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Seasonal changes in the abundance and composition of picophytoplankton in relation to the occurrence of '*Kyucho*' and bottom intrusion in Uchiumi Bay, Japan

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ABSTRACT: Uchiumi Bay experiences intermittent physical events of '*Kyucho*' and bottom intrusion. A *Kyucho* is an intrusion of warm surface water from the Kuroshio in the Pacific Ocean. Bottom intrusion, which contains a large amount of nitrates, phosphates, and silicates, slips through just above the continental shelf. We investigated seasonal changes in the abundance of *Prochlorococcus*, *Synechococcus*, and eukaryotic picophytoplankton while monitoring *Kyucho* and bottom intrusion from March to October 2002. *Kyucho* and bottom intrusion frequently occurred from June to September. Relatively high concentrations of nitrate + nitrite (>0.8 µmol N l^{-1}) and phosphate (>0.1 µmol P l^{-1}) were found when bottom intrusion occurred. The cell densities of *Prochlorococcus* were relatively high (>1×10^4 cells ml^{-1}) when *Kyucho* occurred. Those of *Synechococcus* were high (2 to 30×10^4 cells ml^{-1}) during the period of thermal stratification except in July, when bottom intrusion occurred. The cell densities of eukaryotic picophytoplankton were high (2 to 8×10^4 cells ml^{-1}) in May and July. To examine the effects on picophytoplankton growth of the nutrients supplied by bottom intrusion, we conducted nutrient-enrichment experiments. The growth rates of *Prochlorococcus* and *Synechococcus* were not stimulated by the addition of any kinds of nutrients. The growth rates of *Prochlorococcus* were negative in most cases. In July, the growth rate of eukaryotic picophytoplankton was stimulated by nitrate and phosphate additions. Thus, *Prochlorococcus* detected in Uchiumi Bay might have been transported by *Kyucho* from the Pacific Ocean and could therefore not grow vigorously. *Synechococcus* may have been flushed out by bottom intrusion, and its growth was not limited by the nutrient concentrations. Eukaryotic picophytoplankton was abundant in spring, and its growth might have been limited by the nutrient concentrations in some cases. These results suggest that *Kyucho* and bottom intrusion have different effects on the abundance and growth rate of the 3 picophytoplankton groups.

KEY WORDS: Bottom intrusion · *Kyucho* · *Prochlorococcus* · *Synechococcus* · Eukaryotic picophytoplankton · Growth response · Nutrient supply

INTRODUCTION

Picophytoplankton is an important component of primary producers, especially in oligotrophic marine environments (Stockner & Antia 1986, Weisse 1993). The major components of picophytoplankton are *Prochlorococcus*, *Synechococcus*, and eukaryotic picophytoplankton. The abundance of these picophytoplankton groups is strongly affected by nitrogen and phosphorus supplies (Glover et al. 1988, Del Amo et al. 1997, Agawin et al. 2000, Duarte et al. 2000). For example, Glover et al. (1988) reported a *Synechococcus* bloom after a nanomolar increase of nitrate in the Sargasso Sea. In contrast, Moutin et al. (2002) suggested that *Synechococcus* might grow rapidly after an episodic phosphate increase in phosphate-depleted environments, such as the Mediterranean Sea (Vaulot et al. 1996). In different marine systems, the nutrient supply has a different effect on the abundance of picophytoplankton.

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Recently, a difference in the nitrogen utilization between *Synechococcus* and *Prochlorococcus* was clarified. *Synechococcus* utilizes various forms of nitrogen, such as nitrate, nitrite, ammonium, and urea (Waterbury et al. 1986, Lindell et al. 1998, Moore et al. 2002), but some *Synechococcus* strains do not utilize urea for growth (Waterbury et al. 1986, Collier et al. 1999). *Prochlorococcus* utilize urea and nitrite but not nitrate (Moore et al. 2002). An analysis of the complete genome sequence showed that *Prochlorococcus* does not use nitrate because of a lack of nitrate permease and nitrate reductase genes (Moore et al. 2002). Thus, in addition to dissolved nitrogen concentrations, the available forms of nitrogen may affect the composition of picophytoplankton in marine environments, though the nitrogen utilization of eukaryotic picophytoplankton is currently unknown.

In contrast, few studies have researched the effects of the phosphorus supply on the abundance of picophytoplankton in marine environments, though picophytoplankton is phosphate-limited in some cases (Vaulot et al. 1996, Thingstad et al. 1998). Moutin et al. (2002) reported that *Synechococcus* might be well adapted to exploit the episodic phosphorus supply resulting from physical events such as wind-induced turbulence and upwelling. The effect of the phosphorus supply on the abundance of picophytoplankton and its growth should be further studied.

In Uchiumi Bay, located in the southern part of the Uwa Sea, Japan, there are intermittent physical events of '*Kyucho*' and bottom intrusion. A *Kyucho* is an intrusion of warm surface water from the Kuroshio in the Pacific Ocean (Akiyama & Saitoh 1993, Takeoka et al. 2000). Thus, a *Kyucho* contains scarce amounts of nutrients. Bottom intrusion is deep, cold water that slips through just above the continental shelf (Kaneda et al. 2002a,b); this cold water contains a large amount of nitrates, phosphates, and silicates (Koizumi 1991, Koizumi & Kohno 1994, Koizumi et al. 1997, Takeoka et al. 2000, Kaneda et al. 2002a,b). Koizumi et al. (1997) noted that, after an occurrence of bottom intrusion, the cell density of diatoms in Shitaba Bay of the Uwa Sea increased with the use of the supplied nutrients. Thus, bottom intrusion is an important supply of nutrients for phytoplankton in the Uwa Sea.

