Vol. 68: 107–116, 2013
doi: 10.3354/ame01606

AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol

Published online January 10

Growth characteristics and vertical distribution of *Triparma laevis* (Parmales) during summer in the Oyashio region, western North Pacific

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ABSTRACT: The vertical and regional distribution of *Triparma laevis* (Parmales), a siliceous pico-sized eukaryotic marine phytoplankton species, was investigated during summer off the south coast of Hokkaido, Japan, in the western North Pacific. Growth characteristics were also studied in the laboratory using a recently isolated culture strain. *T. laevis* was abundant in the subsurface layer (30 to 50 m), where water temperature was <10°C, but it was absent above the pycnocline when temperatures were >15°C. Growth experiments revealed that *T. laevis* was able to grow at 0 to 10°C but not higher than 15°C, indicating that its depth distribution mainly depended on temperature. High irradiances resulted in increased growth rates of *T. laevis*, with the highest rates of 0.50 d^{-1} at 150 µmol m^{-2} s^{-1}. Using measured daily incident photosynthetically available radiation and *in situ* light attenuation, the growth rates of *T. laevis* at 30 and 50 m were calculated as 0.02 to 0.34 and −0.01 to 0.08 d^{-1}, respectively. Distribution of *T. laevis* was strongly coupled with the occurrence of Oyashio water. The growth rate of *T. laevis* was lower than that of bolidophytes and diatoms, which are closely related to Parmales. Bloom forming diatoms in this region have higher growth rates and a broader temperature range (0 to 20°C) and, therefore, might be expected to dominate, but Parmales does manage to co-exist in this region, albeit at a moderate abundance compared to the diatoms.

KEY WORDS: *Triparma laevis* · Parmales · Vertical distribution · Growth characteristics · Oyashio region

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). To understand how environmental factors affect their abundance and distribution as well as their ecological role in marine environments, an auto-ecological approach is necessary. However, there have been few auto-ecological studies combining measures of the abundance and distributions of pico-eukaryotic phytoplankton (Guillou et al. 1999b, Not et al. 2005) with growth characteristics determined from culture experiments (Timmermans et al. 2005, Lovejoy et al. 2007).

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). They are widely distributed from tropical to polar waters (Nishida 1986, Takahashi et al. 1986, Booth & Marchant 1987, Kosman et al. 1993), but they are more frequently reported from polar and sub-polar waters (Booth et al. 1981, 1982, Taniguchi et al. 1995, Komuro et al. 2005, Konno et al. 2007). Phylogenetic information

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was very limited until we managed to isolate and characterize a Parmales species, *Triparma laevis*, for the first time from the western North Pacific (Ichinomiya et al. 2011). The molecular phylogenetic analyses of SSU rDNA and the *rbc*L gene revealed that *T. laevis* belonged to the class Bolidophyceae, which are autotrophic pico-sized naked flagellates that lack silica structures and form a sister group of diatoms (Guillou et al. 1999a).

Some morphological features of Parmales, such as the number of plates or plate ornamentation, are species-specific (Booth & Marchant 1987, Konno et al. 2007) and can only be observed with a scanning electron microscope (SEM). There have been some studies on abundance and distributions of Parmales species (Booth et al. 1980, Bravo-Sierra & Hernández-Becerril 2003, Konno & Jordan 2007), but only 2 studies have assessed growth characteristics using natural Parmales assemblages (Taniguchi et al. 1995, Ichinomiya et al. 2010) and no laboratory growth studies using cultures.

The Oyashio Current is located in the southwestern part of the western subarctic gyre in the North Pacific, flowing southwestward along the Kuril Islands and Hokkaido. The flow turns to the east off Honshu, becoming an extension flow. The area surrounded by the Oyashio Current and its extension is called the Oyashio region. 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 (Itoh & Yasuda 2010).

In the Oyashio region, a massive phytoplankton bloom (mainly diatoms) normally occurs in spring due to the nutrient-rich Oyashio water, but this terminates with the development of water stratification in summer (Isada et al. 2009). Parmales abundance increases from late spring, reaching a peak in summer–autumn in the Oyashio region (H. Hattori pers. comm.). However, the spatial distribution of Parmales across the Kuroshio-Oyashio transition region as well as the effects of hydrographic features are still unknown.

Triparma laevis belongs to the family Triparmaceae and is widely distributed in the North Pacific (Booth et al. 1982, Konno et al. 2007). In the Oyashio region, Ichinomiya et al. (2010) reported that this species was dominant (>90%) among the Parmales assemblage in winter. The aim of the present study was to investigate what controls the vertical and horizontal distributions of *T. laevis* during summer by combining field data collected from across the Oyashio and Kuroshio-Oyashio transition regions with laboratory investigations of growth characteristics under varying temperatures and irradiances.

