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The Relationships of Green Euglenoids to Environmental Variables in Jeonjucheon, Korea

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전주천의 녹색 유글레나조류와 환경요인과의 상호관계. 김준태 · 부성민* (충남대학교 자연과학대학 생물학과)

녹색 유글레나조류와 수환경 요인과의 상호 관계를 규명하기 위하여, 전주시내 도심 하천에서 1996년 9월부터 1997년 8월까지 유글레나조류의 종조성과 밀도 및 환경요인을 매월 조사하였다. 질산염 농도는 6월에 3.22 mg/l로 높았고, 인산염은 겨울철에 0.71 mg/l 이상으로 높았다. 녹색 유글레나조류는 5속 71종이 출현하였으며, 봄에서 초여름 사이에 35～42종으로 높고, 겨울에는 20종 이하였다. 출현종 수는 수온과는 정의 상관관계를 보여주었다. 녹색 유글레나조류의 밀도는 겨울과 초여름에 각각 최대가 되는 전형적인 bimodal형을 보여주었다. 겨울의 밀도는 5,394 cells/ml로, *Euglena caudata*, *E. geniculata*와 *E. viridis*의 활발한 생장에 기인하였으며, 인산염에 정의 상관 관계를 보였다. 초여름의 밀도는 4,823 cells/ml로, *E. deses*, *Lepocinclis ovum*과 *Phacus trypanon* 등의 활발한 생장에 기인하며, 암모늄염과 질산염에 정의 상관을 보였다. 녹색 유글레나조류의 생태적 특징은 전주천과 같이 오염된 수계에서 환경요인의 변화를 예측하는 생물학적 자료로 활용할 수 있다.

Key words : Density, Correlation, Ecology, Environmental factors, Euglenophyceae, Eutrophic water, Species number

INTRODUCTION

Members of *Euglena*, *Lepocinclis*, *Phacus*, *Strombomonas*, and *Trachelomonas*, commonly occurring in freshwaters, are green euglenoids with chlorophyll a and b in chloroplasts and reserve the photosynthetic products as paramylons (Walne and Kivic, 1989). These green euglenoids are unicellular, free−living, and contribute to the phytoplankton community in various waters. Many of euglenoids are cosmopolitan and ubiquitous in swamps, pools, ponds, and lakes as well as flowing waters of rivers (Lackey, 1968; Zakrys, 1986; Tell and Conforti, 1986; Kim and Boo, 2000). In particular, the green euglenoids develop and bloom in eutrophic waters with a high organic and inorganic content (Hutchinson, 1975; Fjerdingatad, 1971; Munawar, 1972; Xavier, 1985; Conforti, 1998; Kim and Boo, 1996). Although green euglenoids are reported to be abundant in winter (Kim and Boo, 1996), early spring (Braarud *et al*., 1958), or summer (Munawar, 1972) in eutrophic waters and these growths are related to nitrogenous matter (Munawar, 1972; Xavier, 1985), there are scarce studies on such temporal changes of green euglenoids and their relationships to environmental variables in worldwide waters.

We found an urban drainage water in Korea, showing a diverse and abundant flora of green euglenoids, and studied monthly composition and density changes of the species depending on the habitat conditions. Our research also focused

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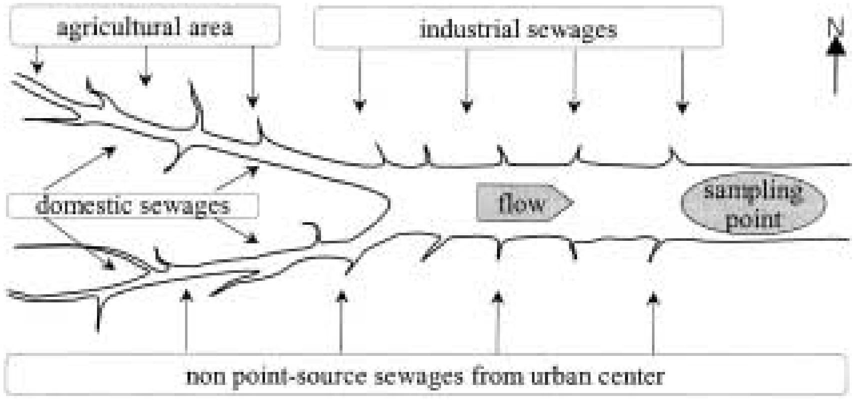

Fig. 1. Schematic drawing of the Jeonjucheon urban drainage and the surveying site, Jeonju, Korea.

on ecological relationships between green euglenoids and environmental variables.

