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Seasonal variation in abundance and species composition of the Parmales community in the Oyashio region, western North Pacific

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ABSTRACT: Seasonal variation in abundance and species composition of the Parmales community (siliceous pico-eukaryotic marine phytoplankton) was investigated off the south coast of Hokkaido, Japan, in the western North Pacific. Growth rates under various temperatures (0 to 20°C) were also measured using 3 Parmales culture strains, *Triparma laevis* f. *inornata*, *Triparma laevis* f. *longispina* and *Triparma strigata*. Distribution of Parmales abundance was coupled with the occurrence of Oyashio water, which originates from the cold Oyashio Current. In March and May, the water temperature was usually low (<10°C) and the water column was vertically mixed. Parmales was often abundant (>1 × 10^2 cells ml^{-1}) and evenly distributed from 0 down to 100 m. In contrast, when water stratification was well developed in July and October, Parmales was almost absent above the pycnocline at >15°C, but had an abundance of >1 × 10^2 cells ml^{-1} in the subsurface layer of 30 to 50 m at <10°C. The seasonal variations in the vertical distributions of the 3 dominant species (*Triparma laevis*, *Triparma strigata* and *Tetraparma pelagica*) were similar to each other. Growth experiments revealed that *Triparma laevis* f. *inornata* and *Triparma strigata*, and *Triparma laevis* f. *longispina* were able to grow at 0 to 10°C and 5 to 10°C, respectively, but not at over 15°C, indicating that their vertical distribution was mainly determined by temperature. The Parmales community grew in the surface layer before becoming evenly distributed with depth in winter and spring. During summer and into autumn, the population survived under the pycnocline.

KEY WORDS: Parmales · Seasonal variation · Species composition · Growth · Oyashio Current

INTRODUCTION

Photosynthetic pico-eukaryotes (≤3 µm) account for a large fraction of the biomass and primary production in marine ecosystems (Li 1994, Worden et al. 2004, Massana 2011). They are highly diverse, comprising several algal classes (Moon-van der Staay et al. 2001, Fuller et al. 2006, Vaulot et al. 2008). While the importance of pico-eukaryotes has been shown in oligotrophic tropical or subtropical waters, they are also abundant in the polar and sub-polar cold waters (Not et al. 2004, Isada et al. 2009). In these high latitude areas, the abundance and species composition of the pico-eukaryotic phytoplankton community might be expected to change seasonally, but information at present is limited to a few species, such as the Mamiellophyceae *Micromonas*, *Bathycoccus* and *Ostreococcus* (Not et al. 2004, Collado-Fabbri et al. 2011).

Parmales (Heterokonta) is a pico-sized eukaryotic marine phytoplankton group of small solitary cells that are surrounded by variously shaped silica plates (Booth & Marchant 1987). Fortunately, the number and morphology of these plates are species-specific

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(Booth & Marchant 1987, Konno & Jordan 2007, Konno et al. 2007), enabling their identification to species level under scanning electron microscopy (SEM). While Parmales species do not have flagellae of unequal length, they have a characteristic ultrastructure with 2 chloroplast endoplasmic reticulate membranes, a girdle lamella and 3 thylakoid lamellae, which places them within the heterokont lineage (Marchant & McEldowney 1986). However, the more detailed taxonomic position of Parmales is uncertain, due to lack of cultures, and it has been assumed that they belong to the Chrysophyceae (Booth & Marchant 1987). Recently, we isolated and characterized a Parmales strain, *Triparma laevis*, for the first time from the western North Pacific (Ichinomiya et al. 2011). Surprisingly, the molecular phylogenetic analyses of small subunit rDNA (SSU rDNA) and the *rbc*L gene revealed that the *Triparma* strain is positioned within the class Bolidophyceae, which is a group of autotrophic pico-sized naked flagellates with no silica structures (Guillou et al. 1999). Similar results were obtained for *T. laevis* f. *longispina*, *T. strigata* and *T.* cf. *verrucosa*, which had been newly cultured from the western North Pacific (M. Ichinomiya et al. unpubl.). Bolidophyceae has also been recognized as a sister group of diatoms, based on molecular phylogeny.

Parmales species are widely distributed from tropical to polar waters (see Takahashi et al. 1986, Booth & Marchant 1987, Guillou 2011). While some are found in subtropical and tropical waters (Kosman et al. 1993, Bravo-Sierra & Hernández-Becerril 2003), they are more frequently reported from polar and sub-polar waters (Booth et al. 1981, 1982, Nishida 1986, Taniguchi et al. 1995, Komuro et al. 2005, Konno et al. 2007, Ichinomiya et al. 2010, Hinz et al. 2012). Parmales are also known to be distributed in both the east and west in the subarctic North Pacific, including the Oyashio region, Bering Sea, Gulf of Alaska and Okhotsk Sea (Booth et al. 1980, 1982, Konno et al. 2007). Compared with biogeographic information, however, ecological studies of Parmales are rare. There are few studies of seasonal variations in abundance and the species composition of the Parmales community (Komuro et al. 2005).

The Oyashio region is an area surrounded by the Oyashio Current, which flows southwestward along the Kuril Islands and Hokkaido in the southwestern part of the western subarctic gyre (Itoh & Yasuda 2010). This turns to flow to the east off Honshu, becoming an extension flow. The Oyashio region is colder and less saline than the southern areas, i.e. the subtropical Kuroshio region and Kuroshio-Oyashio transition region, where warm and cold eddies frequently detach from the Kuroshio and Oyashio regions, respectively. In the Oyashio region, a massive phytoplankton bloom (mainly diatoms) normally occurs in spring due to the nutrient-rich Oyashio water, but this ends with the development of water stratification in summer (Isada et al. 2009, Suzuki et al. 2011). We previously reported the depth distribution of *T. laevis*, which is the dominant species among the Parmales community, across the Oyashio and Kuroshio-Oyashio transition regions during summer (Ichinomiya et al. 2013). We also found that *T. laevis* abundance was high in the Oyashio region but low in the transition regions, indicating that the presence of Oyashio water can affect Parmales abundance. *T. laevis* was also abundant in the subsurface layer (30 to 50 m), where water temperature was <10°C, but it was absent above the pycnocline where temperatures were >15°C. This agreed with results from growth experiments with a *T. laevis* strain, identified as *T. laevis* f. *inornata* (M. Ichinomiya et al. unpubl.), which was able to grow at 0 to 10°C, but not at temperatures over 15°C. Therefore, *T. laevis* is expected to maintain its population under the pycnocline during summer. In the present study, we determined the seasonal variation in abundance, spatial and vertical distribution, and species composition of the Parmales community in the Oyashio region. The aim of the present work was to investigate the seasonal growth and summer survival strategy of the Parmales species in this region.

MATERIALS AND METHODS

Sampling

Oceanographic observations were carried out from March to October 2009 during 4 seasonal cruises of the RV 'Wakataka-Maru' and 'Hokko-Maru', Fisheries Research Agency, along the monitoring route 'A-line', which crosses the Oyashio and Kuroshio-Oyashio transition regions, off the south coast of Hokkaido, Japan, in the western North Pacific (Fig. 1). Sampling was conducted at 16 stations in March, at 4 stations in May and at 21 stations in July and October.

Temperature and salinity were recorded with a CTD (Sea Bird Electronics). Water samples for quantifying nitrate, silicate and chlorophyll *a* concentration were collected with a bucket at the surface and Niskin sampling bottles from 7 depths from 10 to 100 m at Stns A1 to A4 and all the odd numbered

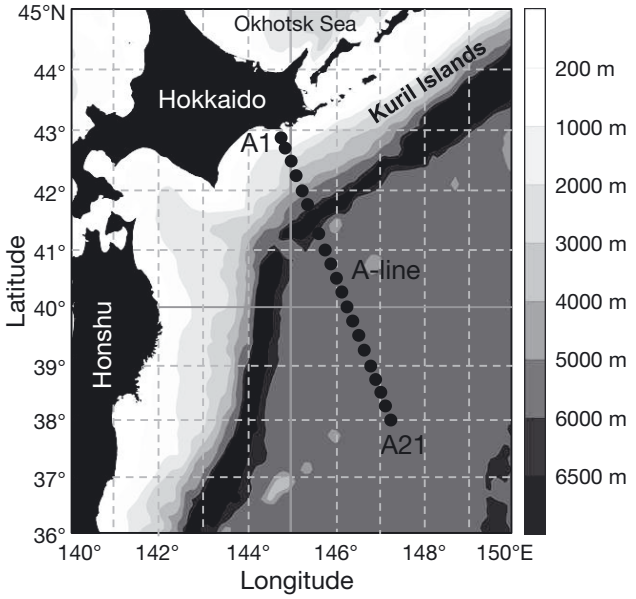


Fig. 1. Location of sampling stations (Stns A1 to A21) along the monitoring line (A-line) in the western North Pacific

stations from A5 to A21. The samples for quantifying abundance of the Parmales community were collected from 7 depths at 0, 10, 20, 30, 50, 80 and 100 m at the same stations sampled for nutrient analysis. At Stn A1, water was collected at 90 m but not at 100 m because of the shallower depths at this station. The 500 ml aliquots of water samples were fixed with acid Lugol's solution (final conc. 1%) and stored in a dark cold room (5°C) until scanning electron microscope (SEM) analysis.

