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Research paper

# Influences of diurnal sampling bias on fixed-point monitoring of plankton biodiversity determined using a massively parallel sequencing-based technique

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ABSTRACT

In this study, we investigated the influence of diurnal sampling bias on the community structure of plankton by comparing the biodiversity among seawater samples (n = 9) obtained every 3 h for 24 h by using massively parallel sequencing (MPS)-based plankton monitoring at a fixed point conducted at Himedo seaport in Yatsushiro Sea, Japan. The number of raw operational taxonomy units (OTUs) and OTUs after re-sampling was 507–658 (558 ± 104, mean ± standard deviation) and 448–544 (467 ± 81), respectively, indicating high plankton biodiversity at the sampling location. The relative abundance of the top 20 OTUs in the samples from Himedo seaport was 48.8–67.7% (58.0 ± 5.8%), and the highest-ranked OTU was *Pseudo-nitzschia* species (Bacillariophyta) with a relative abundance of 17.3–39.2%, followed by *Oithona* sp. 1 and *Oithona* sp. 2 (Arthropoda). During seawater sampling, the semidiurnal tidal current having an amplitude of 0.3 m $s^{-1}$ was dominant, and the westward residual current driven by the northeasterly wind was continuously observed during the 24-h monitoring. Therefore, the relative abundance of plankton species apparently fluctuated among the samples, but no significant difference was noted according to G-test ($p > 0.05$). Significant differences were observed between the samples obtained from a different locality (Kusuura in Yatsushiro Sea) and at different dates, suggesting that the influence of diurnal sampling bias on plankton diversity, determined using the MPS-based survey, was not significant and acceptable.

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## 1. Introduction

Phytoplankton succession is a complex process resulting from local-scale and transitory blooms associated with spatial and temporal environmental heterogeneities (Margalef, 1967; Smayda, 1973). Succession is a change in species composition within a given water mass resulting from changing physical (light and temperature), chemical (nutrients, water quality, and allelopathy), and biological (competition and grazers) factors within that water mass (Smayda, 1980). Growth interactions between phytoplankton species involve allelopathy, which is defined as any direct or indirect inhibitory or stimulatory effect that one plant has on another by the production of chemical secretions (Maestrini and Bonin, 1981). Plankton communities are very sensitive to variation in environmental conditions (Smayda, 1998; Stomp et al., 2008). In changing environments, phytoplanktons compete with each other to acquire their own ecological niche and to adapt to local environmental conditions. Although dense monospecific blooms are common, many species occasionally co-occur as fine-scale blooms in shallow coastal waters. However, the species composition and abundance fluctuate greatly based on their environmental preferences (Smayda, 1980), and dense blooms may suddenly disappear within 1–3 days because of turbulence in water columns caused by winds or tidal currents, resulting in hourly fluctuation in microalgal communities (Margalef, 1978; Kemp and Mitsch, 1979; Brunet and Lizon, 2003; Sharples, 2008; Blauw et al., 2012). The abundance of dinoflagellates, one of the major microalgal components in shallow coastal waters, changes significantly along the water columns within a day because of

*Abbreviations:* MPS, massively parallel sequencing; NMDS, non-metric multidimensional scaling; OTUs, operational taxonomy units.

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their swimming behavior, the so-called "diel vertical migration" (Eppley et al., 1968; Heaney and Eppley, 1981). These environmental and biological factors might greatly influence the reliability of a fixed-point observation during a weekly or monthly monitoring of plankton.

The Yatsushiro Sea is a semi-enclosed, narrow coastal water body located off the western coast of Kyusyu Island, Japan. Its length is ca. 50 km from the northeast to the southwest coast. The bay connects through several narrow straits with the Ariake Sea and the East China Sea. The northeastern area is shallow (<10 m), and it gradually deepens southwestward to about 70 m near the Ushibuka Strait, through which seawater is mainly exchanged between the Yatsushiro Sea and the East China Sea. The midwest–southwest section of the Yatsushiro Sea is a large fish-farming area in Japan. In the summer of 2009 and 2010, a noxious red-tide causative species *Chattonella antiqua* formed a dense red tide in the Yatsushiro Sea and caused severe damage to aquacultured fish (Onitsuka et al., 2011). The red tide killed about 1.0 and 1.8 million cultured yellowtail, striped jack, and great amberjack in 2009 and 2010, respectively, and the amount of damage accounted for losses of ¥2,900,000,000 and ¥5,300,000,000 (US$290,000,000 and 53,000,000, respectively; exchange rate of 100 ¥ = $1; Fisheries Agency, 2010, 2011). Intensive field observations and different laboratory experiments have been conducted from to determine the growth mechanism of *Chattonella* under oceanographic conditions in the Yatsushiro Sea (e.g., Sakurada et al., 2008; Shikata et al., 2011; Onitsuka et al., 2011; Aoki et al., 2012). To elucidate the biological interaction between *Chattonella* species and other plankton species or bacteria during the development of the red tide, i.e., appearance, exponential growth, decline, and disappearance, we conducted a massively parallel sequencing (MPS)-based monitoring of plankton and bacteria at four sampling sites in the Yatsushiro Sea once a week in the summer between 2011 and 2014. We investigated the influence of diurnal sampling bias on the community structure of plankton by comparing the biodiversity among seawater samples obtained every 3 h for 24 h (n = 9) by using MPS-based plankton monitoring at a fixed point in Himedo seaport in the Yatsushiro Sea, Japan.

