Full-depth profiles of prokaryotes, heterotrophic nanoflagellates, and ciliates along a transect from the equatorial to the subarctic central Pacific Ocean

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Abstract

Studies in epipelagic waters report higher heterotrophic microbial biomass in the productive high latitudes than in the oligotrophic low latitudes, however, biogeographical data are scarce in the deep ocean. To examine the hypothesis that the observed latitudinal differences in heterotrophic microbial biomass in the epipelagic zone also occur at depth, abundance and biomass of heterotrophic prokaryotes, nanoflagellates (HNF), and ciliates were determined at depths of 5–5000 m in the central Pacific between August and September of 2005. Heterotrophic microbial biomass increased from the tropical to the subarctic region over the full water column, with latitudinal differences in prokaryotic biomass increasing from 2.3-fold in the epipelagic zone to 4.4-fold in the bathypelagic zone. However, the latitudinal difference in HNF and ciliate biomass decreased with depth. In the mesopelagic zone, the vertical attenuation rate of prokaryotic abundance, which was calculated as the linear regression slope of log-log plot of abundance versus depth, ranged from –0.55 to –1.26 and was more pronounced (steeper slope) in the lower latitudes. In contrast, the vertical attenuation rate of HNF in the mesopelagic zone (–1.06 to –1.27) did not differ with latitude. In the subarctic, the attenuation rate of HNF was 1.7 times steeper than for prokaryotes. These results suggest the accumulation of prokaryotes in the deep subarctic Pacific, possibly due to low grazing pressure. Although the vertical attenuation rate of ciliates was steepest in the bathypelagic zone, HNF abundance did not further decrease at depths below 1000 m, except for at 2000 m where HNF was lowest across the study area. Ciliate abundance ranged 0.3–0.8 cells l^{-1} at 4000 m, and were below the detection limit (<0.1 cells l^{-1}) at 5000 m. To our knowledge, this study presents the first data for ciliates below 2000 m.

Keywords: prokaryotes, heterotrophic nanoflagellates, ciliates, deep water, biogeography, central Pacific; 10°S–53°N /160°W

This MS has a supplementary data file in Excel format.

1. Introduction

Heterotrophic microorganisms play an important role in biogeochemical cycling in the ocean (Azam et al., 1983; Legendre and Rivkin, 2008). One of the first steps toward understanding the functioning of an ecosystem is to identify the biomass distribution. Several previous studies report higher biomass in the productive, high latitudes compared to the oligotrophic low latitudes with respect to prokaryotes, nanoflagellates (HNF), and ciliates (Jiao and Ni, 1997; Vázquez-Domínguez et al., 2008). However, most such studies are from the epipelagic waters, while studies of deeper waters are scarce (Nagata et al., 2010).

From a biological perspective, the deep sea is a cold and high hydrostatic pressure system with limited bioavailable organic matter (Barber, 1968). However, mesopelagic and bathypelagic Pacific waters contain a vast number of heterotrophic prokaryotes, and their biomass and production increase toward the north in relation to the northward increase in the sinking flux of particulate organic carbon (POC) (Nagata et al., 2000). Likewise, a strong trophic linkage between heterotrophic prokaryotes, HNF, and ciliates is suggested for the meso- and bathypelagic zones (Tanaka and Rassoulzadegan, 2002). Given these studies, one can predict that latitudinal differences in heterotrophic microbial biomass should be propagated to the deep ocean; however, we are still far from a consensus on the extent of the vertical attenuation of biomass, or on the magnitude of latitudinal differences in biomass in the deep ocean. Nagata et al. (2000) showed a similar vertical attenuation rate of prokaryotic abundance between the subtropical and the subarctic North Pacific below 1000 m, whereas Yamaguchi et al. (2002) reported steeper vertical attenuation in the subtropical than in the subarctic of the western North Pacific for the biomass of phytoplankton, prokaryotes, microzooplankton,

and metazooplankton below 100 m. The latter implies an increase in the latitudinal differences in biomass with depth and corresponds to the pattern of sinking POC flux, which attenuates more rapidly in the subtropical than in the subarctic North Pacific (Buesseler et al., 2007). Abundance and biomass of higher trophic-level microorganisms are apparently more attenuated with depth at high latitudes, which results in an increase in prey:predator ratios with depth (Tanaka and Rassoulzadegan, 2002; Yamaguchi et al., 2002). However, we have very limited information regarding differences in the vertical attenuation rate among microorganisms at low latitudes. In order to fully understand how matter and energy are transferred to the ocean's interior and utilized there, deep-sea expeditions covering large geographic variations are needed. In this study, we determined the abundance and biomass of heterotrophic prokaryote, HNF, and ciliates at depths of 5–5000 m along a meridional transect down the central Pacific to test the hypotheses that (I) latitudinal differences in heterotrophic microbial biomass increase with increasing depth, and (II) the vertical attenuation of heterotrophic microorganisms is steeper toward lower latitudes, corresponding with the more rapid attenuation of their substrate or prey.

2. Materials and methods

2.1. Sampling

Sampling of microorganisms was conducted at 14 stations along the 160°W meridian between 10°S and 53°N in the central Pacific in August–September, 2005 during the KH-05-2 cruise of the R/V *Hakuho-maru* (Table 1, Fig. 1). At an additional 14 stations along the transect temperature and salinity were measured by XCTD (Table 1). The stations were classified as the equatorial region (Stn. 3) and separate climatic

zones based on the location of the Subtropical Front, Subarctic Front (PICES, 2004), and the North Pacific Tropical Water (Suga et al., 2000) (Fig. 2). Samples of heterotrophic microorganisms were collected with Niskin bottles mounted on a CTD-rosette sampler at 5, 10, 20, 50, 75, 100, 200, 500, 800, 1000, 1500, 2000, 3000, 4000, and 5000 m, but ciliates samples could not be obtained at 800 m or 1500 m. Samples were gently dispensed into sampling bottles using silicon tubes to avoid bursting delicate HNF and ciliates. A maximum 30 ml (for prokaryotes) and 100 ml (for HNF) of seawater were collected, and 0.5 l (5–20 m) to 10 l (2000–5000 m) seawater were collected for ciliates. Samples were immediately fixed with neutralized formalin (final conc., 2%), 20% glutaraldehyde (electron microscopic grade; final conc., 1%), or acid Lugol's solution (final conc., 2%) for prokaryotes, HNF, and ciliates, respectively.

2.2. Abundance and biomass of prokaryotes, HNF, and ciliates

Prokaryotic abundance was determined using the method of Porter and Feig (1980). In brief, 10–30 ml of the preserved sample was stained with 4′,6-diamidino-2-phenyl indole (DAPI; final conc., 0.1 μg ml^{-1}) and filtered on Irgalan black-stained 0.2-μm pore-size Nuclepore© filters within a few days after collection. The filters were mounted on glass slide and kept frozen until counting under an epifluorescence microscope (BX51, Olympus) on land. The precision of counting was 20% on average. Prokaryotic abundance was converted to biomass using carbon (C) conversion factors directly measured in the Pacific basins by Fukuda et al. (1998), *i.e.*, 5.9 fg C $cell^{-1}$ (equatorial and tropical), 12.8 fg C $cell^{-1}$ (subtropical), and 13.3 fg C $cell^{-1}$ (transitional and subarctic).

Abundance of HNF was determined by epifluorescence microscopy after cells

were stained with DAPI (final conc., 0.1 μg ml^{-1}) and fluoresceinisothyocyanate isomer-I and collected on 0.8-μm pore-size Nuclepore© filters using funnels with a filtration diameter of ca. 3 mm (Fukuda et al., 2007). We filtered 10–100 ml of sample, depending on depth, and counted at least 50 cells without autofluorescence. Cell images were captured by a CCD camera (DP70, Olympus) under blue excitation, and the size of 20 cells was measured using Image-Pro Plus version 6 (Media Cybernetics). The effect of the "halo" was compensated for by calibration with TetraSpeck Fluorescent Microspheres Size Kit (Molecular Probes). Biovolume of HNF was calculated by assuming a spherical cell, and biomass was calculated by multiplying abundance by the mean biovolume and 220 fg C μm^{-3} (Børsheim and Bratbak, 1987).

Ciliate samples were preconcentrated to 100 ml on board by settling the cells for 24 h and siphoning the supernatant and then stored at 4 °C in the dark until enumeration on land. Cells were then further concentrated in an Utermöhl chamber for 24 h and counted in the whole area of the chamber under the inverse microscope (BX71, Olympus). The length and width of ciliate cells were sized with eyepiece scale. Their biovolume was calculated by assuming standard geometric shapes, and the cell size was expressed as equivalent spherical diameter (ESD). Ciliate biomass was estimated from the multiplication of abundance, biovolume, and 190 fg C μm^{-3} (Putt and Stoecker, 1989). We excluded the lorica from size measurements and carbon biomass calculations. Applying the empirical factors of Verity and Langdon (1984) and Gilron and Lynn (1989), the biomass including loricae should amount to 93%–129% of our estimated loricate ciliate biomass.

Because it took 6 months to measure all the ciliate samples, we compared the abundance and cell size of ciliates between two different storage periods. Additionally,

151 we compared the recovery of the concentrated sample on board with the same on land.
152 Samples were taken at 3 discrete depths (50, 75, and 100 m) at Stns. 12 (45°N) and 13
153 (50°N), where the ship pitched and rolled most heavily during the cruise. Each of the six
154 samples were divided into three aliquots and treated as follows: (1) concentrated to 100
155 ml on board and measured after 6 months, (2) concentrated on land and measured
156 within 2 weeks, and (3) concentrated on board and measured within 2 weeks. Paired
157 Student's *t*-test between two treatments on each 6 samples showed that neither
158 abundance nor cell size was significantly different between treatment (2) and (3) or
159 between treatment (1) and (3) ($p > 0.05$), implying that the ciliates were successfully
160 recovered on board and their morphology was maintained for 6 months.
161
162 2.3. Phytoplankton biomass
163 Chlorophyll *a* (Chl *a*) was measured for depths ≤300 m with a fluorometer
164 (10-AU-005, Turner Designs) after extraction with N,N-dimethylformamide (Suzuki
165 and Ishimaru, 1990). The detection limit of Chl *a* was 0.007 μg l^{-1}. The Chl *a*
166 concentration was converted to phytoplankton carbon biomass using C:Chl *a*
167 conversion factors calculated from equation 1 in Behrenfeld et al. (2005). According to
168 this formula, C:Chl *a* is a function of mixed layer light level (Ig), and C:Chl *a* at low
169 light and at light-saturated conditions. We chose these three variables at the site closest
170 to our station in Table 1 of Behrenfeld et al. (2005), but since the Ig values were
171 reported only as ranges, the maximum Ig was applied for depths above or equal to the
172 1% PAR level and the minimum Ig was applied for depths below the 1% PAR level. If
173 the mixed layer depth (MLD) was deeper than the 1% PAR level, the boundary between
174 the maximum and minimum Ig was set at the MLD. MLD was calculated according to