The nitrate and silicate concentrations were determined with a TRAACS 800 autoanalyzer following the methods of Strickland & Parsons (1972). For chlorophyll *a* analysis, 128 ml aliquots of seawater were GF/F filtered. The filters were soaked in N, N-dimethylformamide to extract chlorophyll *a* (Suzuki & Ishimaru 1990) and concentrations were determined with a Turner Designs fluorometer (10-AU-005).

The counting method for the Parmales community was similar to that used by Booth et al. (1981) and Taniguchi et al. (1995). To quantify Parmales abundance, 286 ml aliquots of the fixed samples were filtered through 0.6 µm polycarbonate filters using low vacuum pressure (<10 kPa). Some samples with quite high chlorophyll *a* concentrations (approximately >5 µg l^{-1}) were prefiltered using 20 µm mesh to remove large dense phytoplankton cells. The filters were air-dried at room temperature after desalting with a drop of Milli-Q water and mounted on the SEM stubs. All filters were coated with Pt/Pd and examined with a SEM (Hitachi S-2550N). The Parmales cells were randomly counted on the stubs at 4000× magnification in up to 400 fields, representing 0.38% of the mounted area of the stubs with a detection limit of 0.9 cells ml^{-1}. Species identification was according to Booth & Marchant (1987), Marchant & Scott (2005) and Konno et al. (2007). In the present study, *Triparma columacea* and *T. laevis* were identified to species level, but some subspecies have been also reported, with variations in spines and cell wall ornamentation (Booth & Marchant 1987, Kosman et al. 1993, Konno et al. 2007). While almost all cells of these species were tightly covered with silica plates, a few broken plates were observed that were probably caused by vacuum pressure. This means that our counts may include some underestimation.

The Oyashio region was defined as the region where seawater temperature at 100 m depth was less than 5°C in March and May, 6°C in July and 7°C in October, following Kawai (1972) and Shimizu et al. (2009). At Stn A1, water temperature at 90 m was used for this definition. The water temperature that characterizes the Kuroshio waters is over 14°C at 200 m depth (Kawai 1969). Since there was no station where the water temperature at 200 m was >14°C in the present study, the areas outside the Oyashio region are categorized as belonging to the Oyashio-Kuroshio transition region.

Culture experiments in the laboratory

Incubation experiments used the *T. laevis* f. *longispina* and *T. strigata* strains that were isolated from the Oyashio region for the first time (Fig. 2). These strains were deposited in the culture collection of the National Institute of Environmental Science in Japan, with culture numbers NIES-3699 and 3701, respectively. They were maintained at 5°C under an irradiance of ca. 30 µmol m^{-2} s^{-1} with a 14 h light:10 h dark photoperiod in f/2 medium (Guillard & Ryther 1962). Incubation experiments were conducted in triplicate at 0, 5, 10 and 15°C under approximately 50 µmol m^{-2} s^{-1} from a white light-emitting diode (LED) (14 h light:10 h dark photoperiod), as in our previous study of *T. laevis* f. *inornata* (= *T. laevis* in Ichinomiya et al. 2013). Neither *T. laevis* f. *longispina* nor *T. strigata* grew at 15°C in pre-cultural experiments.

Each experiment was carried out using a 70 ml disposable sterilized culture bottle (Nunc). Inoculation was with a small amount of culture in the exponential growth phase at an initial cell concentration of 10^4 cells ml^{-1}. Incubation periods were 11 to 30 d,

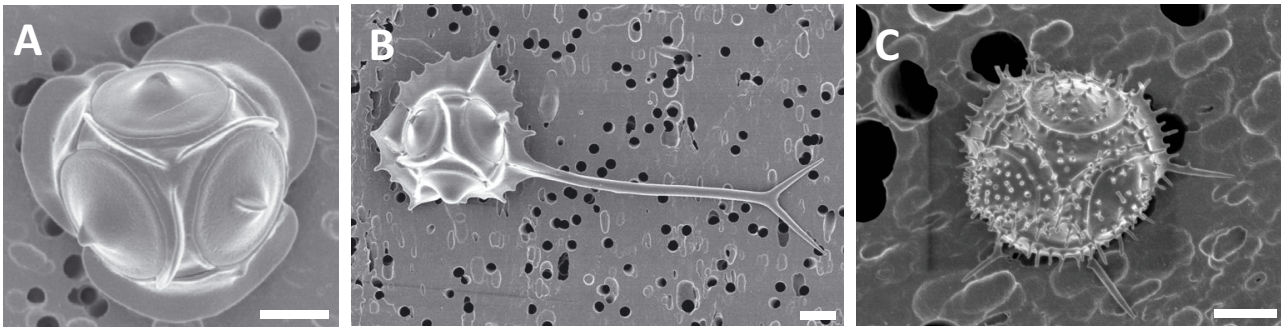


Fig. 2. Parmales strains used in culture experiments. (A) *Triparma laevis* f. *inornata*, (B) *T. laevis* f. *longispina*, (C) *T. strigata*. Scale bars = 1 µm

with subsampling every day. At each sampling time, a 1 ml aliquot of algal culture was fixed with acid Lugol's solution (final concentration of 1%) and stored at 5°C in dark. Cell concentrations were determined with a Burker-Turk cell-counting chamber until >400 cells had been counted in randomly selected fields. Growth rates (µ; d^{-1}) under varying temperature were determined from the changes in cell number with time by log-line regression during exponential growth.

RESULTS

Environmental conditions

According to horizontal temperature mapping offshore of Hokkaido, the cold area was distributed at nearly 39°N on the A-line (see the time series of isotherms at http://tnfri.fra.affrc.go.jp/kaiyo/temp/temp.html). Therefore, the Kuroshio-Oyashio transition region was distributed south of approximately 39°N. A slightly warmer water mass above 5°C at about 40 to 42°N migrated seasonally northward to the Oyashio region.

In March, the water column was vertically mixed (Fig. 3A). Stns A1 to A6, A15 and A16 were recognized as being within the Oyashio region from the water temperature at 90 or 100 m. The water temperature was <10°C throughout the water column at Stns A1 to A6, A9, A15 and A16, but >10°C at the other stations (Stns A7, A8 and A17 to A21) with relatively high salinity (>34). At Stn A1, water temperature and salinity were quite low at <0°C and <33, respectively. Nutrient concentrations were high in the Oyashio region with >10 µM nitrate and >20 µM silicate, except at Stn A1. Chlorophyll *a* concentrations were >1.0 µg l^{-1} at Stns A1 and A7, with a maximum of 4.9 µg l^{-1} at 20 m at Stn A1.

In May, the water temperature was <10°C throughout the water column at 4 stations, of which only Stn A7 was not recognized as being within the Oyashio region (Fig. 3B). High chlorophyll *a* concentrations of >5 µg l^{-1} were often observed at Stns A1 and A3, with a massive phytoplankton bloom of 11.4 µg l^{-1} at the surface at Stn A3. Nitrate remained >5 µM at all stations except at the surface at Stn A1.

In July, vertical stratification of water temperature was evident between 20 and 40 m at almost all stations (Fig. 3C). In the surface layer down to 20 m, water temperature reached >10°C or >15°C, except at Stn A1. Under the pycnocline, the water temperatures were <10°C at 50 m at many stations and <5°C at Stns A1, A2, A4 and A11 to A15. At the southern Stns A20 and A21, relatively low temperatures of <10°C were observed at 50 m, while water temperatures at the surface reached 20°C. The water temperature at 90 or 100 m was <6°C at Stns A1 to A4, A6 to A16 and A20 to A21, which were recognized as being within the Oyashio region. Nitrates were <5 µM or very low in the surface layers down to 20 m at stations to the south of Stn A5. Relatively high chlorophyll *a* concentrations (>1 µg l^{-1}) were observed from the surface down to 20 m at Stns A5 to A11 and A15.

In October, the water temperature further increased to >15°C in the surface layer down to approximately 40 m at many stations (Fig. 3D). However, the water temperature remained <10°C at 50 m at 11 stations (Stns A2 to A5, A7, A8, A10, A12 and A14 to A16). Stns A2 to A5, A7 to A12 and A14 to A18 were identified as belonging to the Oyashio region. Extremely low salinity at the surface (<33) was due to a typhoon just before the cruise. Nitrates were <5 µM or very low in the surface layers down to 20 m at stations further south than Stn A7. The chlorophyll *a* concentrations also decreased to <1 µg l^{-1} at stations further south than Stn A7.