MATERIALS AND METHODS

Field study

Oceanographic observations were carried out from 7 to 22 July 2009 at 21 stations 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. 1A). Temperature and salinity were recorded with a CTD. Water samples for quantifying nutrients and chlorophyll *a* concentrations were collected with a bucket at the surface and Niskin sampling bottles from 7 depths from 10 to 100 m at every station of A1 to A4 and odd stations of A5 to A21. The samples for quantifying the abundance of *Triparma laevis* were collected from 7 depths at 0, 10, 20, 30, 50, 80 and 100 m at the same stations collected for nutrient analysis. At Stn A1, the water was collected at 90 m but not at 100 m because the depth at this station was <100 m. Aliquots (500 ml) of water samples were fixed with acid Lugol's solution (final conc. 1%) and stored in a dark cold room (5°C) until SEM analysis.

Incident photosynthetically available radiation (PAR, 400 to 700 nm) above the sea surface was continuously measured on deck with a PAR sensor (ML-020P, EKO Instruments) and recorded in a data logger during the investigation period. During daytime sampling, vertical profiles of water PAR were obtained with a profiling reflectance radiometer PRR-600/610 (Biospherical Instruments). From the vertical profiles, attenuation coefficients (k, m^{-1}) were determined to approximate the following equation:

$$I_z = I_0 \exp^{-kz} \quad (1)$$

where I_z and I_0 are the irradiance at each depth (z, m) and the surface.

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

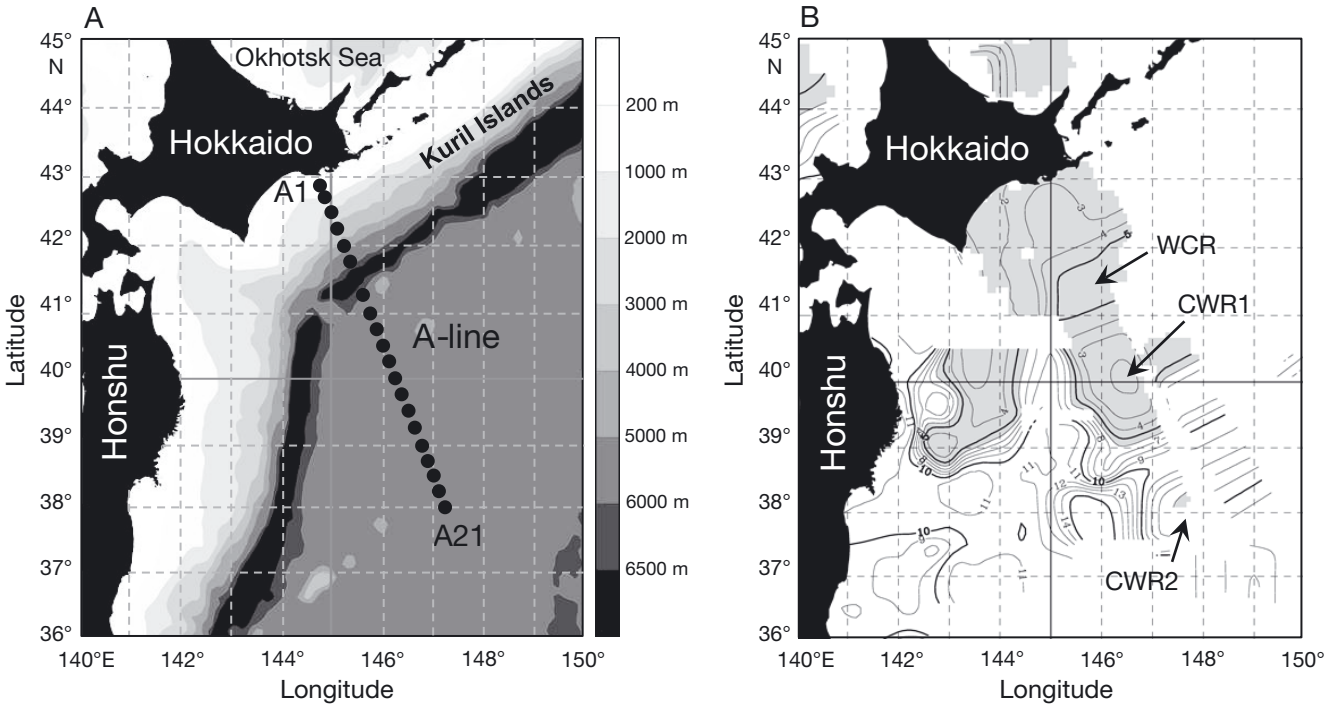

Fig. 1. (A) Sampling stations along the monitoring section A-line with the bottom topography and (B) the isotherms (°C) at 100 m depth in July 2009. The temperature map was the color-modified one of the monthly publication by Tohoku National Fisheries Research Institute (http://tnfri.fra.affrc.go.jp/kaiyo/temp/data/200907_100.gif), which was drawn based on present observations and the other cooperative organizations' data. The area with temperature <6°C at 100 m depth, which was regarded as the Oyashio region according to Kawai's (1972) indicator isotherm, is shaded, and the locations of a warm core ring (WCR) and the cold water regions (CWR1 and CWR2) along the A-line are denoted in panel B

The counting method for *Triparma laevis* was similar to that used by Booth at al. (1981) and Taniguchi et al. (1995). To quantify *T. laevis* abundance, 286 ml aliquots of the fixed samples were filtered through 0.6 µm polycarbonate filters using low vacuum pressure (<10 kPa). 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 numbers of *T. laevis* cells were randomly counted on the stubs at 4000× with a SEM in up to 400 fields, representing 0.38% of the mounted area of the stubs, with a detection limit of 9.1×10^2 cells l^{-1}. In the present study, *T. laevis*, which has 3 subspecies and 1 form based on the existence of spines and cell wall ornamentation (Booth & Marchant 1987, Kosman et al. 1993, Konno et al. 2007), was identified to species level. While almost all *T. laevis* cells 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.