MATERIALS AND METHODS

The drainage of Jeonjucheon (35° 81′N, 127° 15′E) traverses urban area of Jeonju city, Korea. After preliminary surveys, one sampling site with heavy inputs of industrial and domestic sewage was selected, as in Fig. 1. Water depth in the sampling site ranged between 60 and 100 cm during the study period and nearly stagnant except during the rainy spell in July 1997. The substrata of drainage were covered with a dark green film of organic sediments from which bubbles arouse. Surveys were conducted every month for measurements of phytoplankton density and environmental variables from August 1996 to September 1997.

Phytoplankton samples were collected using plankton net (20 μm in mesh size) over which water was poured 30 times with a 2,000 ml gourd dipper. To establish species composition, fresh samples were analyzed under light microscope (Olympus VANOX AHBT3). Because of limited observations of cells due to many chloroplasts, the samples were starved in a cooled dark refrigerator and observed every five days during one month. Identification of species was based on previous papers studied by the authors (Kim and Boo, 1998a; Kim *et al.*, 1998, 2000a, b). Taxonomic reports by Huber−Pestalozzi (1955), Zakrys (1986), and Tell and Conforti (1986) were also referenced.

An aliquot of algal suspension was used for counting green euglenoids (Kim and Boo, 1998b). For algal suspension preparation, 1,000 ml water sample was fixed with Lugol's iodine solution. The samples were precipitated in a 1,000 ml volumetric flask for a week under cooled dark conditions and concentrated to 100 ml. The number was determined using Sedgwick−Rafter (S−R) counting cell under inverted microscope (Olympus IX70) at 400× or 1,000× magnifications. All 50 strips (10 strips multiplied 5 times) in the S−R cell were counted to derive the cell number per milliliter. The mean cell measurement for each count was used to assign a density of each species.

Surface water temperature was measured in the field. Water samples were collected using a 100 ml bottle at a depth of 20 cm. Samples were filtrated within 48 hours using a precombusted Whatman GF/C filter and analyzed for pH and dissolved nutrients. Dissolved nitrogen and phosphate were analyzed by the persulfate oxidation and colorimetric method, respectively. Detailed information on the analytical procedures is available in U. S. Environmental Protection Agency (1987). Correlation or regression analyses (Sokal and Rohlf, 1995) were performed to determine relationships between environmental variables and green euglenoids.

RESULTS

Water temperature and nutrient contents

The environmental variables of the urban drainage Jeonjucheon were indicated in Table 1. Surface water temperature measured synchronously

Table 1. Seasonal changes of environmental factors in Jeonjucheon.

	1996				1997							
	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.
WT (°C)	15.0	12.0	8.0	7.5	4.0	9.0	10.0	20.0	20.5	27.0	30.0	31.0
pH	6.98	7.01	7.46	7.73	7.60	6.78	7.27	7.11	6.83	7.22	7.81	7.88
NH_4^+ (mg/ml)	0.56	0.43	0.57	0.39	0.15	0.23	0.08	0.23	0.91	1.03	0.74	0.43
NO_2^- (mg/ml)	0.30	0.28	0.14	0.44	0.15	0.14	0.33	0.30	0.01	0.34	0.45	0.28
NO_3^- (mg/ml)	1.21	1.05	1.38	2.25	1.82	1.21	1.61	1.59	2.29	3.22	2.51	1.23
PO_4^{3-} (mg/ml)	0.15	0.38	0.71	0.73	0.91	0.22	0.60	0.05	0.01	0.17	0.17	0.18