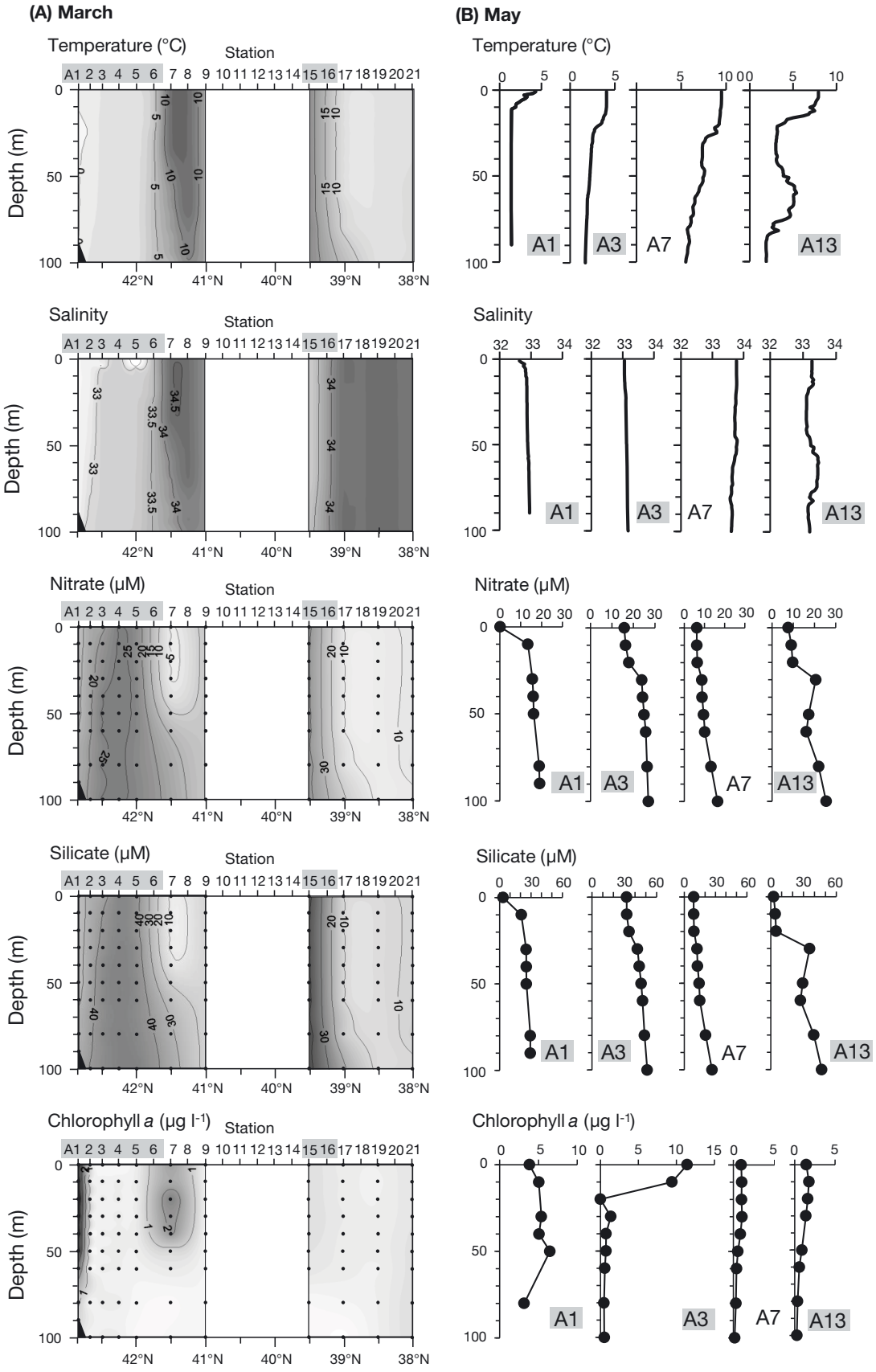
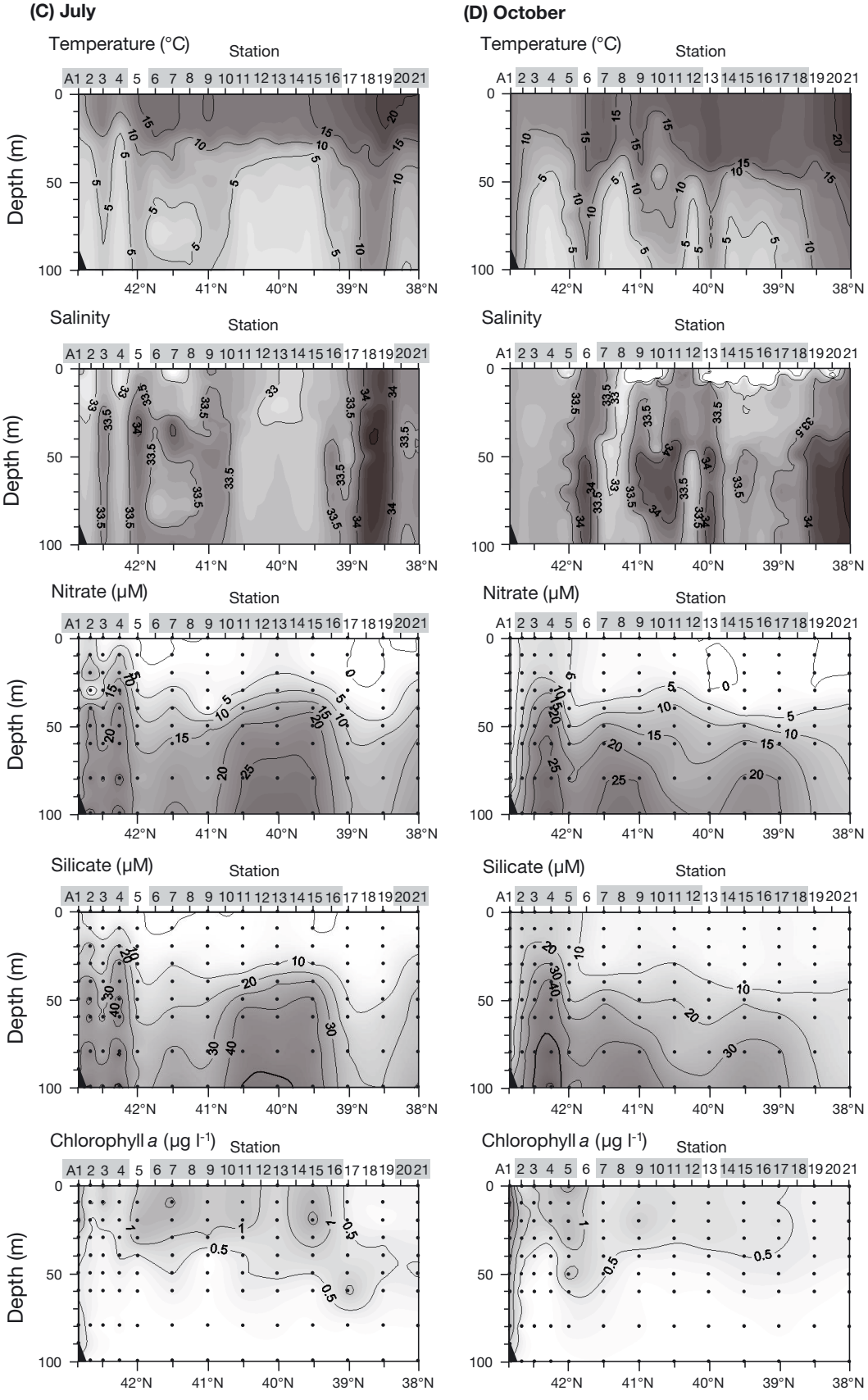


Fig. 3. (Above and following page.) Seasonal variation in depth distributions of temperature, salinity, nitrate, silicate and chlorophyll *a* concentration along the A-line in (A) March, (B) May, (C) July and (D) October 2009. Shaded station numbers indicate the Oyashio region



