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Morphological Variation and Density of *Euglena viridis* (Euglenophyceae) Related to Environmental Factors in the Urban Drainages

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도시하천의 환경요인과 *Euglena viridis*의 형태 변이 및 밀도와의 관계. 김준태 · 부성민 (충남대학교 자연과학대학 생물학과)

*Euglena viridis*의 형태변이, 밀도 및 환경요인과의 관계를 규명하기 위하여, 무심천, 미호천, 대전천, 전주천, 광주천 및 금호강의 도시하천에서 1996년 12월 25일에서 1997년 1월 4일 사이에 *E. viridis*의 형태와 밀도 및 수환경 요인들을 조사하였다. 각 하천에서 *Euglena viridis*는 형태적으로 두 가지 형태형으로 구분되었으나, 엽록체가 별 모양인 점에서 동일종으로 동정되었다. 형태형 I은 *E. viridis*의 일반적인 형태와 일치하였고, 대부분의 정점에서 대발생하여 광주천에서 최대 5386 cells · mL^{-1}이었다. 형태형 I의 밀도는 암모늄과 아질산염에 각각 정의 상관관계 ($r = 0.80$과 0.68), 질산염과는 부의 상관관계에 있었다. 형태형 II는 엽록체들의 가장자리가 거치형이며, 세포질 소립들이 불규칙하게 분산되어 있는 점 등이 특징이었다. 형태형 II의 밀도는 질산염과 정의 상관관계 ($r = 0.98$)를 보인 반면, 암모늄이나 아질산염과는 각각 부의 상관관계에 있었다. 그러나 두 가지 형태형은 각각 인산염, 수온 및 pH는 밀도와 유의한 상관을 보이지 않았다. 이 결과는 *E. viridis*의 두 가지 형태형들이 서로 다른 질소성 영양염들을 이용하므로서 동 시기 및 동일 지소에서 공존하고 있다는 것을 보여준다.

Key words : Density, ecology, Euglena, Euglenophyceae, Eutrophic waters, Morphotypes, Nitrogenous nutrients

INTRODUCTION

Green euglenoids are reported to show morphological and ecological plasticity in the field (Kiss *et al*., 1986; Conforti, 1998). The natural habitats are not consistent and make a diverse condition of environmental factors, which, in turn, bring about the diversity of natural populations of some euglenoids (Lackey, 1968; Kim and Boo, 2000). However, there are few studies on relationships of morphological variation of euglenoids related to environmental factors in the field (Kim and Boo, 1998).

Euglena viridis Ehrenberg has been known as the typical species having one star-like cluster of chloroplast lobes among the genus *Euglena* members (Pringsheim, 1956; Zakrys, 1986). It is unicellular, free-living, and cosmopolitan, inhabiting very wide range of water environments (Kim *et al*., 1998). The species often predominates in eutrophic waters including a high organic and inorganic content (Munawar, 1972; Kim and Boo, 1996, 2001). However, *E. viridis* is very changeable in various waters, and many varieties are described on minor differences of chloroplast morphology, shape and size of the cells (Zakrys, 1986; Kim *et al*., 1998). In addition,

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E. stellata Mainx is treated as a synonym of *E. viridis* because of identity in structure of chloroplast under transmission electron microscopy (Dragos *et al.*, 1979; Zakrys and Walne, 1998) and because of genetic similarity between *E. stellata* and *E. viridis* (Zakrys *et al.*, 1997). However, additional research on the '*Euglena viridis*' group in the field is necessary for understanding morphological variation (Kim *et al.*, 1998).

We have preliminarily observed that *Euglena viridis* predominated in the winter season in urban waters in Korea (Kim and Boo, 2001). The urban waters are usually turbid and are suitable for studying euglenoids response to the water environments, since urban waters budget a high degree of nitrogenous or phosphorus nutrients, derived from waste waters of domestic or industrial activities (Lackey, 1968; Kim and Boo, 2000). The present study was aimed to know 1) whether morphologically different populations of *E. viridis* occur in several waters, 2) which type of population is more abundant than others, and 3) if there are some relationships between densities of populations and environmental factors.