The trophic state of the water in Uchiumi Bay is oligo- to mesotrophic based on its chlorophyll level (Tomaru et al. 2002). The concentration of chlorophyll *a* in the <2 µm fraction compared to the total fraction ranged between 5 and 60% (Hirose et al. unpubl. data). Thus, picophytoplankton might be an important primary producer in the bay. *Prochlorococcus*, *Synechococcus*, and eukaryotic picophytoplankton have been detected using flow cytometric analysis (T. Hirose et al. unpubl.). As described above, nutrients supplied by bottom intrusion strongly affect the cell densities of diatoms in the Uwa Sea (Koizumi & Kohno 1994). However, the effects of the nutrient supply on the abundance and composition of picophytoplankton have not yet been evaluated. The differences in the growth rate responses of the 3 picophytoplankton groups to the supply of nitrate and phosphate might cause changes in the picophytoplankton composition.

The objective of the present study was to clarify the seasonal changes in the abundance and composition of picophytoplankton in relation to the occurrence of *Kyucho* and bottom intrusion. In addition to the investigation of seasonal changes in picophytoplankton, we conducted monthly *in situ* incubation experiments to evaluate the effects of nutrient addition on the growth rate of the 3 picophytoplankton groups.

MATERIALS AND METHODS

Water temperature monitoring and sampling. To monitor the occurrence of *Kyucho* and bottom intrusion, the water temperature was measured every 30 min with a thermistor chain at 2, 10, 20, 30, 40, 50, and 60 m depths at Stn Ut (Fig. 1). Field monitoring and *in situ* incubation experiments were conducted once a month from March to October 2002 at Stn Ub in Uchiumi Bay (Fig. 1). Water samples were collected from depths of 0, 2, 5, 10, 15, 20, 30, 40, and 50 m using a 6 l Van-Dorn water sampler. The water temperature was measured vertically with a CTD profiler (Arec Electronics, Japan). The samples were stored in a 10 l polyethylene tank and brought to the laboratory.

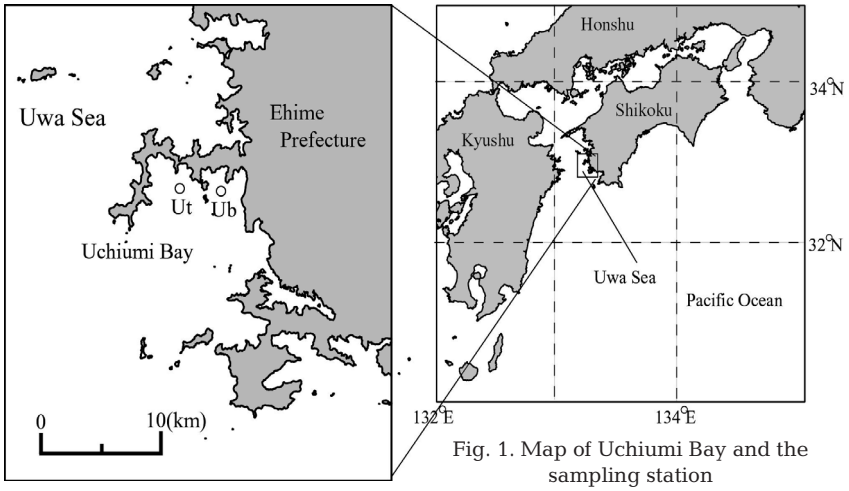

Fig. 1. Map of Uchiumi Bay and the sampling station

The water samples were filtered through 0.2 or 2.0 µm Nuclepore filters (Whatman). The filtrate of the former was used for the determination of nutrient concentrations, and that of the latter was used for the enumeration of the cell density of picophytoplankton. Nitrate + nitrite, ammonium, and phosphate concentrations were determined with an autoanalyzer (TRAACS 800, BRAN+LUEBEE).

Flow cytometric counts. The cell density of picophytoplankton was determined with a flow cytometer (FACSvantage SE, Becton Dickinson) equipped with a water-cooled Argon laser (488 nm, 1 W, Coherent). The forward scatter detector of the flow cytometer was changed from a photodiode to a photomultiplier tube to increase the sensitivity. Thus, our flow cytometer, in conjunction with the high-power water-cooled Argon laser, had enough sensitivity to detect small phytoplankton, such as *Prochlorococcus* (Fig. 2).

Flow cytometry sheath fluid was made by filtering seawater through a 0.1 µm pore-size membrane filter (Millipore). The forward scatter, side scatter, and 3 fluorescence intensities (red, 675 to 715 nm; orange, 564 to 586 nm; and green, 515 to 545 nm) were recorded on each picophytoplankton cell. The instrument threshold was set to red fluorescence in order to not detect heterotrophic organisms. All parameters were normalized with 2 µm fluorescent beads (Polyscience). The beads were also used as the internal standard for the enumeration of *Prochlorococcus*, *Synechococcus*, and eukaryotic picophytoplankton cells (Olson et al. 1993).