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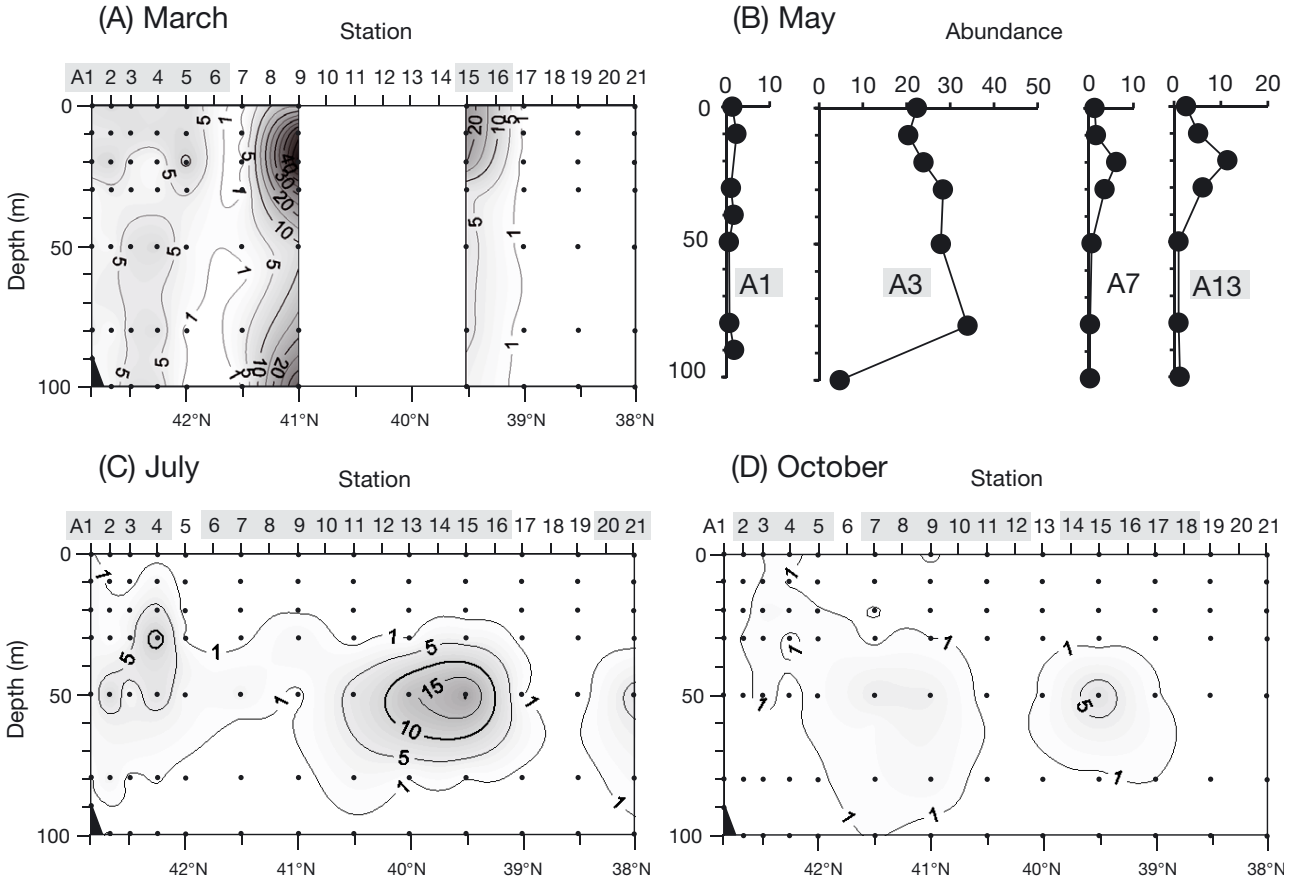

Fig. 4. Seasonal variation in the abundance (×10 cells ml^{-1}) of the Parmales community along the A-line in (A) March, (B) May, (C) July and (D) October 2009. Shaded station numbers indicate the Oyashio region

Seasonal variation of the Parmales community

The Parmales community was evenly distributed from 0 down to 100 m in March and abundant at Stns A1 to A5, A9 and A15, where the water temperature was low, i.e. <10°C (Figs. 3A & 4A). At these stations, the abundances of total Parmales were often >1 × 10^2 cells ml^{-1}, but <10 cells ml^{-1} at the other stations. The maximum abundance was 5.7 × 10^2 cells ml^{-1} at 20 m at Stn A9, followed by 2.4 × 10^2 cells ml^{-1} at the surface at Stn A15 (Fig. 4A).

In May, the abundance was >1 × 10^2 cells ml^{-1} at Stns A3 and A13, which was typical for the Oyashio region (Figs. 3B & 4B). At Stn A3, Parmales was also evenly distributed from 0 down to 80 m, with an abundance of 2.0 × 10^2 to 3.4 × 10^2 cells ml^{-1} (Fig. 4B).

In July, Parmales abundance had peaks in depths at 30 or 50 m at many stations, but with low abundances above and below this (Fig. 4C). The abundance was highest (2.1 × 10^2 cells ml^{-1}) at 50 m at Stn A15. An abundance of >1 × 10^2 cells ml^{-1} was observed at Stns A4, A13 and A15, typical for the Oyashio region. An abundance of 68 cells ml^{-1} was recorded at 50 m at the most southern Stn A21. In October, the abundance generally decreased to <10 cells ml^{-1}, but a slightly higher abundance of 74 cells ml^{-1} remained at 50 m at Stn A15 (Fig. 4D).

Within the Parmales community, 6 species (*Tetraparma catinifera*, *Tetraparma pelagica*, *Triparma columacea*, *Triparma laevis*, *Triparma strigata* and *Triparma verrucosa*) were recognized during the investigation (Fig. 5). The average percentage over the investigation was 64 ± 22% for *Triparma laevis*, followed by 11 ± 8% for *Triparma strigata* and 10 ± 16% for *Tetraparma pelagica*, and <5% for the other species. *Triparma laevis* was the dominant species and showed only small regional and seasonal difference in its relative presence. Occurrence of *Triparma strigata* was also constant at almost all stations through the year. On the other hand, a relatively high percentage of *Tetraparma pelagica* was observed at the coastal Stns A1 to A4 from March to July, with a high of 60% at Stn A1 in July.

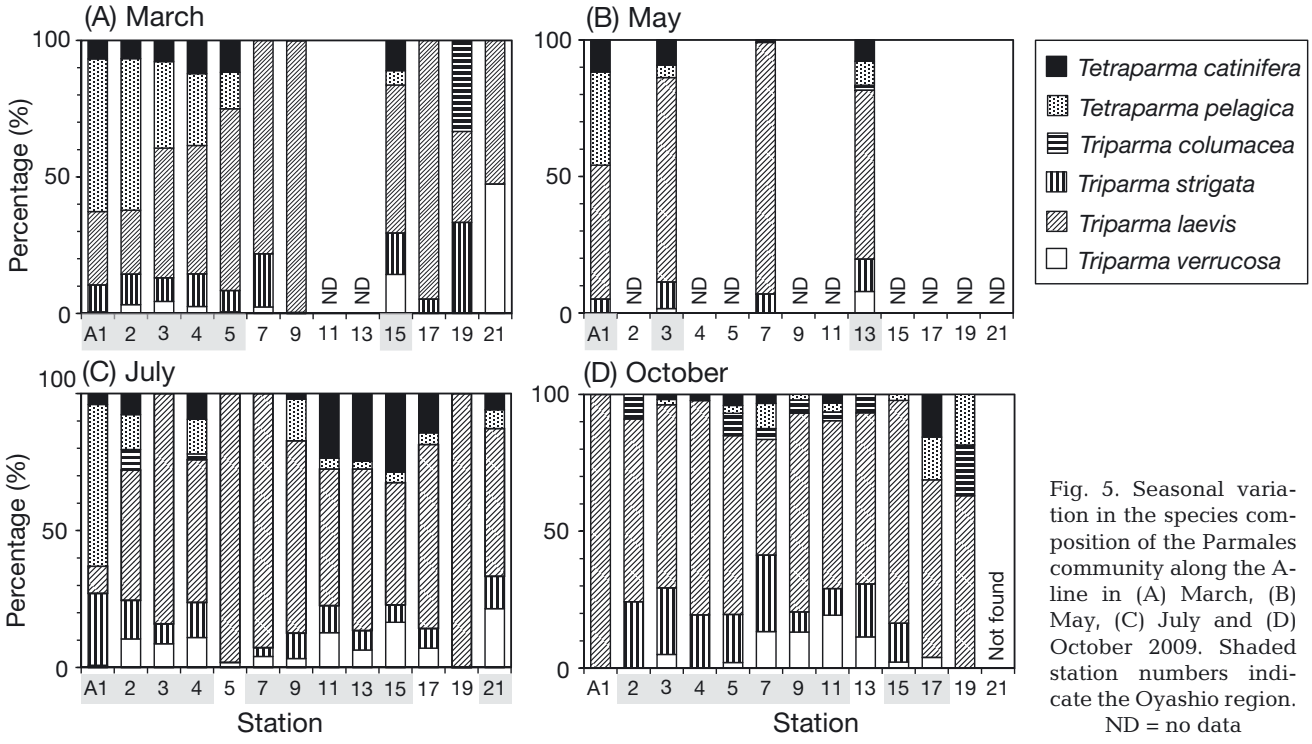

Fig. 5. Seasonal variation in the species composition of the Parmales community along the A-line in (A) March, (B) May, (C) July and (D) October 2009. Shaded station numbers indicate the Oyashio region. ND = no data

