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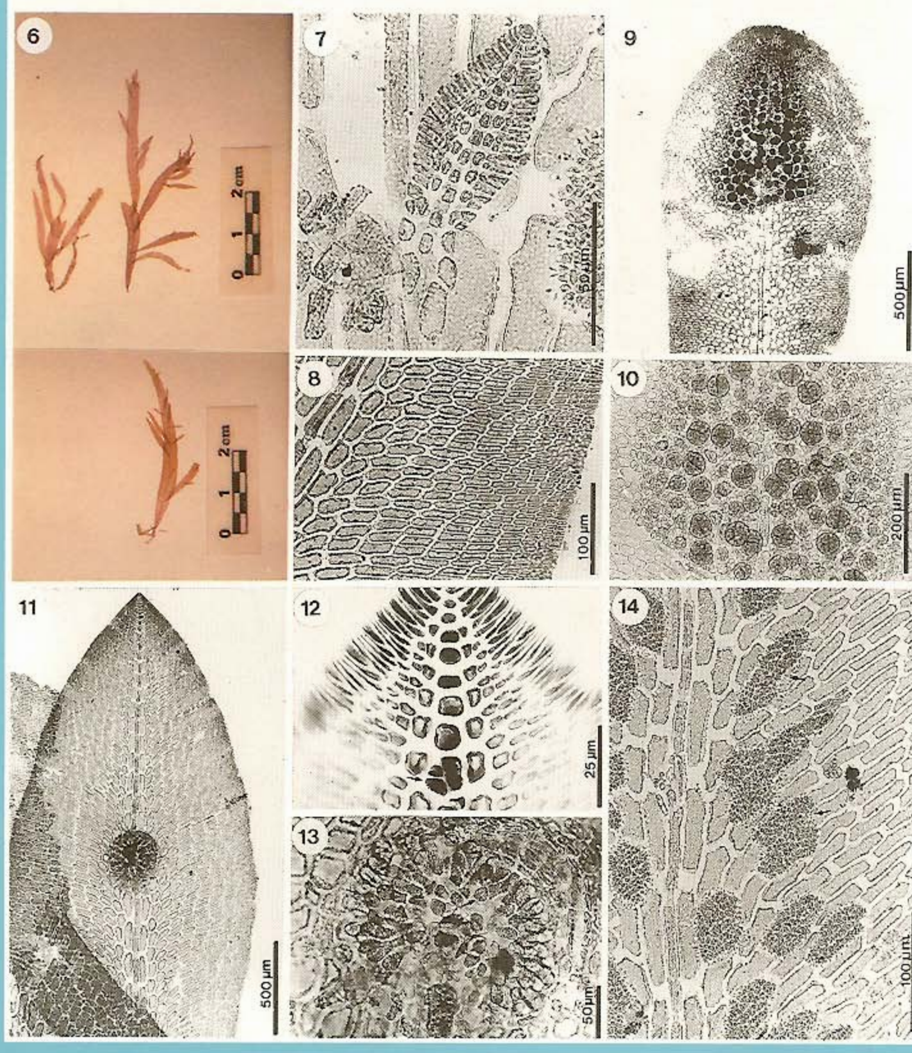

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Diel Vertical Distribution of Phytoflagellates in a Small Artificial Pond

Han Soon Kim* and Noriko Takamura[1]

Deptartment of Biology, Kyungpook National University, Daegu 702-701, Korea and
[1]National Institute for Environmental Studies, Tsukuba 305, Japan

Diel vertical distribution of phytoflagellates and interactions between the phytoplankton components and environmental and biological factors were studied in a small artificial pond for three days on the December 18, 1998 and April 9 to 10, 1999. The phytoplankton population was dominated by *Mallomonas akrokomos* of chrysophytes and *Cryptomonas marssonii* and *Chroomonas* sp. of cryptophytes. The vertical distribution of these phytoflagellates taxa exhibited clear diel migration pattern. Moreover their migration patterns are showed differential fluctuation between *M. akrokomos*, *C. marssonii* and *Chroomonas* sp. The later two species upward migrated in the evening as well as night, whereas the former species migrated downward. Their distinctive migration pattern was found during the night but was not observed in the morning. During daytime *C. marssonii* and *Chroomonas* sp. showed maximum density above 2 m depth but *M. akrokomos* below 2 m depth. The diel vertical distribution of the dominant phytoflagellates did not show significant correlation between physical, chemical and biotic factors.

Key Words: *Chroomonas*, *Cryptomonas marssonii*, diel migration, *Mallomonas akrokomos*, phytoflagellates, vertical distribution.

INTRODUCTION

The members of the phytoplankton community have a specific ranges of their preference environmental factors such as light, nutrients and temperature. Motile phytoflagellate is capable of vertical migration to the most favorable depth of water column. A number of studies of the vertical distribution and diel migration pattern of phytoplankton have been done in the last several decades (Wetzel 1983; Reynolds 1984; Sandgren 1988). Specific vertical distribution patterns of phytoflagellates generally are well known as a result from their vertical migration in correlation with the environmental gradient formed with depth in strongly stratified lakes. However, the mechanism of the phytoflagellate migration was not completely understood. Recently, many studies have been carried out to elucidate the effects of variable environmental factors in controlling the vertical distribution and migration of phytoflagellates. There was increasing evidence that the vertical distribution and diel migration of phytoflagellates was caused by such as light intensity (Tilzer 1973; Georgeand Heaney 1978) and nutrients (Cloern 1977; Sommer 1982; Salonen *et al.* 1984) or both (Heaney and Eppley 1981; Arvola *et al.* 1987). Additionally, it may be caused by zooplankton grazing (Arvola 1984; Arvola *et al.* 1987; Jones 1988) and endogenous rhythms (circadian) (Sournia 1974; Weiler and Karl 1979; Heaney and Furnass 1980; Frempong 1984). However, a generalization of the vertical migration patterns of phytoflagellates has not been available for yet because the vertical distribution patterns appears to be involved complicated interactions between variable external factors with species. Therefore, individual speces within the phytoplankton community does not uniformly respond to external environmental factors, and might be not shown to synchronized distribution patterns due to the difference of sampling time and regions.