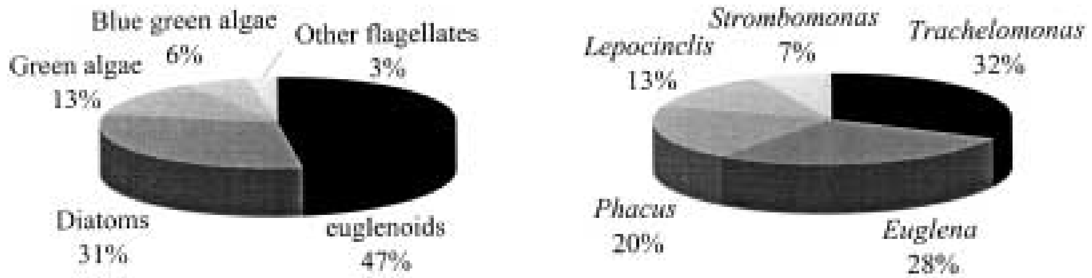

Fig. 2. Composition of planktonic algae occurred in Jeonjucheon. Left panel shows the percentages of the representative planktonic algal groups throughout the year. Right panel shows the percentages of the five representative genera of green euglenoids throughout the year.

with the sampling showed a seasonal trend with the temperate zone. It was over 30°C in July to August and below 4°C in January. Value of pH fluctuated within a neutral zone from 6.78 to 7.88 during the entire year.

Concentrations of nitrogenous nutrients were high during summer. Ammonium concentration was high at 1.03 mg/l in June and low at 0.08 mg/l in March. Nitrite concentration was below 0.45 mg/l during the year. Nitrate fluctuated within a wide range, varying from a high of 3.22 mg/l in June to a low of 1.05 mg/l in October. Phosphate concentration was over 0.71 mg/l from November 1996 to January 1997 corresponding to the winter season, and below 0.20 mg/l during September 1996 and April to August 1997.

Species composition of green euglenoids

During this study, 149 algal taxa were found in Jeonjucheon (Fig. 2). Of these, green euglenoids had the highest number of taxa (71), followed by diatoms (46) and green algae (19). Blue green algae (9) and other flagellates (4) was low in the number of taxa. Because of the high number of the green euglenoids and ecological importance in turbid waters, green euglenoids only were included for further analyses.

Green euglenoids belonging to the five genera were diverse in our study site (Table 2). Fig. 2 shows the relative proportions of the representative genera. Genus *Trachelomonas* was the most species−rich group with 23 taxa (32%), followed by *Euglena* with 20 (28%), *Phacus* with 14 (20%), *Lepocinclis* with 9 (13%), and *Strombomonas* with 5 (7%).

The species number of green euglenoids (Fig. 3) increased in March (35 taxa), April (42 taxa), and May to June (36 taxa). Compared to this, they decreased in October to November (20 taxa), December (19 taxa), and January (16 taxa), when *Euglena* mostly occurred. Although many euglenoids fluctuated temporally, *Euglena acus*, *E. caudata*, *E. deses*, *E. geniculata*, *E. viridis*, *Lepocinclis ovum*, *Trachelomonas hispida*, and *T. volvocina* were constantly found throughout the year.

Density of green euglenoids

Total cell density of the green euglenoids in Jeonjucheon peaked with 4,718 cells/ml in December and 5,394 cells/ml in January and 3,711 cells/ml in May and 4,823 cells/ml in June

Table 2. Green euglenoids found in Jeonjucheon during a year of study, with their mean density (cells/ml). +indicates cell number below 10 per milliliter.