## 2. Materials and methods

### 2.1. Sampling and DNA extraction

Seawater samples were obtained using a 5-m-long hose (diameter, 25 mm) every 3 h from 1630 on Oct. 3, 2012 to 1630 on Oct. 4, 2012 (nine samples in total) from Himedo seaport (32°43.8′N, 130°41.2′E) in the Yatsushiro Sea (Kumamoto Prefecture, Japan; Fig. 1). The variation in plankton biodiversity at Himedo seaport was evaluated by obtaining two samples from Kusuura (station KM1, 32°23.8′ N, 130°13.5'E) in the Yatsushiro Sea (Fig. 1) on Aug. 17 and 24, 2011. All the plankton in the seawater samples was trapped by filtering 500 mL seawater through an 8-μm pore-size polycarbonate filters (Nuclepore membrane; GE Healthcare, Tokyo, Japan), followed by filtering through 1-μm pore-size filters (GE Healthcare, Japan). The filters were stored in a deep freezer (−80 °C) until use. For DNA extraction, a 5% Chelex® suspension (Chelex 100 Molecular Biology Grade Resin; Bio-Rad Laboratories Inc., Richmond, CA, USA) was prepared by dispersing the resin into ultra-pure water. For effective DNA extraction from plankton components trapped on the filters, the filters were cut in half, placed in 1.5-mL tubes (A.150; Assist, Tokyo, Japan), and extracted in 150 μL of 5% Chelex buffer. The plankton cells were crushed using a pellet pestle motor (Kontes Glass, Vineland, NJ, USA) for 60 s, and 350 μL of buffer was added to make the final volume of 500 μL. DNA was extracted by heating the 1.5-mL tubes at 95 °C for 20 min (Nagai et al., 2012). DNA extracted from the 8-μm and 1-μm filters was mixed in equal amounts (50 μL + 50 μL) and used as template DNA.

### 2.2. Polymerase chain reaction amplification and 454 pyrosequencing

A set of primer pairs for 18S rRNA gene (SSU-F1,289, F: TGGAGYGATTTGTCTGGTTDATTCCG; SSU-R1,772, R: TCACCTACGGAAACCTTGTTACG; modified from Nishitani et al., 2012) were used as universal primers to amplify the V7–9 hypervariable regions; unique 6-bp tags were added to the 5′-end of the primers for recognition of each sample in the metagenome analysis. Polymerase chain reaction (PCR) was performed using a thermal cycler (PC-808; ASTEC, Fukuoka, Japan) in a reaction mixture (50 μL) containing 2.0 μL template DNA (<1 ng); 0.2 mM of each dNTP; 1× PCR buffer; 1.5 mM $Mg^{2+}$; 1.0 U KOD-Plus-ver.2 (TOYOBO, Osaka, Japan), which has intensive 3′ → 5′ exonuclease activity; and 1.0 μM of each primer. The PCR cycling conditions were as follows: initial denaturation at 94 °C for 2 min, followed by 25 cycles at 94 °C for 15 s, 56 °C for 30 s, and 68 °C for 1 min. PCR amplification was verified by agarose gel electrophoresis. The PCR products were purified using a High-Pure PCR Product Purification Kit (Roche, Basel, Switzerland) and eluted in 35 μL of elution buffer following the manufacturer's protocol. Amplified PCR products were quantified using a NanoDrop system (NanoDrop Technologies, Wilmington, USA) and pooled together in equal quantities. Amplicon sequencing was performed using a Roche 454 GS-XLR70 Titanium sequencing platform. A 454-pyrosequencing library was constructed using a GS Titanium Rapid Library Preparation Kit (Roche Diagnostics, Branford, CT, USA),

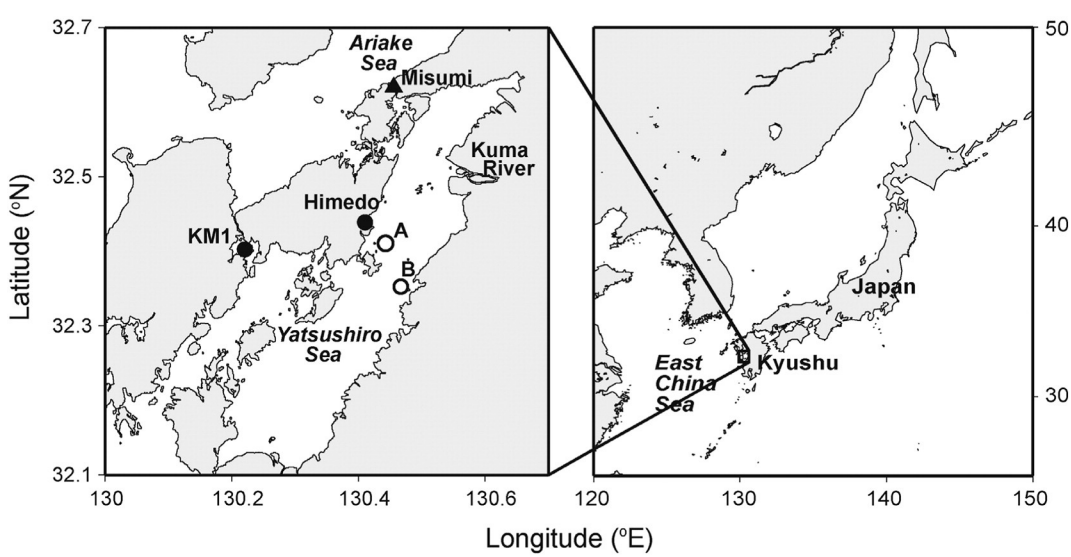


**Fig. 1.** Sampling locations at the Himedo seaport and Kusuura (station KM1) in Yatsushiro Sea, Japan.

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