The present study describes the diel vertical distribution of phytoflagellates and the relative importance of physical, chemical and biological factors controlling the vertical distribution of the phytoplankton in a small artificial pond.

*Corresponding author (kimhsu@knu.ac.kr)

Table 1. Data for the water temperature (W.T., °C), pH and dissolved oxygen (DO, mg · l^{-1}) concentration during the study

Date	Time	W.T.					pH					DO				
		0m	1m	2m	3m	4m	0m	1m	2m	3m	4m	0m	1m	2m	3m	4m
18. Dec. 98	5:00	6.5	6.5	6.5	6.5	6.5	6.4	6.6	6.7	6.8	6.8	9.5	6.9	6.6	7.7	5.5
	8:00	6.4	6.4	6.4	6.4	6.4	6.6	6.6	6.8	6.8	6.8	9.5	6.9	6.6	7.7	5.5
	11:00	8.5	6.7	6.5	6.4	6.4	6.2	6.4	6.5	6.7	6.8	7.7	7.2	6.4	6.1	6.4
	14:00	7.3	6.8	6.6	6.4	6.4	6.9	6.7	6.8	6.8	6.7	6.9	6.1	6.4	6.4	6.1
	17:00	6.7	6.7	6.5	6.5	6.6	6.9	6.7	6.8	6.8	6.7	6.9	6.7	6.6	5.3	5.3
	23:00	6.3	6.7	6.6	6.5	6.5	7.2	6.9	6.9	7.0	6.9	10.9	7.1	6.3	6.2	6.1
09. Apr. 99	5:00	11.0	11.1	11.0	10.9	10.8	7.1	7.2	7.3	7.4	7.7	6.7	6.8	6.6	6.8	6.1
	8:00	11.0	10.9	10.9	10.7	10.7	8.1	8.0	8.0	7.9	7.9	6.8	7.0	7.2	6.7	5.7
	11:00	11.9	11.3	11.0	10.8	10.7	7.9	7.7	7.8	7.7	7.7	6.7	6.5	6.5	7.1	6.4
	14:00	14.1	11.7	11.2	11.0	10.9	7.7	7.6	7.6	7.6	7.6	7.3	6.7	6.9	6.9	6.3
	17:00	15.0	12.3	11.4	11.0	10.9	7.8	7.7	7.6	7.7	7.6	7.0	7.2	6.9	6.9	7.2
	23:00	12.5	12.3	11.4	11.1	10.9	7.8	7.3	7.4	7.5	7.5	7.2	7.0	7.3	7.5	7.2
10. Apr. 99	5:00	11.6	11.6	11.4	11.0	10.9	7.3	7.3	7.4	7.4	7.5	7.0	6.5	6.2	6.4	5.6
	8:00	11.7	11.7	11.5	11.1	11.0	7.8	7.7	7.7	7.7	7.6	6.2	6.0	5.3	5.2	5.5
	11:00	12.4	11.9	11.6	11.1	10.9	7.8	7.7	7.6	7.6	7.6	6.0	5.8	5.6	5.7	5.4
	14:00	12.8	12.0	11.3	11.1	11.0	7.7	7.4	7.5	7.4	7.5	9.2	9.3	9.9	9.9	9.1
	17:00	12.4	12.2	11.6	11.1	11.0	7.3	7.2	7.1	7.2	7.3	8.8	9.0	7.5	7.7	7.3
	23:00	12.5	12.0	11.8	11.2	11.1	7.3	7.2	7.1	7.1	7.1	9.1	7.3	7.3	7.8	8.0

MATERIAL AND METHODS

This study was carried out from October, 1998 to July, 1999. The diel observations were undertaken during three days on December 18, 1998 and April 9 to 10, 1999. Water samples were collected with a polyvinyl chloride tube (3 cm in inner diameter) using vacuum pump from five depths (surface, 1, 2, 3 and 4 m). Phytoplankton samples were preserved with Lugol's iodine solution and counted at magnification of 400x using an inverted microscope after sedimentation (Uterm hl 1958). Ciliates in all the field of chamber was counted at magnifications of 100x and 200x using the same phytoplankton sample. Two zooplankton samples (each 8 *l*) were collected with the same method as that used for phytoplankton sampling and were filtered through a plankton net (mesh size 40 μm). Collected zooplankton was fixed with sugar-formalin (Heaney and Hall 1973) and counted at magnifications of 100x and 200x using with a binocular microscope. Water temperature, dissolved oxygen, pH and irradiance were measured with oxygen electrode (YSI Model 57), a pH meter (MP120) and a quantum sensor (LI-COR, UWQ-190), respectively. Total nitrogen, total phosphorus, nitrite and nitrate nitrogen, ammonium nitrogen, phosphate-phosphorus and silica were measured according to the standard methods (APHA 1992). Correlation coefficents were calculated using the SPSS ver.7.0 program.