Taxa	1996				1997							
	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
Euglena acus	+	35	+	+	+	+	+	54	49	30	+	+
E. agilis	+							+				
E. caudata	21	73	1028	1361	3006	51	59	45	+	96	30	25
E. deses var. *deses*	86	16	31	36	66	12	15	149	822	989	166	23
E. deses var. *intermedia*	+			+	+	+	+	38	452	99	+	+
E. ehrenbergii	+						+	29	333	+		
E. geniculata	+	53	520	1284	689	15	22	14	15	58	25	85
E. gracilis						+	+				+	
E. granulata							+	+				
E. haemichroata			+	+	+	+	+	+	79	+		
E. mesnilii				+	+	+	145	+				
E. obtusa							87	+	+	40		
E. oxyuris	+					+	+					
E. proxima			+	+								
E. spirogyra	+				+	+	+	+	38			+
E. texta					+	+	+					
E. tripteris			+			+						
E. truncata				+	+		+					
E. variabilis						+	+		+			
E. viridis	55	48	98	1720	1205	97	36	15	23	13	86	36
Lepocinclis cylindrica										+	+	
L. gracilicauda				+				+	+		+	
L. marsonii var. *inflata*							+	+				
L. ovum var. *angustata*						+		+				
L. ovum var. *dimidio-minor*			+				+	+	+	+	79	50
L. ovum var. *ovum*	258	19	31	25	15	69	50	56	631	862	87	60
L. salina		+									+	+
L. steinii	+							+		+	55	60
L. wangi							+		+			
Phacus acuminatus				+			+					
P. anomalus	+						+	176	+	+	+	
P. carinatus		+							+	+		
P. circumflexus			+						+	+	24	
P. horridus							+			+	+	
P. logicauda	+	20					+			+		+
P. mirabilis			+						+	+	+	255
P. obolus				+						+		
P. pleuronectes	+	+					+	+		+	55	+
P. suecicus	+	+										
P. torta	53		+					+	+			45
P. triqueter	+											
P. tryphanon						35	21	74	886	1536	70	66
P. undulatus	+	+									+	
Strombomonas maxima									+	+		
S. napiformis	874	46	18				25	489	56	27	28	37
S. rotunda									+		+	
S. subcurvata					+							
S. urceolata	63	+	+						+	+		
Trachelomonas abrupta			+			+	+		+			
T. acanthostoma						+	30	+	+			+
T. armata	+							+	+	26	30	+
T. australica							+	+				

Table 2. Continued.

Taxa	1996				1997							
	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
T. bacillifera	19	+	+	+					+	+	+	+
T. curta	+			+			45		+	+		
T. cylindrica var. *decollata*				21	+							
T. flava								+	+		+	+
T. hispida	186	645	61	42	18	27	20	75	105	759	+	16
T. intermedia						+		+			+	
T. kelloggi var. *nana*		+										+
T. lefevrei	+	+							+	+		
T. oblonga var. *australica*								+				
T. oblonga var. *oblonga*		+	+			+	+	+				
T. obovata var. *klebsiana*					+	21						
T. pavlovskoensis							+	20				
T. planktonica			+			+	+	+				
T. robusta	+											+
T. similis						+						
T. volvocina	19	+	16	120	305	386	948	521	21	+	26	13
T. volvocinopsis										+	+	
T. woycickii		+							+			
T. zorensis			+					+	+	61	67	218

(Fig. 3). *Euglena* species induced the winter maximum, whereas the mixed development of *Euglena*, *Lepocinclis*, *Phacus*, and *Trachelomonas* species constituted the early summer maximum. On the contrary, the total density was low with 862 cells/ml in February, 1,025 to 1,107 cells/ml in July to August, and 1,064 cells/ml in October.

With respect to the dominant species, green euglenoids in Jeonjucheon showed a series of seasonal patterns. *Euglena caudata*, *E. geniculata*, and *E. viridis* showed density peaks in the winter (Fig. 3). *Euglena caudata* was abundant with 1,028 cells/ml in November, 1361 cells/ml in December, and 3,006 cells/ml in January. *Euglena geniculata* was abundant with 520 cells/ml in November, 1,284 cells/ml in December, and 680 cells/ml in January. *Euglena viridis* was abundant with 1,720 cells/ml in December and 1,205 cells/ml in January.