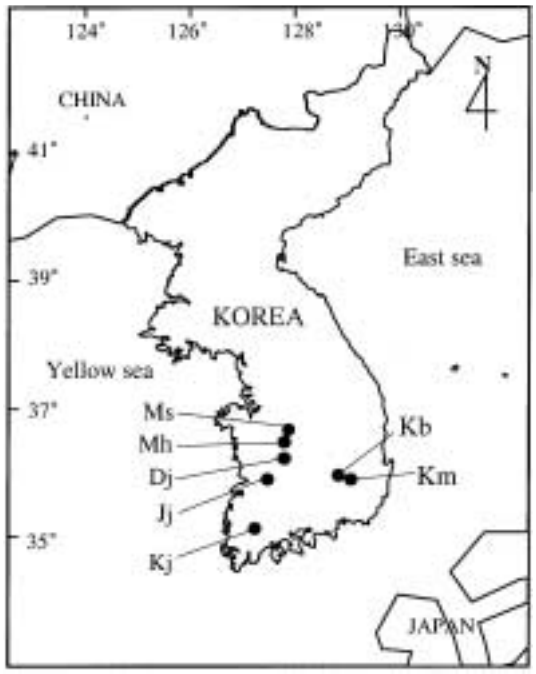

Fig. 1. Stations of urban drainages sampled in this study. Dj = Daejoncheon, Jj = Jeonjucheon, Kb = Keumhogang–Bisan, Kj = Kwangjucheon, Km = Keumgogang–Mangwoo, Mh = Mihocheon, and Ms = Mushimcheon.

MATERIALS AND METHODS

A total of seven stations were selected in the urban waterways receiving heavy inputs of industrial or domestic sewage, after the preliminary survey (Fig. 1). All samplings were completed during 11 days from 25 December 1996 to 4 January 1997 to minimize the temporal variations of environmental factors such as water temperature and dissolved nutrients. Data of surface water temperature, pH and dissolved inorganic nutrient were from Kim and Boo (1998).

Two aliquots of algal suspension were collected using 1,000 mL bottle from each station. One aliquot was concentrated for observing the morphology of *Euglena viridis*. Half of this sample was put under cooled dark conditions (below 4°C) and observed at an interval of five days. The other half was fixed with Lugol's solution. Both samples were used for scrutinizing the intracellular details because of difficulty in observations due to many chloroplasts in live cells. The remaining 1,000 mL aliquot for cell counting was fixed with the fixative (Lugol's solution) just after sampling. The number was determined using the Sedgwick–Rafter (S–R) counting cell and an inverted microscope (Olympus IX70) at 400× or 1,000× magnifications. A total of 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 the density of *Euglena viridis* cells in each station. Correlation and linear regression analyses (Sokal and Rohlf, 1995) were performed to determine relationships between the cell density and dissolved inorganic nutrients.

RESULTS

Morphology of *Euglena viridis*

Euglena viridis cells from the seven urban waters were morphologically separated into two morphotypes (Fig. 2). Morphotype I was spindle, 55 to 68 μm in length and 11 to 19 μm in width. The posterior part of cell was sharply or bluntly pointed on movement. The cytoplasmic granules were spirally arranged beneath the pellicle. The pellicles were finely and spirally striated and the number of striae was 15 to 17 per a 10–μm leng-

