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Morphology and Ecology of *Peridinium bipes* var. *occultatum* Lindem. (Dinophyceae) Forming Freshwater Red Tides in Korean Dam Reservoirs

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This study was performed to understand the morphological and ecological characteristics of *Peridinium bipes* var. *occultatum* Lindem., which is the organism responsible for freshwater red tides in Sangsa dam and Miryang dam reservoirs. The samples were collected from April to July 2004 when the freshwater red tides occurred. In the study, we were able to differentiate *P. bipes* var. *occultatum* from *P. bipes*, a closely related species, by its smaller antapical horn size. In Miryang dam reservoir the red tide occurred only within the area of the upstream, but it was well developed in all of the water area in Sangsa dam reservoir. In 2004 average LTSI (Lake Trophic Status Index; Yang and Dickman, 1993) of Miryang dam reservoir was 3.53 of mesotrophic state and Sangsa dam reservoir was 8.59 of eutrophic state. It was determined, through culture experiments under various conditions that vitamins, trace elements, phosphorus and nitrogen were important contributing factors to the growth of *P. bipes* var. *occultatum*. A rapid toxic effect of *P. bipes* var. *occultatum* on aquatic organisms such of *Daphnia magna* and *Oryzias latipes* was not identified in this study.

Key Words: freshwater red tide, Miryang, *Peridinium bipes* var. *occultatum*, Sangsa

INTRODUCTION

Water blooms can be defined as the phenomenon occurring when phytoplankton, protists, and microorganisms increase abruptly to great numbers or accumulate biologically or physically, causing the color of the water to change and harming other organisms (Lee 1999; MOMAF 2000). The term red tide refers to the phenomenon of by which water bodies turn red due to the high numbers of Dinophyceae

In recent times, repeated occurrences of freshwater red tides in domestic dam reservoirs, including Imha dam reservoir, have caught the attention of scientists, and in the years 2003 and 2004 serious red tides occurred in dam reservoirs all over the country, including Sangsa, Chuam, Gucheon and Miryang dam reservoirs, causing great worries not just to ecologists, but through all levels of society in Korea (KOWACO 2003).

Korean freshwater red tides have been reports by Kim *et al.* (1987), Kang *et al.* (1989) and Ahn (1990). However, the first two were in fact very short abstracts rather than dissertations or reports, and the report by Ahn (1990) simply conducted research on the bacterial variation caused by red tides rather than the red tide itself. Thus we can say that there have been no actual Korean research projects directly examining freshwater red tides. Also, because the research projects mentioned above were all conducted on Soyang dam reservoir, it is also true that the amount of research done is much limited to be able to achieve complete understanding of the phenomenon of freshwater red tides in Korean dam reservoirs as a whole. However, work such as the investigation on the classification and the genotypic analysis of the organisms that cause freshwater red tides in Chuam dam reservoir, Sangsa dam reservoir and the Togyo dam reservoir by Ki *et al.* (2005a, b) showed that, in recent times, research on freshwater red tides in Korea has been gradually progressing. Nevertheless, research on the present level of freshwater red tide incidents and their causes is still insufficient. Therefore, it is critical that we examine the seriousness and causes of freshwater red tides in the dam reservoirs where they occur, devise appropriate countermeasures against freshwater red tides and investigate the subsequent hindrances to utilization of water resources, such as conducting bioassays

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to evaluate rapid toxicity levels.

This research investigated Miryang dam and Sangsa dam reservoirs, where freshwater red tides have occurred, and focused the identification and the ecological characteristics on the causative organisms of red tides. Also we used cultivation to research the relationship between of red tide causing organisms and environmental factors, and conducted bioassays to evaluate the rapid toxicity of freshwater red tides to other organisms.

MATERIALS AND METHODS

This research was conducted on each one station where red tides occurred in Miryang dam reservoir, located in Gyenonsangnam-do Miryang-shi, and Sangsa dam reservoir, located in Jeonranam-do Suncheon-shi (Fig. 1). Biological samples were collected from April to July 2004, when freshwater red tides had occurred. These collections were on an irregular basis, in keeping with the characteristics of the freshwater red tides.