Seasonal variation of the dominant Parmales species

Seasonal variation of *Triparma laevis* was similar to that of total Parmales (Fig. 6, Table 1). High abundances of >1.0 × 10^2 cells ml^{-1} were observed at Stns A9 and A15 in March, and at Stns A3 and A13 in May (Fig. 6A,B). In July and October, *Triparma laevis* was less abundant (<10 cells ml^{-1}) in the surface layer down to 30 m at almost all stations, but with abundances of >50 cells ml^{-1} at 50 m at Stns A13 to A15 in July and at 50 m at Stn A15 in October (Fig. 6C,D). *Triparma strigata* was also abundant in March and May, with a maximum abundance of

Table 1. Abundance (cells ml^{-1}) of the Parmales species during the 4 seasonal cruises. T: temperature; S: salinity; *Tc*: *Tetraparma catinifera*; *Tp*: *Tetraparma pelagica*; *Tl*: *Triparma laevis*; *Ts*: *Triparma strigata*; *Tv*: *Triparma verrucosa*

Depth (m)	T (°C)	S	*Tc*	*Tp*	*Tl*	*Ts*	*Tv*
Stn A9 on 6 March 2009 during the WK0903 cruise							
0	7.57	34.035	0	0	1.3×10^2	2.7	0.9
10	7.56	34.035	0	0.9	4.9×10^2	0	0
20	7.56	34.035	0	0	5.7×10^2	0.9	0
30	7.57	34.037	0	0.9	3.3×10^2	0	0
50	7.56	34.036	0	0	87	0	0
80	7.57	34.037	0	0	1.8×10^2	1.8	0
100	7.57	34.038	0	0	3.7×10^2	0	0.9
Stn A3 on 13 May 2009 during the WK0905 cruise							
0	3.80	33.057	43	30	2.2×10^2	85	15
10	4.10	33.056	26	26	2.0×10^2	24	26
20	3.88	33.064	56	35	2.4×10^2	57	6.5
30	2.58	33.101	36	6.5	2.8×10^2	23	6.5
50	2.24	33.120	43	15	2.8×10^2	41	0
80	1.93	33.146	19	13	3.4×10^2	30	1.8
100	1.74	33.171	5.5	2.7	47	1.8	0

Depth (m)	T (°C)	S	*Tc*	*Tp*	*Tl*	*Ts*	*Tv*
Stn A15 on 7 July 2009 during the WK0907 cruise							
0	15.28	33.102	0	0	0	0	0
10	15.26	33.115	0	0	0	0	0
20	14.70	33.176	1.8	0	0	0	0
30	7.15	33.038	4.6	4.6	6.4	0.9	15
50	3.49	33.054	64	6.4	92	15	29
80	2.21	33.098	1.8	0	4.6	0	0.9
100	2.08	33.147	0	0	4.6	0	0
Stn A15 on 14 October 2009 during the HK0910 cruise							
0	17.46	32.544	0	0	0	0	0
10	17.46	32.988	0	0	0	0	0
20	17.46	32.990	0	0	1.8	0	0
30	17.48	33.026	0	0	0.9	0	0
50	8.39	33.504	0	1.8	60	10	1.8
80	5.32	33.466	0	0	2.7	1.8	0
100	4.17	33.443	0	0	9.1	0	0

85 cells ml^{-1} at the surface at Stn A3 in May (Fig. 6A,B). *Triparma strigata* also had abundance peaks at 30 m at Stn A4 and at 50 m at Stns A13 to A15 in July, and at 50 m at Stn A7 in October (Fig. 6C,D). *Tetraparma pelagica* was found at the coastal stations with abundances >10 cells ml^{-1} at Stns A1 to A5 and A15, with a maximum abundance of 59 cells ml^{-1} at 20 m at Stn A2 in March (Fig. 6A). *Tetraparma pelagica* was observed with abundances of >10 cells ml^{-1} at Stn A1 in May and July, where the other Parmales species were less abundant (Fig. 6B,C). In October, the abundance decreased to <10 cells ml^{-1} at all stations, with a maximum abundance of 5.5 cells ml^{-1} at 50 m at Stn A7 (Fig. 6D).

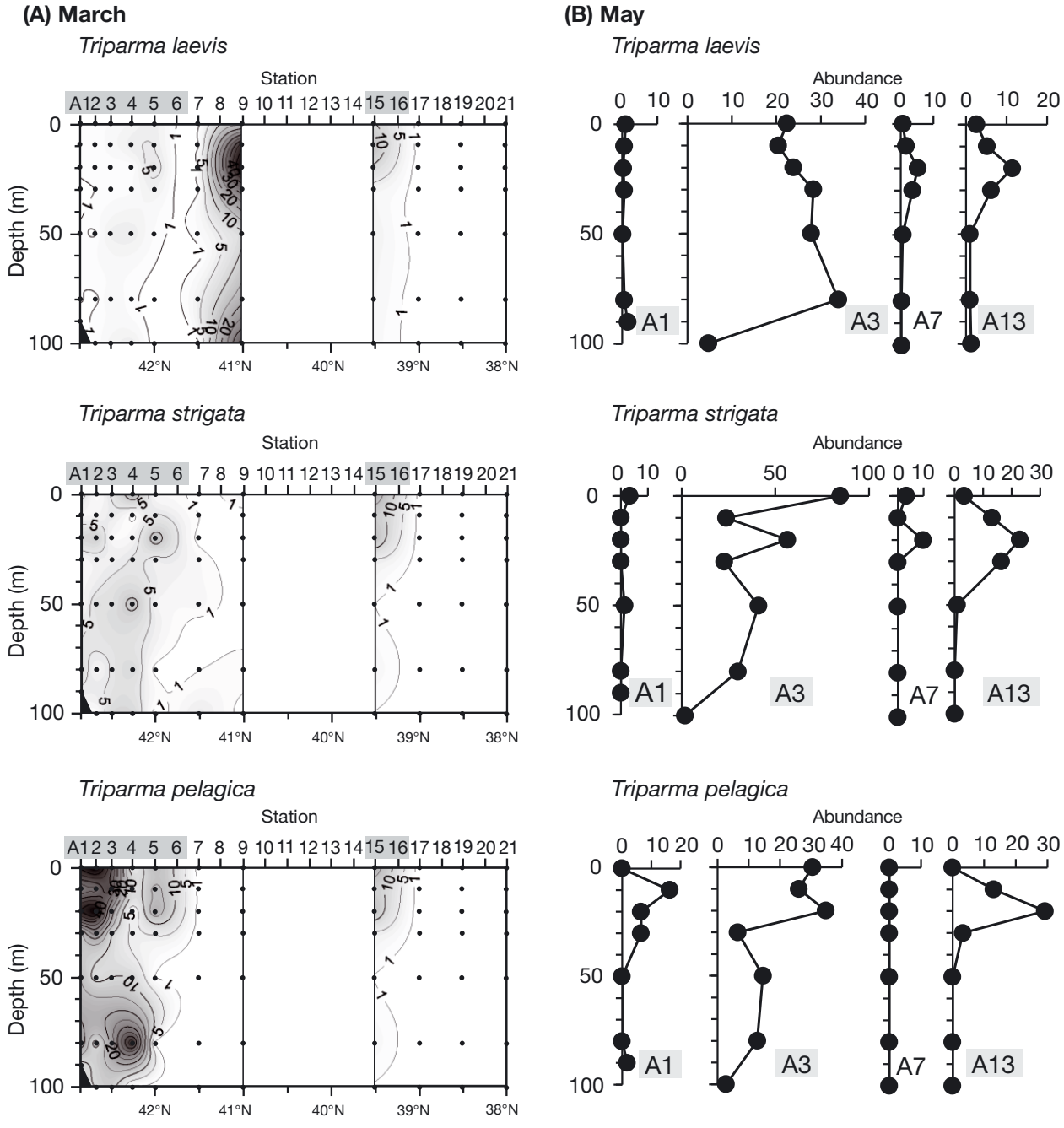

Fig. 6. (Above and following page.) Seasonal variation in the abundance of the dominant Parmales species, *Triparma laevis* (×10 cells ml^{-1}), *Triparma strigata* (cells ml^{-1}) and *Tetraparma pelagica* (cells ml^{-1}), along the A-line in (A) March, (B) May, (C) July and (D) October 2009. Shaded station numbers indicate the Oyashio region

During the investigation, temperature and salinity at the water sampling depths for quantifying Parmales abundance in the water column down to 100 m ranged from −0.1 to 22.2°C and from 32.2 to 34.5, respectively (Fig. 7). While *Triparma laevis* was found in waters with temperatures over the wide range of −0.1 to 18.4°C, high abundances of >1.0 × 10^2 cells ml^{-1} were limited to waters with temperatures of 1.9 to 7.6°C in March and May (Fig. 7A,B). For *Triparma strigata* and *Tetraparma pelagica*, abundances of >10 cells ml^{-1} were also limited to waters with temperatures of −0.1 to 9.9°C and −0.1 to 8.4°C, respectively. *Tetraparma pelagica* occurred at high abundances of >50 cells ml^{-1} when there were low temperature (<1°C) and low salinity (<33) ranges. However, abundances of >50 cells ml^{-1} were found when the temperature was >1.4°C for *Triparma laevis* and >3.8°C for *Triparma strigata*.

