

Phytoplankton assemblages in Onagawa Bay from 2012 to 2013 determined by DNA sequencing and pigment analysis

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Abstract: The phytoplankton assemblages in Onagawa Bay were investigated by photosynthetic pigment analysis and DNA sequencing over two consecutive years, from January 2012 to December 2013. Chlorophyll *a* (Chl *a*) concentrations tended to be high from winter to spring, and fucoxanthin, the source of which is mainly diatoms, was also high. Chlorophyll *b* concentration, which is retained in picoprasinophytes, tended to be higher in June. Cyanobacteria tended to appear in the summer, although, at less than 2%, in relatively small amounts. Since picoeukaryotes and cyanobacteria are small, shellfish filters cannot trap them efficiently, so the amount of nutrition obtained from them is relatively low and inefficient. *Dinophysis norvegica* (the causative agent of diarrheal shellfish poisoning) was the dominant dinoflagellate species throughout the study. Two species of dominant cryptophyte that were found are food sources of ciliate *Myrionecta rubra* which is a food source for *Dinophysis* spp., so unfortunately, they render Onagawa Bay susceptible to the growth of *Dinophysis* spp.. *Phaeocystis* spp. was the dominant haptophyte. The combination of pigment analysis by HPLC and DNA Next Generation Sequencing provided good data on seasonal phytoplankton variation, which is necessary to understand the detailed feeding environment for shellfish raised in Onagawa Bay.

Keywords : NGS, HPLC, Photosynthetic pigments analysis, Sanriku coast

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1. Introduction

Onagawa Bay is located just to the north of Sendai Bay, in the southern part of the Sanriku coast of northeastern Honshu, Japan (FUJII *et al.*, 2018). It is an important area for the cultivation of scallops, oysters, and ascidians (KATAYAMA *et al.*, 2018), along with important coastal fisheries for various finfish species. Appropriate management of shellfish aquaculture is a necessary contribution to maintaining a sustainable fishery industry in Onagawa. Since the food source for shellfish aquaculture is mainly natural phytoplankton in the seawater, feeding is not required. However, low phytoplankton levels can sometimes lead to insufficient feeding of the shellfish being cultivated, leading to poor growth, death, and a consequent decrease in shellfish production.

In order to understand changes in the feed quantity of shellfish, Chlorophyll *a* (Chl *a*), an indicator of phytoplankton abundance, is frequently measured on a regular basis at aquaculture farms (NAGASAWA *et al.*, 2016; OKUMURA *et al.*, 2017). However, various types of phytoplankton grow within the coastal waters, including diatoms (FURUYA *et al.*, 1993; KUDO *et al.*, 2000; WATANABE *et al.*, 2017), which are generally considered excellent feed for shellfish (IRIGOIEN *et al.*, 2002); dinoflagellates (MASUDA *et al.*, 2014), which are considered unsuitable (TURNER *et al.*, 1998); and small cyanobacteria (FLOMBAUM *et al.*, 2013; TANIUCHI *et al.*, 2017). Some dinoflagellates are poisonous (OFFICER and RYTHER, 1980; SUZUKI *et al.*, 2017), and if the poison accumulates in the shellfish through feeding on these dinoflagellates, harvesting is restricted voluntarily (TANABE and KAGA, 2017). In addition, cyanobacteria and picoeukaryotes, which are smaller than diatoms and dinoflagellates, cannot be filtered by bivalve gills and so they pass through (RISGÅRD, 1988). Lowered filtration efficiency re-

duces feeding efficiency, leading to poor growth. Therefore, the data on phytoplankton species composition is important for shellfish aquaculture. Recently, attempts have been made to understand the feeding environment in more detail, for example searching for shellfish feed organisms using stable isotope ratios (KATAYAMA *et al.*, 2018).

Onagawa Bay was affected by the 2011 tsunami, along with other coastal areas in northeastern Honshu ('Tohoku' in Japanese). In order to understand the impact of the disaster and the extent of post-disaster recovery, various surveys have been conducted, such as a post-disaster sediment environment survey (YOKOYAMA *et al.*, 2018), a survey of the benthos growth environment (KANEKO *et al.*, 2018), and construction of habitat maps (FUJII *et al.*, 2018). Seasonal changes in constituents of the phytoplankton (TANIUCHI *et al.*, 2017) and the distribution of diatoms (WATANABE *et al.*, 2017) in Sendai Bay were not affected by the tsunami. However, the incidence of dinoflagellates causing shellfish poisoning is reported to have increased in Sendai Bay (KAMIYAMA *et al.*, 2014), Kesenuma Bay (ISHIKAWA *et al.*, 2015), Ofunato Bay (TAKEHIKO *et al.*, 2016), and in southern Hokkaido Funka Bay (NATSUIKE *et al.*, 2014). Therefore, the effects of the tsunami differed, affecting some taxonomic groups (such as dinoflagellates) but not others (such as diatoms).