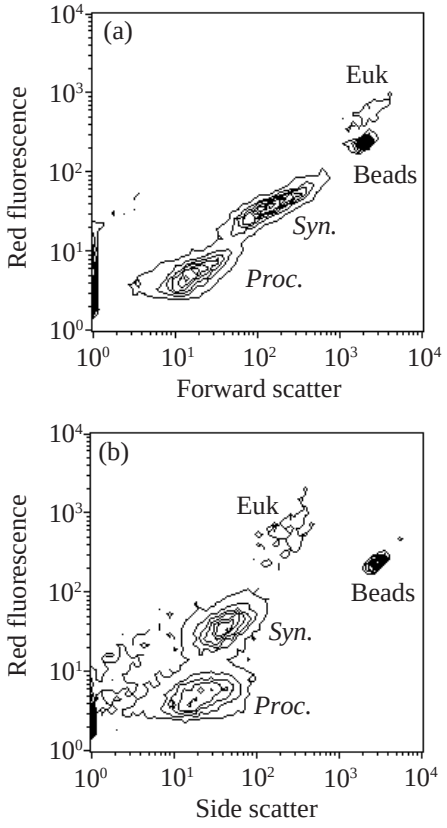

Fig. 2. Contoured plots of flow cytometric analysis for *Prochlorococcus* (Proc.), *Synechococcus* (Syn.), and eukaryotic picophytoplankton (Euk); (a) forward scatter vs. red fluorescence, and (b) side scatter vs. red fluorescence. The lines in the panels show a linear density scale. The noise on the forward scatter is visible at <2, and hence, is well below the signal of *Prochlorococcus*

Ocean Data View software was used to make contoured plots for water temperature, salinity, nutrient concentrations, and picophytoplankton cell densities (Schlitzer, http://www.awi-bremerhaven.de/GEO/ODV/).

***In situ* incubation experiments.** Samples collected from a depth of 10 m were filtered through a 0.2 µm Gelman culture capsule filter and a 2.0 µm Nuclepore filter. In the <2.0 µm filtrate, most of picophytoplankton grazers were probably removed. The filtrates (2.0 µm:0.2 µm) were mixed with ratios of 3:7 in March and April and 1:9 in May to October; they were then poured into 300 ml polycarbonate bottles. Thus, in our experiments, the removal of grazers and the dilution of the sample resulted in a reduction in the amount of grazing on the phytoplankton (Fahnenstiel et al. 1991), though grazing still occurred at a negligible level. Hence, we estimated gross growth rates rather than net growth rates. KNO_3, NH_4Cl, urea, and/or NaH_2PO_4 were added to each bottle at a final concentration of 15 µmol N l^{-1} and 1 µmol P l^{-1}. Eight nutrient-enrichment treatments were prepared in triplicate: control (no addition), +nitrate, +ammonium, +urea, +phosphate, +nitrate & phosphate, +ammonium & phosphate, and +urea & phosphate. The bottles thus prepared were incubated near the sampling station at a 10 m depth for 1 d. At time zero and at the end of the incubation, subsamples for the enumeration of picophytoplankton were taken and fixed with glutaraldehyde at a final concentration of 1% and stored in liquid nitrogen. The growth rate, µ, was calculated from the equation $\mu = \ln(Nf/N0)/t$, where $N0$ and Nf are the cell densities at time zero and after 1 d of incubation, respectively, and t is the incubation period. One-way ANOVAs were carried out using Kaleida Graph v. 3.5 (Synergy Software) to test for differences among treatments and between treatments; subsequently, multiple comparisons were carried out using Tukey's test with a discrimination level of $p < 0.05$.

RESULTS

Seasonal changes in water temperature and nutrient concentrations

The surface water temperature at Stn Ut gradually increased to 20°C at the end of May. From June to September, the surface water temperature was highly

variable, ranging between 20 and 28°C at a 2 m depth and between 18 and 27°C at a 60 m depth. These large fluctuations were due to the occurrence of *Kyucho* and bottom intrusion (Fig. 3). We found 9 occurrences of *Kyucho* (*K*1 to *K*9) and 8 occurrences of bottom intrusion (B1 to B8). *K*2, *K*6, and *K*9 corresponded to our sampling dates on 12 June, 2 August, and 11 September, respectively, and B4 corresponded to that on 12 July (Fig. 3a).

Changes in the water temperature at Stn Ub (Fig. 3) were similar to those at Stn Ut (Fig. 4a). The water temperature in the whole water column decreased in July because of the occurrence of bottom intrusion and increased again in August. In October, the surface water temperature decreased to 23°C, and the water column became almost isothermal.

Salinity gradually decreased from May (>34.6 PSU) to September (34.0 PSU) and increased from September to October (34.4 PSU, Fig. 4b), indicating that there was an exchange of seawater in Uchiumi Bay attributed to the occurrence of both *Kyucho* and bottom intrusion.

Nitrate + nitrite and ammonium concentrations varied from below the detection limit to 3.74 µmol N l^{-1}