Densities in *Euglena deses* var. *deses* and *E. deses* var. *intermedia*, *E. ehrenbergii*, *Lepocinclis ovum*, *Phacus trypanon*, and *Trachelomonas hispida* peaked in the early summer. *Euglena deses* var. *deses* was abundant with 822 cells/ml in May and 989 cells/ml in June. *Lepocinclis ovum* was abundant with 631 cells/ml in May and 862 cells/ml in June. *Phacus trypanon* was abundant with 886 cells/ml in May and 1,536 cells/ml in June (Fig. 3). *Euglena ehrenbergii* was abundant with 333 cells/ml in May, *Trachelomonas hispida* 759 cells/ml in June and 645 cells/ml in October (Table 2), and *T. volvocina* was abundant with 948 cells/ml in March and 521 cells/ml in April (Table 2). *Strombomonas napiformis* was 489 cells/ml in April and 874 cells/ml in September.

Correlation between parameters of green euglenoids and environmental variables

The species number of green euglenoids positively correlated ($r = 0.587$, $P < 0.05$, Fig. 4 left) with surface water temperature, but negatively correlated ($r = 0.683$, $P < 0.05$, figure not shown) with phosphate concentration. However, the relationship between species number and dissolved nitrogen components was low. The relationship between species number and total density of the green euglenoids was low at −0.081 (figure not shown).

The total density positively correlated ($r = 0.628$, $P < 0.05$, Fig. 4 right) with nitrate−nitrogen concentration, but did not show any significant correlation with other variables such as water temperature, ammonium, nitrite, and phosphate. However, cell densities of dominant euglenoid species correlated to environmental variables. Cell density of winter dominant species such as *Euglena caudata*, *E. geniculata*, and *E. viridis* positively correlated with phosphate concentra-