Study area

The present investigation was performed at a small artificial pond in the National Institute for Environmental Studies (NIES), Tsukuba, Japan (140°07'E, 36°02'N). The pond was constructed in a wetland and filled with ground water in 1980. The maximum volume of this pond is 5,500 m^3, maximum depth 4.2 m, mean depth 1.4 m and surface area of 3894 m^2. The edge of the pond is primarily covered with emergent aquatic macrophytes. Fish has not been found and introduced since the pond construct in 1980. Larvae of *Chaoborus flavicans*, have been maintained a high density every year since its first appearance in July, 1981. The zooplankton community is dominated by two cladoceras, *Daphnia rosea* and *Ceriodaphnia reticulata*, a cyclopoid copepod, *Tropocyclos prasinus* and a calanoid copepod, *Acanthodiaptomus pacificus* (Iwakuma *et al.* 1989; Xie *et al.* 1998).

RESULTS

Environmental conditions

The weather was sunny both on December 18, 1998 and April 9, 1999. However, it was cloudy and some-

Table 2. Nitrite + nitrate nitrogen (NO_2-N + NO_3-N), ammonium nitrogen (NH_4-N), total nitrogen (TN), phosphate phosphorus (PO_4-P), total phosphorus (TP) and silicate (Si) concentration ($\mu g \cdot l^{-1}$) during the study.

Date	Time	NO_2 NO_3 0m	1m	2m	3m	4m	NH_4 0m	1m	2m	3m	4m	TN 0m	1m	2m	3m	4m
18. Dec. 98	5:00	159.0	142.3	142.1	143.2	140.3	98.4	92.6	90.2	91.8	89.7	546.0	537.6	537.6	601.5	506.2
	8:00	143.0	141.4	140.9	142.0	140.3	98.1	74.2	79.0	81.6	88.3	527.7	514.1	501.3	505.4	491.7
	11:00	140.0	139.0	139.3	139.4	139.5	94.6	91.9	93.5	92.7	93.5	505.2	515.1	517.1	545.9	516.9
	14:00	139.0	139.6	138.7	139.4	139.5	109.1	109.4	109.2	109.4	86.9	569.0	642.6	499.2	509.5	498.8
	17:00	140.6	139.4	139.8	140.1	139.9	93.6	88.8	88.5	90.2	91.0	664.8	493.6	494.5	510.5	623.4
	23:00	141.3	140.5	139.7	139.4	139.8	88.3	63.5	90.2	90.5	91.9	505.8	532.7	518.7	503.8	508.1
09. Apr. 99	5:00	56.7	45.3	42.8	43.3	39.4	34.5	33.0	32.4	37.4	27.9	363.3	464.5	391.2	412.1	395.8
	8:00	41.3	38.5	38.7	37.6	34.8	34.0	25.9	26.5	25.4	25.9	458.5	317.1	296.0	300.0	317.7
	11:00	41.2	38.2	38.3	37.3	35.2	42.6	26.3	25.8	24.5	24.6	583.1	307.3	319.3	287.6	302.6
	14:00	44.7	40.1	40.1	38.9	35.8	46.0	29.1	30.4	33.2	26.0	595.2	355.0	361.2	411.3	301.3
	17:00	40.7	39.5	39.7	38.5	37.6	28.7	26.1	25.8	24.7	25.4	292.5	300.0	290.8	287.5	309.1
	23:00	40.2	41.2	40.0	37.3	36.5	27.4	34.0	30.1	27.0	27.5	288.8	329.5	286.5	275.5	282.0

Date	Time	PO4 0m	1m	2m	3m	4m	TP 0m	1m	2m	3m	4m	Si 0m	1m	2m	3m	4m
18. Dec. 98	5:00	1.1	1.5	1.5	1.9	1.4	12.8	13.6	9.9	9.3	11.8	4.1	4.1	4.1	4.1	4.1
	8:00	1.1	0.8	1.1	0.9	1.0	12.5	10.4	10.2	12.6	9.4	4.1	4.1	4.1	4.1	4.1
	11:00	2.9	1.3	1.1	1.3	1.2	8.1	10.3	11.3	12.5	10.2	4.1	4.1	4.1	4.1	4.1
	14:00	1.2	1.1	1.4	1.1	1.7	9.5	10.8	10.3	12.5	17.6	4.1	4.1	4.2	4.1	4.1
	17:00	1.8	0.7	1.7	1.3	1.8	12.3	9.7	10.9	12.4	10.2	4.1	4.1	4.1	4.1	4.1
	23:00	1.7	1.9	1.5	1.8	1.2	10.7	10.9	9.6	10.7	11.6	4.1	4.2	4.2	4.1	4.1
09. Apr. 99	5:00	1.7	1.7	1.5	1.7	1.2	8.4	6.1	7.8	6.9	7.4	2.4	2.4	2.4	2.4	2.4
	8:00	1.8	0.9	1.1	0.9	1.8	8.5	4.7	5.2	4.4	8.4	2.4	2.4	2.4	2.4	2.4
	11:00	2.0	1.3	1.2	1.1	1.1	6.2	4.2	4.6	4.3	4.7	2.4	2.4	2.6	2.4	2.4
	14:00	2.0	1.6	2.1	1.4	1.4	6.3	5.4	6.2	4.0	4.2	2.4	2.4	2.4	2.4	2.4
	17:00	1.9	1.0	1.0	2.3	1.2	10.5	4.9	4.9	4.9	8.7	2.4	2.4	2.4	2.4	2.4
	23:00	1.7	1.6	1.7	1.6	1.5	11.2	10.1	10.2	9.3	14.6	2.4	2.4	2.4	2.4	2.4

what rained in the afternoon April 10, 1999.