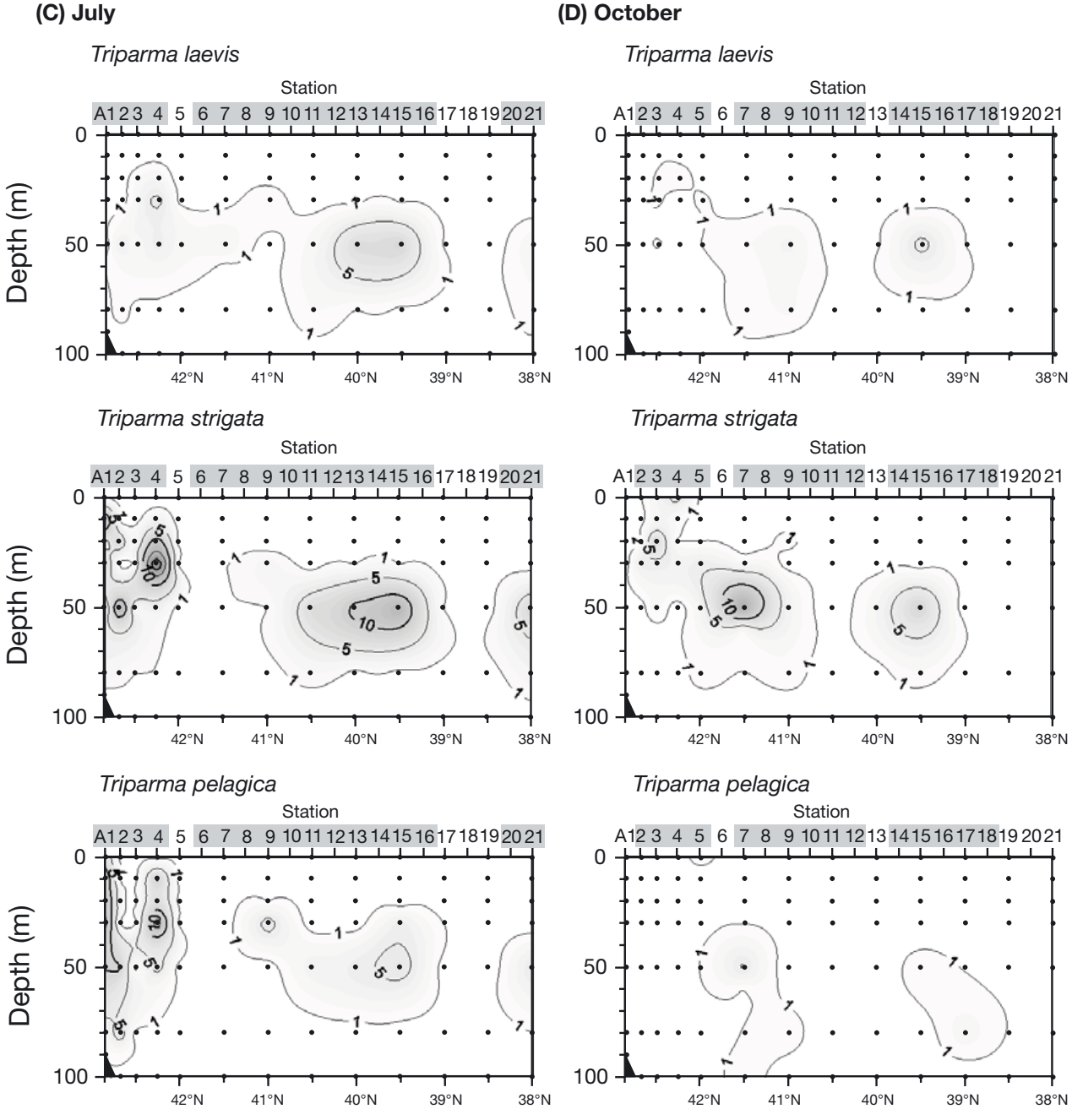

Fig. 6 (continued)

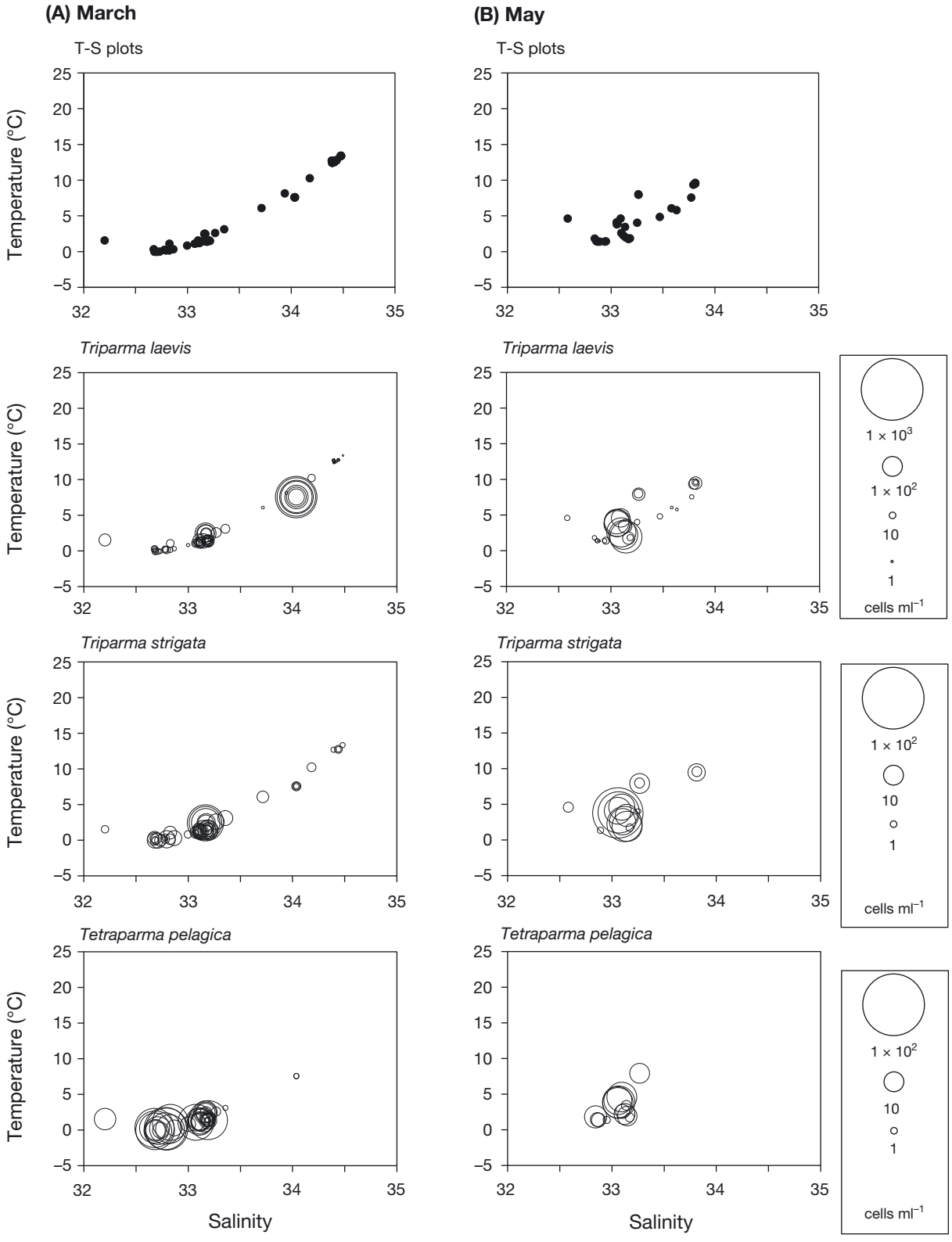

Fig. 7. (Above and following page.) Temperature-salinity (T-S) plots in the 0 to 100 m water columns and the abundance of the dominant Parmales species, *Triparma laevis*, *Triparma strigata* and *Tetraparma pelagica*, with water temperature and salinity in (A) March, (B) May, (C) July and (D) October 2009