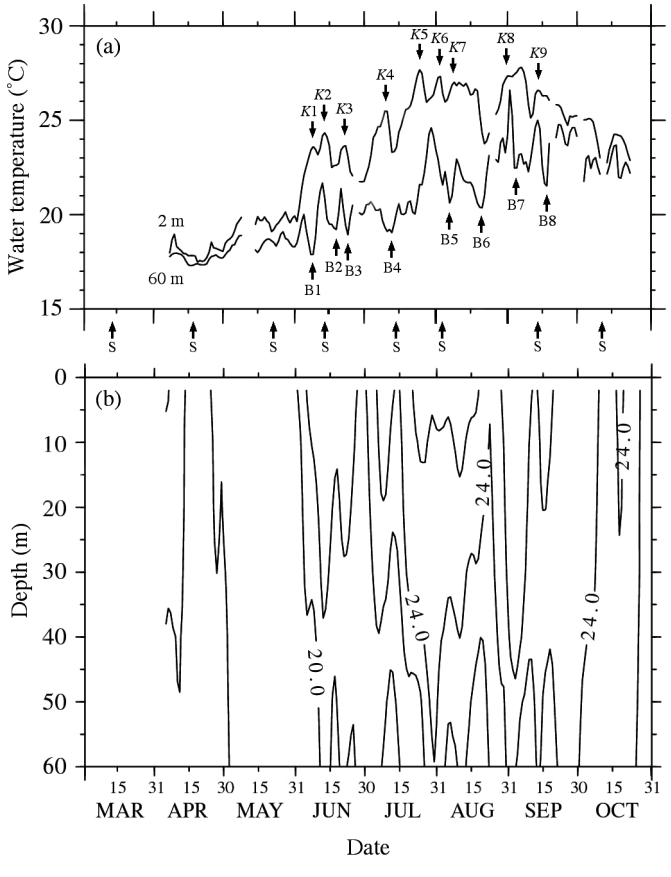

Fig. 3. Seasonal changes in (a) water temperature at depths of 2 and 60 m at Stn Ut, and in (b) vertical distributions of water temperature. The occurrences of *Kyucho* (*K*) and bottom intrusion (B) are shown with arrows. The numbers after *K* and B indicate the times of the occurrences after March 2002. Sampling dates (S) are indicated with arrows at the bottom of (a)

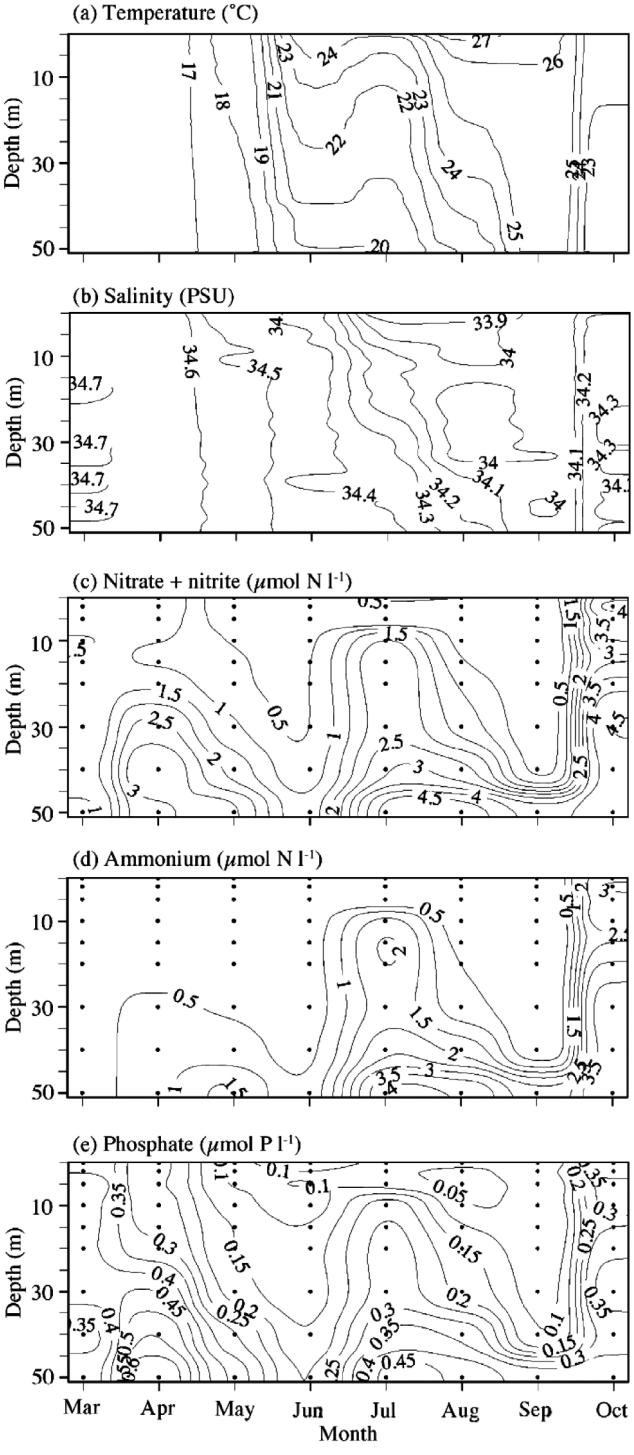

Fig. 4. Seasonal changes in the vertical distributions of (a) water temperature, (b) salinity, (c) nitrate + nitrite concentrations, (d) ammonium concentrations, and (e) phosphate concentrations

and from the detection limit to 5.48 µmol N l^{-1}, respectively (Fig. 4c,d). The concentrations of nitrate + nitrite were relatively high in March (>1 µmol N l^{-1}), decreased to <0.5 µmol N l^{-1} near the surface in June, increased in the deeper layer (2.5 to 4 µmol N l^{-1}) due to the occurrence of bottom intrusion, and decreased again below the detection limit in August. Ammonium concentrations were low in April and May (Fig. 4d). The vertical distributions of the ammonium concentrations were similar to those of the nitrate + nitrite concentrations from June onwards. Seasonal changes in the vertical distribution of phosphate concentrations were similar to those of nitrate + nitrite concentrations (Fig. 4c,e). Relatively high phosphate concentrations (0.4 to 0.3 µmol P l^{-1}) were detected in March and October and in the deeper layer in July. Lower concentrations of both inorganic nitrogen and phosphorus were found when *Kyucho* occurred. When bottom intrusion occurred, these nutrient concentrations increased below a depth of 15 m.