Generally the vertical profile of the water temperature, pH, dissolved oxygen and nutrients did not show stratification on sampling period (Tables 1 and 2). pH generally decreased toward the bottom, but increased toward the bottom early in the morning. Dissolved oxygen gradually decreased toward the bottom, and was maximum at the surface early in the morning and night.

Nitrite and nitrate nitrogen and ammonium nitrogen concentration was maximum at the surface, and decreased from the surface to the bottom; their concentrations on the April 9 were much lower than that of the December 18. However, total nitrogen did not exhibit this pattern. Diel and vertical variation of phosphate and total phosphorus concentrations did not show a clear exhibit pattern and total phosphorus concentration on December 18 appeared to be higher than April 9. Silica concentration did not show variable depends upon observation time and depth, and the concentration on December 18 appeared higher than that of April 9. Light conditions were stable on December 18 and April 9, but were not stable with impact of clouds and rain on the April 10. The light intensity on April 9 was about two-fold higher than that of the other days (Figs. 1 and 2).

Phytoplankton composition

In this study, the phytoplankton community was characterized by *Cryptomonas marssonii* and *Chroomonas* sp. of cryptophytes and *Mallomonas akrokomos* of chrysophytes (Fig. 3). Seasonal variations of the phytoplankton community were dominated by *C. marssonii*, *Chroomonas* sp. and *M. akrokomos* throughout the present study.

Diel vertical distribution of phytoplankton

Cryptophyceae and Chrysophyceae usually contributed to the majority part of the total phytoplankton

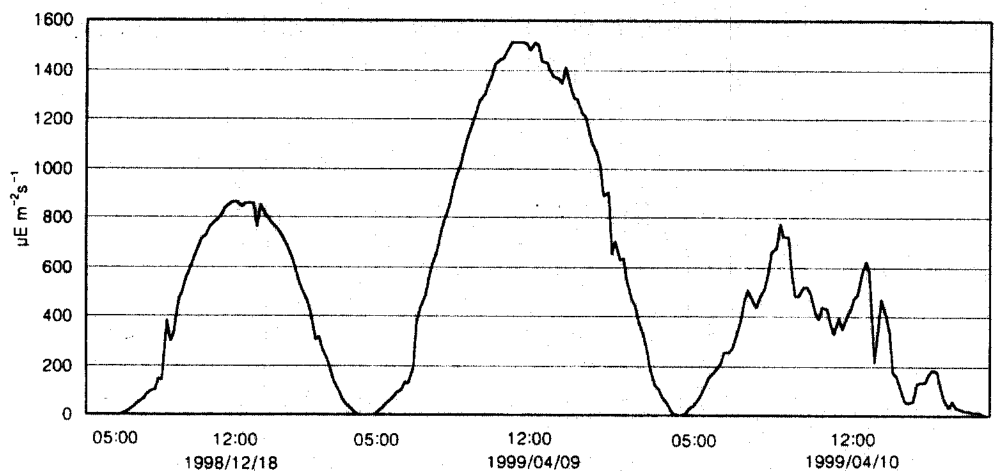

Fig. 1. Variation of the solar radiation during the study.

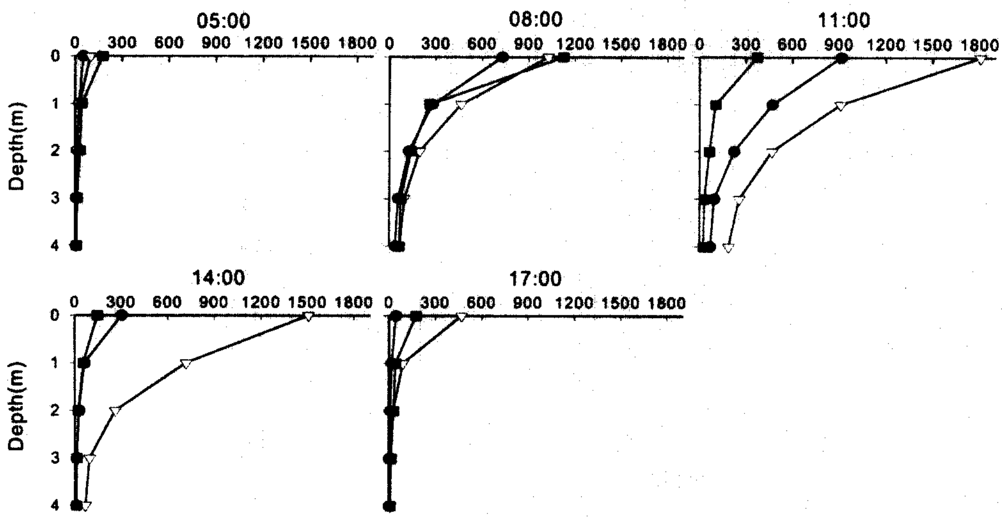

Fig. 2. Depth profiles of the light intensity ($\mu E \cdot m^{-2} \cdot s^{-1}$) during the study. ●: 18 Dec. '98, ▽: 9 Apr. '99, ■: 10 Apr. '99

community in this study. *Mallomonas akrokomos* (1-53%) of chrysophyte and *Cryptomonas marssonii* (5-66%) and *Chroomonas* sp. (8-66%) of cryptophytes were account for most of the total population during the study period between Oct., 1998 and Jun, 1999. During the vertical investigation period on December 18, 1998 and April 9 to 10, 1999 *M. akrokomos, C. marssonii* and *Chroomonas* sp. were accounted for 4-53%, 2-40% and 5-61% of the total phytoplankton density, respectively.