Culture experiments in the laboratory

Incubation experiments used the *Triparma laevis* strain that was isolated from the Oyashio region and deposited in the culture collection of the National Institute of Environmental Science in Japan under number NIES-2565 (Ichinomiya et al. 2011). It was maintained at 5°C under 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, 15 and 20°C at ca. 50 µmol m^{-2} s^{-1} by white LED (14 h light:10 h dark photoperiod), but *T. laevis* did not grow at 15 and 20°C in pre-cultured experiments. Experiments with 21 irradiance levels (3.7 to 200 µmol m^{-2} s^{-1}, 14 h light:10 h dark photoperiod) were conducted at 5°C.

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 10^3 cells ml^{-1}. Incubation periods were 11 to 30 d, with subsampling every day, except at low irradiance levels (3.7 to 9.8 µmol m^{-2} s^{-1}) which were subsampled

every other day. At each sampling time, a 1 ml aliquot of algal culture was fixed with 10% paraformaldehyde (final conc. of 1%) and immediately stored in liquid nitrogen. Cell concentrations of *Triparma laevis* were determined by an Epics XL (Beckman Coulter) flow cytometer equipped with a 15 mW laser at 488 nm excitation with the standard filter set-up for enumeration of picophytoplankton. Red fluorescence (>630 nm) from chlorophyll *a* was measured. Sample acquisition time was set for 5 min and ceased when 10 000 events were counted. The analytical volume at a given time and flow rate was calibrated by running solutions of beads of known concentration. For a size check, internal standard beads of 1.0 and 2.0 µm solid latex (Polysciences) were added to each sample. All data were analyzed using EXPO32 software (Beckman Coulter).

Growth rates (µ; d^{-1}) under varying temperature and irradiance (I; µmol m^{-2} s^{-1}) were determined from the changes in cell number with time by log-line regression during exponential growth. The maximum growth rate (μ_{max}; d^{-1}), half-saturation value (K_s; µmol m^{-2} s^{-1}) and the compensation irradiance (I_{comp}; µmol m^{-2} s^{-1}) under varying irradiance (I; µmol m^{-2} s^{-1}) were calculated with the following equation of Yamaguchi et al. (1991), modifying that of Lederman & Tett (1981):

$$\mu = \mu_{max} \times (I - I_{comp}) \frac{\mu_{max} \times (I - I_{comp})}{(K_s - I_{comp}) + (I - I_{comp})} \quad (2)$$

RESULTS

Environmental conditions

Horizontal temperature mapping at 100 m depth showed that the cold area (<6°C), which roughly represented the Oyashio region (the precise distribution will be discussed in the next subsection), was distributed offshore from Hokkaido, reaching to nearly 39° N on the A-line (Fig. 1B). Therefore, the Kuroshio-Oyashio transition region was distributed south of ~39° N. In the Oyashio region, a slightly warmer water mass above 5°C at about 41 to 42° N and 146° E was judged to be a warm eddy that migrated northward from the Kuroshio-Oyashio transition region (see the time series of isotherms at http://tnfri.fra.affrc.go.jp/kaiyo/temp/temp.html). In the Kuroshio-Oyashio region, a cold water mass existed around 38° N on the A-line, which was a cold eddy that originated from the Oyashio region and was modified by the Kuroshio water.

Vertical stratification of water temperature and sigma-t was evident between 20 and 40 m at almost all stations (Fig. 2A,C). In the surface layer down to 20 m, water temperature was >10°C, except for at Stns A1, A2 and A4. Low temperatures (<5°C) were observed below 20 to 30 m at Stns A1, A2 and A4, ~70 m at Stns A6 to A8 and ~40 m at Stns A11 to A15. At the most southern station (Stn A21), relatively low temperatures (<10°C) were observed below 50 m, while water temperature was >10°C down to 100 m at Stns A18 and A19. Changes in the depth distribution of salinity were similar to those of temperature, and cold waters were generally less saline. Salinity under 33.5 was observed at Stns A1 to A2, A4 and A11 to A15, except at the surface at some stations (Fig. 2B). Salinity above 33.5 occurred at Stns A3, A5 to A9 and A17 to A21.