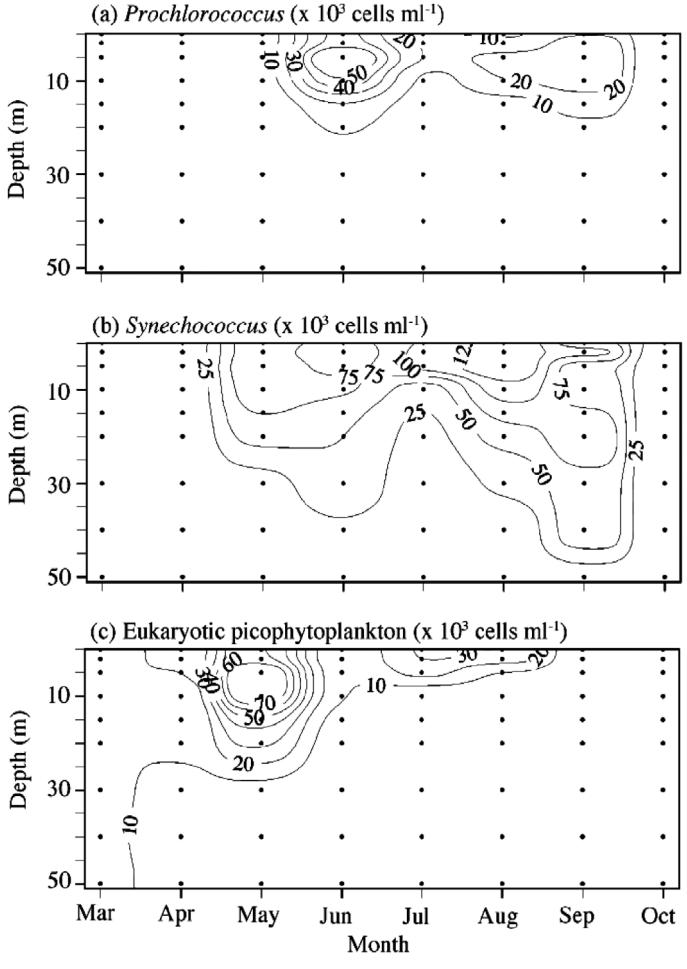

Fig. 5. Seasonal changes in the vertical distributions of (a) *Prochlorococcus*, (b) *Synechococcus*, and (c) eukaryotic picophytoplankton

Seasonal changes in the abundance of picophytoplankton in relation to *Kyucho* and bottom intrusion

The abundance of picophytoplankton was high during the thermal stratification period (Fig. 5), though the vertical distributions of picophytoplankton were different among the 3 groups. The cell densities of *Prochlorococcus* were low (<10^3 cell ml^{-1}) before June, and high cell densities were detected at 0 to 15 m depths in June, with the highest value of 76.2 × 10^3 cells ml^{-1} occurring at a 5 m depth (Fig. 5a). In August and September, relatively high cell densities were found at 5 to 15 m depths (>22 × 10^3 cells ml^{-1}). Overall, the high densities corresponded to the occurrence of *Kyucho*.

Synechococcus was abundant from May to September, except for July (Fig. 5b). In June, August, and September, *Synechococcus* was distributed between depths of 0 to 30 m (41.5 to 154.9 × 10^3 cells ml^{-1}), though the vertical distribution of *Prochlorococcus* was limited to the upper 15 m. The highest cell density (154.9 × 10^5 cells ml^{-1}) of *Synechococcus* was detected in August at a depth of 5 m.

The maximum abundance of eukaryotic picophytoplankton (93.9 × 10^3 cells ml^{-1}) was found in May (Fig. 5c). In June, August, and September, when *Kyucho* occurred, the cell densities of eukaryotic picophytoplankton were low. Relatively high densities (23.5 to 32.5 × 10^3 cells ml^{-1}) were found at 0 and 5 m depths in July.

Effects of nutrient addition on the growth rate of picophytoplankton

The growth rates of *Prochlorococcus* were below 0 d^{-1} except for October (Table 1). We could not determine the growth rate from March to May because of the low cell densities (<10^3 cells ml^{-1}, Fig. 5a). In October, positive growth rates ranging between 0.02 and 0.23 d^{-1} were found except in the urea and phosphate treatments. The growth rate in the urea treatment in October was relatively high (0.23 ± 0.13 d^{-1}), though no significant difference in the growth rate between the urea and control treatments could be detected.

The growth rates of *Synechococcus* in the control treatments varied from 0.04 d^{-1} in April to 1.05 d^{-1} in June (Table 1) and were higher than those of the eukaryotic picophytoplankton and *Prochlorococcus*. In most cases, the growth rates in the nutrient treatments were not significantly different from those in the control. The positive effects of ammonium, phosphate, urea, and phosphate addition were only detected in April.

The growth rate of eukaryotic picophytoplankton ranged from −0.10 to 0.59 d^{-1} in the control treatments (Table 1). In April and July, the cell density of eukary-

Table 1. Growth rates (d^{-1}) of *Prochlorococcus*, *Synechococcus* and eukaryotic picophytoplankton in each enrichment treatment. Values show mean ± SD, and letters in parentheses denote significant differences at 5% among treatments as determined by Tukey's test. nd = not determined