For Onagawa Bay, there have been reports of changes to the temporal and horizontal distribution of Chl *a* (FUJII *et al.*, 2018), size fractionated Chl *a* from 2007 to 2009 (ABE *et al.*, 2011), and the occurrence of phytoplankton species between 2018 and 2019 (MASUDA *et al.*, 2022). However, to our knowledge, there have been no reports so far on the composition of taxonomic groups of phytoplankton in the years immediately just after the tsunami. An outbreak of

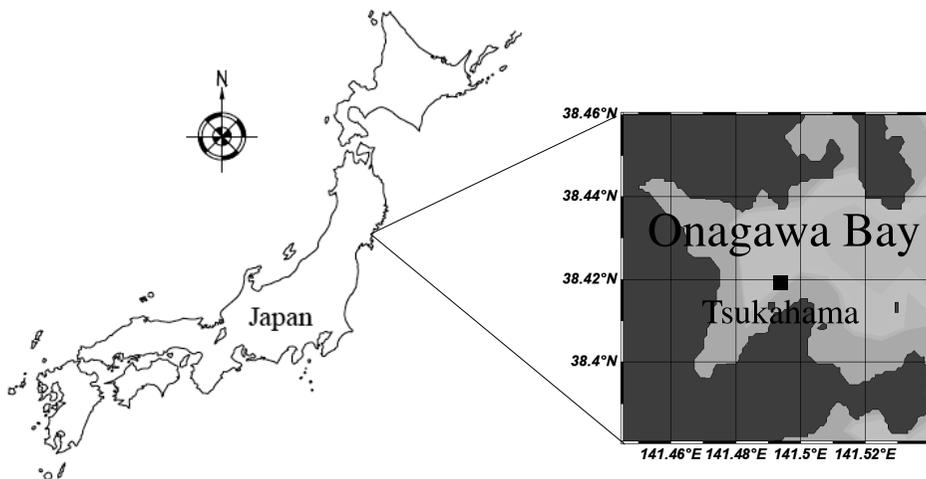


Fig. 1 Sampling site at Tsukahama (black square).

paralytic shellfish poisoning was caused in Onagawa Bay in 2018 by an increase in numbers of *Alexandrium* spp. (MASUDA *et al.*, 2022). There were also more cysts of *Alexandrium* spp. after 2018 in comparison with before the disaster and in 2013 (TANABE and KAGA, 2017). If a data series on phytoplankton species can be collected, it can be used as a medium- to long-term comparison to judge whether or not the coastal ecosystem has been restored to pre-tsunami conditions.

In the present study, pigment analysis and DNA sequencing were conducted on seawater collected between 2012 and 2013 from a site in Onagawa Bay: the Tsukahama area, which is one of the sampling points monitored for the occurrence of shellfish poisoning by Miyagi Prefecture Fisheries Technology Institute (MASUDA *et al.*, 2014). This study had the following objectives: to (1) follow the annual variation of phytoplankton assemblages in detail, (2) consider possible effects of phytoplankton fluctuations on natural feeding aquaculture, and (3) preserve a record of information on the phytoplankton in Onagawa Bay from 2012 to 2013 during the second year

following the tsunami.

2. Materials and methods

2.1. Sample collections and pretreatments

A map of the sampling site is shown in Fig. 1. Water was collected at Tsukahama in Onagawa Bay at approximately monthly intervals from January 2012 to December 2013, using a sampling bucket and Van Dorn water sampler. The sampling depths were 0, 5, 10, 15, 20 m, and the bottom minus 1 m (set to 25 m in the contour figure).

2.2. Photosynthetic Pigment analysis

To quantify phytoplankton photosynthetic pigments [Chlorophyll *a* (Chl *a*), Fucoxanthin (Fuco), Chlorophyll *b* (Chl *b*), Alloxanthin (Allo), Peridinin (Perid)], 150 ml samples of seawater were filtered through Whatman GF/F glass microfiber filters (GE Healthcare UK Ltd., Buckinghamshire, UK). The phytoplankton pigments were extracted from the filter with 1 ml methanol and allowed to settle for at least 24 h in a freezer at -20°C . After removing the filter and centrifugation at $17,000 \times g$ for 10 min, pigments

in the supernatant were analyzed by high-performance liquid chromatography (HPLC; Shimadzu, Kyoto, Japan) using the method of ZAPATA *et al.* (2000). Contour plots of the annual variation for each major pigment detected by HPLC were made using Ocean Data View software (AWI <https://odv.awi.de/>).

2.3. DNA extraction, polymerase chain reaction (PCR), Next Generation Sequencing (NGS), and data analysis

NGS was conducted only on surface seawater samples. The DNA extraction method was basically the same as reported previously (OKUMURA *et al.*, 2020). Briefly, seawater samples were filtered using a Durapore filter and DNA was extracted from each filter using 1 mL SNET minus NaCl buffer (0.3% SDS with 5 mM EDTA, and 20 mM Tris HCl pH 8.0) and 40 μ L of 5 mg/mL proteinase K (SHIMADZU CORPORATION, 2012), for at least 1 hour at 37°C. After removal of the Durapore filter, DNA extract solutions were centrifuged at $17,000 \times g$ for 10 min and the supernatants were used as PCR templates. PCR was conducted using KOD FX of DNA polymerase according to the manufacturer's protocol (TOYOBO, 2019). The primers used in PCR were the same as those used previously (OKUMURA *et al.*, 2020), with an adaptor for Genome Sequencer-FLX (ROCHE, 2009, ROCHE APPLIED SCIENCE, 2013).

The *psb A* gene, which encodes the D1 polypeptide of the photosystem II reaction center complex, was amplified. PCR was performed under the following conditions: initial denaturation at 94°C for 2 min; followed by 40 cycles consisting of denaturation at 98°C for 10 s, annealing at 55°C for 30 s, and extension at 68°C for 1 min; and a final extension step at 68°C for 10 min. DNA sequencing was conducted by Genome Sequencer-FLX (Roche, Basel, Switzerland). Af-

ter sequencing, phytoplankton species were identified by a homology search using pipeline software QIIME (Quantitative Insights into Microbial Ecology; CAPORASO *et al.*, 2010).