Diel vertical distribution patterns of these three phytoflagellates varied between species (Fig. 4). In general, constant vertical distribution patterns of these three species did not show during the observation period. *M. akrokomos* showed maximum density at different water depths during the day time; from the evening to sunrise it showed maximum density at the bottom except early in the morning on April 9 and was evenly distributed throughout the water column just after sunrise. In general, *M. akrokomos* showed avoidance tendency at the surface of strong irradiance during the day time and clearly showed downward migration pattern at night.

Chroomonas sp. exhibited a maximum density at the variable depths of the water column just before and after sunrise of the observation day; however, its cell density was nearly and evenly distributed throughout the water column. After 14:00, *Chroomonas* sp. gradually migrated to the surface; maximum density was generally occurred at the surface at 23:00 during the first sampling day on December 18 and was evenly distributed throughout the water column (Fig. 4). The diel vertical distribution of *C.*

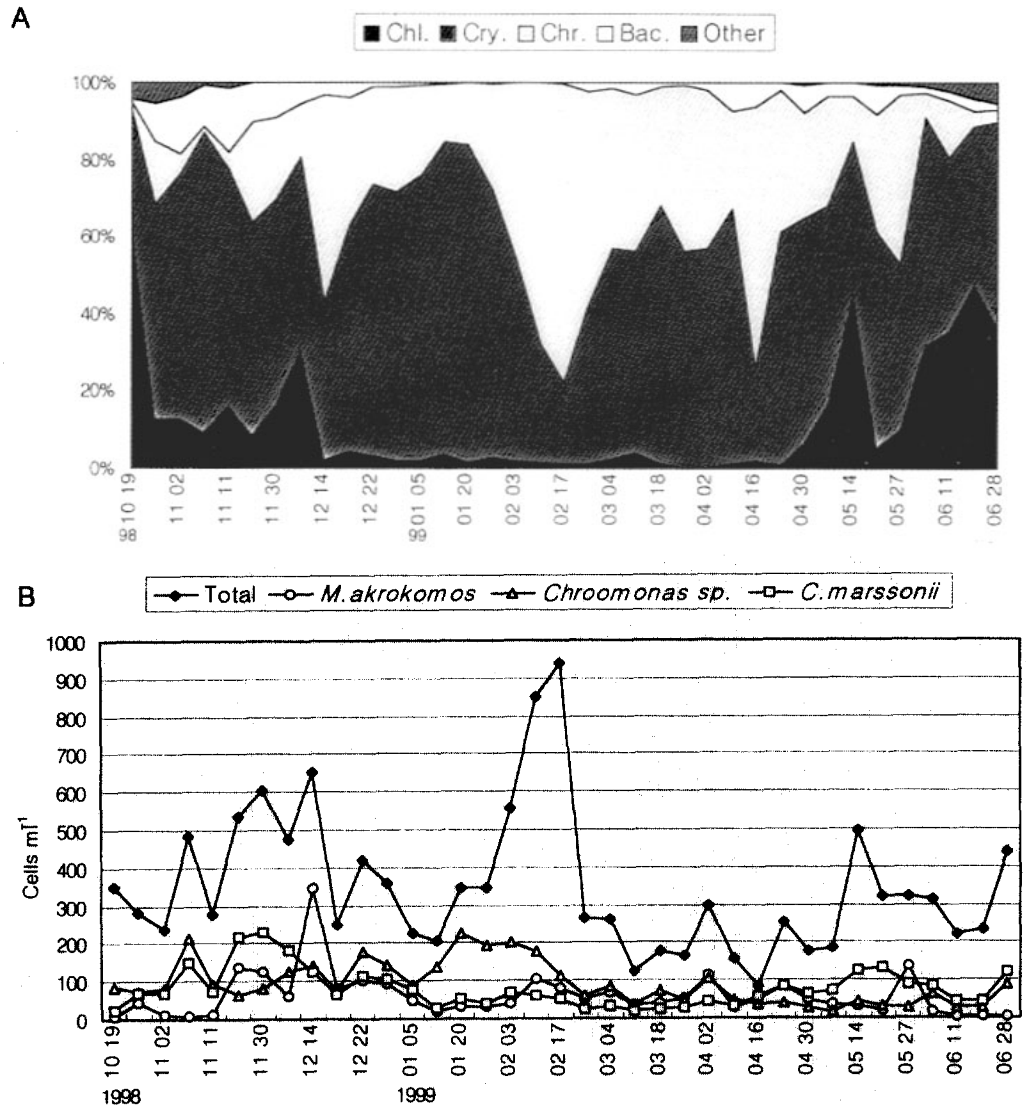

Fig. 3. Contribution of main algal groups (A) and seasonal variations of the dominant species and total phytoplankton density (B) in a artificial pond during the study. (Chl. = Chlorophyceae, Cry. = Cryptophyceae, Chr. = Chrysophyceae, Bac. = Bacillariophyceae)

marssonii generally showed some degree of similarity to that of *Chroomonas* sp. However, the maximum density was appeared at the 1 m or 2 m depth from 14:00 to night compared with *Chroomonas* sp., which showed the maximum density at the surface layer. Additionally, at 08:00 and 23:00 on April 10 the maximum density occurred at the surface and bottom respectively in contrast with that of *Chroomonas* sp. The vertical distribution of each species showed different patterns clearly at different sampling time.