	Control	+nitrate	+ammonium	+urea	+phosphate	+nitr&phos	+amm&phos	+urea&phos
Prochlorococcus								
Mar	nd	nd	nd	nd	nd	nd	nd	nd
Apr	nd	nd	nd	nd	nd	nd	nd	nd
May	nd	nd	nd	nd	nd	nd	nd	nd
Jun	−0.55 ± 0.26 (a)	−0.11 ± 0.18 (a)	−0.27 ± 0.37 (a)	−0.99 ± 0.82 (a)	−0.05 ± 0.36 (a)	−0.07 ± 0.20 (a)	−0.11 ± 0.35 (a)	−0.26 ± 0.31 (a)
Jul	−1.51 ± 0.50 (a)	−1.21 ± 0.37 (a)	−0.98 ± 0.50 (a)	−1.36 ± 0.32 (a)	−1.10 ± 0.20 (a)	−1.23 ± 0.32 (a)	−1.36 ± 0.32 (a)	−1.43 ± 0.15 (a)
Aug	−1.08 ± 0.11 (a)	−1.07 ± 0.19 (a)	−1.00 ± 0.06 (a)	−0.90 ± 0.20 (a)	−0.93 ± 0.12 (a)	−0.90 ± 0.13 (a)	−0.94 ± 0.12 (a)	−0.82 ± 0.02 (a)
Sep	−0.20 ± 0.24 (b)	−0.42 ± 0.13 (ab)	−0.36 ± 0.50 (ab)	−0.50 ± 0.11 (ab)	−0.40 ± 0.23 (ab)	−0.39 ± 0.05 (ab)	−0.42 ± 0.09 (ab)	−0.87 ± 0.41 (a)
Oct	0.05 ± 0.05 (ab)	0.05 ± 0.09 (ab)	0.09 ± 0.06 (ab)	0.23 ± 0.13 (b)	0.15 ± 0.05 (ab)	0.18 ± 0.08 (ab)	0.02 ± 0.14 (ab)	−0.05 ± 0.10 (a)
Synechococcus								
Mar	0.42 ± 0.04 (a)	0.46 ± 0.25 (a)	0.42 ± 0.03 (a)	0.39 ± 0.14 (a)	0.39 ± 0.07 (a)	0.39 ± 0.08 (a)	0.35 ± 0.03 (a)	0.38 ± 0.01 (a)
Apr	0.04 ± 0.07 (a)	0.10 ± 0.08 (ab)	0.33 ± 0.14 (b)	0.22 ± 0.07 (b)	0.35 ± 0.02 (b)	0.37 ± 0.04 (bc)	0.31 ± 0.17 (ab)	0.35 ± 0.09 (b)
May	0.44 ± 0.05 (a)	0.45 ± 0.10 (a)	0.50 ± 0.02 (a)	0.46 ± 0.10 (a)	0.35 ± 0.10 (a)	0.31 ± 0.05 (a)	0.36 ± 0.08 (a)	0.34 ± 0.07 (a)
Jun	1.05 ± 0.12 (a)	1.05 ± 0.08 (a)	1.05 ± 0.08 (a)	1.00 ± 0.20 (a)	1.15 ± 0.04 (a)	1.12 ± 0.03 (a)	1.06 ± 0.02 (a)	1.07 ± 0.06 (a)
Jul	0.62 ± 0.09 (a)	0.69 ± 0.11 (a)	0.60 ± 0.11 (a)	0.66 ± 0.04 (a)	0.78 ± 0.05 (a)	0.73 ± 0.03 (a)	0.64 ± 0.12 (a)	0.63 ± 0.09 (a)
Aug	0.48 ± 0.13 (a)	0.38 ± 0.04 (a)	0.44 ± 0.12 (a)	0.42 ± 0.04 (a)	0.37 ± 0.03 (a)	0.36 ± 0.03 (a)	0.35 ± 0.04 (a)	0.39 ± 0.05 (a)
Sep	0.84 ± 0.15 (a)	0.79 ± 0.03 (a)	0.77 ± 0.03 (a)	0.79 ± 0.04 (a)	0.82 ± 0.06 (a)	0.84 ± 0.08 (a)	0.78 ± 0.03 (a)	0.73 ± 0.07 (a)
Oct	0.88 ± 0.13 (b)	0.87 ± 0.06 (b)	0.91 ± 0.03 (b)	0.82 ± 0.26 (ab)	0.66 ± 0.14 (ab)	0.50 ± 0.07 (a)	0.84 ± 0.06 (ab)	0.80 ± 0.01 (ab)
Eukaryotic picophytoplankton								
Mar	0.36 ± 0.05 (a)	0.47 ± 0.28 (a)	0.27 ± 0.06 (a)	0.40 ± 0.11 (a)	0.34 ± 0.02 (a)	0.35 ± 0.05 (a)	0.25 ± 0.10 (a)	0.39 ± 0.03 (a)
Apr	−0.03 ± 0.04 (a)	0.07 ± 0.04 (a)	0.20 ± 0.11 (ab)	0.16 ± 0.08 (ab)	0.24 ± 0.08 (ab)	0.26 ± 0.12 (ab)	0.16 ± 0.17 (ab)	0.30 ± 0.07 (b)
May	0.30 ± 0.06 (a)	0.34 ± 0.07 (a)	0.26 ± 0.01 (a)	0.35 ± 0.08 (a)	0.26 ± 0.15 (a)	0.19 ± 0.06 (a)	0.07 ± 0.04 (a)	0.12 ± 0.09 (a)
Jun	0.14 ± 0.15 (a)	0.24 ± 0.24 (a)	0.06 ± 0.13 (a)	0.07 ± 0.33 (a)	0.37 ± 0.14 (a)	0.31 ± 0.08 (a)	−0.09 ± 0.12 (a)	0.22 ± 0.08 (a)
Jul	−0.10 ± 0.17 (ab)	0.07 ± 0.28 (ab)	0.05 ± 0.07 (ab)	−0.21 ± 0.10 (a)	0.32 ± 0.17 (b)	0.36 ± 0.17 (b)	0.18 ± 0.23 (ab)	−0.05 ± 0.13 (a)
Aug	0.33 ± 0.41 (ac)	−0.00 ± 0.28 (a)	0.50 ± 0.15 (ac)	0.71 ± 0.19 (c)	0.59 ± 0.08 (ac)	0.08 ± 0.09 (ab)	0.37 ± 0.11 (ab)	0.67 ± 0.13 (c)
Sep	0.59 ± 0.26 (c)	0.36 ± 0.16 (bc)	−0.53 ± 0.13 (a)	0.52 ± 0.14 (c)	0.48 ± 0.35 (c)	0.49 ± 0.11 (c)	−0.33 ± 0.19 (ab)	0.17 ± 0.22 (bc)
Oct	0.46 ± 0.11 (c)	0.49 ± 0.12 (c)	0.06 ± 0.19 (ac)	0.41 ± 0.29 (bc)	0.10 ± 0.18 (bc)	0.02 ± 0.09 (ac)	−0.12 ± 0.36 (ab)	−0.47 ± 0.07 (a)