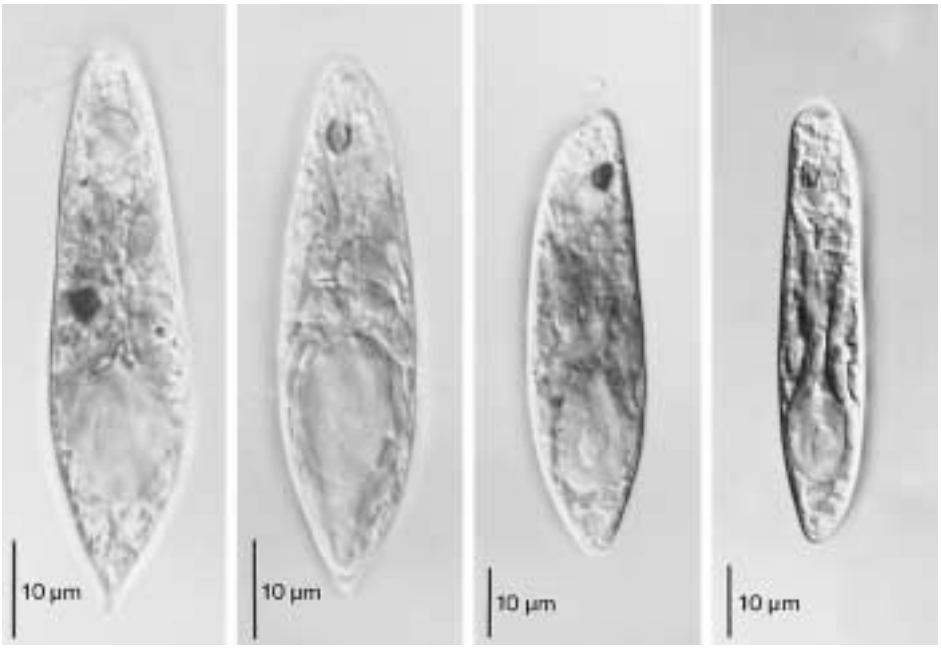

Fig. 2. Two morphotypes of *Euglena viridis* occurred in urban drainages in Korea. A–B. Morphotype I cells. C–D. Morphotype II cells.

th of cell surface. The chloroplasts, band–shaped and even–margined lobes, were aggregated into the star–like form with centrally located pyrenoid. This aggregate was located before the nucleus. The paramylon grains were small rods or discoid in shape. They were densely clustered in the aggregation center of chloroplast lobes and some were freely scattered in the cytoplasm. In the cells starved in dark, the central pyrenoid space sometimes occurred and the paramylon grains massed in the chloroplast center were detached. The emergent flagellum was similar to the body or slightly shorter than the body length.

In the morphotype II, the cells were 38 to 49 μm in length and 9 to 19 μm in width. The posterior of cells was not pointed but elongated to round on movement. The cytoplasmic granules were randomly scattered beneath the pellicle. The chloroplast lobes were uneven–margined. The number of pellicular striae was 20 to 22 per a 10–μm length of cell surface. The flagellar length was about twice of body length. Other features are similar to those of the morphotype I.

A morphological comparison of the above–mentioned two morphotypes is given in Table 1.

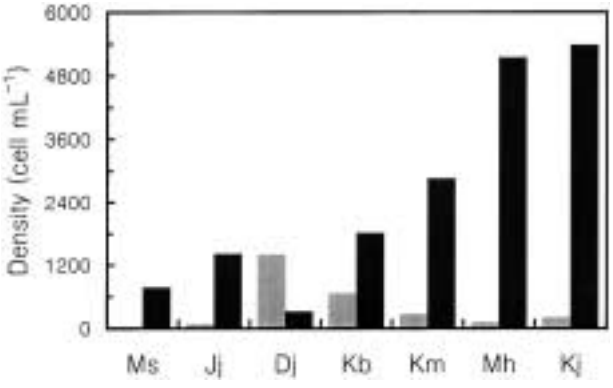

Fig. 3. Comparison of densities of two *Euglena viridis* morphotypes in urban drainages. Left column in each station indicates morphotype I and right column indicates morphotype II. Abbreviations in figure are same in Figure 1.