Concentrations of nitrate and silicate were lower than 5 and 10 µM, respectively, down to 20 m, except at Stns A1 to A4 (Fig. 2D,E). High concentrations (>10 µM in nitrate and >30 µM in silicate), were observed below 50 m at Stns A2, A4 and A11 to A15. In general, concentrations of chlorophyll *a* were high at depths from 0 to 20 m at almost all stations, with no clear evidence of subsurface chlorophyll maxima (Fig. 2F). Concentrations of chlorophyll *a* >1 µg l^{-1} were observed at the surface at Stns A1, A5 to 11 and A15.

The daily incident PAR above the sea surface was in the range of 7.5 to 54.6 mol m^{-2} d^{-1} with an average of 34 mol m^{-2} d^{-1} during the investigation period (Table 1). Vertical profiles of PAR were measured at 4 stations, and k was in the range of 0.109 to 0.150 m^{-1}. From this k value, the irradiances at 30 and 50 m were equivalent to 1.1 to 3.8 and 0.05 to 0.43% of the surface irradiance, respectively.

Table 1. Sampling date, daily incident photosynthetically available radiation (PAR) and attenuation coefficient

Date of sampling (2009)	Sampling location	Daily incident PAR (mol m^{-2} d^{-1})	Attenuation coefficient (m^{-1})
12 July		30.7	
13 July			
14 July	A1		0.138
15 July		7.5	
16 July		53.5	
17 July	A15	46.4	0.150
18 July	A17	22.3	0.131
19 July	A19	24.7	0.109
20 July		54.6	
21 July		36.9	
Average		34.6	

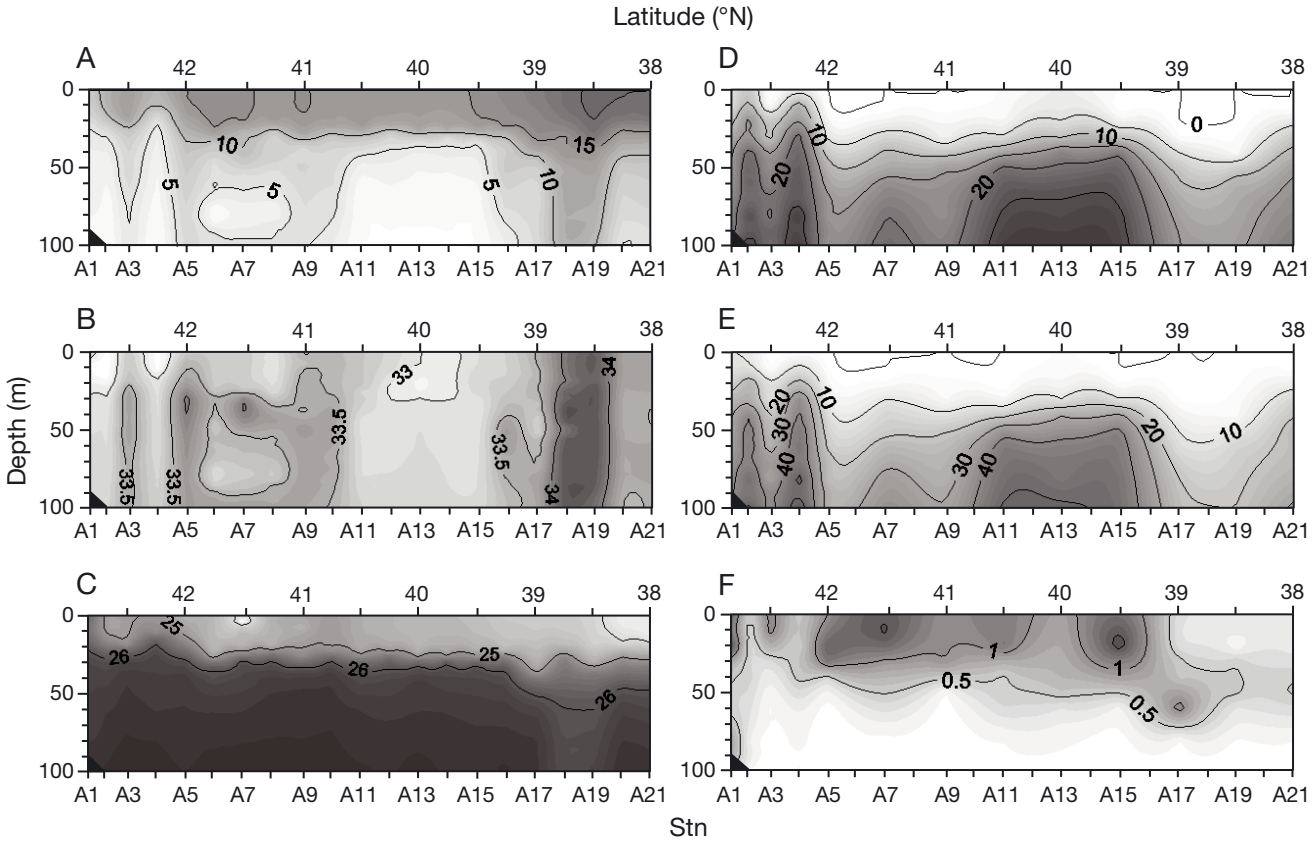

Fig. 2. Vertical distributions of (A) temperature (°C), (B) salinity, (C) sigma-t, (D) nitrate (µM), (E) silicate (µM) and (F) chlorophyll *a* concentration (µg l^{-1}) in July 2009 along the A-line