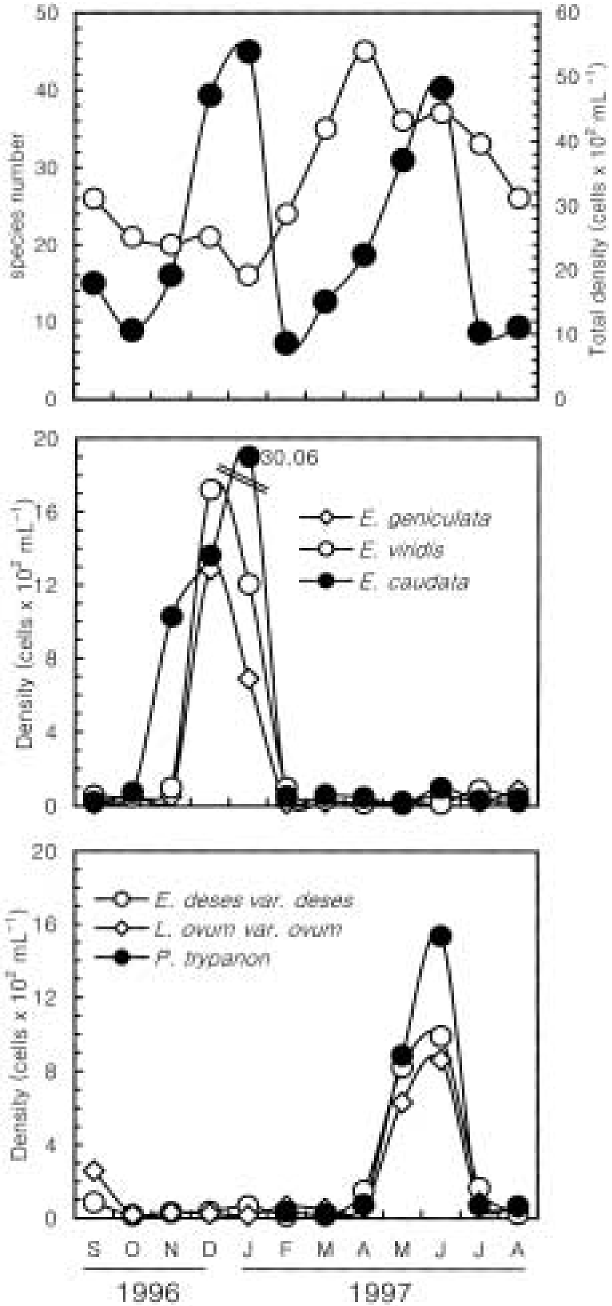

Fig. 3. Seasonal changes of green euglenoids in Jeonjucheon. Upper panel shows the change of species number (white−circle line) and total density (dark −circle line). Middle panel shows the change of density of representatives in winter. Lower panel shows the change of density of representa-tives in early summer.

tion (r = 0.821, P < 0.01, Fig. 5 upper right). The cell density negatively correlated with water temperature having r = −0.594 at the 95% confidence level (Fig. 5 upper left). Cell density of early summer dominant species such as *Euglena deses*, *Lepocinclis ovum*, and *Phacus trypanon* positively correlated with ammonium concentration (r = 0.794, P < 0.01, Fig. 5 lower left), and rather well correlated with nitrate concentration (r = 0.746, P < 0.05, Fig. 5 lower right). However, *Euglena deses* var. *intermedia*, *E. ehrenbergii*, and *Trachelomonas hispida* showed no significant relations to environmental variables.

Trachelomonas volvocina showed a significant negative relationship with ammonium concentration (r = −0.719, P < 0.01). *Strombomonas napiformis* and *Trachelomonas hispida* showed no statistical correlations with the environmental variables.

DISCUSSION

The high occurrence of the green euglenoids (48%) recorded in this study, compared to that of diatoms (31%), green (13%) and blue green algae (6%), indicates that green euglenoids are an important contributor for the phytoplankton community in the Jeonjucheon urban drainage. The green euglenoids in Jeonjucheon are also highly diverse with 71 taxa, of which most are known as the common eutrophic species (Sládecek and Perman, 1978; Sládecek, 1989). Compared to previous reports in other waters (e.g. Forberg and Ryding, 1980; Harper, 1992), the budgets of dissolved nutrients indicate that Jeonjucheon drainage is eutrophic. These results accord with previous reports (Munawar, 1972; Xavier, 1985; Kim and Boo, 1998b) that the green euglenoids were abundant in locations rich in organic and inorganic matter. The Jeonjucheon drainage is, to our knowledge, one of the eutrophic waters worldwide, housing highly diverse green euglenoids.

The second finding is that the green euglenoids in the Jeonjucheon drainage showed a bimodal pattern of density, being maximal in the winter and the early summer. In winter, *Euglena caudata, E. geniculata*, and *E. viridis* were abundant and the total density was the highest throughout the year. Our correlation analyses showed that the abundance of *Euglena caudata*, *E. geniculata*, and *E. viridis* significantly related to the low temperature and high concentration of