3. Results

3.1. Annual variation of major pigments

Chl *a* concentrations tended to be higher during winter and spring, with a maximum concentration of 9.9 μ g/L at 25 m depth on April 6, 2012, and 7.5 μ g/L at 20 m on February 12, 2013 (Fig. 2). Fuco showed similar fluctuations to Chl *a*, with a maximum of 13.5 μ g/L on April 6, 2012, and 6.7 μ g/L on April 30, 2013. Chl *b* concentrations tended to be higher in June, with a maximum of 1.2 μ g/L on June 26, 2012, and 1.1 μ g/L on June 24, 2013. Perid concentrations tended to be sporadically higher in spring and summer, with a maximum of 0.2 μ g/L on March 2, 2012, and 0.43 μ g/L on July 29, 2013. Allo concentrations tended to be high during summer and fall, with a maximum of 0.53 μ g/L on July 29, 2013, and 0.21 μ g/L on October 9, 2012.

The fluctuation of each pigment concentration was related to that of Chl *a* concentration: significant correlations were observed between Chl *a* concentrations and major pigment concentrations (Fuco, Allo, Perid, and Chl *b*; Fig. 3), for all of R^2 values are above 0.28 (when the sample number is > 100 and $R^2 > 0.26$, the result is significant; OKUNO, 1978). The correlation ($R^2 = 0.67$) between Chl *a* and Fuco was the highest among the major pigments.

3.2. Phytoplankton relative abundance detected at the sea surface by NGS

Although the dominant phytoplankton taxa varied with the season, in general, the order was as follows: diatoms (51% of the total number of sequences) $>$ green algae (27%) $>$ haptophytes (9%) $>$ dinoflagellates (7%). These four taxon

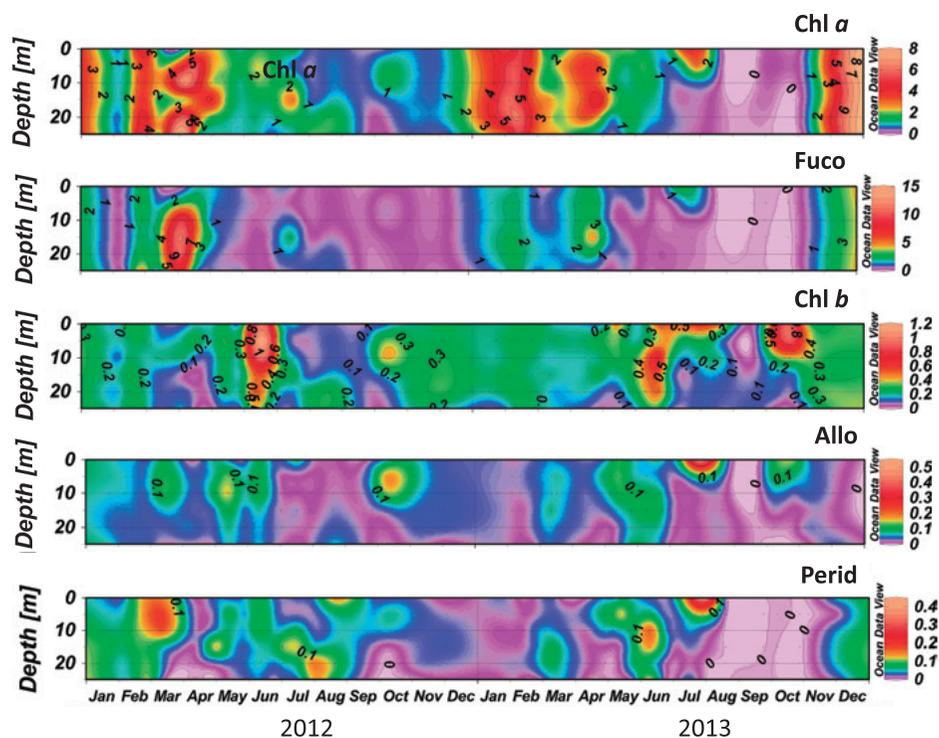


Fig. 2 Depth and temporal variation in pigment concentration at Tsukahama, Onagawa Bay, during the study period. (Color key at right indicates concentration, in $\mu\text{g/L}$).

groups accounted for more than 90% of the total (Fig. 4).

A total of 35 operational taxonomic units (OTUs) were detected for diatoms, which was the highest of all the phytoplankton groups. The proportion of diatoms tended to increase during periods of high Chl *a* concentration. Chl *a* in the surface layer exceeded $5 \mu\text{g/L}$ on both February 28, 2012, and February 12, 2013, and the number of diatom sequences was $> 70\%$ of the total number of sequences detected at those times. The relative abundance among the diatoms (Fig. 5a) was as follows: *Skeletonema costatum* (20% of the total number of sequences) $>$ *Asterionella* spp. (8%) $>$ *Chaetoceros* spp. (7%) $>$ *Thalassiosira* spp. (7%). The proportion of *S. costatum* tended to increase in spring when it sometimes accounted for 77% of all sequences. From June to

August, when the relative abundance of *S. costatum* decreased, the proportion of *Chaetoceros* spp. tended to increase, sometimes up to 60% of the total number of sequences. In winter, the proportion of *Asterionella* spp. increased, up to 81%. *Thalassiosira* spp. show less seasonality than other diatoms, with a maximum of 28%. An approximately seasonal trend was observed, with an increase in the proportion of *S. costatum* in spring, *Chaetoceros* spp. in summer, and *Asterionella* spp. in winter.