otic picophytoplankton in the control treatments decreased during incubation. In July, the addition of phosphate and nitrate and phosphate significantly stimulated the growth rate.

DISCUSSION

Effects of *Kyucho* on the abundance of picophytoplankton

In many coastal waters, *Prochlorococcus* is not generally detected (Partensky et al. 1999). In the present study, *Prochlorococcus*, which is considered oceanic (Partensky et al. 1999), was detected in the upper layer of Uchiumi Bay from June to October, when *Kyucho* occurred (Figs. 3a & 5a). Some researchers also detected *Prochlorococcus* in coastal seas other than the Uwa Sea, such as the plume of the Rhone River in the Mediterranean Sea (Veldhuis & Kraay 1990) and Suruga Bay, Japan (Shimada et al. 1995). It is controversial whether *Prochlorococcus* grows actively in coastal seas or is simply advected from the open ocean (Partensky et al. 1999). The present study provides an important insight into the controversy.

There are 2 possible reasons for our finding. One is the growth of *Prochlorococcus* in the bay, and the other, advection from the Pacific Ocean. In our incubation experiments, *Prochlorococcus* could not grow in control treatments (Table 1). Thus, the former possibility cannot fully explain the presence of *Prochlorococcus* in the bay. In contrast, the seawater of the Kuroshio, where *Kyucho* originates, contains an abundance of *Prochlorococcus* (Jiao et al. 2002). Moreover, the water temperature in the bay during winter usually decreases to 15°C (data not shown), which is the lower limit for the distribution of *Prochlorococcus* (Partensky et al. 1999). Moore et al. (1995) showed that a *Prochlorococcus* strain (SS120) could not grow at 12.5°C. Thus, it is possible that *Pro-*

chlorococcus in the bay cannot maintain its abundance in winter and serve as seed population for the next summer. Hence, the *Prochlorococcus* detected in the present study probably had been advected by *Kyucho* from the surface water of the Pacific Ocean.

The occurrence of *Kyucho* was also reported in Suruga Bay (Inaba et al. 2003), where *Prochlorococcus* was detected (Shimada et al. 1995). In addition to Uchiumi Bay and Suruga Bay, *Kyucho* also occurred in Sagami Bay (Matsuyama et al. 1999) and Sukumo Bay (Akiyama & Saitoh 1993). These bays face the Kuroshio in the Pacific Ocean. Thus, there is a possibility that the transportation of *Prochlorococcus* by *Kyucho* occurred in the bays. *Prochlorococcus* may be detected in the bays after the occurrence of *Kyucho*.

In contrast, changes in the cell densities of *Synechococcus* after the occurrence of *Kyucho* (Figs. 3a & 5b) were different from those of *Prochlorococcus* (Fig. 5a). A high abundance of *Synechococcus* was found in May, and the cell densities increased after the occurrence of *Kyucho*. Moreover, in June and September, a high abundance was found in the deeper layers, where the effects of *Kyucho* were negligible. In general, *Synechococcus* is ubiquitous from coastal waters to the open ocean (Olson et al. 1988, Campbell et al. 1998, Brown et al. 1999). In incubation experiments, high growth rates of *Synechococcus* in control treatments were observed during the investigation period. Thus, *Synechococcus* is transported by *Kyucho* and grows actively in Uchiumi Bay.

Eukaryotic picophytoplankton showed a different response to the occurrence of *Kyucho* (Figs. 3a & 5c). The largest abundance during the investigation period was found in May. From June to October, when *Kyucho* occurred frequently, the abundance decreased. It is possible that the cell density of eukaryotic picophytoplankton in the Kuroshio is lower than that in the bay. In August 2001, we determined that eukaryotic picophytoplankton was abundant along a transect from the bay to offshore and found that the abundance of eukaryotic picophytoplankton in offshore areas was low relative to those in the bay (T. Hirose et al. pers. comm.). This result supports the possibility. It is likely that the low cell densities of eukaryotic picophytoplankton in the bay during the *Kyucho* were due to the flushing out of the picophytoplankton by the *Kyucho* from the bay.

Effects of bottom intrusion on the abundance and growth of picophytoplankton

The cell densities of *Prochlorococcus* decreased during the occurrence of bottom intrusion (Figs. 3a & 5a), though bottom intrusion supplies nutrients to the phytoplankton in the Uwa Sea (Koizumi & Kohno 1994, Takeoka et al. 2000). In our incubation experiments, the growth of *Prochlorococcus* was not enhanced either with or without the addition of nitrogen and phosphorus nutrients (Table 1). Ammonium and urea, which are available forms of nitrogen for *Prochlorococcus* (Moore et al. 2002), also failed to stimulate *Prochlorococcus* growth (Table 1). Hence, it is likely that the growth of *Prochlorococcus* is limited by some environmental variables other than the supply of available nitrogen and phosphorus.