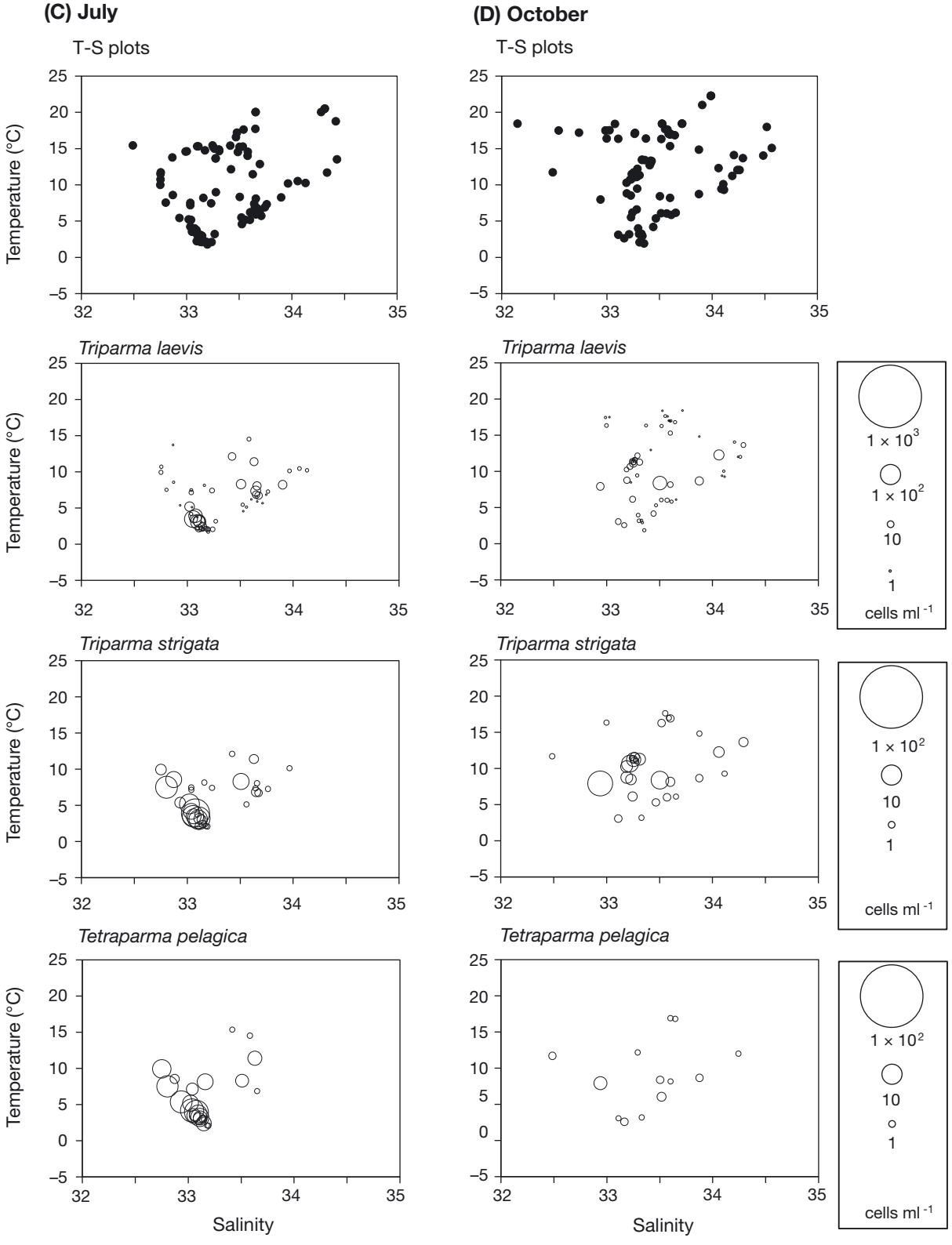

Fig. 7 (continued)

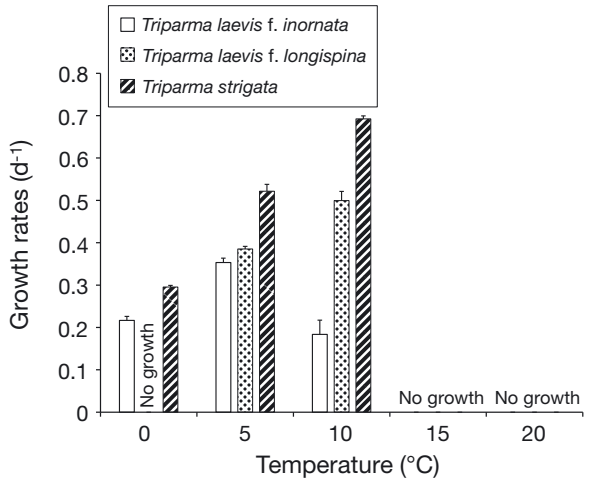

Fig. 8. Growth rates of *Triparma laevis* f. *inornata*, *T. laevis* f. *longispina* and *T. strigata* at 0, 5, 10, 15 and 20°C at an irradiance of ca. 50 µmol m^{-2} s^{-1} (14 h light:10 h dark cycle). Data for *T. laevis* f. *inornata* are the same as those for *T. laevis* reported by Ichinomiya et al. (2013). *T. laevis* f. *longispina* did not grow in pre-cultural experiments at 0°C and none of the strains grew at 15 or 20°C. Error bars are SD (n = 3)

Culture experiments

In culture experiments, *Triparma laevis* f. *inornata*, *Triparma laevis* f. *longispina*, and *Triparma strigata* showed exponential growth in batch culture over the range of 0 to 10°C, 5 to 10°C, and 0 to 10°C, respectively, under an irradiance of approximately 50 µmol m^{-2} s^{-1} (Fig. 8). The optimal temperatures were 5°C for *T. laevis* f. *inornata*, 10°C for *T. laevis* f. *longispina* and 10°C for *T. strigata*, with growth rates of 0.35 ± 0.18 d^{-1}, 0.50 ± 0.02 d^{-1} and 0.69 ± 0.01 d^{-1}, respectively.

DISCUSSION

Distribution of the Parmales community in the Oyashio region

The present study shows that the spatial distribution of the Parmales community is closely linked to water temperature, with high abundances in the Oyashio region (Fig. 4), generally occurring at the time when the water temperatures were below 10°C (Fig. 3). This was confirmed by culture experiments that showed that the optimal growth rates of each Parmales species were observed at temperatures lower than 10°C (Fig. 8). At the most southern Stn A21 in July, relatively low temperatures of <10°C were observed below 50 m and Parmales abundance was slightly higher than 74 cells ml^{-1} (Fig. 4C). This is likely the result of the presence of cold water at Stns A20 and A21, defining it as Oyashio water. On the other hand, Parmales showed low abundance or was absent in the warm waters north of 39° N (Fig. 3). During the present study, warm waters that probably detached from the Kuroshio Currents were observed at around 40 to 42° N in March, July and October. In the southern part of the Oyashio region, variously sized cold and warm areas of water originate from the extensions of the Oyashio and Kuroshio Currents, which then detach and migrate temporally (Itoh & Yasuda 2010). Parmales abundance appears to be related to these hydrographic conditions, which are affected by localized physical parameters and large-scale oceanographic currents. Therefore, the primary factor influencing Parmales abundance in this region is water temperature.

In the present study, 6 Parmales species were recognized (Fig. 5), i.e. *Tetraparma catinifera*, *Tetraparma pelagica*, *Triparma columacea*, *Triparma laevis*, *Triparma strigata* and *Triparma verrucosa*, all of which are common in the North Pacific (Booth et al. 1981, Konno et al. 2007). The dominant species was *Triparma laevis* in all seasons, with only a small variation in the species composition. The dominance of *Triparma laevis* suggests that the ambient environments in this region are favorable for this species. This contrasts with the report that *Tetraparma pelagica* was usually dominant at Stn KNOT in the Western Subarctic Gyre of the western North Pacific, at a higher latitude than the present study area (Komuro et al. 2005). Using shipboard incubations in the western North Pacific, Taniguchi et al. (1995) observed that *Triparma* spp. could grow actively at 10°C but not *Tetraparma* spp. They suggested that *Triparma* spp. may prefer higher temperatures than *Tetraparma* spp. In the present study, high percentages of *Tetraparma pelagica* were observed at low temperature ranges only at coastal stations in March, May and July (Figs. 3, 5 & 7). From January to July, relatively low temperature and salinity water (<33.0), which mainly originated from the Okhotsk Sea, appeared along the Hokkaido coast (Kono et al. 2004), and it could be distinguished from the offshore Oyashio water by its salinity. This is shown at Stns A1 and A2 in March, May and July, where salinity was below 33.0 (Fig. 3A–C). At Stn A1 from March to July, the abundance of *Tetraparma pelagica* was often >10 cells ml^{-1}, but the other species were rare (Fig. 7A–C). Therefore, low temperature and salinity conditions originating from the Okhotsk Sea may be appropriate for *Tetraparma pelagica*. This suggests

that the abundance and species composition of the Parmales community are also affected by environmental conditions, including water temperature, salinity and water origins.