175 Levitus (1982), and the 1% PAR level was cited from Figure 1 of Harimoto et al. (1999)
176 which reported PAR level along 175°E between April and June. Our calculated C:Chl *a*
177 ranged between 36 and 163 (Table 2).
178
179 2.4. Statistical analyses
180 We applied ANOVA and Scheffe's test or Student's *t*-test to examine the
181 difference in mean microorganism biomass among the depth strata and climatic zones,
182 assuming a normal distribution. The vertical attenuation rate of microorganisms was
183 calculated by linear regression as the slope of a log-log plot between abundance (N) and
184 depth (z) using the model $\log N = b \times \log z + a$ (null hypothesis: $b = 0$) within the
185 epipelagic, mesopelagic, and bathypelagic zones. We also applied a semi-log linear
186 regression model ($\log N = bz + a$), but obtained a slope significantly different than zero in
187 only 63 of all 126 cases tested (r = –0.9996 to 0.56), whereas the slope $b \neq 0$ was
188 obtained in 82 cases using the log-log linear regression model (r = –0.995 to 0.64). Thus,
189 we applied the log-log linear regression model.
190
191 3. Results
192 3.1. Distribution of loricate ciliates
193 Loricate ciliates ranged from <2 to 64 cells l^{-1} in the epipelagic zone, and their
194 abundance was maximum near the surface at all stations (Fig. 3). Loricate ciliates were
195 more abundant in the equatorial and subarctic regions. Their contribution to the sum of
196 naked ciliates and loricate ciliates was highest in the subarctic, ranging 6%–28%
197 (abundance), and 4%–22% (biomass) in the epipelagic zone, and 5%–13% (abundance)
198 and 4%–16% (biomass) in the mesopelagic zone (Fig. 3). Abundance of loricate ciliates

199 decreased with depth and was below detection (<0.1 cells l^{-1}) at depths of ≥2000 m.

200

201 3.2. Cell size of HNF and ciliates

202 The mean cell size of HNF (diameter) and ciliates (ESD) ranged from 1.5 to
203 3.1 μm and from 17.1 to 26.7 μm, respectively, and generally increased toward the
204 north (Fig. 4). Cell size varied randomly with depth; further, there was no evidence of
205 larger cell sizes at the depth of the Chl *a* maximum. Coefficient of variation among the
206 depths was 6.2%–14.4% and 3.2%–12.1% for HNF and ciliates, respectively.

207

208 3.3. Distribution of microbial abundance and biomass in the epipelagic zone (5–100 m)

209 Chl *a* exhibited a clear latitudinal trend with a higher concentration in the
210 equatorial and subarctic than in the tropical and subtropical regions in the upper 75 m
211 (Fig. 5). The deep Chl *a* maximum (DCM) reached a depth of 100 m in the subtropical
212 region and became shallow toward the subarctic region. Chl *a* was not detected (<0.007
213 μg l^{-1}) deeper than 300 m at any station. Generally, heterotrophic microorganisms were
214 more abundant in the surface waters than in the DCM, while ciliates at Stns. 12 (45°N)
215 and 13 (50°N) were densest near the DCM (Fig. 5). Naked ciliates were mostly
216 responsible for these subsurface maximum values (Fig. 3).

217 Depth-integrated biomass in surface waters varied in the range of 1380–6150
218 mg C m^{-2} (phytoplankton), 350–1060 mg C m^{-2} (prokaryotes), 40–179 mg C m^{-2} (HNF),
219 and 10–41 mg C m^{-2} (ciliates) (Table 3). Integrated biomass was highest at the most
220 northern station (Stn. 14) and lowest at the tropical stations (Stn. 1 or 4) regardless of
221 the organism. The depth-integrated biomass of prokaryotes, HNF, and ciliates was thus
222 significantly higher in the subarctic than in the tropical region (ANOVA and Scheffe's

test, $p < 0.05$) and positively correlated with increasing north latitude ($r = 0.62–0.92$, $n = 14$, $p < 0.05$; Table 3). We thus define the 'latitudinal difference' as the ratio between the mean depth-integrated biomass values at the subarctic and tropical stations. Latitudinal differences ranged from 1.8-fold (phytoplankton) to 2.9-fold (ciliates) (Table 3).

3.4. Distribution of microbial abundance and biomass in the mesopelagic (100–1000 m) and bathypelagic (1000–5000 m) zones

Prokaryotes, HNF, and ciliates exhibited unique vertical profiles. Abundance of HNF and ciliates rapidly decreased with depth between 100 and 200 m, following the decrease in Chl *a*, and the rate of vertical attenuation decreased between 200 and 1000 m (Fig. 6). Below 1000 m, vertical attenuation became more rapid for ciliates, where their abundance decreased to 0.3–0.8 cells l^{-1} at 4000 m and was below the detection limit at 5000 m (Fig. 5). In contrast, HNF did not further decrease with depth below 1000 m, except at 2000 m, where HNF abundance was minimum (~6000 cells l^{-1}) at most stations (Figs 5 and 6). Prokaryote abundance attenuation with depth was relatively constant from 100 m to 5000 m as compared to attenuation in HNF and ciliates. Microbial abundance did not significantly increase near the bottom (Fig. 5), though stimulation of prokaryotic production by POC was reported in the other regions (Boetius et al., 2000)

Depth-integrated biomass in the mesopelagic zone was in the range of 523–2260 mg C m^{-2} (prokaryotes), 55–171 mg C m^{-2} (HNF), and 11–38 mg C m^{-2} (ciliates) (Table 3). Similar to that in the epipelagic zone, the depth-integrated microbial biomass in the mesopelagic zone was positively correlated with increasing north latitude

247 (r = 0.70–0.94, n = 14, p < 0.01) and was significantly higher in the subarctic
248 (prokaryotes and ciliates) or transitional (HNF) than in the tropical region (ANOVA and
249 Scheffe's test, p < 0.05). In the bathypelagic zone, depth-integrated biomass was in the
250 range of 347–1830 mg C m^{-2} (prokaryotes), 45–119 mg C m^{-2} (HNF), and 5–17 mg C
251 m^{-2} (ciliates). Similar to that in the upper two zones, depth-integrated microbial biomass
252 was positively correlated with increasing north latitude (r = 0.53–0.93, n = 14, p < 0.05),
253 but only prokaryotic biomass was significantly higher in the subarctic than in the
254 tropical region (ANOVA and Scheffe's test, p < 0.05). Latitudinal differences in the
255 depth-integrated prokaryotic biomass increased with depth from 2.3-fold in the
256 epipelagic to 3.6-fold and 4.4-fold in the meso- and bathypelagic zones, respectively
257 (Table 3), which agrees with our hypothesis I. In contrast, depth-integrated biomass of
258 HNF and ciliates decreased with depth between the epipelagic and bathypelagic zones
259 from 2.7-fold to 1.3-fold for HNF and from 2.9-fold to 1.7-fold for ciliates.
260
261 3.5. Vertical attenuation rate of prokaryotes, HNF, and ciliates within each depth strata
262 Due to the clear changes in the rate of vertical attenuation of microbial
263 abundance with depth (Fig. 6), we calculated attenuation for each depth stratum
264 separately (Table 4). In the epipelagic zone, prokaryotes decreased with depth only in
265 the equatorial and subarctic regions where Chl *a* was highest near the surface (Figs. 5
266 and 6, Table 4). Prokaryotes significantly decreased with depth at all the stations in the
267 mesopelagic zone, and the vertical attenuation rate was positively correlated with
268 increasing north latitude between Stn. 7 (20°N) and Stn. 14 (53°N) (r = 0.90, n = 8, p <
269 0.01), *i.e.*, the rate was steeper toward lower latitudes, which agrees with our hypothesis
270 II. The attenuation rate in the subtropical region (–1.09 to –1.25) was 1.7-fold that of the

subarctic region (–0.55 to –0.73) (Table 4). In contrast, the attenuation rate of prokaryotes in the bathypelagic zone (–0.63 to –0.94) did not significantly differ between climatic zones (ANOVA, $p = 0.6$) and was statistically identical to the rate in the mesopelagic zone (paired Student's *t*-test, $p = 0.06$, $n = 14$).

In contrast to prokaryotes, HNF abundance significantly decreased with depth in the epipelagic zone at most stations and the vertical attenuation rate was relatively higher in the subarctic (–0.23 to –0.33) compared to other regions (–0.11 to –0.23) (Table 4). In the mesopelagic zone, attenuation of HNF increased up to 10-fold (–1.06 to –1.27) (Table 4) due to a rapid decrease between 100 and 200 m (Fig. 6). The attenuation of HNF in the mesopelagic zone was not significantly different among the climatic zones (ANOVA, $p = 0.3$), but its relation to attenuation of prokaryotes differed with latitude. The attenuation was similar between HNF (–1.18 to –1.20) and prokaryotes (–1.09 to –1.25) in the subtropical region, whereas the attenuation of HNF (–1.14 to –1.27) was 1.7-fold higher than that of prokaryotes (–0.55 to –0.73) in the subarctic region (Table 4). In the bathypelagic zone, HNF abundance did not significantly decrease with depth at most stations.

Although ciliates were generally most abundant near the surface (Fig. 5), the vertical decrease was not significant in the epipelagic zone at most stations (Table 4). This result implies that ciliates occurred at the DCM to a greater extent than did HNF (Fig. 6). Ciliates did not significantly decrease with depth in the mesopelagic zone either. This result may be due to the small mesopelagic sample size (4 depths; Table 4); however, despite an identical sample size, the highest attenuation of all microorganims (–1.34 to –2.43) was seen for ciliates in the bathypelagic zone (paired Student's *t*-test, $p < 0.001$, respectively). The attenuation of ciliates in the bathypelagic zone did not

295 significantly differ among the climatic zones (ANOVA, $p = 0.3$).

