (II) *Actinoptychus senarius*, (III) *Thalassiosira* sp., (IV) *Odontella aurita*, and (V) *Leptocylindrus danicus*. Scale bars are 20 μ m.



Figure 6. Photomicrographs of representative pennate diatoms by direct microscopic observation; (I) Naviculoid type diatom, (II) *Haslea* sp., (III) *Nitzschia* sp., (IV) *Surirella* sp., (V) *Fragilariopsis* sp., (VI) unidentified pennate diatoms. Scale bars are 20 μ m.

Table 3. Taxa and concentrations of the diatom resting stages in the bottom sediment at A1, A2, and A5, determined by direct microscopic observation. Cells of species other than pennate diatoms and unidentified diatoms were identified as resting stages in the present study.

Species	Cell Concentration (Cells g ⁻¹ Wet Sediment)		
	A1	A2	A5
<i>Actinocyclus</i> spp.	0	0	40
<i>Actinopterychus senarius</i>	60	80	20
<i>Chaetoceros affinis</i> or <i>vanheurckii</i>	20	840	20
<i>Chaetoceros contortus</i>	90	2560	150
<i>Chaetoceros coronatus</i>	0	20	0
<i>Chaetoceros debilis</i>	10	1000	10
<i>Chaetoceros diadema</i>	60	360	80
<i>Chaetoceros didymus</i>	10	180	10
<i>Chaetoceros radicans</i>	80	2480	190
<i>Coscinodiscus</i> spp.	0	20	0
<i>Leptocylindrus danicus</i>	0	40	0
<i>Odontella aurita</i>	30	0	0
<i>Thalassiosira</i> spp.	60	180	30
Pennate diatoms	50	260	130
Unidentified	30	800	20
<i>Chaetoceros</i> spp.	30	800	20
Unidentified diatoms	60	0	140

3.2.2. Abundance of Viable Cells Estimated by MPN

The diatom species confirmed by MPN belonged to four genera (*Chaetoceros* spp., *Odontella* spp., *Skeletonema* spp., and *Thalassiosira* spp.) which were fewer than those with direct observation. However, the MPNs of viable cells at A1, A2, and A5 were 1735, 9920, and 741 cells g⁻¹ wet sediment, respectively (Figure 7), and the highest value calculated by MPN at A2 showed close agreement with the direct observation. On the other hand, the contribution of *Chaetoceros* spp. was relatively low in the species composition of the MPN, unlike the result of the direct observation. The pennate diatoms were generally dominant in the composition of each station, accounting for 74.9%, 23.2%, and 72.9% at A1, A2, and A5, respectively. In the species composition of A2, however, unidentified spherical cells of a size of about 10 µm were abundantly observed (49.4%). The MPN value of viable diatom cells at A2 was more than 13 times that at the non-reef station (A5).

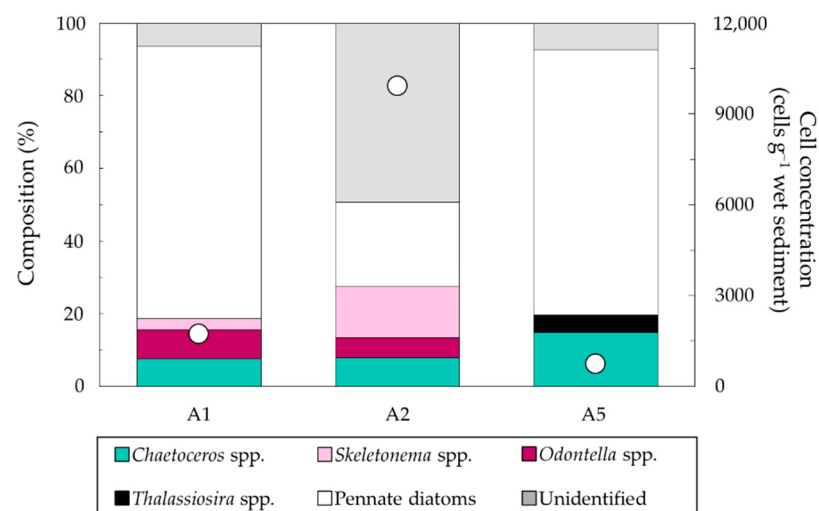


Figure 7. MPN of viable cells and species composition of diatoms in the bottom sediment at A1, A2, and A5; an open circle represents cell concentration.