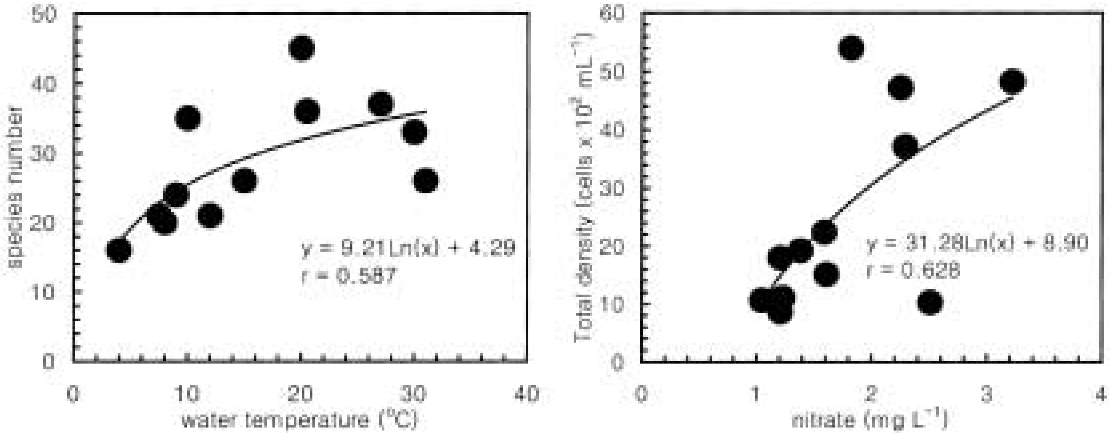

Fig. 4. Relationship of species number of green euglenoids to water temperature and their total density to nitrate in Jeonjucheon.

phosphate. The fact that these winter representatives exhibited their greatest diapauses during spring to autumn strongly supports their special preferences for high concentration of phosphorus nutrient in Jeonjucheon. We, therefore, hypothesize that the preferred responses of three species may be due to phosphate rather than water temperature. These results are consistent with previous reports (Saywer, 1947; Round, 1981; Kim and Boo, 1998) that the green euglenoids become abundant with the increase of phosphate concentration, although no statistical analyses were given.

The density peak in the early summer is attributed to the active growths of *Euglena deses* var. *deses* and *E. deses* var. *intermedia*, *E. ehrenbergii*, *Lepocinclis ovum*, *Phacus trypanon*, and *Trachelomonas hispida*. Ammonium and nitrate increased from April to June in Jeonjucheon. Our correlation analyses showed that the early summer abundances of those species positively related to high concentration of ammonium and nitrate nitrogen, as shown in Fig. 5. The results indicate that the early summer representatives are well adapted to a combination of high concentration of ammonium and nitrate, and high water temperature in Jeonjucheon. Our results are consistent with previous reports that euglenoids become diverse and abundant in summer (Zafar, 1959; Barone and Flores, 1994; Kim and Boo, 1996) and high concentration of nitrogenous nitrogen (Munawar, 1972; Xavier, 1985). No significant relation of *Euglena deses* var. *intermedia*, *E. ehrenbergii* and *Trachelomonas hispida* to environmental variables is difficult to explain here.

The negative relationship of *Trachelomonas volvocina* to ammonium nitrogen is not consistent with the positive relationship of other euglenoids, as discussed in the above. The peaks of *Strombomonas napiformis* in September and April and *Trachelomonas hispida* in October and June are noticeable, but the densities of both species were low and not related to any of environmental variables. It is probable that biological competition produces the small peaks in the density of *Trachelomonas hispida*, *T. volvocina*, and *Strombomonas napiformis* because interspecific relationships tend to be strong at low nutrient levels (Hutchinson, 1975; Levandowsky, 1972). The small-sized species like *Trachelomonas* and *Strombomonas*, having the high surface to volume ratio, are favored in oligotrophic waters compared to other large-sized planktonic algae (Sourina, 1981).

Water temperature and irradiance of sunlight, although the latter being not measured in this study, also exhibit marked effects on the euglenoids because of a diversity of species (36 to 42 species) in April to June together with the secondary peak of density, when such two climatic factors are optimal for growth of planktonic algae (see Round, 1981), while the low number of species (16) in January, the coldest month in Korea. Some biological interactions may also contribute to the small peaks of *Trachelomonas* and *Strombomonas* species in the absent period of blooming by other species. The low density of green eugle-