296

297 3.6. Latitudinal differences in microorganism biomass ratios

298 Ratios of depth-integrated biomass among microorganisms are shown in Table
299 5. In the epipelagic zone, ratios of prokaryotes:phytoplankton and HNF:phytoplakton
300 were significantly lower in the equatorial and tropical than in the other regions
301 (Student's *t*-test, $p = 0.008$ and 0.02, respectively), but were not lower for
302 ciliates:phytoplankton (Student's *t*-test, $p = 0.9$). The ciliates:phytoplankton biomass
303 ratio was higher at Stn. 3 (0°), Stn. 12 (45°N), and Stn. 13 (50°N) than at the other
304 stations. The ciliates:HNF ratio was also significantly higher in the equatorial than in
305 the other regions (ANOVA and Scheffe's test, $p < 0.05$).

306 The prokaryotes:HNF and prokaryotes:ciliates ratios in the subarctic increased
307 by 2.8-fold and 4.6-fold, respectively, from the epipelagic to the bathypelagic zone, and
308 this increase was greater than in the other regions (1.1-fold and 1.6-fold, respectively,
309 on average). This result clearly indicates a greater accumulation of prokaryotes than of
310 HNF and ciliates in the deep subarctic waters. On the other hand, the ciliates:HNF ratio
311 decreased on average by 0.7-fold from the epipelagic to the bathypelagic zones; this
312 change did not significantly differ among the climatic zones (ANOVA, $p = 0.2$).

313

314 4. Discussion

315 4.1. Distribution of loricate and naked ciliates

316 Ciliate abundance ranged 0.3–0.8 cells l^{-1} at 4000 m (Fig. 5). To our knowledge,
317 this is the deepest record of marine ciliate abundance, since ciliates were not detected
318 (Yamaguchi et al., 2004) or enumerated (Tanaka and Rassoulzadegan, 2002) below

2000 m. Contribution of loricate ciliates to the sum of naked ciliates and loricate ciliates was high in the equatorial and subarctic regionsFig. 3, which is consistent with the suggestion of Suzuki and Taniguchi (1998) that loricate ciliates are adapted to high Chl *a* environments. As loricate ciliates, such as tintinnids, consume larger prey (2–20 μm) than the prey of oligotrichous naked ciliates (0.5–10 μm; Rassoulzadegan et al., 1988), a high abundance of loricate ciliates in the equatorial and subarctic regions may be related to a high abundance of HNF (Fig. 3) and autotrophic eukaryotes there (Suzuki et al., 1997). Similar to HNF and prokaryotes, loricate ciliates were most abundant near the surface across the study areaFig. 3. In contrast, Suzuki and Taniguchi (1998) and Gómez (2007) noticed the maximum abundance of loricate ciliates was near or below the DCM between the subtropical and subarctic regions of the western Pacific. Contribution of loricate ciliates in the mesopelagic zone was also higher (5–13%) in the subarctic than in the other regionsFig. 3, which is consistent with extensive attachment of tintinnid cells with sinking detrital aggregates, as reported on the west coast of Sweden (Jonsson et al., 2004).

4.2. Cell size of HNF and ciliates

The mean cell size of HNF (ESD_{HNF}) and ciliates ($ESD_{ciliates}$) increased toward the north over the water column (Fig. 4). We cannot ignore the effects of fixation (Jerome et al., 1993) and hydrostatic pressure on our estimated cell size, but we consider the latter as minor because several strains of HNF and ciliates maintained their morphology before and after brief exposure to hydrostatic pressure equivalent to a depth of 6000 m (Kitching, 1957). The cell size of HNF and ciliates is dependant upon the individual species (Esteban and Finlay, 2007), prey concentration (Weisse et al., 2002),

343 temperature (Weisse et al., 2002; Atkinson et al., 2003), and size-selective grazing by
344 predators (Fenchel, 1982). We applied regression analyses to test the hypothesis that the
345 effects of temperature and prey biomass on ESD differed among the depth strata. In the
346 epipelagic zone, ESD_{HNF} and $ESD_{ciliates}$ negatively correlated to temperature (r = –0.43
347 and –0.77, respectively, n = 84, $p < 0.001$) and positively correlated to their potential
348 prey– prokaryote biomass (r = 0.49) and HNF biomass (r = 0.54), respectively (n = 84,
349 $p < 0.001$). Multiple linear regression analysis of ESD indicated that a combination of
350 temperature and prey biomass as independent variables explains 32% of the variation in
351 ESD_{HNF} and 69% of variation in $ESD_{ciliates}$ (n = 84, $p < 0.001$ for all regression
352 coefficients and constants). The standard partial regression coefficient (β) showed a
353 similar contribution of temperature (β = –0.32) and prokaryotic biomass (0.40) to
354 ESD_{HNF}, whereas the contribution of temperature to $ESD_{ciliates}$ (–0.66) was twice of that
355 of HNF biomass (0.33). This result indicates a strong temperature control on $ESD_{ciliates}$
356 in the epipelagic zone.

357 In the meso- and bathypelagic zones, ciliates appear to feed on prokaryotes
358 rather than on HNF (see section 4.5.). In the mesopelagic zone, ESD_{HNF} and $ESD_{ciliates}$
359 were positively correlated to prokaryotic biomass (r = 0.47 and 0.53, respectively, n =
360 42, $p < 0.001$), whereas only $ESD_{ciliates}$ were negatively correlated to temperature (r =
361 0.39, n = 42, $p < 0.001$). The combination of temperature and prokaryotic biomass
362 explained the variation in $ESD_{ciliates}$ by 50% (n = 42, $p < 0.001$ for all regression
363 coefficients and constants) with a similar contribution of temperature (β = –0.47) and
364 prokaryotic biomass (0.59). In the bathypelagic zone, only $ESD_{ciliates}$ and prokaryotic
365 biomass were significantly correlated (r = 0.45, n = 40, p = 0.004). Insignificant
366 correlation between ESD and temperature was likely due to the small temperature

367 difference (0.8–1.2°C). Overall, we observed that $ESD_{ciliates}$ depended more on
368 temperature and prey biomass than did ESD_{HNF} and that $ESD_{ciliates}$ was more strictly
369 controlled by prey biomass than by temperature in the deep water.
370
371 4.3. Comparison of the epipelagic microbial biomass with previous studies
372 We compared depth-integrated microbial biomass in this study to previous
373 studies of the epipelagic open ocean (Table 6). Biomass of prokaryotes and HNF in this
374 study was almost equal to or lower than that observed in previous studies, and ciliate
375 biomass was lower than in previous studies using acid Lugol's or Bouin's solution as a
376 fixative by one-half (equatorial and tropical) to one-tenth (subarctic). Several factors
377 may explain low ciliate biomass in this study. The first is temporal differences. We
378 performed the survey in the stratified, most oligotrophic conditions of the year. Gómez
379 (2007) reported lower ciliate abundance in summer than in spring in the western North
380 Pacific, and our results are close to theirs in summer. Low microbial biomass was
381 reported in the central equatorial Pacific in El Ninõ conditions (Stoecker et al., 1996),
382 however, our study period (August–September, 2005) was not during an El Ninõ.
383 Second, microbial biomass varies on the mesoscale, such as among semi-permanent
384 mesoscale eddies (Karayanni et al., 2005) and between the outside and inside of
385 cyclone-induced mesoscale eddies (Brown et al., 2008). Considering the small biomass
386 variations within each climatic zone (Table 3), it is unlikely we encountered mesoscale
387 productive events. Third, methodological artifacts resulting from fixation may have
388 lowered ciliate counts, especially for naked ciliates which are easily damaged
389 (Karayanni et al., 2004). The contribution of loricate ciliates in the epipelagic zone
390 (0%–28%; Fig. 3) was similar to that observed in previous studies using acid Lugol's in

391 the North Pacific (10%–20%; Yang et al., 2004; Gómez, 2007). Significant ciliate cell
392 loss was reported on use of 2% acid Lugol's (Stoecker et al., 1994) and long term (2
393 weeks) preservation (Zinabu and Bott, 2000), but ciliates biomass can hardly be
394 underestimated to one-tenth by these factors , and by exclusion of loricae from biomass
395 estimation (see section 2.2.). Thus, we could not completely rule out the relationship
396 between the ciliate biomass observed in the current study and the relative oligotrophic
397 status of the sampling sites. This conclusion is supported by the fact that the biomass
398 ratios of ciliates:HNF:prokaryotes in this study are similar to those previously reported
399 (Table 7). For example, the ratios of ciliates:HNF in this study (0.05–0.51) are similar to
400 those in the Mediterranean Sea (0.02–0.62) (Tanaka and Rassoulzadegan, 2002).
401 Likewise, the ratios of prokaryotes:HNF in this study (3.8–22.9) are within the range of
402 those in the North Pacific (1.3–200).
403
404 4.4. Latitudinal differences in microbial biomass in the epipelagic zone
405 The data of depth-integrated microbial biomass in the epipelagic zone
406 compiled from previous studies showed considerable variability, but tended to be higher
407 in the subarctic than in the tropical and subtropical regions, as shown in this study
408 (Tables 3 and 6). In this study, biomass ratios of prokaryotes:phytoplankton and
409 HNF:phytoplankton were low in the equatorial and tropical regions (Table 5). Previous
410 studies also show low biomass and production ratios of heterotrophic
411 prokaryotes:phytoplankton in the central equatorial Pacific (Kirchman et al., 1995;
412 Nagata et al., 2000). From an insignificant correlation between dissolved organic carbon
413 (DOC) and Chl *a* between 10°S–10°N of the central Pacific (Taki and Suzuki, 2001),
414 we infer a deficiency of fresh DOC which could limit production of the microbial food

415 web in the equatorial and tropical central Pacific. On the other hand, the biomass ratio
416 of ciliates:phytoplankton was high at the equator and at high latitudes in this study (0°,
417 45°N, and 50°N; Table 5) and others (Table 7). This result suggests an effective energy
418 transfer to ciliates in the equatorial and subarctic regions; for example, the grazing of
419 ciliates on phototrophic eukaryotes besides transfer through the microbial food web (*i.e.*,
420 DOC→prokaryotes→HNF→ciliates). This is consistent with significant grazing of
421 ciliates on phototrophic eukaryotes in the central equatorial Pacific (Latasa et al., 1997).
422 It should be noted that many studies showed an excess of heterotrophic prokaryotic
423 biomass over phytoplankton biomass in the oligotrophic waters (*e.g.*, Fuhrman et al.,
424 1989; Ducklow and Carlson, 1992), but the prokaryotes:phytoplankton biomass ratio
425 was <1 in this study (Table 5). This is likely due to higher C:Chl *a* (36–163; Table 2)
426 and lower prokaryotic C:cell (5.9–13.3 fg C cell^{-1}, see section 2.2.) applied in this study
427 versus previous studies (*e.g.*, C:Chl *a* = 30–60 and prokaryotic C:cell = 15 fg C cell^{-1} in
428 Caron et al.,1995).
429
430 4.5. Potential nutrient sources for HNF and ciliates in the meso- and bathypelagic zones
431 HNF and ciliate abundance attenuated most rapidly from 100 m to 200 m (Fig.
432 6). This implies a bottom-up control on HNF and ciliates by *Prochlorococcus* and
433 *Synechococcus*, which are their preferential prey (Christaki et al., 1999) and which
434 steeply decrease in these depths (Suzuki et al., 1995, 1997).
435 To test the prey-predator relationship, we examined correlations of
436 depth-integrated biomass between microorganisms (Table 8). Depth-integrated biomass
437 of HNF was significantly positively correlated to that of prokaryotic biomass in the
438 mesopelagic zone, whereas the correlation was insignificant in the bathypelagic zone.