Density of local populations

The total density of *Euglena viridis* cells was high at 5,592 cells · mL^{-1} in Kwangjucheon and low at 780 cells · mL^{-1} in Mushimcheon (Fig. 3). The density of morphotype I remarkably bloomed with 5,149 cells · mL^{-1} in Mihocheon and 5,386 cells · mL^{-1} in Kwangjucheon, while the density

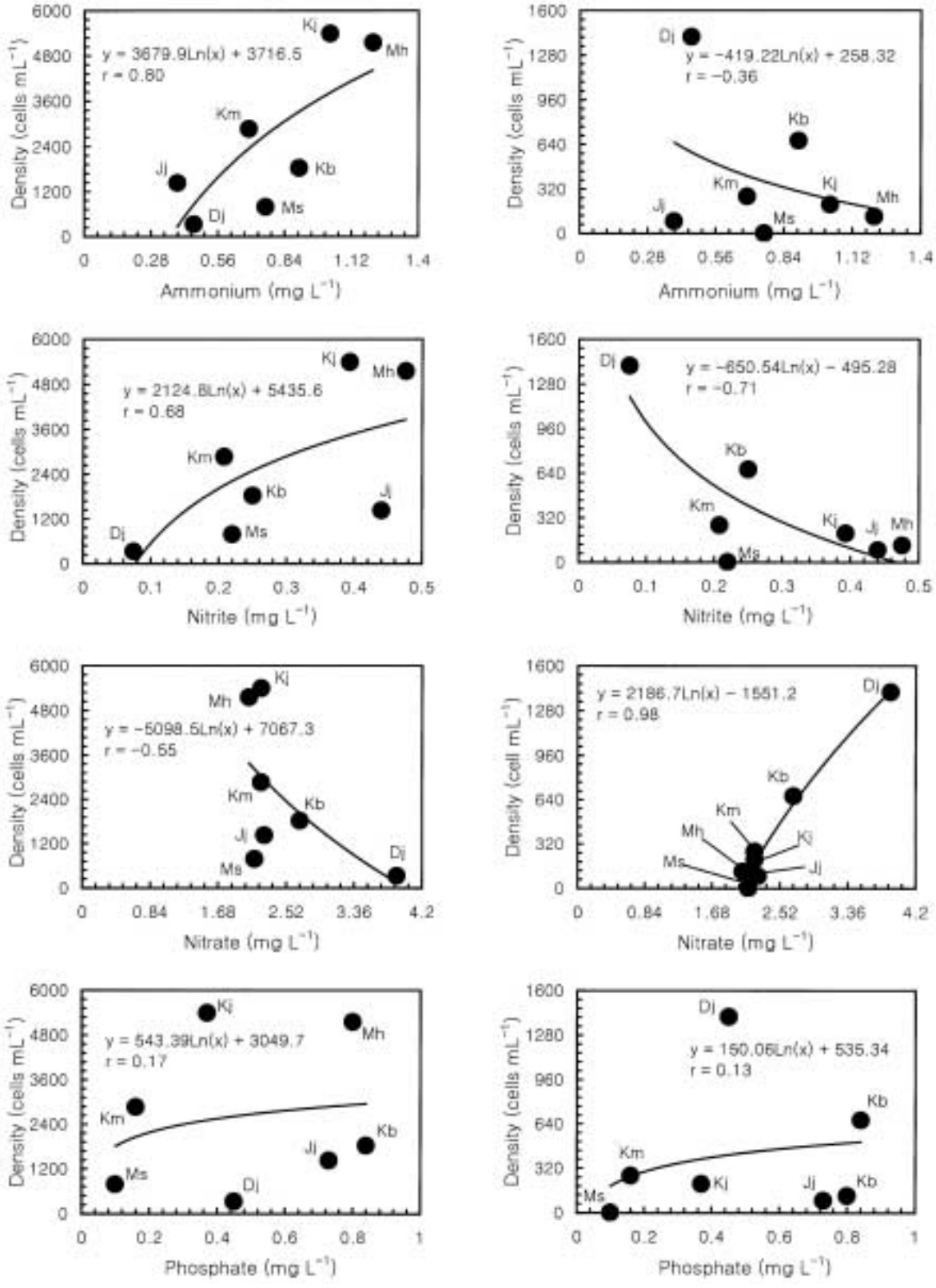

Fig. 4. Relationships between densities and dissolved inorganic nutrients. Left panel corresponds to morphotype I and right panel to morphotype II. Abbreviations in figure are same in Figure 1.