4. Discussion

The previous studies reported that the deployment of ARs resulted in increases in primary production in the surrounding environment, enhancing the feeding environment for marine organisms induced by the promotion of settling suspended particles, thus contributing to the augmentation of fishery productivity [10,29,30]. The primary productivity increment is often explained as the upwelling generated by ARs bringing nutrient-rich water, which enhances primary production in the overlying water [9,10]. Diatoms, the major marine primary producer [31], are classified into two types, namely centric and pennate [23], and the key ecophysiological characteristic of the most centric planktonic diatom species is their dormant stages [13]. These dormant stages can be of two types: resting cells [32] or resting spores [33]. Once produced, they settle down to the bottom, serving as seed populations for a future diatom bloom [18]. Thus, if enhancement of primary production by ARs is contemplated, it is essential to also integrate information on the distribution of diatom dormant cells and the physical effect of ARs such as the wake region, known to accumulate biodeposits [19,20]. The present study has demonstrated that the concentration of the diatom resting stages at A2 (north end of the cube reef area) reached 8920 cells g^{-1} wet sediment with the direct microscopic observation method, mainly composed of *Chaetoceros*, the dominant phytoplankton group distributed worldwide [34], and the value was more than ten times the concentration at the non-reef station (A5). On the other hand, interestingly, the cell concentration at A1, also a station near ARs but located at the south end, was the same level as that at the non-reef station. From the point of view of the environmental variables observed in this study, there were no distinct differences in the vertical environmental profiles (Figure 2) and temporal changes in water temperature at the seafloor. On the west coast of Hokkaido, where the study area is located, the Tsushima Warm Current flows northward in the surface layer, and northward flows were also observed at a depth of 100 m [35]. The dominant flow direction observed near the seafloor at A5 during the study period was also northerly (Figure 3). A2 was located approximately 55 m north of A1, and multiple cubic reefs were densely distributed to the south of A2, while only tower reefs were distantly located to the south of A1 (Figure 1c,d). In general, TOC concentration is inversely proportional to the particle size of the sediment; however, both the TOC concentration and LOI at A2 showed the highest values, even though the particle size at A2 was much larger than at A5 (Table 2). In general, soft sediments such as clay and silt are not suitable substrates for AR placement because ARs may sink into these sediments; thus, hard substrates are recommended as a deployment site for ARs [36]. The original substratum of the AR system in this study had mainly been composed of gravel before its deployment. However, although the proportion of gravel (>2.0 mm) was still high, accounting for 58.4% and 44.4% at A1 and A2, respectively, the proportion of clay and silt smaller than 0.075 mm was also relatively high (A1: 20.4%, A2: 28.5%), and the particle size at A2 was also smaller than at A1. It is therefore assumed that A2, which is positioned on the downstream side of A1, was within the range of the wake region that spreads out northward. Assuming the same amount of supply, the highest concentration of diatom resting stages at A2 can reasonably be explained by the positional relationship between the stations and the dominant flow direction. In other words, our results suggest that ARs do not necessarily accumulate diatom resting stages in their vicinity, but the wake region generated by ARs traps settling diatom resting stages and accumulates in their vicinity.

In this study, similar values to the direct observation values were also obtained by the MPN method, indicating that diatom cells in the sediment collected at a depth of about 90 m were mostly viable (Figures 4a and 7). However, the species composition obtained with the MPN method showed a much lower ratio of *Chaetoceros* compared to that with direct observation (Figure 7). In contrast, the ratio of pennate diatoms was confirmed to be high at all stations with the MPN method. For the direct microscopic observation, the sediment subsamples were consecutively sieved through 100, 20, and 10 μm mesh and the remaining diatoms on the 20 and 10 μm mesh were observed, while the sediment samples only diluted with the medium were used for the MPN method. The above-mentioned