These results can be explained by a requirement for a threshold prey concentration, above or equal to which predator meets a net growth. For example, the threshold was reported as 0.2–5 × 10^8 prokaryotes l^{-1} for HNF in laboratory studies (Eccleston-Parry and Leadbeater, 1994; John and Davidson, 2001). Prokaryotic abundance in the mesopelagic zone (0.26–8.3 × 10^8 cells l^{-1}; Fig. 5) exceeded the threshold, but the abundance in the bathypelagic zone (0.11–0.82 × 10^8 cells l^{-1}) was close to the lower value. Thus, HNF may utilize a source of nutrition other than prokaryotes in the bathypelagic zone, such as colloids. Colloids are denser by more than one order of magnitude than prokaryotes from the epipelagic to the bathypelagic zones (Wells and Goldberg, 1994; Nagata and Koike, 1995) and can be utilized by HNF (Sherr, 1988; Tranvik, 1994). HNF abundance was minimum at 2000 m, where prokaryotes or ciliates were not at their minimum (Figs 5 and 6). The depth of 2000 m corresponds to the North Pacific Deep Water, which is considered the oldest water mass in the world ocean (Matsumoto and Key, 2004) with very low DOC concentration (36 μmol C l^{-1}; Lang et al., 2006). Possibly, HNF production might be repressed at 2000 m by a low concentration of organic colloids that are included in the DOC fraction.

Depth-integrated biomass of ciliates positively correlated with that of prokaryotes but not with HNF in the meso- and bathypelagic zones (Table 8). Ciliates might consume prokaryotes rather than HNF in the meso- and bathypelagic zones because of a lower HNF abundance (0.11–2.6 × 10^5 cells l^{-1}; Fig. 5) than the threshold (4.3 × 10^5–6 × 10^9 HNF l^{-1}; Montagnes, 1996). However, ciliates may graze less effectively on small prokaryotes in the meso- and bathypelagic zones than on HNF in the epipelagic zone. According to the empirical relationship of cell size between ciliates and their prey (Hansen et al., 1994), and $ESD_{ciliates}$ in the meso- and bathypelagic zones

463 (Fig. 4), ciliates can ingest prey larger than 0.7 μm at ≥10% of the optimum clearance
464 rate. We did not measure prokaryotic cell size, but the mean cell size of prokaryotes in
465 the meso- and bathypelagic zones is estimated to be 0.32–0.47 μm (Patching and Eardly,
466 1997; Fukuda et al., 2007), which is smaller than the potential prey size of ciliates.
467 Ciliate abundance attenuated most rapidly with depth in the bathypelagic zone, more
468 rapidly than prokaryotes and HNF (Table 4 and Fig. 6). A steep decline in ciliates was
469 unlikely to be related to hydrostatic pressure because of their known resistance to high
470 hydrostatic pressure (Kitching, 1957). We posit that a steep decline in ciliates reflects a
471 decrease in actively growing prokaryotes with increasing depth because ciliates
472 selectively feed on them (Turley et al., 1986) and also because the turnover time of
473 prokaryotes becomes longer with increasing depth in the bathypelagic zone (Fig. 2 in
474 Nagata et al., 2000).
475
476 4.6. Latitudinal differences in microbial biomass in the meso- and bathypelagic zones
477 Prokaryotic biomass increased from low to high latitudes, by 2.3-fold in the
478 epipelagic zone, and 4.4-fold in the bathypelagic zone (Table 3), which supports our
479 hypothesis I. Previous studies have also reported 2–7 times greater abundance and
480 biomass of bathypelagic prokaryotes in the subarctic than in the subtropical region
481 (Patching and Eardly, 1997; Nagata et al., 2000). An increase in prokaryotic abundance
482 from the subtropics to the subarctic was also reported in the North Pacific, with a 2-fold
483 increase in the epipelagic zone and a 9-fold increase in the mesopelagic zone (Steinberg
484 et al., 2008).
485 An increase in prokaryotic biomass with increasing depth is related to a higher
486 vertical attenuation rate of prokaryotic abundance toward low latitudes in the

mesopelagic zone (Table 4), which supports our hypothesis II. The vertical attenuation rate of prokaryotes in the subarctic (–0.55 to –0.73) and the subtropical regions (–1.09 to –1.25) in this study is close to that in the subarctic (–0.37) and subtropical (–1.05) western North Pacific below 100 m (Yamaguchi et al., 2002). Interestingly, our rates are comparable to the vertical attenuation rate of sinking POC flux in the mesopelagic zone in the subarctic (–0.50 and –0.52) and the subtropical (–1.29 and –1.38) North Pacific, which was calculated in the same manner as our rate by Steinberg et al. (2008). Although several studies conclude that dissolution from sinking POC flux was insufficient to meet the prokaryotic carbon demand in the mesopelagic zone (Steinberg et al., 2008, and references therein), the similarities in the vertical attenuation rate and in the magnitude of latitudinal differences between prokaryotic biomass and sinking POC flux (Steinberg et al., 2008) imply a strong linkage between prokaryotes and sinking POC flux in the mesopelagic zone.

In the bathypelagic zone, the vertical attenuation rate of prokaryotes did not differ with latitude (Table 4). Nagata et al. (2000) report a similar vertical attenuation rate of prokaryotic abundance among the equatorial, subtropical, and subarctic North Pacific regions below 1000 m, and the slope in this study (–0.63 to –0.94) is within their range (–0.427 to –1.19). Considering the similarity in the vertical attenuation rate between prokaryotic biomass and sinking POC flux in the bathypelagic zone (Nagata et al., 2000), prokaryotes may be tightly coupled with the sinking POC flux not only in the mesopelagic zone but also in the bathypelagic zone.

Although depth-integrated prokaryotic biomass increased by 3.6-fold from the tropical to the subarctic in the mesopelagic zone, HNF and ciliates increased only by 1.8-fold and 2.1-fold, respectively (Table 3). Tanaka and Rassoulzadegan (2002) noticed

511 an increase in the prokaryote:HNF abundance ratio from 10^3:1 at the surface to 10^4:1 at
512 2000 m in the Mediterranean Sea, and they inferred greater bottom-up control on HNF
513 and ciliates at the deeper depths. In this study, the ratio increased from $10^{3.5}$:1 to $10^{4.5}$:1
514 in the upper 2000 m of the subarctic but not in the other regions (data not shown). Our
515 results suggest an accumulation of prokaryotes rather than a bottom-up control on HNF
516 in the deep subarctic region. Hansell and Ducklow (2003) also mentioned a high
517 prokaryotic abundance in the bathypelagic zone of the subarctic North Pacific,
518 speculating that it might result from high growth rate of prokaryotes and/or low removal
519 of prokaryotes by grazing or viral lysis. The former supports Nagata et al. (2000) who
520 showed a significantly shorter turnover time of prokaryotes in the subarctic than in the
521 subtropical region of the North Pacific, and the latter at least does not contradict our
522 result of high prokaryotes:HNF ratios in the subarctic (Table 5). Although we do not
523 ultimately know why, possible explanations for low grazing pressure on the deep
524 subarctic prokaryotes are as follows: First, a negative effect of low temperature (by up
525 to 25°C between the stations in the mesopelagic zone; Fig. 2) on the grazing rate of
526 HNF, which was confirmed by grazing experiments of mesopelagic HNF conducted at
527 *in situ* temperature and atmospheric pressure (Cho et al., 2000). However, previous
528 studies in the epipelagic zone indicated a similar Q_{10} between the prokaryotic
529 production rate and microzooplankton ingestion rate (Rivkin et al., 1999 and references
530 therein); thus, we assume a constant grazing pressure on prokaryotes along the
531 temperature gradient in the mesopelagic zone. Second, mesozooplankton such as
532 copepods might heavily graze on HNF and ciliates in the meso- and bathypelagic zones
533 of the subarctic. Copepods feed on ciliates, but unlikely on HNF in the surface waters of
534 the subarctic Pacific (Liu et al., 2005; Ide et al., 2008). If ciliates were grazed in the

535 deep subarctic, one would expect to observe a decrease in ciliates, an increase in HNF,
536 and a decrease in prokaryotes; the latter two of which are inconsistent with our results.
537 Third, viral lysis of HNF (Garza and Suttle, 1995) may occur in the deep subarctic.
538 However, our knowledge of the abundance of viruses that infect protists is very limited,
539 especially for deep waters (Nagata et al., 2010). Further studies on viral infection and *in*
540 *situ* grazing rates of microzooplankton are key for a better understanding of trophic
541 transfer in the deep ocean.
542
543 5. Conclusion
544 The results of this study support our hypotheses, in that (I) latitudinal
545 difference in prokaryotic biomass increased with depth from 2.3-fold in the epipelagic
546 to 4.4-fold in the bathypelagic zones (Table 3) and that (II) the vertical attenuation of
547 prokaryotic abundance was steeper toward lower latitudes in the mesopelagic zone
548 (Table 4) in accordance with the latitudinal differences in the attenuation of sinking
549 POC flux (Buesseler et al., 2007; Steinberg et al., 2008). However, the vertical
550 attenuation rate of HNF or ciliates did not differ with latitude, and the latitudinal
551 differences in their biomass decreased with increasing depth. The highest biomass ratios
552 of prokaryotic:HNF and prokaryotes:ciliates occurred in the meso- and bathypelagic
553 zones of the subarctic; we therefore hypothesize a weak trophic linkage between
554 prokaryotes and HNF/ciliates in the deep subarctic Pacific. Although ciliates decreased
555 sharply at depths below 1000 m, HNF did not further decrease, with the exception of
556 exhibiting a minimum at 2000 m. From these profiles and the correlation of
557 depth-integrated biomass, it is inferred that ciliates might graze on prokaryotes rather
558 than on HNF in the meso- and bathypelagic zones, and that HNF in the bathypelagic

559 zone might gain their nutrition from sources other than prokaryotes, such as organic
560 colloids.
561
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569

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Table 1. Sampling site in the KH-05-2 cruise. Station name with "X" represents the station whrere XBT was conducted.