Vertical and regional distribution of *Triparma laevis* along the A-line

Triparma laevis abundance was high (>1.0 × 10^4 cells l^{-1}) in the subsurface layers of 30 to 50 m at almost all stations, but was low in the shallower layers down to 20 m and in the deeper layers below 80 m (Fig. 3). The highest abundance was 9.6 × 10^4 cells l^{-1} at 50 m at Stn A15. At Stn A4, the maximum of 6.0 × 10^4 cells l^{-1} was observed at 30 m. At Stns A1, A17 and A19, the abundance was <1.0 × 10^4 cells l^{-1} through the water column. At Stn A21, a moderate abundance of 2.0 × 10^4 cells l^{-1} was recorded at 50 m.

During the investigation period, temperature and salinity in the water column down to 100 m ranged from 1.7 to 20.5°C and from 32.8 to 34.4, respectively (Fig. 4). *Triparma laevis* was found in waters with temperatures in the range of 1.7 to 14.5°C but not at >15°C and occurred in a salinity range of 32.8 to 34.1. Relatively high abundances of >2 × 10^4 cells l^{-1} were limited to waters with temperatures of 2.4 to 11.4°C and salinities of 33.0 to 33.9.

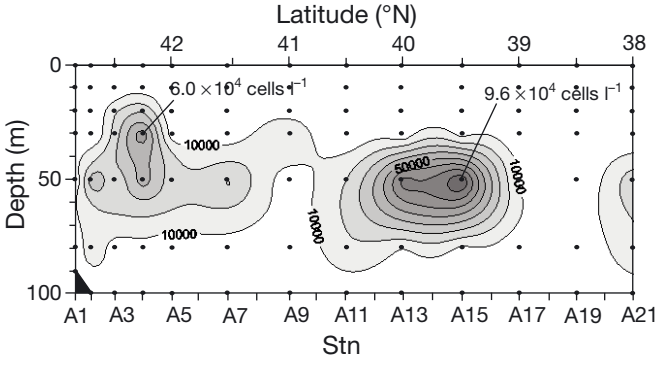

Fig. 3. Vertical distributions of abundance of *Triparma laevis* in July 2009 along the A-line

Culture experiments

In culture experiments, *Triparma laevis* showed exponential growth in batch culture over the range of 0 to 10°C (Fig. 5). The optimal temperature was 5°C, with a growth rate of 0.35 d^{-1}, and was significantly different from 0.22 d^{-1} at 0°C and 0.18 d^{-1} at 10°C at ca. 50 µmol m^{-2} s^{-1}.

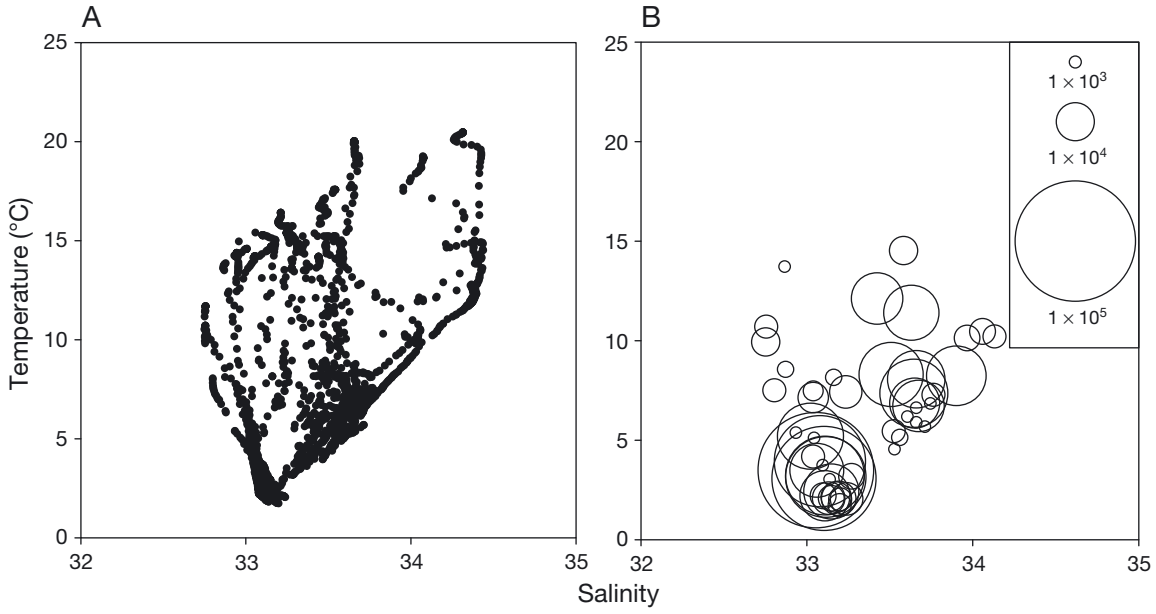

Fig. 4. (A) Temperature-salinity plots of 1 m intervals from the CTD data in the 0 to 100 m water columns and (B) the distribution of *Triparma laevis* (cells l^{-1}) with water temperature and salinity