There are 2 possible reasons for the suppression of growth in *Prochlorococcus*, namely, the trace metal requirement and the metal toxicity. Saito et al. (2002) reported that *Prochlorococcus* has a cobalt requirement. In their experiments, zinc could not serve as a substitute for cobalt, though this is not the case for other phytoplankton species (Saito et al. 2002). With regard to the metal toxicity, Mann et al. (2002) demonstrated that the growth rate of *Prochlorococcus* was more susceptible than that of *Synechococcus* to a low level of the free cupric ion (free Cu^{2+}). Contamination by toxic metals may be due to toxic elements from seawater or to those from incubation bottles that may have been inappropriately washed before experiments. However, the positive growth of *Prochlorococcus* in the control treatment was detected in October (Table 1), suggesting that our estimation seemed to be equivalent to the gross growth rate. Since there are many pearl oyster culture farms (Tomaru et al. 2002, Hashimoto & Nakano 2003), Uchiumi Bay is heavily impacted by human activities. Thus, it is likely that the presence of toxic metal in seawater suppresses *Prochlorococcus* growth; however, we do not have any information on the metal concentrations in the bay. For more accurate estimation by bottle incubation, very stringent trace metal techniques are needed.

The positive growth found in October means that *Prochlorococcus* can grow in certain cases. *Prochlorococcus* in Uchiumi Bay may proliferate in cases where oceanic water entering the bay retains its qualities as a result of less mixing with the coastal water. Further studies will be needed to clarify the relationship between trace metal concentrations and the growth of *Prochlorococcus*.

Synechococcus decreased in abundance after bottom intrusion (Figs. 2a & 5b). In the incubation experiments, the growth of *Synechococcus* was relatively high compared to that of other picophytoplankton groups and was not limited by the nutrient concentrations in most cases (Table 1). Nitrate and phosphate, both of which are major nutrients supplied by bottom intrusion (Koizumi & Kohno 1994), did not stimulate the growth of *Synechococcus* (Table 1). It is well known that picophytoplankton has a high affinity for

low levels of dissolved nutrients because of its high surface to volume ratio (Raven 1998). However, the growth rate of *Synechococcus* did not seem high enough to compensate for the loss of cells flushed from the bay.

In the present study, we could not find any difference in the growth rates of *Synechococcus* among various nitrogen forms (Table 1). It is known that different strains of *Synechococcus* respond differently to various nitrogen forms (Waterbury et al. 1986, Collier et al. 1999) and that *Synechococcus* consists of a diverse group of strains (Ferris & Palenik 1998, Urbach et al. 1998, Honda et al. 1999). The pigmentation of *Synechococcus* in coastal waters differs from that of oceanic waters (Olson et al. 1990, Lantoine & Neveux 1997, Wood et al. 1998), suggesting oceanic and coastal *Synechococcus* are genetically different. Thus, oceanic *Synechococcus*, which uses different nitrogen form(s), may be advected from Kuroshio to Uchiumi Bay by *Kyucho*. Further studies are needed to generalize the nitrogen utilization of *Synechococcus* in Uchiumi Bay.

Among the 3 groups of picophytoplankton, the growth response of eukaryotic picophytoplankton to bottom intrusion differed from those of *Synechococcus* and *Prochlorococcus*. The cell densities of eukaryotic picophytoplankton increased after bottom intrusion at depths of 0 to 5 m (Fig. 5b). In July, the growth rate of eukaryotic picophytoplankton was significantly stimulated in the phosphate and nitrate and phosphate treatments (Table 1). Nutrients supplied by bottom intrusion may stimulate the growth rate of eukaryotic picophytoplankton. A similar result was obtained in the Arabian Sea (Campbell et al. 1998). The abundance of eukaryotic picophytoplankton increased during the southwest monsoon, and thereafter, the contribution of eukaryotic picophytoplankton in depth-integrated carbon biomass reached 56.9% of total microbial biomass (Campbell et al. 1998). Thus, the response of eukaryotic picophytoplankton to the nutrient supply was similar to that of larger phytoplankton, such as diatoms. However, the growth of eukaryotic picophytoplankton was not significantly enhanced by nutrient additions in July (Table 1), though their cell densities increased after the occurrence of bottom intrusion. Our incubation was conducted at a 10 m depth, where the light intensity would be significantly reduced relative to that at the surface. Indeed, we did not detect high cell densities of eukaryotic picophytoplankton below a depth of 10 m in July (Fig. 5c), though the densities became high above 10 m. Hence, it is likely that the light environment was not favorable to the growth of eukaryotic picophytoplankton.

In April, when the phosphate concentration was relatively high (0.28 µmol P l^{-1}, Fig. 4e), positive effects of phosphate addition on the growth rates of *Synechococcus* and eukaryotic picophytoplankton were detected (Table 1). By contrast, the growth rates of the 2 picoplankton were not stimulated by the addition of phosphate in June and August, when the phosphate concentration was low (0.03 µmol P l^{-1}). One possible reason for this was the difference in species, though no information on the species was available in the present study. Further analyses of the species composition of these picophytoplankton are needed.

In conclusion, the present study clearly demonstrates that the responses of the growth rate and abundance of picophytoplankton to the occurrence of *Kyucho* and bottom intrusion are different among the 3 groups. Drastic changes in the abundance of picophytoplankton and its composition due to these events may strongly affect the food linkage and flow of matter in the bay.

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