For green algae, 24 OTUs were detected, the second highest number after diatoms (Fig. 5b). The proportion of green algae increased as the proportion of diatoms decreased, the former exceeding 70% in June, when Chl *b* increased, in both 2012 and 2013. The relative abundance of green algae genera, dominated by

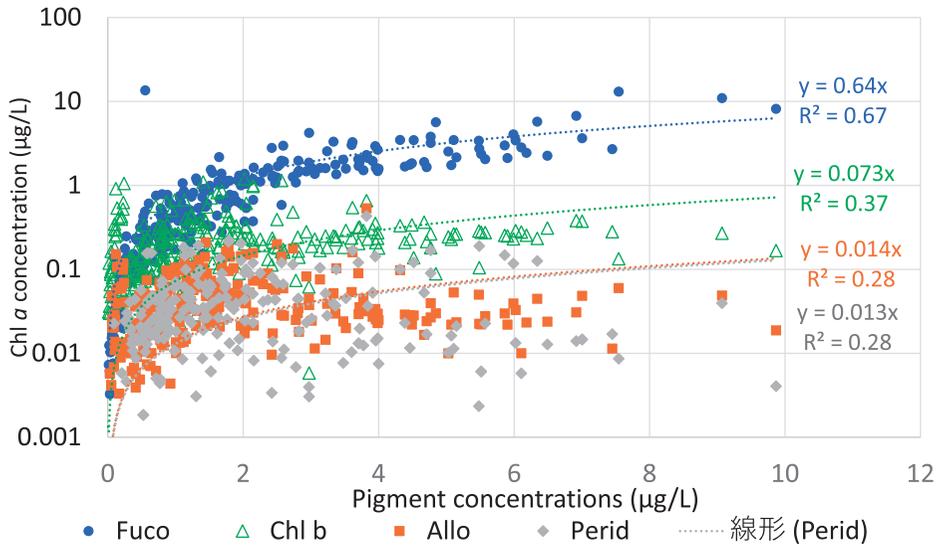


Fig. 3 Scatter plot and correlation equations between Chl *a* (ordinate) and four other pigment concentrations (abscissa). The plots are each pigment concentration by the sampling depths for all observation dates. There were 252 samples of each pigment. Correlation coefficients were calculated by Microsoft Excel. When the sample number is > 100 and $R^2 > 0.26$, the result is significant (i.e., all the values are significant).

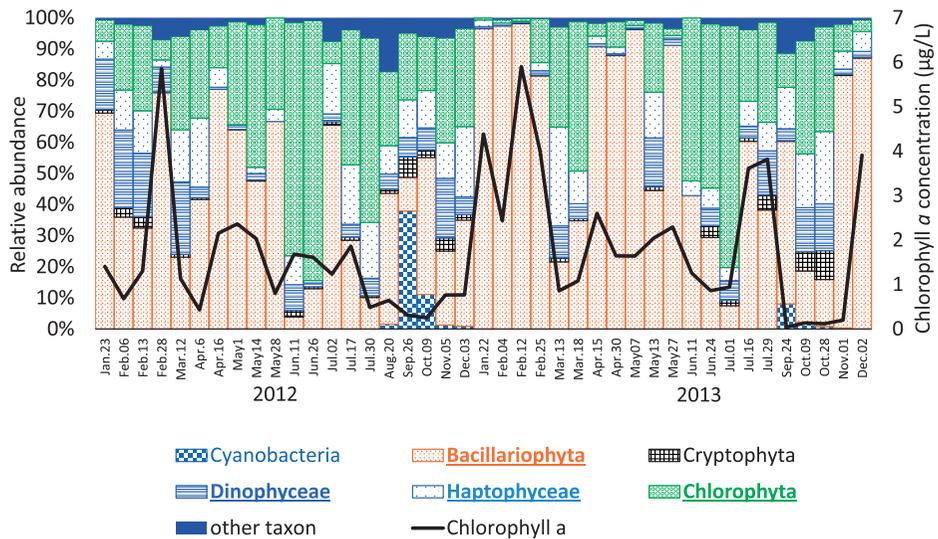


Fig. 4 Chlorophyll *a* concentration and relative abundance of phytoplankton assemblages at the surface at Tsukahama during the years 2012 and 2013. The legends of major phytoplankton groups are shown in bold and underlined. The legend "other taxa" includes Bolidophyceae, Phaeophyceae, Pinguiphyceae, Xanthophyceae, Chrysophyceae, Compsopogonophyceae, Dictyochophyceae, Florideophyceae, Foraminifera, Pelagophyceae, Raphidophyceae, and Synurophyceae.

Prasinophyceae, was as follows: *Micromonas* spp. (14% of the total number of sequences) > *Ostreococcus* spp. (7%) > *Bathycoccus* spp. (2%). In June, the proportion of *Ostreococcus* spp. and *Bathycoccus* spp. also increased with *Micromonas* spp.

The relative abundance of Haptophyta tended to increase in the period when the Chl *a* concentration decreased, sometimes up to 32% (Fig. 5c). The relative abundance of the Haptophyta was as follows: *Phaeocystis* spp. (7% of total leads) > *Emiliania huxleyi* (1%), *Phaeocystis* spp. was the dominant species.

The relative abundance of dinoflagellates changed sporadically and did not show the seasonality observed with diatoms, but the proportion of dinoflagellates to the total number of sequences increased up to a maximum of 25% (Fig. 5d). *Dinophysis norvegica* was the dominant dinoflagellate throughout the observation period: *Dinophysis norvegica* (5% of the total number of sequences) > *Peridinium foliaceum* (0.3%) > *Dinophysis acuminata* (0.2%).