Higher irradiances resulted in increased growth rates at 5°C (Fig. 6). The highest growth rate was 0.50 d^{-1} at 150 µmol m^{-2} s^{-1} and the lowest was 0.08 d^{-1} at 3.8 µmol m^{-2} s^{-1}. The μ_{max}, K_s and I_{comp} for irradiance were calculated as 0.51 d^{-1}, 21.1 µmol m^{-2} s^{-1} and 0.7 µmol m^{-2} s^{-1} ($r^2 = 0.97$), respectively (Table 2).

DISCUSSION

The depth distribution of *Triparma laevis* during summer across the Oyashio and Kuroshio-Oyashio regions as well as the growth characteristics in culture are reported. *T. laevis* was mainly distributed in the subsurface layer at 30 to 50 m, where water temperature was <10°C (Figs. 2 & 3). *T. laevis* was less abundant from the surface down to 20 m and at temperatures >15°C that were above the pycnocline. The depth distributions of *T. laevis* corresponded well with that predicted from the growth experiments for temperature (Fig. 5). *T. laevis* was able to grow at 0 to 10°C at ca. 50 µmol m^{-2} s^{-1} but not at >15°C. Optimum growth (0.35 d^{-1}) was at 5°C. Since this is the first report of a growth rate of a parmalean alga and is based on a single strain of *T. laevis* in culture, the present results may not represent growth rates of *T. laevis* populations *in situ*. However, the present result was consistent with a previous report that the natural Parmales assemblage was able to grow with rates of 0.29 to 0.38 d^{-1} at 5 to 12°C but not at 15°C on a shipboard incubation in the western North Pacific (Taniguchi et al. 1995). At Stn A4, the peak of *T. laevis* (5.7×10^4 cells l^{-1}) was observed at 30 m, which was shallower than at the other stations (Fig. 3). This was probably because the pycnocline was shallow and the temperature was <10°C at depths below 10 m at this station (Fig. 2A,C). In general, the vertical distribution of *T. laevis* in summer was strictly limited to depths where the prevailing water temperature was suitable for its growth.

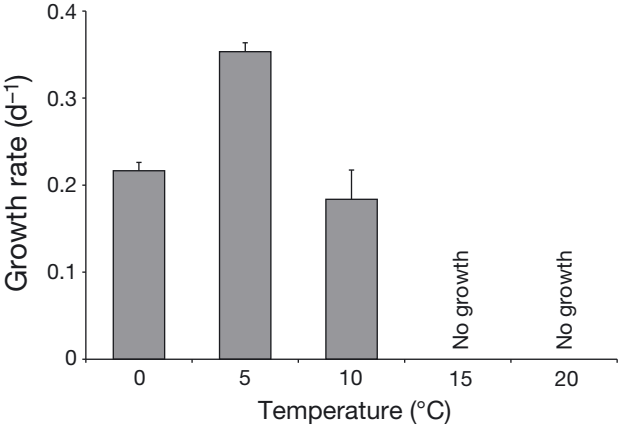

Fig. 5. Growth rates of *Triparma laevis* at 0, 5, 10, 15 and 20°C at ca. 50 µmol m^{-2} s^{-1} (14 h light:10 h dark photoperiod). *T. laevis* did not grow at 15 and 20°C in pre-cultural experiments. Bars are standard deviations (n = 3)

Light conditions also influenced the depth distribution of *Triparma laevis* (Fig. 6). During the investiga-

Table 2. Maximum growth rates (μ_{max}), half-saturation values (K_s) and compensation irradiance (I_{comp}) for cultures of algal taxa with respect to different irradiance levels. μ_{max} and K_s were calculated using the equations of Monod (1950) or Yamaguchi et al. (1991). –: not available