Zooplankton

The vertical distribution pattern of zooplankton did not show constant according to the sampling time (Fig. 5). Ciliates during the morning and night on December 18, 1998 were evenly distributed throughout water column. Density of surface layer appeared to below in daytime. On April 9 to 10, maximum density occurred at the mid-depth of 2-3 m and a large population appeared to be above 2 m depth. The minimum density appeared at the bottom except at 23:30 on April 10 with maximum density. The most abundant members of zooplankton were rotifer *Polyarthra*, a cladocera *Daphnia*, and cyclopoid copepods. In general, the vertical distribution of *Daphnia* population showed their maximum density at the bottom in the morning and in the upper part in the afternoon, but in the afternoon of the second investigation on April 9 its density was very low and showed an

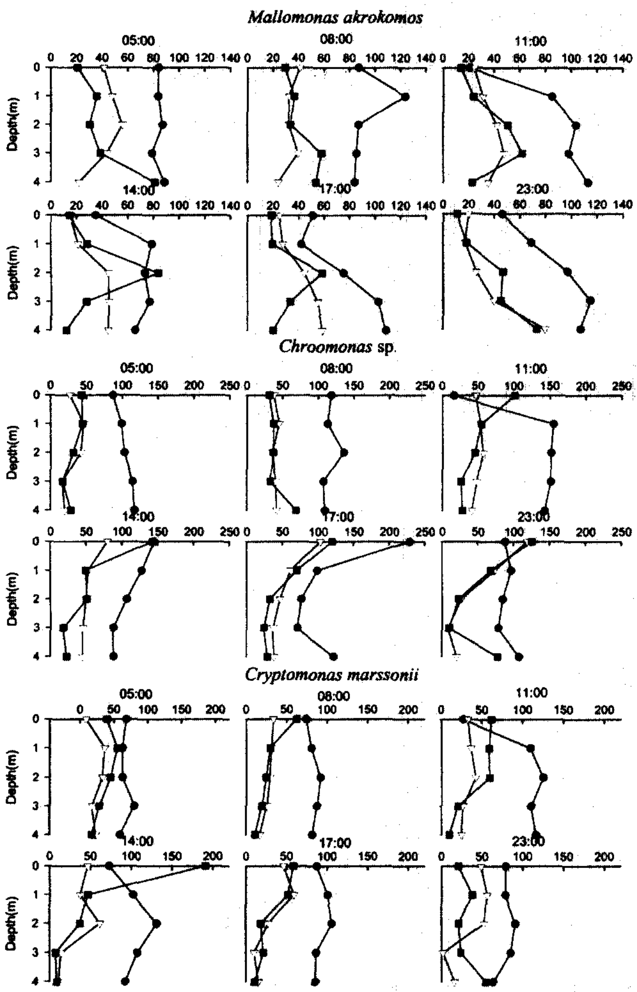

Fig. 4. Diel vertical distributions of the phytoflagellates (individuals l^{-1}) during the study. ●: 18 Dec. '98, ▽: 9 Apr. '99, ■: 10 Apr. '99

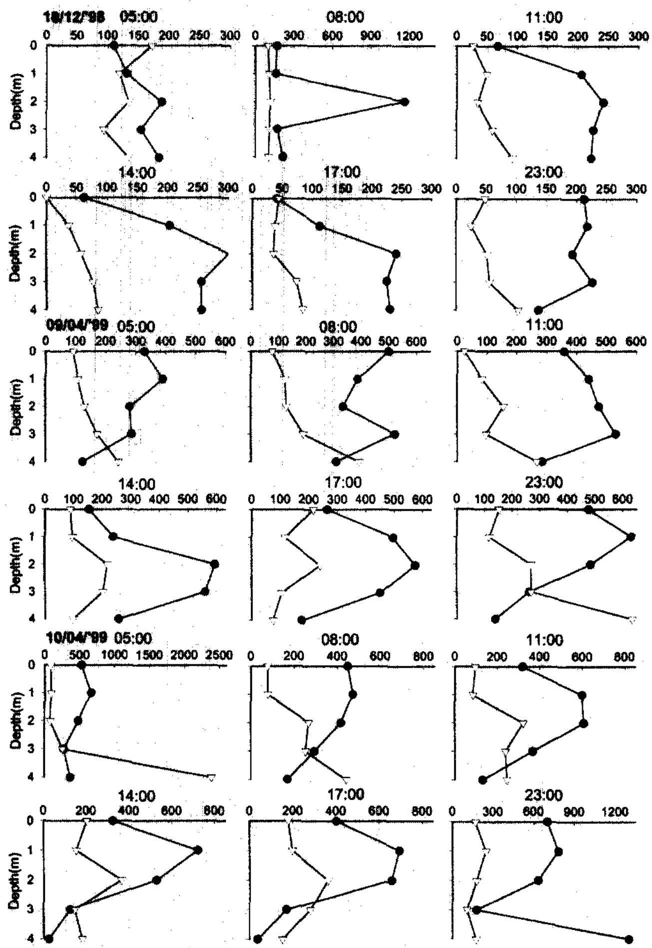

Fig. 5. Diel vertical distributions of the ciliates and total zooplankton density (individuals l^{-1}) during the study. ●: ciliates, ▽: total zooplankron

opposite distribution pattern that during the night on April 10 (Fig. 6). Copepods did not show definite diel vertical distribution. *Polyarthra* were similar to that of ciliates; however, during the morning and night of the second investigation on April 9 maximum density occurred at the bottom and appeared at the 2 m depth during the afternoon.