The relative abundance of Cryptophyta was as follows: *Teleaulax amphioxiea* (0.7%) and Cryptophyta sp. CR-MAL11 (0.7%) was dominant regardless of the season (Fig. 5e).

Although the proportion of Cyanobacteria was less than 2% of the total number of sequences for most of the year, there was a maximum of 38% on September 26, 2012 (Fig. 5f). Cyanobacteria also increased in the summer of 2013, reaching approximately 8% in September. The most common species of cyanobacteria detected was *Prochlorococcus marinus*.

4. Discussion

Chl *a* concentration in Onagawa Bay tended to be high during winter and spring. Maximum concentrations were 9.9 $\mu\text{g/L}$ at 25 m depth in 2012 and 7.5 $\mu\text{g/L}$ at 20 m in 2013 (Fig. 2). Phy-

toplankton growth (TANIGUCHI, 1983; DONEY, 2006) is regulated by water temperature (TROMBETTA *et al.*, 2019), light intensity (GLE *et al.*, 2007), nutrient concentrations (COLIJN and CADEE, 2003), and blooms begin when solar radiation is sufficient, and the mixing depth becomes shallower by stratification (SVERDRUP, 1953). Along the Pacific coasts of Tohoku and Hokkaido, phytoplankton blooms have been observed in early spring in Funka Bay (KUDO *et al.*, 2000), Otsuchi Bay (FURUYA *et al.*, 1993), Sendai Bay (TANIUCHI *et al.*, 2017), and in Onagawa Bay in 2007–2009 (ABE *et al.*, 2011) and in 2018–2019 (MASUDA *et al.*, 2022). The timing of blooms in Onagawa Bay from 2012 to 2013 was similar to these other records.

Chl *a* concentration in Onagawa Bay from 2012 to 2013 was similar to, or slightly lower than, the previous reports of slightly above 10 $\mu\text{g/L}$ (FURUYA *et al.*, 1993; KUDO *et al.*, 2000; ABE *et al.*, 2011; TANIUCHI *et al.*, 2017). While, Chl *a* concentration of about 4 $\mu\text{g/L}$ was observed in July, 2013. Although the increase of Chl *a* concentration during summer was observed in Onagawa Bay in the past (ABE *et al.*, 2011), it differed from the general trends in other parts of the Pacific coasts of Tohoku and Hokkaido. In addition to nutrient and solar radiation limitations, phytoplankton growth also is reduced due to grazing by higher trophic organisms (ABE *et al.*, 2011). The mouth of the Shin-Kitakami River (an 'A' class river managed by the Japanese government) is located approximately 15 km north of Onagawa Bay, and the water from this river tends to flow southward along the coast (GOMI *et al.*, 2021). This river water contributes nutrients to Onagawa Bay, which enriches phytoplankton growth there.

The correlations between Chl *a* concentration and each major pigment concentration were all significant (Fig. 3). In particular, the R^2 value for

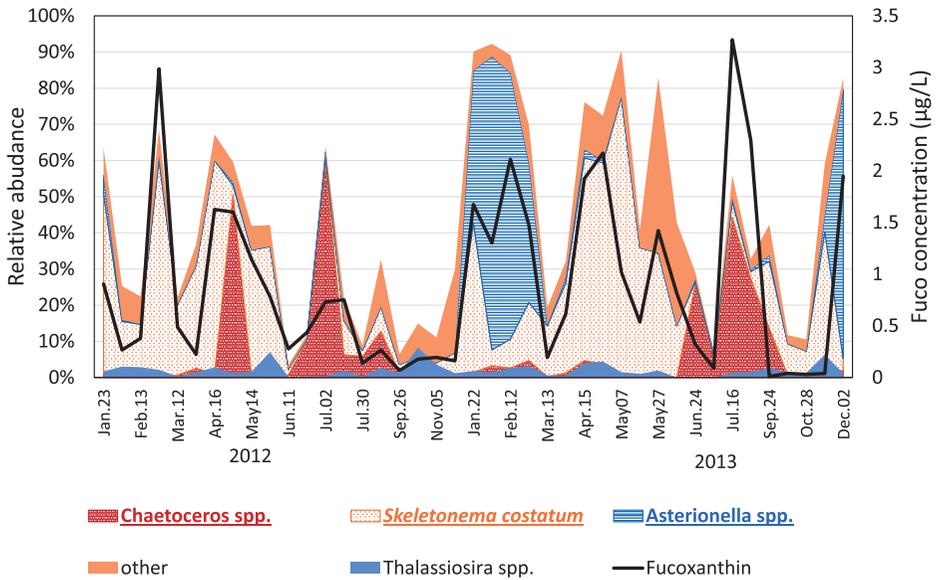


Fig. 5a Fucoxanthin (Fuco) concentration (bold black line) and relative abundance of diatoms during the years 2012 and 2013. The species in bold and underlined were predominant.