Taxon	Species	Incubation temperature (°C)	Photo-cycle (h light:h dark)	μ_{max} (d^{-1})	K_s ($\mu mol\ m^{-2}\ s^{-1}$)	I_{comp} ($\mu mol\ m^{-2}\ s^{-1}$)	Source
Nano- and micro-phytoplankton							
Diatoms	*Asterionellopsis glacialis*	25	12:12	2.9	52.2	17.3	Shikata et al. (2010)
	Chaetoceros debilis	25	12:12	2.6	47.1	10.4	Shikata et al. (2010)
	Skeletonema costatum	25	12:12	1.9	34.6	8.0	Shikata et al. (2010)
	Skeletonema costatum	20	12:12	1.9	139	8.6	Shikata et al. (2008)
Dinoflagellates	*Cochlodinium polykrikoides*	25	12:12	0.35	45.1	10.4	Kim et al. (2004)
Raphydophytes	*Chattonella antiqua*	25	12:12	1.0	61.1	20.2	Shikata et al. (2010)
	Heterosigma akashiwo	25	12:12	1.3	153	33.8	Shikata et al. (2008)
Pico-phytoplankton							
Cyanobacteria	*Prochlorococcus marinus*	24±1	14:10	0.53 ± 0.06	–	1	Moore et al. (1995)
	Prochlorococcus marinus	24±1	14:10	0.63 ± 0.06	–	4	Moore et al. (1995)
	Synechococcus sp.	24±1	14:10	1.0 ± 0.1	–	5	Moore et al. (1995)
	Synechococcus sp.	24±1	24:0	1.1 ± 0.02	–	2	Six et al. (2004)
	Synechococcus sp.	20	16:8	0.67	19.7	–	Timmermans et al. (2005)
Parmales	*Triparma laevis*	5	14:10	0.51	21.1	0.7	Present study
Pelagophytes	*Pelagomonas calceolata*	20	16:8	0.52	3.1	–	Timmermans et al. (2005)
Prasinophytes	*Prasinomonas capsulatus*	20	16:8	0.39	5.8	–	Timmermans et al. (2005)

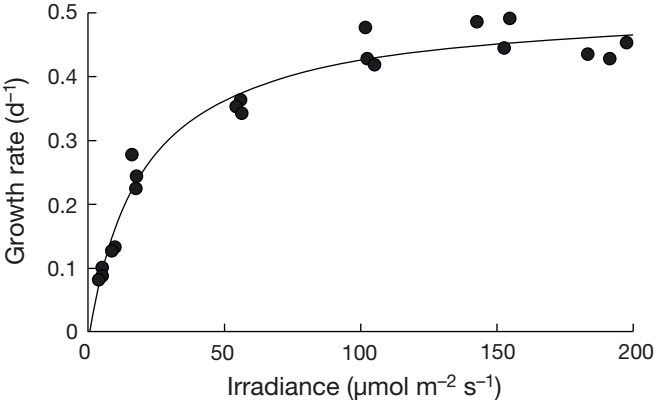

Fig. 6. Variation in growth rates with irradiance in cultures of *Triparma laevis*. The line is fitted to an equation of Yamaguchi et al. (1991)

tion period, the daily incident PAR ranged from 7.5 to 54.6 mol m^{-2} d^{-1}, and the attenuation coefficient ranged from 0.109 to 0.150 m^{-1} in this region (Table 1). From these data, we calculated that the growth rate of *T. laevis* at 50 m ranged from –0.01 to 0.08 d^{-1}, with μ_{max} = 0.51 d^{-1}, K_s = 21.1 µmol m^{-2} s^{-1} and I_{comp} = 0.7 µmol m^{-2} s^{-1} for the *T. laevis* strain (Table 2), assuming that 4% of the incident PAR reflected back at the sea surface (Lalli & Parsons 1993). Such low growth rates resulted from the PAR at 50 m having decreased to 0.05–0.43% of the surface PAR, equivalent to 7.5–218 mmol m^{-2} d^{-1} (=0.08–4.5 µmol m^{-2} s^{-1} in 14 h light:10 h dark). The average daily PAR during the present study period was within the range of the previous studies around the Oyashio region (Shiomoto 2000). Based on the laboratory studies, *T. laevis* would not be expected to grow at 50 m depth. The growth rate of *T. laevis* at 30 m was calculated as 0.02–0.34 d^{-1} when the irradiance at 30 m was equivalent to 1.1–3.8% of surface irradiance. This was similar to Stn A4, where the pycnocline was shallow and *T. laevis* could grow. In contrast, the absence of *T. laevis* below 80 m could be due to low irradiance (0.001–0.02% to the surface irradiance).

K_s and I_{comp} values of *Triparma laevis* were lower than those of other nano- and micro-sized phytoplankton species, such as diatoms, dinoflagellates and raphydophytes, but equivalent to or higher than those of pico-phytoplankton, including cyanobacteria, pelagohytes and prasinophytes (Table 2). This means that *T. laevis* would be better adapted to low light conditions. *Prochlorococcus* (Cyanophyceae) and *Ostreococcus* (Prasinophyceae) have been shown to have diverse ecotypes with distinct adaptations to particular light environments (Moore at al. 1998,

Rodríguez et al. 2005, Six et al. 2008). The strains of these algae isolated from deeper layers showed positive growth at low irradiance, while those isolated at the surface did not grow. More physiological information is needed from other species and strains of Parmales to fully understand their vertical distribution and light adaptation.