Correlations between the vertical distribution of phytoflagellates and physico-chemical and biological factors

There was negative correlation between the vertical distribution of the three dominant flagellates phytoplankton and water temperature ($r = -0.53$ - -0.79, $P < 0.05$) and pH ($r = -0.50$ - -0.64, $P < 0.05$), while positive correlation showed between nitrogen concentrations ($r = 0.58$-0.82, $P < 0.05$). The vertical distributions of the three dominant phytoplankton showed relatively high correlations with water temperature and nitrogen, but did not show significant correlations with other physico-chemical factors and zooplankton (Table 3).

DISCUSSION

In the present study, the phytoplankton community was dominated by a chrysophyte species, *Mallomonas akrokomos*, and two cryptophyte species, *Cryptomonas marssonii* and *Chroomonas* sp. The diel vertical distributions and migration patterns of these three phytoflagellates were varied between species. Additionally, even the same species did not showt synchronized distribution (Fig. 4). The diel vertical distribution pattern of *M. akrokomos* showed an opposite migration pattern to that of the other two species at night and was not synchronized on the three sampling days. In general *M. akrokomos* avoided the strongly illuminated surface layer, was

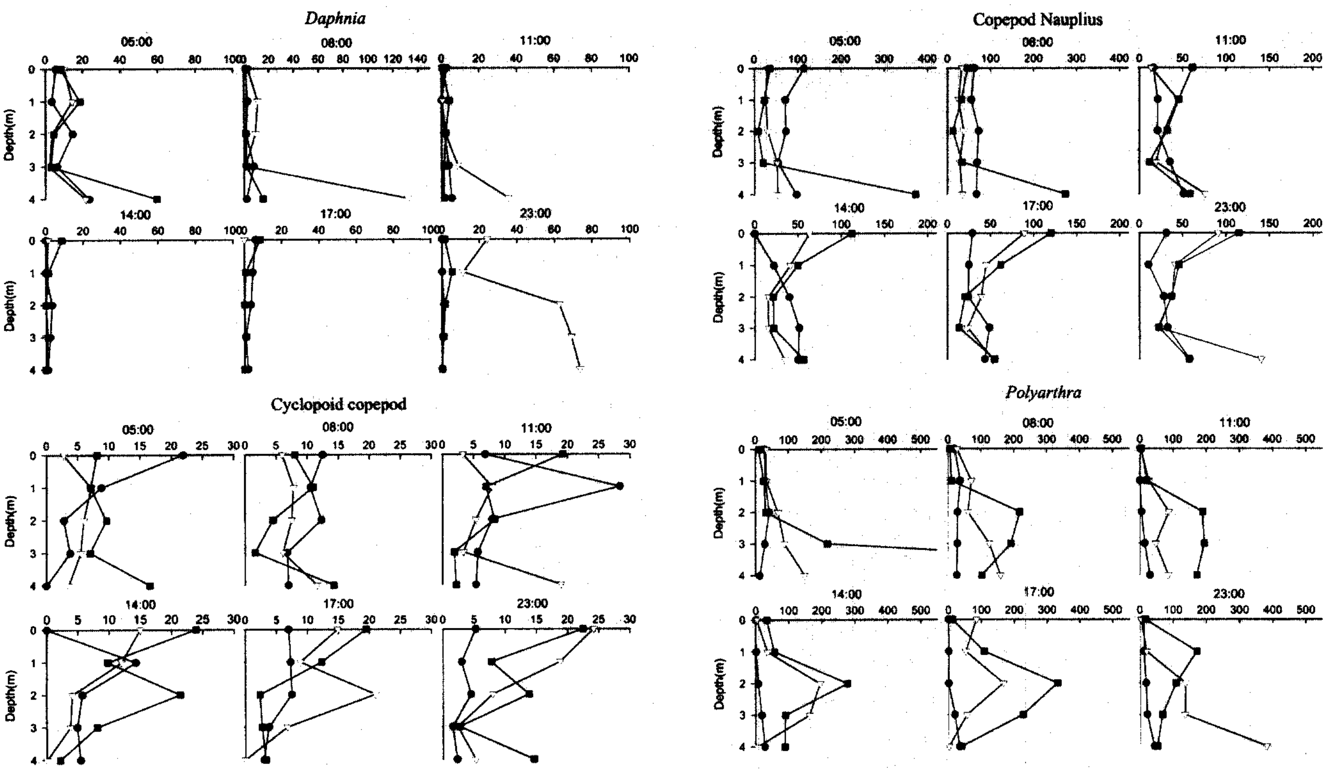

Fig. 6. Diel vertical distributions of the zooplankton biomass (ind. l^{-1}) during the study. ●: 18 Dec., ▽: 9 Apr., ■: 10 Apr.