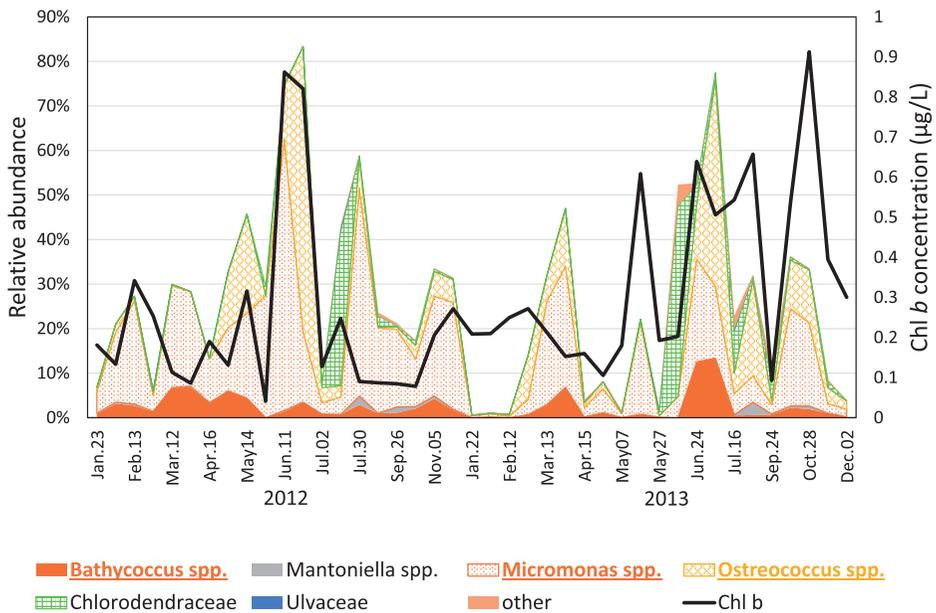


Fig. 5b Chlorophyll *b* (Chl *b*) concentration (bold black line) and relative abundance of green algae during the years 2012 and 2013. The species in bold and underlined were predominant.

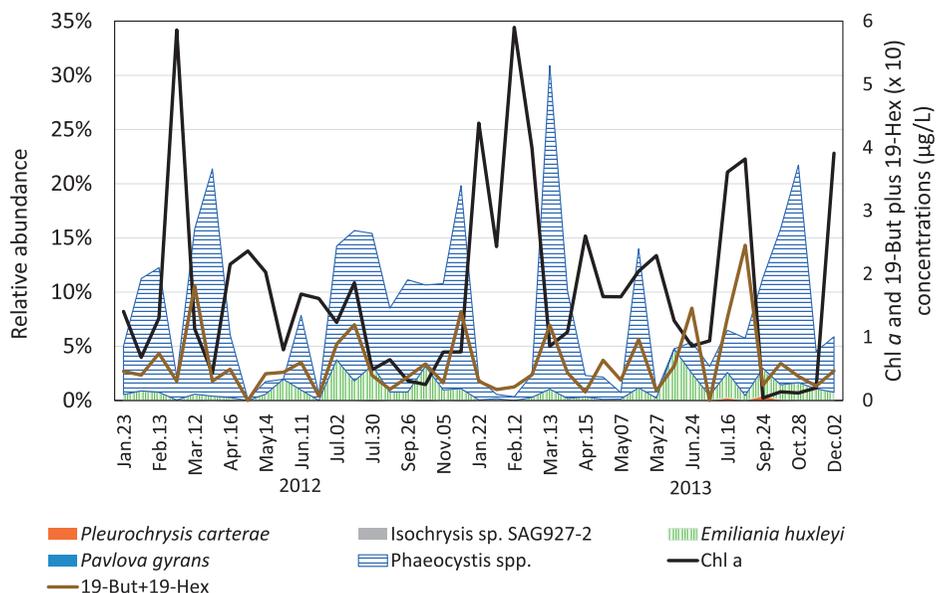


Fig. 5c Chlorophyll *a* (Chl *a*) concentration (bold black line) and relative abundance of haptophytes during the years 2012 and 2013.

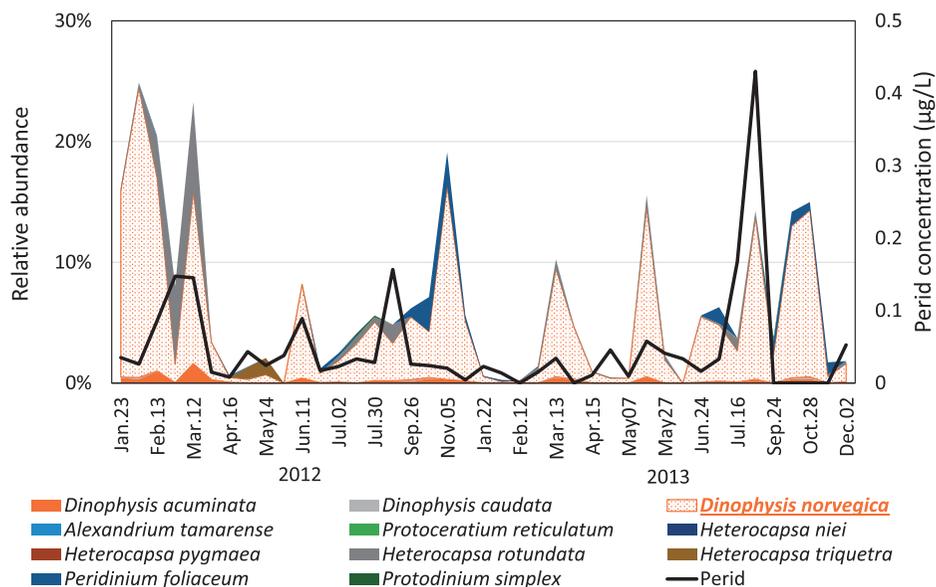


Fig. 5d Peridinin (Period) concentration (bold black line) and relative abundance of dinoflagellates (many species of *Dinophysis* spp. and *Alexandrium* spp. are toxic) during the years 2012 and 2013. The species in bold and underlined were predominant.