Table 1. A comparison of two *Euglena viridis* morphotypes in urban drainages in Korea.

Characters	Morphotype I	Morphotype II
Cell dimension (μm)	55 × 19 to 68 × 11	38 × 19 to 49 × 9
Cell posterior on movement	Sharply or bluntly pointed	Not pointed but elongated to even rounded
Cytoplasmic granules beneath pellicle	Spirally arranged	Randomly scattered
Body to flagellar length	< 1 or = 1	= 2
Striae number per 10 μm	16.3 ± 1.7	20.8 ± 1.4
Chloroplast lobes	even margined	uneven margined

was small with 320 cells · mL^{-1} in Daejoncheon and 780 cells · mL^{-1} in Mushimcheon.

The density of the morphotype II was high at 1,410 cells · mL^{-1} in Daejoncheon, but less than that of morphotype I in most stations (Fig. 3).

Environmental variables

Temperature of surface water was an average of 5.6 ± 1.9°C during the study period. The pH value fluctuated within a neutral zone with an average 7.80 ± 0.14. The ammonium concentration was high at 1.21 mg · L^{-1} in Mihocheon and low at 0.39 mg · L^{-1} in Jeonjucheon. The nitrite concentration was a high of 0.476 mg · L^{-1} in Mihocheon and a low of 0.075 mg · L^{-1} in Daejoncheon. The nitrate concentration was a high of 3.89 mg · L^{-1} in Daejoncheon and a low of 2.06 mg · L^{-1} in Mihocheon. The phosphate concentration was below 0.84 mg · L^{-1} in every station. All contents of this section were described in Kim and Boo (1998) and should be changed just as its citation.

Relationships between the population size and environmental variables

The density of each type of *Euglena viridis* correlated with the dissolved ammonium, nitrite, and nitrate, respectively (Fig. 4). The density of the morphotype I positively correlated to ammonium ($r = 0.80$, $P < 0.01$) and nitrite ($r = 0.68$, $P < 0.05$), while negatively with nitrate concentration ($r = -0.55$, $P < 0.05$). The density of the morphotype II positively correlated with nitrate, and the correlation was highly significant ($r = 0.98$, $P < 0.01$). However, the density negatively correlated with ammonium ($r = -0.36$) and nitrite ($r = -0.71$, $P < 0.05$).

The relationships between the density of each morphotype and inorganic phosphate concentration were not significant. The density of each morphotype was not significantly related with temperature and pH of surface water measured in the sampling time (data not shown).

DISCUSSION

Although all *Euglena viridis* cells in the urban waterways in Korea are same in having single star-like cluster of chloroplast lobes, the detailed observations show that *E. viridis* consists of two morphotypes, as seen in Table 1. The morphotype I cells agree well with the typical form of *E. viridis* on the basis of the traditionally known diagnostic features (Pringsheim, 1956; Zakrys, 1986; Kim *et al*., 1998). On the other hand, the morphotype II may be recognized as *E. stellata* Mainx (Pringsheim, 1956) because of small dimension of cells, randomly scattered cytoplasmic granules beneath pellicle, and uneven-margined lobes of chloroplasts. However, we consider that the morphotype II should be defined as *E. viridis* because the features such as cell dimension, mucoplasts, and shape of chloroplast lobes are variable in the field and are not sufficient to distinguish *E. stellata* and *E. viridis*. So both morphotypes of *E. viridis* are recognized as ecological clones, not different species. This wide conception of *E. viridis* concurs with the view of Zakrys *et al.* (1997) that *E. viridis* and *E. stellata* are not different species but rather clones of the same species based on studies of the cell size and the DNA polymorphism among clones of both species. Similar result was obtained for a very common and changeable species such as *E. geniculata* Dujardin (Kim and Boo, 1998), as in other phytoplanktonic microalgae (Wood and Leatham, 1992; Mann, 1999).