Region	Station	Date (2005)	Latitude	Longitude	Water depth (m)
Tropical	1	20 Aug	10°10.41'S	160°16.57'W	4988
	2	21 Aug	4°59.84'S	160°00.52'W	5319
Equatorial	3	22 Aug	0°00.07'N	160°14.65'W	5057
Tropical	4	24 Aug	7°09.83'N	159°59.65'W	4158
	X1	24 Aug	8°57.36'N	160°00.05'W	-[a]
	5	25 Aug	10°04.57'N	160°01.63'W	5148
Subtropical	X2	25 Aug	12°03.18'N	160°00.06'W	-
	6	26 Aug	15°02.37'N	160°02.43'W	5471
	X3	26 Aug	17°28.86'N	160°00.00'W	-
	7	25 Aug	20°00.06'N	160°01.63'W	4515
	8	4 Sep	26°20.32'N	160°00.31'W	5147
	X4	4 Sep	28°00.29'N	160°00.08'W	-
Transitional	9	5 Sep	30°00.39'N	159°57.97'W	5729
	X5	5 Sep	32°30.02'N	159°59.98'W	-
	10	6 Sep	35°00.73'N	159°59.81'W	5772
	X6	6 Sep	36°59.15'N	160°00.00'W	-
	X7	6 Sep	38°30.35'N	160°00.07'W	-
	11	7 Sep	40°00.12'N	160°01.63'W	5468
	X8	8 Sep	41°00.15'N	159°59.94'W	-
Subarctic	X9	8 Sep	43°30.00'N	160°00.00'W	-
	X10	8 Sep	44°00.01'N	159°59.99'W	-
	12	9 Sep	45°00.16'N	159°59.91'W	5282
	X11	9 Sep	46°00.46'N	159°59.93'W	-
	X12	9 Sep	47°30.15'N	159°59.71'W	-
	X13	9 Sep	48°59.94'N	160°00.41'W	-
	13	10 Sep	49°59.80'N	159° 59.98'W	4952
	X14	11 Sep	51°30.51'N	160°00.02'W	-
	14	11 Sep	53°34.77'N	160°00.31'W	6474

[a] -:not measured.

Table 2. C:Chl *a* conversion factors applied in this study (g g^{-1}).

Station	Shallow		Deep	
	Depth (m)	C:Chl *a*	Depth (m)	C:Chl *a*
1, 2	0 - 100	163	150 - 300	53
3	0 - 100	77	150 - 300	36
4, 5	0 - 100	130	150 - 300	45
6, 7, 8	0 - 100	157	150 - 300	36
9	0 - 100	130	150 - 300	45
10	0 - 75	91	100 - 300	63
11, 12	0 - 50	91	75 - 300	63
13	0 - 30	91	50 - 300	63
14	0 - 20	91	30 - 300	63

Table 3. Depth-integrated biomass (mg C m^{-2}) along the 160°W meridian in the central Pacific.

Region	Station	Phytoplankton	Prokaryotes			HNF			Ciliates		
		5-100 m	5-100 m	100-1000 m	1000-4000 m	5-100 m	100-1000 m	1000-4000 m	5-100 m	100-1000 m	1000-4000 m
Equatorial	3	1990	410	585	497	53	59	86	27	19	11
Tropical	1	1380	420	679	347	67	55	90	10	11	5
	2	3100	480	589	407	52	68	45	15	18	13
	4	2120	350	523	415	40	89	52	14	19	8
	5	1390	350	580	382	56	63	94	11	17	9
Subtropical	6	1910	707	1130	606	79	92	71	11	17	8
	7	2030	782	1400	565	97	80	100	12	17	8
	8	1930	633	1350	708	104	123	109	10	13	7
Transitional	9	2230	959	1730	844	113	113	91	11	16	10
	10	1990	1060	2020	1200	134	132	84	16	18	12
	11	2310	614	2110	1600	134	171	119	18	18	9
Subarctic	12	2680	1010	2260	1630	126	99	92	30	29	13
	13	1890	674	2050	1690	129	129	103	35	38	13
	14	6150	1060	2040	1830	179	148	90	41	35	17
subarctic/tropical [a]		1.8	2.3	3.6	4.4	2.7	1.8	1.3	2.9	2.1	1.7
r with latitude [b]		0.45	0.76**	0.94***	0.93***	0.92***	0.84***	0.53*	0.62*	0.70**	0.57*

[a] Ratio between the mean values at the subarctic and tropical stations.

[b] Correlation coefficient between depth-integrated variable and the north latitude, where the south latitude was transformed to negative number. Significant correlation was indicated with asterisks: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Table 4. Slope (b) of linear regression on the model of log N = b × log z+a, where N is abundance and z is depth, range of correlation coefficient (r) and sample size (*i.e.*, number of depth; n) in each depth stratum. b, r and n are shown only for significant case (null hypothesis, b = 0; $p < 0.05$).

Statistics	Region	Station	Prokaryotes			HNF			Ciliates		
			5-100 m	100-1000 m	1000-4000 m	5-100 m	100-1000 m	1000-4000 m	5-100 m	100-1000 m	1000-4000 m
b	Equatorial	3	-0.38	-0.72	-0.81	-0.20	-1.06				
	Tropical	1		-1.26	-0.63		-1.24				-2.01
		2		-1.02	-0.63	-0.13	-1.15				-2.05
		4		-0.66	-0.80	-0.18	-1.16	-0.25		-1.25	-1.61
		5		-1.03	-0.69	-0.15	-1.14	-0.23			-2.10
	Subtropical	6		-1.21	-0.66		-1.18				-1.67
		7		-1.25	-0.76		-1.19		-0.17		-1.46
		8		-1.09	-0.67	-0.23	-1.20				-2.33
	Transitional	9		-1.09	-0.65	-0.11	-1.21		-0.11		-1.63
		10		-0.80	-0.92	-0.19	-1.15		-0.25	-0.845	-1.83
		11		-0.76	-0.76	-0.17	-1.18		-0.21	-1.07	-1.34
	Subarctic	12	-0.45	-0.55	-0.94	-0.23	-1.14				-2.43
		13		-0.73	-0.60	-0.32	-1.13			-1.26	-1.60
		14	-0.75	-0.69	-0.81	-0.33	-1.27				-2.00
	Subarctic/tropical		-[a]	0.66	1.1	1.9	1.0	-	-	1.0	1.0
r			-0.88－-0.97	-0.91－-0.99	-0.91－-0.995	-0.89－-0.99	-0.90－-0.98	-0.95, -0.97	-0.82－-0.89	-0.96－-0.98	-0.93－-0.99
n			6	5 or 6	3－5	6	6	5	6	4	4

[a] -: not caliculated due to insufficient data set.

Table 5. Ratios of depth-integrated biomass among ciliates, HNF, prokaryotes and phytoplankton. The ratios were normalized to phytoplankton (Phytoplankton = 1), HNF (HNF = 1) and ciliates (Ciliates = 1), respectively.

Region	Station	Phytoplankton = 1					HNF = 1						Ciliates = 1		
		5-100m					5-100m		100-1000m		1000-4000m		5-100m	100-1000m	1000-4000m
		Ciliates	:	HNF	:	Prokaryotes	Ciliates	: Prokaryotes	Ciliates	: Prokaryotes	Ciliates	: Prokaryotes	Prokaryotes		
Equatorial	3	0.013	:	0.026	:	0.21	0.51	: 7.9	0.33	: 9.9	0.13	: 5.8	15	30	46
Tropical	1	0.007	:	0.048	:	0.30	0.15	: 6.3	0.21	: 12.4	0.05	: 3.8	42	59	73
	2	0.005	:	0.017	:	0.15	0.29	: 9.1	0.26	: 8.6	0.30	: 9.0	32	33	30
	4	0.006	:	0.019	:	0.17	0.34	: 8.8	0.21	: 5.9	0.15	: 8.0	26	28	53
	5	0.008	:	0.040	:	0.25	0.20	: 6.3	0.27	: 9.2	0.09	: 4.1	32	34	43
Subtropical	6	0.006	:	0.041	:	0.37	0.15	: 9.0	0.19	: 12.3	0.12	: 8.5	62	66	73
	7	0.006	:	0.048	:	0.39	0.12	: 8.0	0.21	: 17.6	0.08	: 5.6	66	85	69
	8	0.005	:	0.054	:	0.33	0.10	: 6.1	0.11	: 10.9	0.07	: 6.5	61	100	98
Transitional	9	0.005	:	0.051	:	0.43	0.10	: 8.5	0.14	: 15.3	0.12	: 9.3	84	111	81
	10	0.008	:	0.067	:	0.53	0.12	: 7.9	0.14	: 15.4	0.14	: 14.4	68	114	103
	11	0.008	:	0.058	:	0.27	0.14	: 4.6	0.10	: 12.3	0.08	: 13.5	33	120	180
Subarctic	12	0.011	:	0.047	:	0.38	0.24	: 8.0	0.30	: 22.9	0.14	: 17.8	34	77	125
	13	0.019	:	0.068	:	0.36	0.27	: 5.2	0.29	: 15.9	0.13	: 16.5	19	54	128
	14	0.007	:	0.029	:	0.17	0.23	: 5.9	0.24	: 13.8	0.19	: 20.3	26	58	109

Table 6. Summary of depth-integrated biomass of prokaryotes, HNF and ciliates in the open ocean.