Seasonal variation in vertical distribution of the Parmales community

The Parmales community showed a clear seasonal variation in abundance and vertical distribution (Fig. 4). The community was mainly composed of *Triparma laevis*, which was evenly distributed from 0 down to 100 m, with a seasonal maximum of 5.7 × 10^2 cells ml^{-1} in March and May when the water column was well mixed or weakly stratified (Fig. 3A,B). In contrast, in July and October during stratification, the Parmales community was mainly distributed under the pycnocline at 30 to 50 m, where water temperature was <10°C (Figs. 3C,D & 4C,D). Parmales was less abundant from the surface down to 20 m, where temperatures were >15°C above the pycnocline. This seasonal variation in depth distribution was common among the dominant Parmales species *Triparma laevis*, *Triparma strigata* and *Tetraparma pelagica* (Figs. 3 & 6). Komuro et al. (2005) also reported similar seasonal variations in depth distributions of Parmales from 0 to 100 m in January and May in the Western Subarctic Gyre of the western North Pacific, but they were limited to the subsurface layer of 30 to 100 m in August. Our previous study of the temperature response of a Parmales culture revealed that *Triparma laevis* was able to grow at 0 to 10°C, with optimum growth at 5°C (growth rate of 0.35 d^{-1}), but it could not grow at temperatures higher than 15°C (Ichinomiya et al. 2013). The distribution of the Parmales community corresponded well with that predicted from the growth experiments at different temperatures (Fig. 8). Three *Triparma* strains were able to grow at 0 to 10°C or 5 to 10°C, but not at >15°C. Therefore, the Parmales community could grow actively during winter and spring, but only quite slowly or not at all in summer and autumn, because the temperature is too high in the surface layer. The broad vertical distribution from 0 to 100 m in March and May was the result of vertical mixing.

The Parmales community maintained its population under the pycnocline in July (Fig. 4C). Similar subsurface Parmales populations have been reported in the Southern Ocean (Nishida 1986) and in the western North Pacific (Komuro et al. 2005). Ichinomiya et al. (2013) concluded that a *Triparma laevis* population at 50 m depth during summer would not be expected to increase in abundance when it had low growth rates of −0.01 to 0.08 d^{-1} due to low light conditions. The growth rates were calculated from experiments using a Parmales culture under various irradiances, including the photosynthetically available radiation (PAR) at this depth, which was estimated to be 0.05 to 0.43% of the surface PAR. Ichinomiya et al. (2013) also suggested that the growth rate of *Triparma laevis* at 30 m, where the irradiance is equivalent to 1.1–3.8% of surface irradiance, will be 0.02–0.34 d^{-1} if the pycnocline is shallow. However, the subsurface abundance peaks at 50 m in July would be the result of a decrease in abundance above the pycnocline, and not from active growth in the subsurface layers. These subsurface populations remained even into October, because the water temperature remained under 10°C at 50 m depth at some stations (Figs. 3D & 4D). This is very important to enable the Parmales community to maintain its population in the water column throughout the year. It is probable that some of the Parmales cells in the subsurface layers are returned to the surface by vertical mixing in winter and these provide the inoculum for the next winter-spring bloom in the surface layer. Concurrently, they can avoid unfavorable conditions, such as high temperature and low nutrient concentrations in the surface layer during summer (Fig. 3). The Parmales populations under the pycnocline may play a role as a 'seed' population.

Ecological role of Parmales

In the western North Pacific, the dominant phytoplankton groups are diatoms in spring but picophytoplankton, including prokaryotes and eukaryotes, in summer (Liu et al. 2002). Chlorophyll *a* concentrations in the Oyashio region are also highest in spring (reaching 10 µg l^{-1}), but low (<1 µg l^{-1}) in winter and summer (Saito et al. 2002). Therefore, the relative contribution of Parmales to primary production increased in winter, because the abundance of the Parmales community was higher in winter and spring than in summer and autumn (Fig. 4). However, the abundance of the Parmales community was generally low, in the order of <10 to 100 cells ml^{-1} (Fig. 4), compared with that of the pico-eukaryotic phytoplankton, which reaches 10^3 to 10^5 cells ml^{-1} (Liu et al. 2002, Isada et al. 2009, Suzuki et al. 2011). From the A-line monitoring data, the average abundance of pico-eukaryotic phytoplankton in the surface layer (0 to 100 m) in the Oyashio region was also in the range of 2000 to 7000 cells ml^{-1} in 2004 to 2008

(A. Kuwata et al. unpubl.). Apart from only one study report (Hinz et al. 2012), in which the maximum abundance of *Tetraparma pelagica* reached up to 1900 cells ml^{-1} in the Southern Ocean, our values for Parmales abundance are similar to those reported for communities in a number of habitats, including 140 cells ml^{-1} off the Aleutian Islands (Nishida 1979), 700 cells ml^{-1} in the Gulf of Alaska (Booth et al. 1980), 100 cells ml^{-1} in the Southern Ocean (Nishida 1986) and 2 studies in the Weddell Sea reporting 5862 cells ml^{-1} (Silver et al. 1980) and 900 cells ml^{-1} (Buck & Garrison 1983). Also, in addition to abundance, the growth rates of the Parmales reported here (Fig. 8), as well as those of 0.29 to 0.38 d^{-1} at 5 to 12°C for natural Parmales populations (Taniguchi et al. 1995), are similar to the rate of 0.55 d^{-1} at 6 to 8°C for a cold water phytoplankton species *Micromonas pusila* (Prasinophyceae) (Lovejoy et al. 2007) that is dominant in Arctic waters (Not et al. 2005). Therefore, we believe that Parmales abundance is probably not limited by growth rate, but by other environmental factors that we do not presently understand. However, 1 contributory factor in the western North Pacific may be that because of their small cell size, only a small fraction of the Parmales population sinks to below the euphotic zone (Komuro et al. 2005). Also, some studies have reported parmalean siliceous plates in the fecal pellets or gut contents of zooplankton (Booth et al. 1980, Marchant & Nash 1986, Urban et al. 1992, Konno & Jordan 2012) and the relatively low values of Parmales abundance might be explained by preferential grazing by zooplankton in winter and early spring when other phytoplankton are scarce.

In the present study, we investigated the seasonal dynamics of the Parmales community in the subarctic Oyashio region. Guillou (2011) found a lot of eukaryotic environmental SSU rDNA sequences that were closely associated with known Bolidophyceae derived from samples taken from equatorial to polar waters, even including 1 sequence from freshwater. This leads to the conclusion that the Parmales or bolidophycean algae are widely distributed, since 4 strains of *Triparma* species are positioned within the class Bolidophyceae (M. Ichinomiya et al. unpubl.). Parmales have also been observed in various environments, such as equatorial deep waters (Bravo-Sierra & Hernández-Becerril 2003) and Antarctic sea ice (Garrison et al. 2005). However, there are few ecological studies on Parmales except those carried out in subarctic waters (Komuro et al. 2005, Ichinomiya et al. 2013).

Parmales and Bolidophyceae share a common ancestor with diatoms (Ichinomiya et al. 2011). Diatoms are one of the most successful phytoplankton groups, providing 40% of primary production and showing high diversity (Armbrust 2009). In the Oyashio region, diatoms grow faster in the spring and are grazed by mesozooplankton, but their large size results in most cells sinking at the end of spring (Takahashi et al. 2008). As a result, the species composition of diatoms changes seasonally (Suzuki et al. 2011). In contrast, the seasonal variation of the species composition of the Parmales community was small, with a predominance of *Triparma laevis* (Fig. 5). A major contributory factor to this is that Parmales can stay in suspension in the subsurface layer due to their small cell size, where we now know they can also continue to grow in conditions where silica concentrations are low (Yamada et al. 2014). This difference between diatoms and Parmales communities makes it likely that they have quite different roles within marine ecosystems. Therefore, we need more ecological information on Parmales to get a better understanding of why their distribution is more restricted to cooler waters in higher latitudes than the much more widely distributed diatoms.

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