Another interesting results are that the two morphotypes occupy the same habitats in the urban drainages but the density of each morphotype is limited by different nutrients, as seen in Fig. 3. The positive relationship between the morphotype I and ammonium and nitrite indicates that the morphotype I cells may take up both nutrients efficiently even at low ammonium and nitrite concentrations. However, the prefer-

ence of the morphotype I cells for ammonium nitrogen is shown by the great decline of the density in Daejoncheon and Mushimcheon having a low concentration of ammonium. These results agree with previous reports that ammonium is preferentially assimilated by euglenoids (Munawar, 1972; Kim and Boo, 1998, 2001) as well as other freshwater algae (e.g. Liao and Lean, 1978). On the other hand, the fact that the morphotype II positively correlated with nitrate while negatively with ammonium and nitrite shows that the density of the morphotype II is dependent on the concentration of nitrate. This result is in accordance with the reports of Munawar (1972) and Kilham and Kilham (1978) that some euglenoids require the unique resource requirements of nitrate nitrogen.

Inorganic phosphate concentration is a key element controlling the algal blooms in many waters (Kim and Boo, 2000). In previous study (Kim and Boo, 1998) carried out in the same place and period with this research, the density of *Euglena geniculata* is positively related to phosphate. It is unexpected that the density of each morphotype of *E. viridis* was not significantly related with inorganic phosphate. This result indicates that *E. viridis* is more dependent on the concentrations of nitrogenous nutrients than that of phosphate. Because *E. viridis* is favored in nitrogenous nutrients while *E. geniculata* is in phosphate, two species appear to coexist in the same place and period.

It is certain that *Euglena viridis* is common in the urban drainages in Korea and is morphologically and ecologically separated into two types. The morphological differences between two morphotypes are considered to be responses to different sources of dissolved nitrogenous nutrients. The morphotype II cells are small-sized and have a larger surface area/volume ratio than the large-sized morphotype I cells, giving a better capacity for nutrient uptake and a competitive advantage in conditions of ammonium nitrogen decline (Banse, 1977; Foy, 1980). However, the densities of morphotype II are less than those of the morphotype I. This may be due to the fact that the morphotype I cells use both ammonium and nitrite while the morphotype II cells use only nitrate. The significant correlations of the densities of both morphotypes with nitrogenous nutrients also indicate that the morphotype I is quite different from the morphotype II. Such a morphological and ecological difference in *E. viridis* in Korea may question the wide concept of the *E. viridis* species. Before the morphotype II may be appraised as an infraspecific category or even an independent species, taxonomic evidences such as laboratory culture and DNA taxonomy should be supplemented.

ABSTRACT

The morphological variation and density of the *Euglena viridis* cells and environmental factors of urban waterways of Daejoncheon, Jeonjucheon, Kwangjucheon, Kumhogang, Mihocheon, and Musimcheon, Korea were studied from 25 December, 1995 to 5 January, 1997 in order to elucidate possible relationships among the biological and abiological factors. All *E. viridis* cells were same in having single star-cluster of chloroplast lobes and included two morphotypes based on other detailed morphology. The morphotype I cells agreed well with the typical form of *E. viridis* and commonly occurred in most of waters and bloomed with 5,386 cells · mL^{-1} in Kwangjucheon. The density of the morphotype I positively correlated with ammonium ($r = 0.80$) and nitrite ($r = 0.68$), while negatively with nitrate concentration. The morphotype II cells were characterized by having randomly scattered cytoplasmic granules beneath pellicle and uneven-margined lobes of chloroplasts. The density of the morphotype II positively correlated with nitrate ($r = 0.98$), while negatively correlated with ammonium and nitrite. However, the density of each morphotype was not significantly related with inorganic phosphate, temperature and pH of surface water. These results indicate that *E. viridis* includes two morphotypes in urban waterways in Korea, that coexist in the same period and station as a response of allocation of nitrogenous nutrients.

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