Table 3. Correlation between the vertical distribution of dominant phytoplankton species and physico-chemical variables, nutrient concentrations and some zooplankton variables (Cil.; Ciliates, Nau.; Nauplius, Pol.; *Polyarthra*, Dap.; *Daphnia*, Cop.; Copepoda, W.T.; water temperature, n = 65-100).

	Cil.	Nau.	Pol.	Dap.	Cop.	W.T.	pH	Light	NO2	NO3	NH4	TN	PO4	TP	Si
M. akrokomos	-0.25	0.07	0.06	-0.04	0.02	-0.79	-0.64	-0.24	0.68	0.75	0.70	0.58	-0.17	0.13	0.76
Chroomonas sp.	-0.07	0.11	-0.30	-0.30	-0.18	-0.53	-0.50	0.08	0.70	0.71	0.71	0.66	-0.18	0.05	0.71
C.marssonii	-0.08	-0.04	-0.27	-0.31	-0.20	-0.61	-0.53	0.05	0.76	0.81	0.82	0.68	-0.13	0.12	0.83

evenly distributed throughout water column below 1 m during the daytime, and migrated to the bottom at night during examined. However, in the third investigation on April 10 surface density was low despite of the irradiance of the surface layer, which was much lower compared to earlier two days; maximum density clearly appeared at 2-3 m depth during the daytime. Several studies have been performed on the vertical distribution of the *Mallomonas* species (Nygaard 1977; Maulood *et al.* 1978; Smol *et al.* 1983; Arvola 1984; Eloranta 1987; Croome and Tyler 1988; Jones 1988; Hoffmann and Wille 1992). Most studies of these species may be shade plankters attaining their maximum densities in the metalimnion, hypolimnion or below the surface. However, Maulood *et al.* (1978) reported maximum density of *Mallomonas* sp. at the surface during the day. In the present study, diel vertical distribution of *M. akrokomos* supports most earlier reports that *Mallomonas* species may be shade plankter attaining their largest densities below subsurface depths of 2-3 m or in the metalimnion and hypolimnion. Additionally, the diel migration pattern was similar to the results of Jones (1988) and Croome and Tyler (1988) that *M. akrokomos* migrated downward in the evening in response to decreased irradiance and migrate upward in the morning response to increasing irradiance. However, in the present study, *M. akrokomos* did not show upward migration pattern in the morning.

In this study, the vertical distributions of *Chroomonas* sp. and *Cryptomonas marssonii* showed quite similar patterns, but the diel vertical distribution patterns of these two species were not synchronized during the study. These two species clearly showed upward migration pattern in the evening and night in contrast to *M. akrokomos*. However, *C. marssonii* showed slight migration pattern to the surface layer in the morning (08:00) during the second investigation. Arvola (1984), Arvola *et al.* (1987) and Salonen *et al.* (1984) reported that *Cryptomonas* (*C. marssonii* and *C. ovata*) showed regular vertical migrations in stratified humic lakes, moving below the thermocline at night and returning to surface waters in the early morning. They reported that complex interactions between light intensity and nutrient concentration seemed to be affect the vertical distribution of flagellates. In this study, diel vertical distribution of the *C. marssonii* and *Chroomonas* sp. during the evening and night was similar to Tilzer's report (1973) that phytoflagellates usually ascend in the evening and at night and migrate downward with increasing light intensity. However, it showed an opposite pattern compared with other previous reports that phytoflagellates including several species of *Cryptomonas* migrate downward in the evening and migrate upward in the morning (Happey-Wood 1976; Cloern 1977; Jones and Ilmavirta 1978; Heaney and Eppley 1981; Arvola 1984; Salonen *et al.* 1984; Arvola *et al.* 1987; Jones 1988). Generally, a distinct migration was not observed except in the second investigation on April 9, during which *C. marssonii* exhibited a slight upward migration pattern in the morning.

Most previous studies on the diel vertical distribution and migration of phytoflagellates have reported that it might be a direct response to changing light intensity, temperature, nutrient condition and a complex interaction of these factors (George and Heaney 1978; Heaney and Talling 1980; Heaney and Epply 1981; Salonen *et al.* 1984). Moreover, the vertical distribution of phytoplankton may be affected by their horizontal patchness or herbivore grazing (Heaney 1976; Lehman and Sandgren 1985; Arvola *et al.* 1987; Jones 1988), and may be ccontrolled by endogenous rhythms (circadian) (Sournia 1974; Weiler and Karl 1979; Heaney and Furnass 1980; Frempong 1984).

In the present study, the correlations between the vertical distributions of dominant phytoplankton and physico-chemical and biological factors were not significant. However, it showed relatively significant correlations between water temperature (r = -0.53 - -0.79) and nitrogen concentration (r = 0.58 - 0.82). The physicochemical factors and nutrient concentrations with water depth were quite similar during the study (Tables 1 and 2). Additionally, considerable variation of the population density of each species was appeared during different times of the days, and diel vertical distribution of the three phytoflagellates showed an opposite pattern at some sampling times on December 18, 1998 and April 9, 1999. These results could be due to the phytoflagellates's horizontal migration during sampling periods and endogenous rhythms. To understand the vertical migration patterns of the phytoflagellates, it is required to have more frequent sampling intervals as well as collect samples at the more narrow range of water depth.

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