differences may be attributed to the compositions derived from different methods, notably the pennate diatoms smaller than 10 μm and unidentified spherical cells (10 μm in diameter) by the MPN method. Shinada et al. [37] calculated the concentration of diatom resting stages in sediment at a depth of 90 m in Funka Bay on the Pacific coast of Hokkaido, reporting that the concentration of *Chaetoceros* (*C. sociale*, *C. compressus*, and *Chaetoceros* spp.) was 18,000 cells g^{-1} wet sediment in July 1995, corresponding to the timing of the present study (late July 2018). Although the concentration of *Chaetoceros* at A2 in the present study was 8240 cells g^{-1} wet sediment determined with direct microscopic observation, which was less than half of the value in Funka bay, it was more than fifteen times the *Chaetoceros* concentration in Funka Bay in August 1995 (523 cells g^{-1} wet sediment) [37]. However, diatom resting stages are generally more abundant in coastal than offshore regions [15] and the Sea of Japan off Hokkaido, where the present study was conducted, is also characterized as oligotrophic compared to other regions of Hokkaido [38]. Considering all the circumstances, the concentration of diatom resting stages at A2 was sufficiently high, subject to the effect of ARs. The resting stages of *Chaetoceros* accounted for a large proportion (>50%) of the species composition at all of the stations. In the coastal waters of the Japan Sea and the Okhotsk Sea off Hokkaido, *Chaetoceros* species are frequently observed and are dominant from April/May to November [39,40]. This is consistent with the results that *Chaetoceros* are the most dominant in the sediment collected in the present study. Light is considered a critical factor for germination of diatom resting stages [13,15,41], and the minimum light intensity required for germination is reported to be 1.3 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for resting spores of *Chaetoceros* spp. [41]. Although the light intensity measured during the present study was mostly below 1.0 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, the light intensity monitored at the same location in September 2019 was often confirmed to be over 1.3 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ during the daytime, indicating that the minimum light intensity for germination reached the sampling stations (ca. 90 m) although not always. The germinated cells may not effectively enhance primary production on a regular basis if the cells are not carried to the euphotic zone by an upward flow such as upwelling and/or vertical mixing [16,17]. According to previous studies, the resting stages of *Chaetoceros* spp. usually keep their viability for approximately 500 to 700 days [15,18,41], while a recent study succeeded with the germination of several millennia-old ancient *Chaetoceros* resting spores from the Baltic Sea [42]. The advantageous conditions for the survival of diatom resting stages are cool temperatures and dark conditions [15], and diatom resting stages were accordingly confirmed to be in a good state of preservation in this study (Figures 4c and 5), indicating that the environmental conditions at A2 (north end of the cube reef area) were favorable for the survival of resting stages, potentially serving as a “seed bank” for many years.

Interestingly, viable pennate diatoms with chloroplasts were constantly observed in the bottom sediments at a depth of about 90 m both with direct observation and the MPN method (Figures 6 and 7). Live benthic pennate diatoms have been found in sediments at a depth of 191 m in Onslow Bay, North Carolina [43], as well as in sediments at a depth of more than 100 m in the Beaufort Sea according to several studies using the MPN method [44,45], implying that the pennate diatoms adapted chromatically [43,46] and grew autotrophically under low light or hetero-/mixotrophic living conditions [47]. On the other hand, since some pennate diatoms are known to form resting stages [14,48,49], they may be in a dormant period on the seafloor, even though the formation of resting stages is not common for pennate diatoms [13,23,50]. However, one pennate diatom species exhibited growth at light intensity controlled by about 1 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$, which was isolated from the bottom sediment of the study area [51]; thus, it is likely that these pennate diatoms were living as vegetative cells on the seafloor of the study area, suggesting novel benthic food webs around ARs deployed at a dysphotic seafloor starting from these diatoms. Few studies have investigated such benthic microalgal production below a depth of 60 m [43,52], and further investigation of the ecology and physiology of pennate diatoms in the dysphotic zone is required to understand their ecological role in an offshore AR ecosystem.

5. Conclusions

This study investigated the distribution of diatom resting stages in sediments around ARs at a depth of about 90 m for the first time, suggesting that the AR has a novel function of accumulating diatom resting stages in its vicinity. Diatom resting stages can survive for years in sediment and serve as a seed population for a future diatom bloom. Thus, the ARs may function as a reservoir of diatom resting stages over the years, contributing to enhancing the primary production around it. However, further investigations are needed to test the universality of the accumulation effect of diatom resting stages by ARs. Moreover, motile pennate diatoms with chloroplasts were constantly observed in the bottom sediments of the study area, suggesting that the pennate diatoms are alive as vegetative cells, serving as a base for novel food webs around ARs in a dysphotic zone. However, their ecology and physiology remain largely unknown. We hope that potential primary productions of diatom resting stages and those benthic diatoms are taken into account for AR researches in the future.

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