Climatic zone	Sector	Period	Depth (m)	Biomass (mg C m^{-2})	Reference
Prokaryotes					
Equatorial	Pacific	Oct	0-100	740	Nagata et al. (2000)
	C Pacific	Aug, Sep	5-100	413	this study
Tropical	NE Atlantic	Jul, Aug	0-100	1586	Buck et al. (1996)
	C Pacific	Aug, Sep	5-100	351-489	this study
Subtropical	Sargasso Sea	Mar, Apr, Aug	0-100	450	Caron et al. (1999)
		Aug, Nov	0-150	990-1340	Fuhrman et al. (1989)
	NE Atlantic	Jul, Aug	0-100	1707	Buck et al. (1996)
	NE Pacific	Jan-Dec	0-200	910-2200	Campbell et al. (1997)
	Pacific	Oct, Nov	0-100	620-2410	Nagata et al. (2000)
		Jul Aug	0-100	890-2700	Fukuda et al. (2007)
	C Pacific	Aug, Sep	5-100	633-782	this srudy
Transitional	C Pacific	Aug, Sep	5-100	614-1065	this study
Subarctic	NE Atlantic	Jul, Aug	0-100	1811	Buck et al. (1996)
	Pacific	Jul, Aug, Oct	0-100	620-2950	Nagata et al. (2000)
	C Pacific	Aug, Sep	5-100	674-1063	this study
HNF					
Equatorial	NE Pacific	Jul	0-90 or 0-100	71-109	Yang et al. (2004)
	C Pacific	Aug, Sep	5-100	53	this study
Tropical	C Pacific	Aug, Sep	5-100	40-67	this study
Subtropical	SE Pacific	Aug, Oct	0-100	320, 372	Zeldis et al. (2002)
	Sargasso Sea	Mar, Apr, Aug	0-100	410	Caron et al. (1999)
		Aug, Nov	0-150	90-120	Fuhrman et al. (1989)
	C Pacific	Aug, Sep	5-100	79-104	this study
Transitional	NE Atlantic	Mar, Apr, May, Sep, Nov	5-100	92-840	Karayanni et al. (2005)
	C Pacific	Aug, Sep	5-100	113-134	this srudy
Subbarctic	Pacifc	Jul, Aug	0-100	30-700	Fukuda et al. (2007)
	C Pacific	Aug, Sep	5-100	126-178	this srudy
Subantarctic	SE Pacific	Aug, Oct	0-100	183, 750	Zeldis et al. (2002)
Antarctic	Southern Ocean	Oct	20-100	43-584	Klaas (1997)
Ciliates[a]					
Equatorial	NE Pacific	Jul	0-90 or 0-100	49-100	Yang et al. (2004)
	C Pacific	Aug, Sep	5-100	27	this study
Tropical	NW Indian	Sep, Oct	0-92	25	Leakey et al. (1996)

	C Pacific	Aug, Sep	5-100	9.9-15	this study
Subtropical	Sargasso Sea	Aug	0-150	30	Lessard & Murrell (1996)
	SE Pacific	Aug, Oct	0-100	98, 193	Zeldis et al. (2002)
	Arabian Sea	Jan, Feb, Mar, Apr	0-160	50-97	Dennett et al. (1999)
	C Pacific	Aug, Sep	5-100	10-12	this study
Transitional	Pacific	Jul, Aug, Sep	0-100	89-160	Ito and Taniguchi (2001)
	C Pacific	Aug, Sep	5-100	11-18	this study
Subarctic	Pacific	Jul, Aug, Sep	0-100	360-440	Ito and Taniguchi (2001)
	Pacific	May, Jun, Jul, Aug	0-80	100-200	Strom et al. (1993)
	C Pacific	Aug, Sep	5-100	30-41	this study
Subantarctic	SE Pacific	Aug, Oct	0-100	128, 170	Zeldis et al. (2002)
Antarctic	Southern Ocean	Oct	20-100	17-271	Klaas (1997)

[a] Studies using acid Lugol's or Bouin's solution as a fixative are shown.

Table 7. Summary of biomass ratios among ciliates, HNF and prokaryotes in seawater. Ratio was normalized to HNF biomass. ND means that biomass was not reported.

Location	Depth	Ciliates:HNF:Prokaryotes	Reference
Equatorial NE Pacific	0-100m	(0.69-2.2):1:ND	Yang et al. (2004)
Sargasso Sea	3-173m	ND:1:(0.9-2.6)	Caron et al. (1995)
	0-150m	ND:1:11	Fuhrman et al. (1989)
	150-2600m	ND:1:16	
Subtropical SE Pacific	0-100m	(0.26, 0.60):1:ND	Zeldis et al. (2002)
Chesapeak Bay	surface	ND:1:(4.0-33)	McManus & Fuhrman (1990)
Mediterranean Sea	surface	(0.07-1.27):1:(2.1-33.3)	Rassoulzadegan et al. (1988)
	5-110m	(0.08-0.62):1:(3.9-19.8)	Tanaka and Rassoulzadegan (2002)
	110-1000m	(0.03-0.13):1:(10.6-39.0)	
	1000-2000m	(0.02-0.33):1:(3.0-107)	
Transitional N Atlantic	5-100m	(0.18-1.1):1:ND	Karayanni et al. (2005)
Western N Pacific	0-200m	ND:1:(5.0-14.2)	Yamaguchi et al. (2004)
(subtropical-subarctic)	200-1000m	ND:1:(3.1-22.9)	
	1000-3000m	ND:1:(3.3-40.5)	
	>3000m	ND:1:(1.8-32.8)	
Subarctic Pacific	0-100m	ND:1:(1.3-47)	Fukuda et al. (2007)
	100-1000m	ND:1:(9.0-66)	
	1000m-bottom	ND:1:(20-200)	
Bering Sea	0-100m	ND:1:(2.0, 33)	Fukuda et al. (2007)
	100-1000m	ND:1:(9.1, 36)	
	1000m-bottom	ND:1:(15, 400)	
Subantarctic SE Pacific	0-100m	(0.17, 0.93):1:ND	Zeldis et al. (2002)
Southern Ocean	20m	ND:1:(2.1-3.3)	Becquevort (1997)
	20-100m	(0.14-2.3):1:ND	Klaas (1997)
Weddell Sea	surface	0.29:1:1.9	Mathot et al. (1991)
Greenland Sea	1-35m	(0.8-4.4):1:(7.2-31)	Nielsen et al. (2007)
Central Pacific	0-100m	(0.10-0.51):1:(4.6-9.1)	This study
(equatorial-subarctic)	100-1000m	(0.10-0.32):1:(5.9-22.9)	
	1000-4000m	(0.05-0.30):1:(3.8-20.3)	

Table 8. Coefficients of correlation between depth-integrated biomass. Only significant cases ($p < 0.05$) are shown.

Depth strata	Phytoplankton-HNF	Phytoplankton-Ciliates	Prokaryotes-HNF	Prokaryotes-Ciliates	HNF-Ciliates
5-100 m	0.57	0.65	0.84		0.58
100-1000m			0.82	0.55	
1000-5000m				0.65	

Figure legends

Fig. 1. Sampling stations in the KH-05-2 cruise in the North Pacific (August–September, 2005). Lines indicate the boundary of climatic zones as defined in Fig. 2.

Fig. 2. Cross-sections of temperature (A) and salinity (B) in the upper 500 m along the 160^{o}W meridian. Shown are horizontal, curved, solid isolines between 0-30 ^{o}C (temperature), and 32-36 (salinity). Dashed lines indicate boundary of climatic zones. Vertical solid lines indicate location of sampling and XBT stations (see Table 1 for details). Arrows in (B) indicate fronts. Note that isolines of salinity=34.8 and 34.9 indicate the southern boundary of the North Pacific Tropical Water (NPTW), and the Subtropical Front, respectively. EQ= equator.

Fig. 3. Depth-profiles of loricate ciliate abundance (circle with line) and their contribution to the sum of naked ciliates and loricate ciliates (% abundance; horizontal bars) at representative stations in each climatic zone. Scales are same for each plot.

Fig. 4. The mean cell size of heterotrophic nanoflagellates (HNF; upper panels) and ciliates (lower panels) at representative depths of the representative stations. Scales are same for each plot.

Fig. 5. Cross-sections of Chl *a* concentration, and abundance of ciliates, heterotrophic nanoflagellates (HNF), and prokaryotes in 5–200 m (upper panels) and 200–5000 m (lower panels). Chl *a* and ciliates were below the detection limit at the depths below or equal to the isolines of Chl *a* = 0.007 μg l^{-1} and ciliates = 0.1 cells l^{-1}, respectively.

White vertical lines indicate the boundary of climatic zones.

Fig. 6. Depth-profiles of Chl *a* concentration, and abundance of prokaryotes, heterotrophic nanoflagellates (HNF), and ciliates at representative stations in each climatic zone. Values below the detection limit, *i.e.*, Chl *a* concentration < 0.007 μg l^{-1} and ciliate abundance < 0.1 cells l^{-1} not shown. Chl *a* was measured in the upper 300 m.

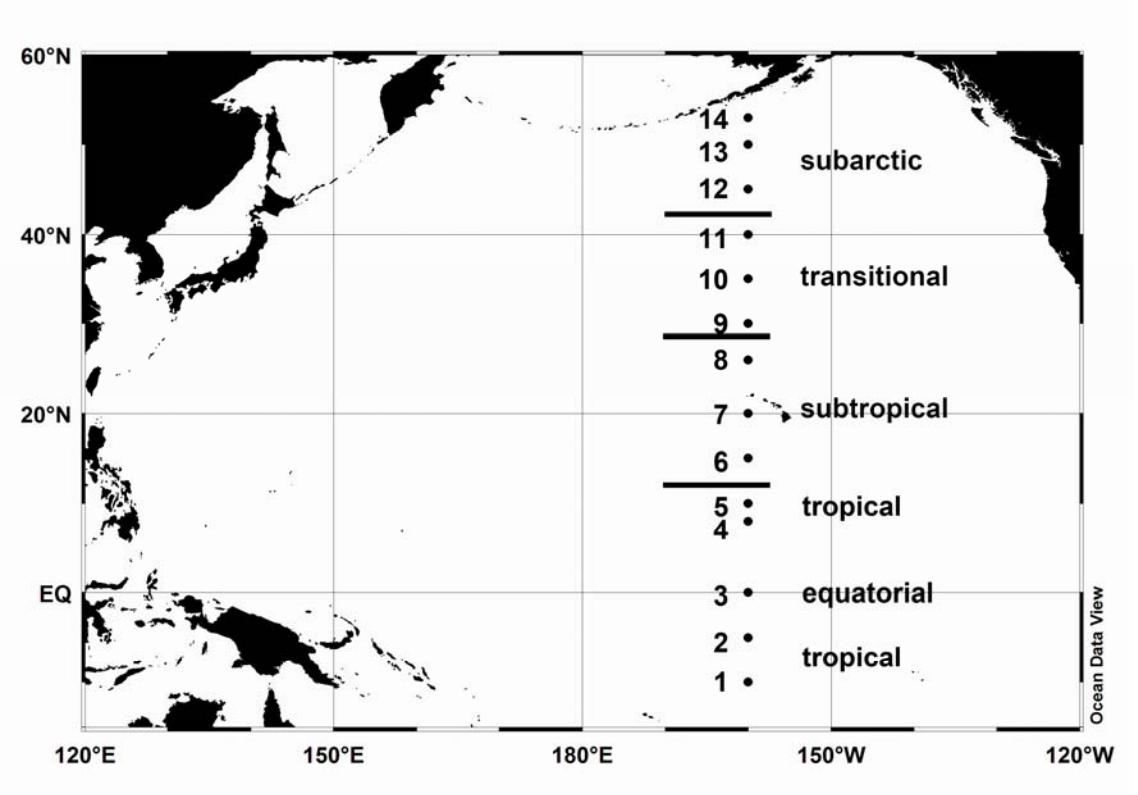
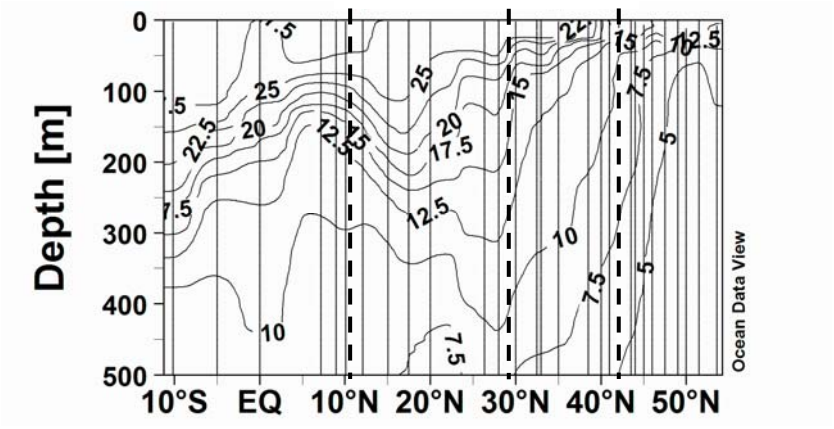