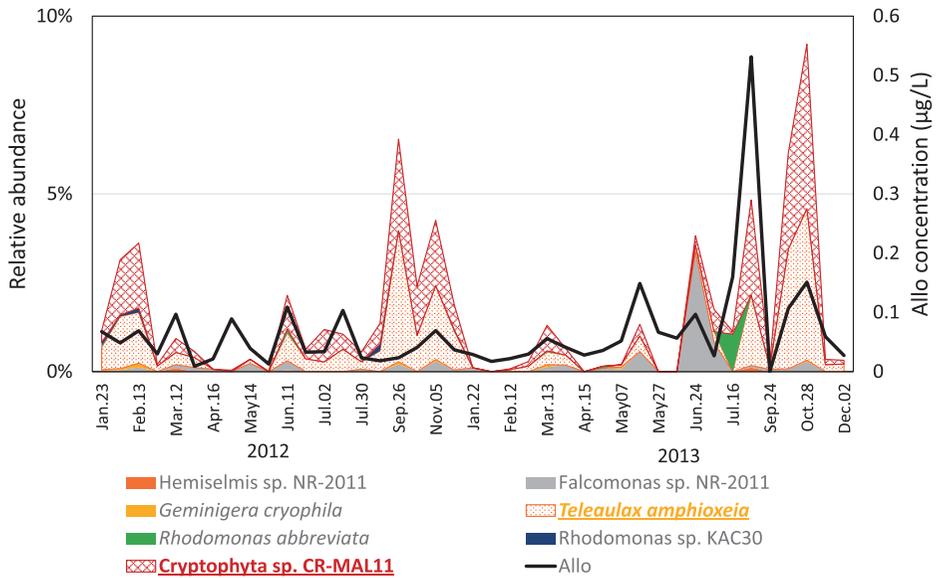


Fig. 5e Alloxanthin (Allo) concentration (bold black line) and relative abundance of cryptophytes during the years 2012 and 2013. The species in bold and underlined were predominant.

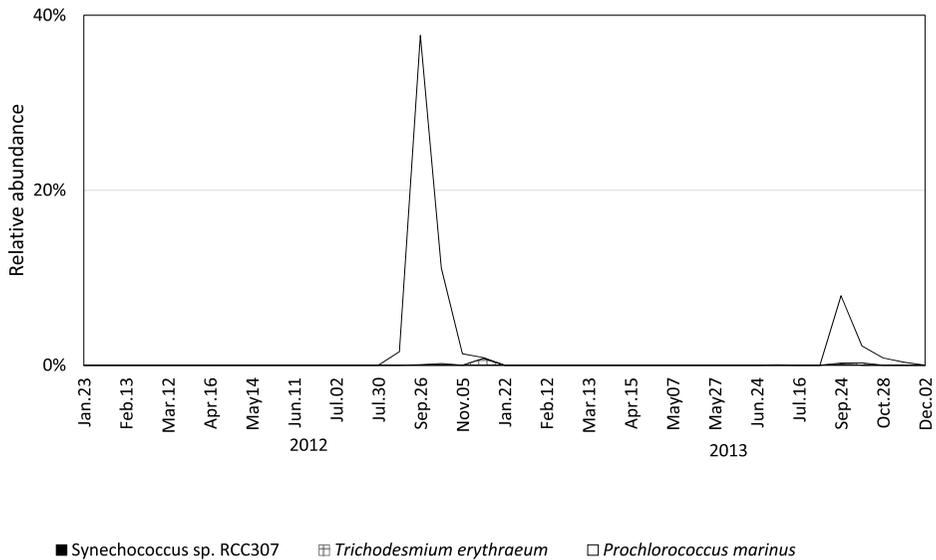


Fig. 5f Relative abundance of Cyanobacteria during the years 2012 and 2013.

Chl *a* versus Fuco was the highest. In general, different groups of phytoplankton have characteristic taxon-specific pigments, such as Fuco in

diatoms, Allo in cryptophytes, Perid in dinoflagellates, and Chl *b* in green algae (JEFFREY and VESK, 1997). The high correlation between Fuco

and Chl *a* suggests that the variation observed in Chl *a* is due mainly to variation in the number of diatoms present. The high proportion of diatoms during the high Chl *a* period from winter to spring suggests that the spring bloom in Onagawa Bay is mainly caused by diatom growth. Diatoms are generally considered to be dominant in temperate zones (IRIGOIEN *et al.*, 2002). The spring blooms in Funaka Bay (KUDO *et al.*, 2000), Otsuchi Bay (FURUYA *et al.*, 1993), and Sendai Bay (TANIUCHI *et al.*, 2017; WATANABE *et al.*, 2017) also have been attributed to the presence of diatoms. Microscopic observations of phytoplankton in Onagawa Bay before the tsunami (ABE *et al.*, 2011), and from 2018 to 2019 (MASUDA *et al.*, 2022) also showed that diatoms were dominant. The spring bloom caused by diatoms in Onagawa Bay from 2012 to 2013 was also considered to be a general seasonal characteristic. However, these microscopic observations, in comparison with the DNA sequencing in the present study from 2012 to 2013 show some inconsistencies. For example, *Pseudo-nitzschia* spp. and *Leptocylindrus danicus* observed by microscope in samples from 2018 to 2019 (MASUDA *et al.*, 2022) were not detected as dominant species by DNA sequences from 2012 to 2013. We speculate that this may be explained by differences in the offshore ocean environment between 2018–2019 and 2012–2013 (Masuda personal communication), or technical problems with DNA sequencing (MEDINGER *et al.*, 2010; XIAO *et al.*, 2014).