The spatial distribution of pico-plankton could be also affected by possible advection due to their limited cell mobility and low sinking rates (Hamilton et al. 2008). High abundance ($>5 \times 10^4$ cells l^{-1}) of *Triparma laevis* was observed at Stns A4 (6.0×10^4 cells l^{-1}), A13 (7.5×10^4 cells l^{-1}) and A15 (9.6×10^4 cells l^{-1}; Fig. 3). According to Shimizu et al. (2009), the region north of the salinity front, with a salinity of 33.6 at 50 to 200 m depths, was defined as the Oyashio region. Based on their definition, Stns A1 to A4, A10 to A16, A20 and A21 were classified as in the Oyashio region. At Stn A21, relatively cold water (<10°C) was observed below 50 m (Figs. 1 & 2A). Such cold water has been considered to originate from the Oyashio water. This explains the moderate abundance of *T. laevis* at the southernmost station (Stn A21; Fig. 3).

The abundance of *Triparma laevis* was low at Stns A1 and A5 to A9, despite the fact that the water temperature seemed suitable for *T. laevis* growth at <10°C throughout the water column (Figs. 2 & 3). A possible explanation for this decrease in abundance in low-temperature water may have been the different origin of the water masses. For example, from January to July, relatively low-salinity water (<33.0), which mainly originated from the Okhotsk Sea, appeared along the Hokkaido coast (Kusaka et al. 2009, see their Fig. 3), and it was distinguished from the offshore Oyashio water by its salinity. At Stn A1, the low-salinity water (<33.0) was distributed from the surface down to 20 m (Fig. 2B), and another Parmales species, *Tetraparma pelagica*, was dominant (data not shown). At Stns A5 to A9, in contrast, salinity under the pycnocline was higher (>33.5) than in the surrounding areas (Fig. 2B), and it corresponded with the warm eddy position (41 to 42° N), as described in the subsection 'Environmental conditions' (Fig. 1B). The water around Stns A5 to A9 must have been a mixture between the Oyashio and Kuroshio waters, which would explain the abundance of *T. laevis*. Our results indicated that the regional distribution of *T. laevis* was also strongly associated with the water currents and warm and cold water masses.

While Parmales is closely related to bolidophytes and diatoms, the growth characteristics of Parmales were different from these algae. *Bolidomonas pacifica*, which have been isolated from the tropical equatorial Pacific and the Mediterranean Sea (Guillou et al. 1999b), prefers warm conditions and has a high growth rate of 0.91 d^{-1} at 20°C (Jacquet et al. 2001). Also, bloom-forming diatom species in the Oyashio region, such as *Thalassiosira nordenskioeldii* and *Chaetoceros debilis*, have much higher maximum growth rates (>0.80 d^{-1}), with a broad temperature range of 0 to 20°C, than that of *Triparma laevis* (Suzuki & Takahashi 1995, A. Kuwata unpubl. data). In this region, small diatoms also form a dense bloom, reaching up to 10^5–10^6 cells l^{-1} and >100 µg C l^{-1} in spring (Isada et al. 2009, Ichinomiya et al. 2010, Suzuki et al. 2011). This would be expected to give them an advantage throughout the euphotic zone in April to May in the Oyashio region. In contrast, *T. laevis*, with its lower maximum growth rate (0.51 d^{-1}) and narrower temperature range (0 to 10°C; Fig. 5), was restricted in its development in the subsurface layer, with a maximum abundance of 9.6×10^4 cells l^{-1} (Fig. 3). This probably explains the relatively low abundances of *T. laevis* compared to diatoms.

A previous study had hypothesized that Parmales, Bolidophyceae and diatoms had a common ancestor (Ichinomiya et al. 2011), but we need more information from comparative studies to fully understand their differentiation and evolution. Diatoms grow faster in the spring and are grazed by mesozooplankton, but they mostly sink out at the end of spring (Takahashi et al. 1990, 2008). In contrast, Parmales stay in suspension in the subsurface layer due to their small cell size (Fig. 3). Only a small fraction of Parmales sink out to below the euphotic zone in the northwestern Pacific (Komuro et al. 2005). These cells in the subsurface layer would likely be grazed by nano- and micro-sized heterotrophic protozoans (Taniguchi et al. 1995) and incorporated into the microbial loop rather than the grazing food chain. However, there is no information on their buoyancy regulation, sinking rates or grazing process. Many other aspects of their ecological role in marine ecosystems are still unclear and, therefore, we need to learn more about what controls their temporal and spatial distributions using combined laboratory and interdisciplinary field studies.

Acknowledgements. We thank Drs. D. Jewson and D. Vaulot for their critical reading of the manuscript. We are grateful to the captain and crew of the RV 'Wakataka-maru' for their cooperation with the sample collection. This study was partially supported by Japan Society for the Promotion of Science Grants-in-Aid for Scientific Research 22657027 (A.K.), 23370046 (A.K.), 19684016 (Y.S.) and 30601918

(M.I.), the Canon Foundation (A.K.), JST, CREST (A.K.) and the MAFF research fund 'Evaluation, Adaptation and Mitigation of Global Warming in Agriculture, Forestry and Fisheries: Research and Development' (A.K. and Y.S.). Special thanks are extended to 3 anonymous reviewers for their critical reading of the manuscript.

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Editorial responsibility: Daniel Vaulot, Roscoff, France

Submitted: April 30, 2012; Accepted: October 28, 2012
Proofs received from author(s): December 10, 2012