Fig. 1 (Sohrin *et al.*)

(A) Temperature [^{o}C]



southern boundary of NPTW

subtropical front

subarctic front

(B) Salinity

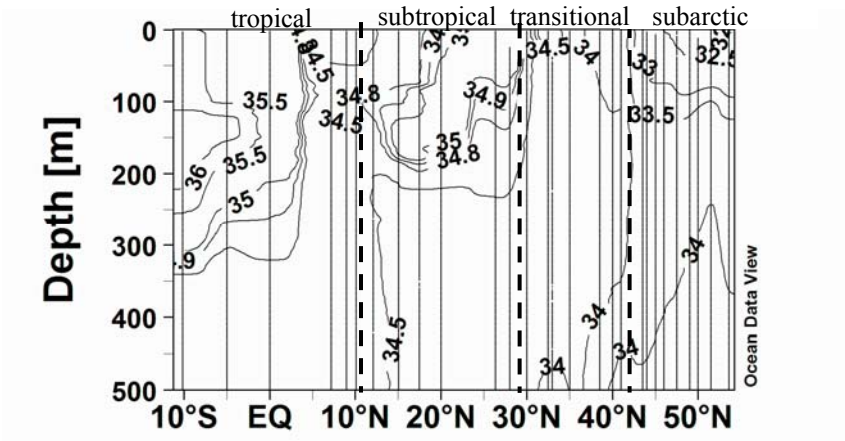


Fig. 2 (Sohrin *et al.*)

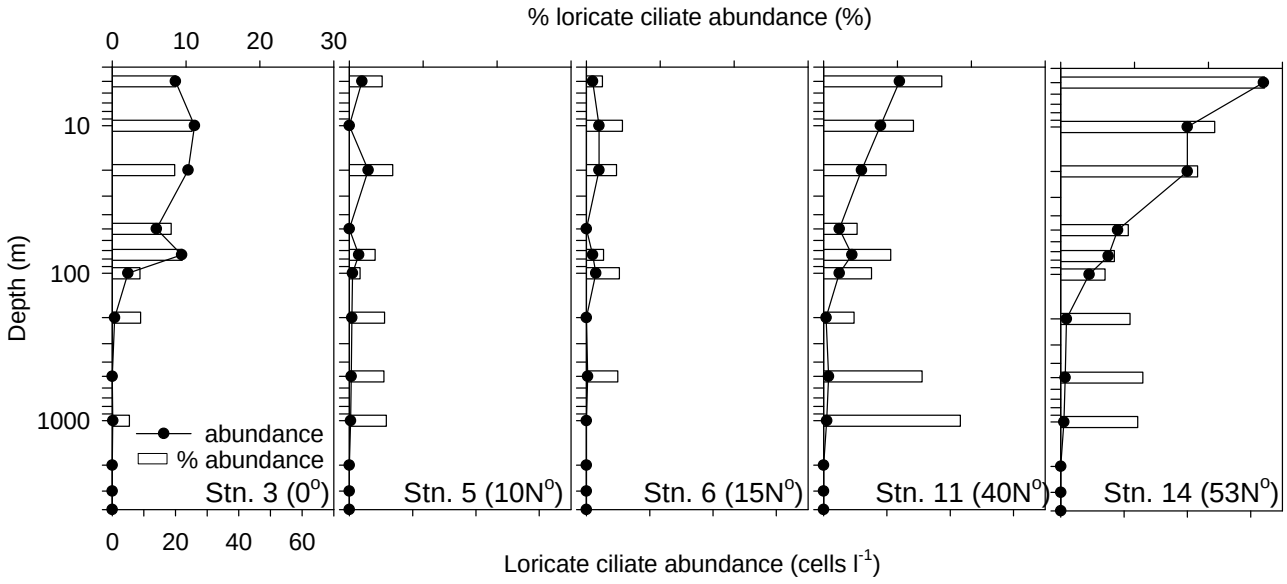


Fig. 3 (Sohrin *et al.*)

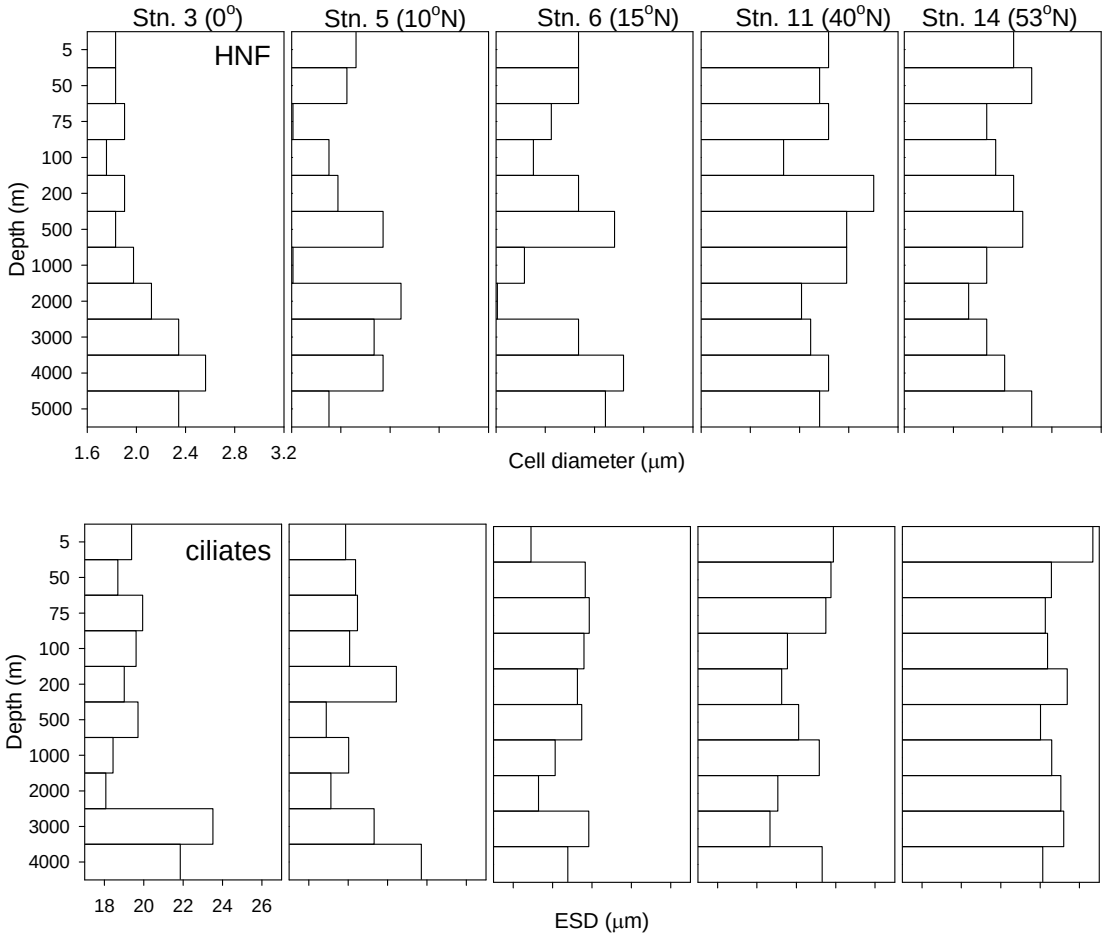


Fig. 4 (Sohrin *et al.*)