Although the correlation between Chl *a* and Chl *b* was lower than that between Chl *a* and Fuco, the number of green algae OTUs was the second highest after those of diatoms. The main species of green algae observed were *Micromonas* spp., *Ostreococcus* spp., and *Bathycoccus* spp.. There are a few reports of green algae from the Pacific Tohoku coast, in

comparison with diatoms (WATANABE *et al.*, 2017), which are useful producers in the marine food web, and dinoflagellates (MASUDA *et al.*, 2014), of which some species are poisonous. In Sendai Bay, picoeukaryotes are as abundant as diatoms (TANIUCHI *et al.*, 2017). In Onagawa Bay, picoeukaryotes are commonly observed in summer by microscopic observation (Masuda, personal communication). As *Micromonas* spp., *Ostreococcus* spp., and *Bathycoccus* spp. are less than 3 μm in diameter (VAULOT *et al.*, 2008), it is deduced that the picoeukaryotes commonly observed in Sendai Bay and Onagawa Bay are these three species of green algae.

The proportion of cyanobacteria, which have a diameter < 1 μm , increases during the summer (Fig. 5f). Both cyanobacteria and the picoeukaryotes, which are smaller than diatoms, have better tolerance to low nutrient conditions (FLOMBAUM *et al.*, 2013). These groups seasonally increase in summer with the rise in water temperature (FLOMBAUM *et al.*, 2013; TANIUCHI *et al.*, 2017). Therefore, it is considered that the proportion of green algae in Onagawa Bay was also higher in summer. The fact that *Prochlorococcus* sp. was detected at much higher levels than *Synechococcus* sp. may have been a characteristic at this time at Tsukahama in Onagawa Bay because *Synechococcus* sp. was frequently detected in larger numbers than *Prochlorococcus* sp. in the surface sediment in Onagawa Bay. (Okumura personal observation). Since the filtration rate of shellfish is considered to decrease due to the passage of small-sized plankton, < 5 μm , such plankton groups (RIISGÅRD, 1988) are presumably of lesser importance in shellfish feeding.

Among haptophytes, there were seven times more *Phaeocystis* spp. present than *Emiliania huxleyi*. *Phaeocystis* spp. are ubiquitous, and sometimes red tide, and some species are

reported to be toxic (SCHOEMANN *et al.*, 2005; WANG *et al.*, 2021). At present, the occurrence of *Phaeocystis* spp. is low in Onagawa Bay, and this species has not been investigated in natural feeding aquaculture making use of natural phytoplankton such as diatoms and dinoflagellates, but it is necessary to continuously monitor these species in the future.

The cryptophyte pigment Allo was often present in high concentrations in summer and autumn. Among the cryptophytes, *Teleaulax amphioxeia* and the cryptophyte species CR-MAL11 were dominant regardless of the season. These two species are food for the ciliate *Myrionecta rubra* (YIH *et al.*, 2004), on which *Dinophysis* spp. feeds (PARK *et al.*, 2006): *Dinophysis* spp. are responsible for diarrheal shellfish poisoning (SUZUKI *et al.*, 2017). However, in Onagawa Bay, the proportions of *Teleaulax amphioxeia* and CR-MAL11 were always high, and therefore provide conditions suitable for the growth of *Dinophysis* spp. If the timing of a high abundance of cryptophytes coincides with the presence of *Myrionecta rubra* and *Dinophysis* spp., diarrheal shellfish poisoning may occur due to the increase in *Dinophysis* spp. Indeed, diarrheal shellfish poisoning caused by *Dinophysis* spp. occurs almost every year in Onagawa Bay (MASUDA *et al.*, 2014).

Analysis of phytopigments by HPLC is a useful technique to determine the amount of each phytoplankton taxon because the concentration of each pigment present can be determined (MACKEY *et al.*, 1996; ZAPATA *et al.*, 2000), even if the phytoplankton species cannot be identified. However, samples cannot be stored for long periods because the pigments are easily degraded (MANTOURA *et al.*, 1997). DNA sequencing by NGS is suitable to identify phytoplankton species, and may sometimes surpass microscopic observation in detecting species in small numbers

(MEDINGER *et al.*, 2010; XIAO *et al.*, 2014). When microorganisms are sequenced, samples can be stored for more than ten thousands of years (LEJZEROWICZ *et al.*, 2013). The disadvantage of sequencing is that it is not quantitative, and may not always agree with the results of microscopic observation (MEDINGER *et al.*, 2010; XIAO *et al.*, 2014). In the present study, it was possible to compensate for the shortcomings of these individual techniques by combining pigment analysis by HPLC and DNA sequencing by NGS. The 454 sequencing by Genome Sequencer-FLX used provides less data than with the latest Miseq sequencer (Illumina, California, U.S.A.), the use of which in the future will enable a more detailed understanding of phytoplankton assemblages.

The present study investigated the entire phytoplankton assemblages in Onagawa Bay from 2012 to 2013. In addition to recognizing the presence of diatoms of use in shellfish feeding and dinoflagellates unsuitable for shellfish feeding, it was possible to understand the temporal trends of phytoplankton assemblages. Such useful information is necessary in order to evaluate the feed environment for aquaculture organisms, relying on natural feeding, such as the presence of picoeukaryotes and cyanobacteria (too small for filter-feeding), cryptophytes (indirect food of *Dinophysis* spp.), and haptophytes (some species of which are poisonous). Continued monitoring and accumulation of temporal data over the medium to long term will aid understanding of the feeding environment for shellfish aquaculture.

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