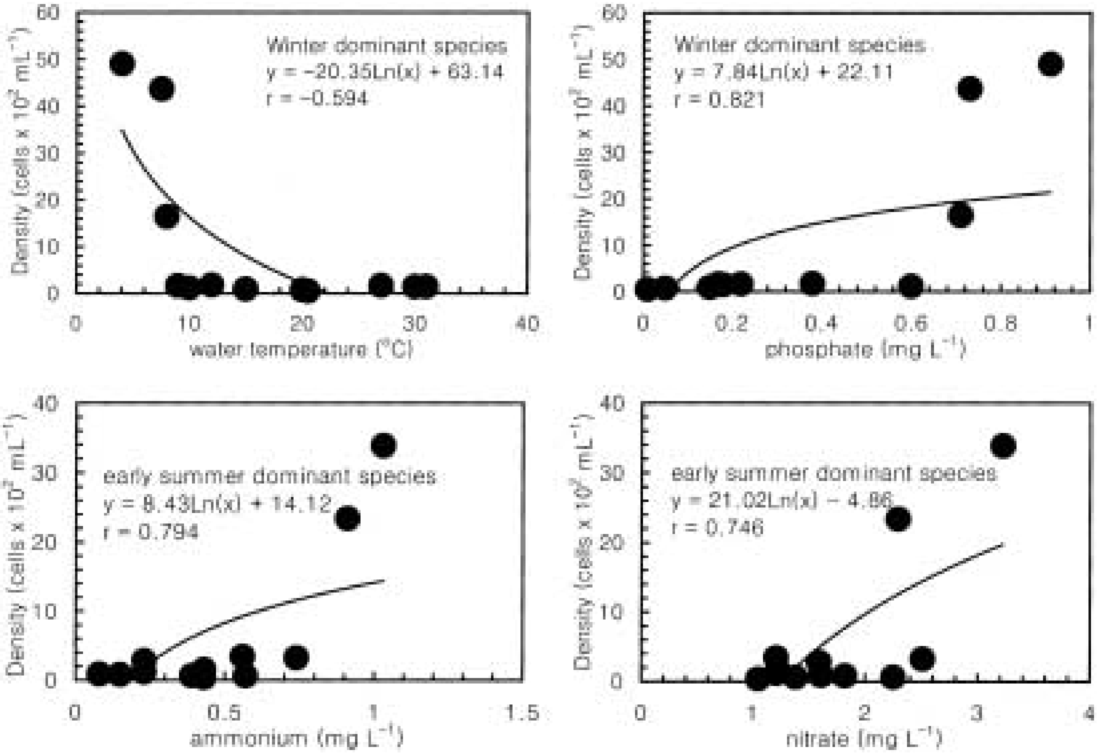

Fig. 5. Relationship of dominant green euglenoids to environmental variables in Jeonjucheon.

noids in July may be a result of washing effects by short water retention time due to the magnitude of monsoon inflow.

It is concluded that nutrients such as ammonium, nitrate, and phosphate, and water temperature contribute to the diversity of green euglenoids and the distinct bimodal pattern of winter and early summer abundance in Jeonjucheon. High phosphate concentration under low water temperature produces winter peak of density attributed by only some species, while high water temperature and high ammonium and nitrate concentration contribute to a diversity of species and the 2^{nd} peak of density in the early summer in Jeonjucheon. Further laboratory work is necessary for testing our field−based hypotheses on the abundance of the green euglenoids in Jeonjucheon.

ABSTRACT

In order to know relationships between green euglenoids and environmental factors, the species composition and density were assessed for 12 months together with environmental variables in the Jeonjucheon urban drainage, Korea. Nitrate was a high of 3.22 mg/l in June and phosphate concentration was over 0.71 mg/l in the winter. The euglenoids totaled 5 genera and 71 species throughout the year, increasing in the early summer (35 to 42 taxa) and decreasing in the winter (below 20 taxa). The number of green euglenoids positively correlated with surface water temperature. The total density of the green euglenoids showed a typical bimodal pattern, being maximal in the winter (5,394 cells/ml in January) and the early summer (4,823 cells/ml in June). The winter peak was a result of active growths of *Euglena caudata*, *E. geniculata* and *E. viridis*, however, each of which positively correlated with the phosphate. The early summer peak was attributed to *Euglena deses*, *Lepocinclis ovum*, and *Phacus trypanon*, each of which positively correlated with the ammonium and nitrate. The complete bimodal spectrum of species number and density of green euglenoids provides a sensitive image in detecting the changes of environmental variables in polluted waters such as Jeonjucheon.

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