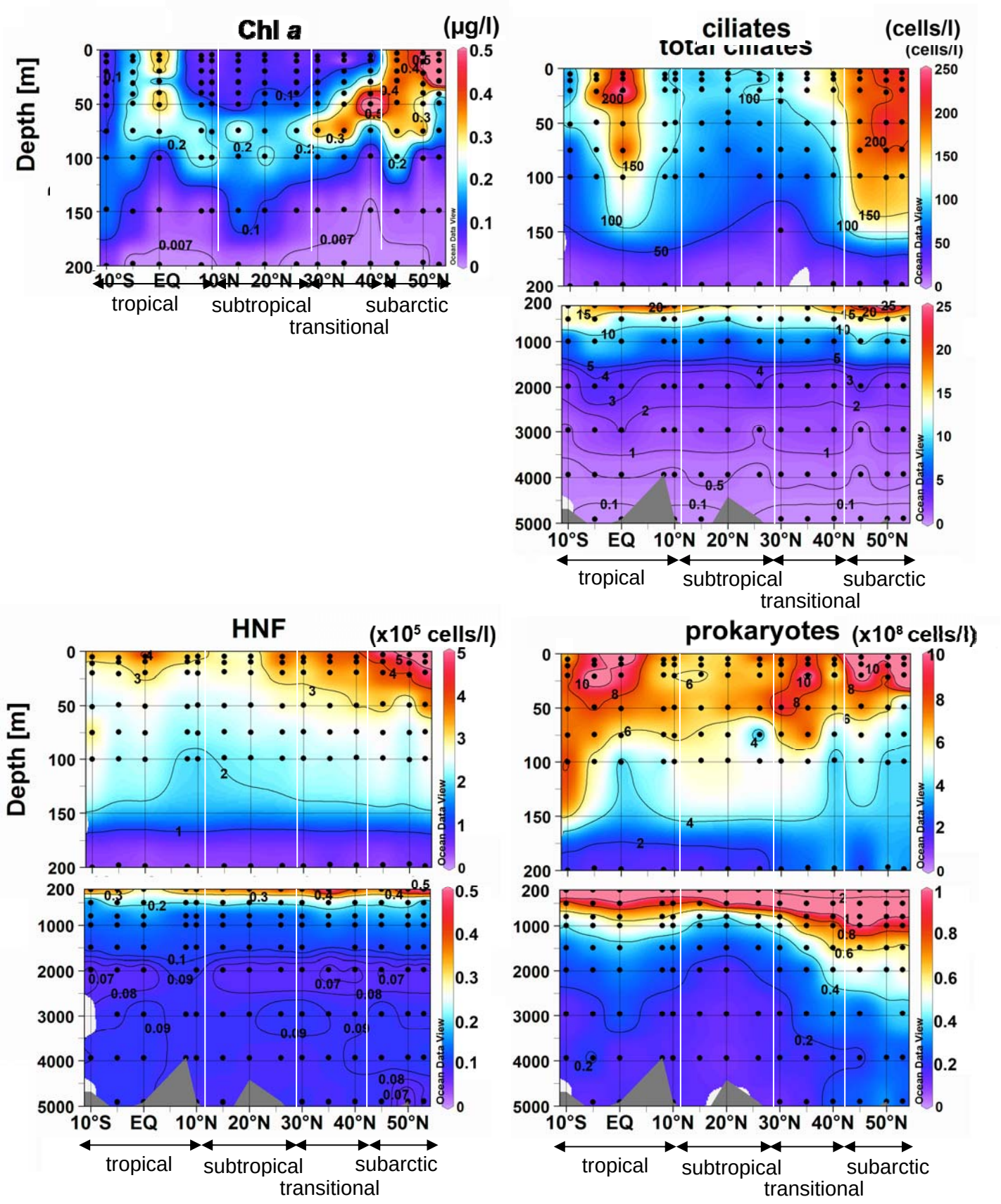


Fig. 5 (Sohrin *et al.*)

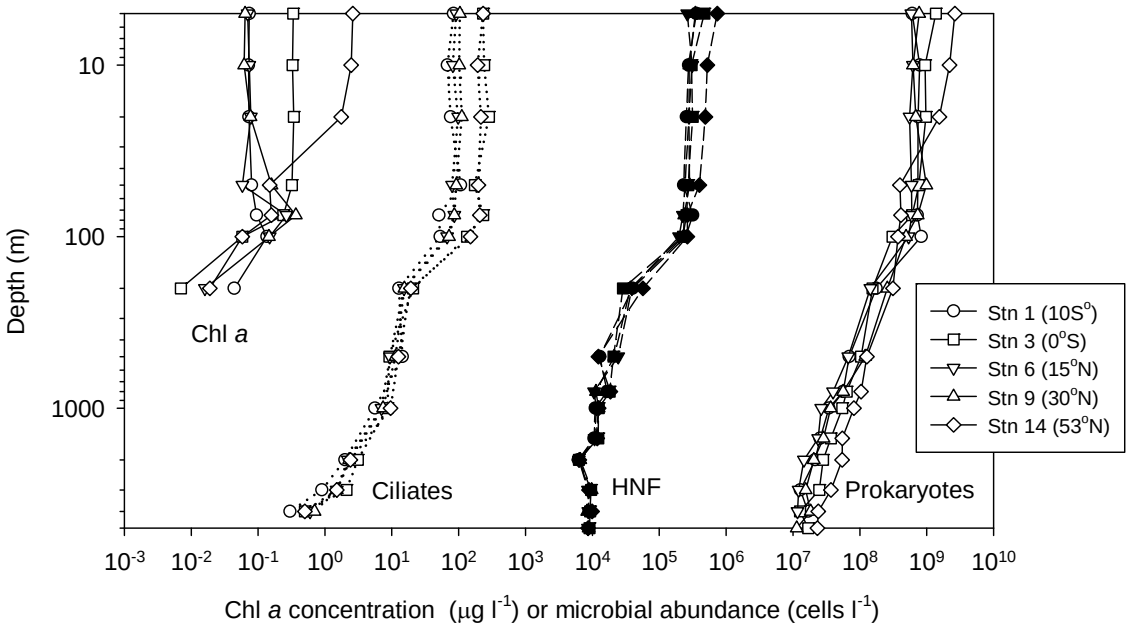


Fig. 6 (Sohrin *et al.*)

Table. Abundance of prokaryotes, HNF and ciliates.

region		equator	tropical				subtropical			transitional			subarctic		
station		Stn. 3	Stn. 1	Stn. 2	Stn. 4	Stn. 5	Stn. 6	Stn. 7	Stn. 8	Stn. 9	Stn. 10	Stn. 11	Stn. 12	Stn. 13	Stn. 14
latitude	depth	0°	10°S	5°S	8°N	10°N	15°N	20°N	26°N	30°N	35°N	40°N	45°N	50°N	53°N
longitude	(m)	160°W	160°W	160°W	160°W	160°W	160°W	160°W	160°W	160°W	160°W	160°W	160°W	160°W	160°W
bottom (m)		5057	4988	5319	4158	5148	5471	4515	5147	5729	5772	5468	5282	4952	6474
Prokaryotes	5	13.7	6.1	9.6	7.5	6.7	5.9	6.1	8.7	7.7	8.5	6.7	19.9	7.4	26.5
(10^8cells l^{-1})	10	9.5	7.8	9.1	6.1	5.5	6.2	6.5	5.8	6.3	8.9	5.1	11.2	4.6	21.9
	20	9.7	7.5	11.8	6.4	5.6	5.5	6.9	6.3	6.9	11.8	4.8	11.2	5.8	15.5
	50	7.6	7.4	8.3	7.9	6.5	5.9	7.3	7.1	9.9	7.4	6.2	7.5	6.7	4.0
	75	6.1	7.3	7.5	6.0	6.8	6.0	5.6	1.6	7.3	8.7	4.0	5.7	4.3	4.1
	100	3.1	8.3	5.4	2.8	6.1	5.4	5.5	5.4	4.9	4.5	3.2	4.4	3.6	3.7
	200	1.6	1.8	1.5	1.3	1.3	1.4	1.8	1.5	2.6	2.6	3.4	2.0	3.5	3.1
	500	1.0	0.69	0.86	0.86	0.80	0.67	1.2	1.1	1.2	1.7	1.5	2.0	1.0	1.3
	800	0.63	0.56	0.56	0.71	0.57	0.40	0.29	0.53	0.56	0.78	0.86	1.5	1.1	1.0
	1000	0.55	0.34	0.41	0.50	0.42	0.26	0.30	0.30	0.37	0.70	0.60	0.87	0.75	0.82
	1500	0.37	0.25	0.32	0.29	0.27	0.24	0.20	0.24	0.29	0.43	0.64	0.58	0.59	0.55
	2000	0.28	0.21	0.21	0.23	0.22	0.15	0.13	-[a]	0.20	0.22	0.45	0.49	0.42	0.54
	3000	0.25	0.13	0.17	0.17	0.17	0.12	0.12	0.14	0.15	0.26	0.30	0.25	-	0.37
	4000	0.13	0.17	0.21	0.17	0.15	0.12	0.11	0.13	0.18	0.17	0.20	0.19	0.27	0.24
	5000	0.17	-	0.13	-	0.14	-	-	-	0.11	0.15	0.24	0.24	0.31	0.23
HNF	5	47	36	34	32	33	26	26	41	35	41	40	54	54	74
(10^4cells l^{-1})	10	30	27	28	33	26	31	26	40	28	38	35	37	41	52
	20	32	25	26	23	26	28	26	32	28	34	32	40	37	49
	50	27	23	24	22	24	24	24	28	28	28	31	33	25	40
	75	26	32	23	21	23	23	25	24	24	26	25	26	21	26
	100	23	25	21	19	18	20	20	20	23	22	23	25	21	26
	200	2.9	4.0	3.8	3.5	3.9	3.9	4.0	4.3	3.7	5.7	4.2	3.7	5.2	5.7
	500	2.1	1.3	2.3	2.0	2.0	2.4	2.0	2.1	2.1	2.4	2.0	2.0	2.1	1.2
	800	1.8	1.7	1.3	1.0	1.1	1.1	1.1	1.2	1.1	1.8	1.7	1.8	1.9	1.9
	1000	1.2	1.1	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.3	1.1	1.2	1.2	1.2
	1500	1.2	1.1	1.2	1.1	1.1	1.1	1.1	1.1	1.1	-	1.1	1.1	1.2	1.2
	2000	0.63	0.62	0.64	-	0.93	0.63	0.63	0.63	0.66	0.63	0.62	0.63	0.65	0.64
	3000	0.96	-	0.89	0.86	0.90	0.86	0.90	0.96	0.98	0.90	0.87	0.96	0.89	0.89
	4000	0.92	0.90	0.95	0.88	0.91	0.87	0.86	0.84	0.84	0.88	0.92	0.91	0.93	0.99
	5000	0.91	-	0.96	-	0.79	0.86	-	-	0.88	0.85	0.87	0.91	0.55	0.86
Ciliates	5	232	84	158	104	90	92	106	102	104	144	150	198	210	232
(cells l^{-1})	10	240	68	146	102	90	82	96	86	102	114	148	182	212	192
	20	284	76	196	98	102	98	98	110	112	142	142	198	188	216
	50	175	107	120	123	95	80	75	85	-	100	110	186	231	197
	75	232	51	101	129	86	86	62	79	86	85	99	203	209	207
	100	133	53	69	102	68	67	-	51	72	56	77	171	160	150
	200	20.8	13	17	26	17	15	16	13	-	-	19	22	32	19
	500	9.2	14	15	10	13	9.4	9.4	13	11	11	12	14	13	13
	800	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1000	8.6	5.6	9.6	5.4	8.0	7.0	4.8	7.4	7.4	7.6	5.4	10.4	8.0	9.6
	1500	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2000	3.1	2	4.1	2.1	2.9	2.2	2.3	3.2	2.3	2.4	1.9	3.2	2.1	2.4
	3000	2.1	0.9	1.5	1.3	1.1	1.5	1.7	0.7	1.5	1.5	1.7	0.7	1.5	1.5
	4000	0.5	0.3	0.5	0.5	0.4	0.6	0.5	0.3	0.7	0.5	0.7	0.4	0.8	0.5
	5000	<0.1	-	<0.1	-	<0.1	<0.1	-	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

[a] No data.