The quantitative samples for examining factors such as the existing amount of phytoplankton were collected from depths of 10-20 cm below the surface water using a Van Dorn water sampler and fixed using Lugol's solution. For qualitative samples needed for classification we used concentrations of the quantitative samples along with the samples collected using the NXXX 25 plankton net. To measure the existing phytoplankton numbers we let the quantitative sample settle for 48-72 hours, removed the upper liquid to concentrate to the appropriate extent, mixed the concentrated samples well and used a Sedgwick-Rafter Chamber and the Schoen (1988) method for calculation.

As for the environmental factors, we used an on-station water quality measuring instrument, the Quanta multiprobe (HYDROLAB), to measure the temperature, dissolved oxygen and conductivity on station, and used the survey data of the Ministry of Environment for values of the levels of total phosphorus, total nitrogen, chlorophyll-a and secchi transparency depth.

To investigate the causative organisms of the freshwater red tides, we naturally dried the samples fixed in 99% ethanol, coated them using an ion-coating instrument (Hitachi Japan, E-1030), examined them using the SEM (Hitachi Japan, S-4300) and classified them using the classification techniques of Pestalozzi (1968) and Pascher (1990).

In order to examine the relativity of freshwater red tide causing organisms and environmental factors, we conducted culture studies. For cultures we used the samples collected from Miryang dam and Sangsa dam reservoirs, and carried out a pure isolation using the dilution method, along with samples we received from the National Institute of Environmental Research of Japan, and the CCAP (England). Culture flasks (250 mL) contained 120 mL of DM, MWC and Carefoot medium (Table 1-3). We then inoculated 20 cells/mL of *Peridinium bipes* var. *occultatum* that we isolated in pure form from

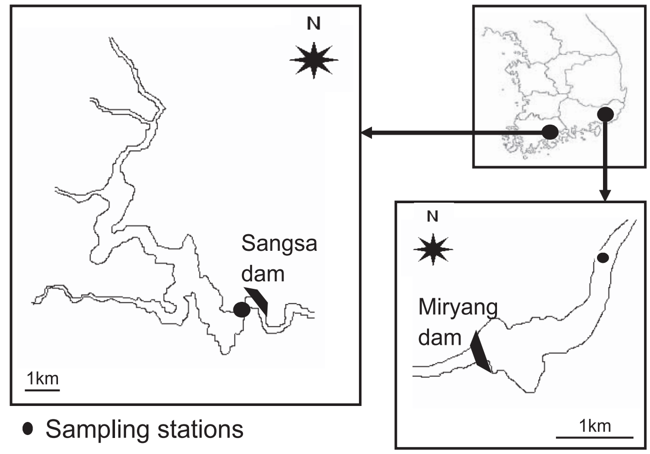

Fig. 1. Map of sampling stations at Miryang and Sangsa dam reservoirs.

Table 1. Composition of DM (Diatom Medium)

Stocks	per 200 mL
① $Ca(NO_3) \cdot 4H_2O$	4.000 g
② KH_2PO_4	2.480 g
③ $MgSO_4 \cdot 7H_2O$	5.000 g
④ $NaHCO_3$	3.180 g
⑤ EDTA solution:	
- EDTA FeNa	0.450 g
- EDTA Na2	0.450 g
⑥ H_3BO_3	0.496 g
$MnCl_2 \cdot 4H_2O$	0.278 g
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.200 g
⑦ Vitamin solution:	
- Thiamine HCl	0.008 g
- Biotin	0.008 g
- Cyanocobalamin	0.008 g
⑧ $NaSiO_3 \cdot 9H_2O$ (Sigma S4392)	11.400 g
Medium	**per liter**
Stock solutions 1-8	1.0 mL each

*Make up to 1 liter with deionized water. Adjust to pH 6.9 with 1M HCl. Dispense to suitable vessels and autoclave at 121°C for 15 minutes.

*Sigma S4392: Sigma Chemical Co., Fancy Road, Poole, Dorset BH17 7TG, UK.

Table 2. Composition of MWC (Modified WC Medium)

Stocks	per liter
① $CaCl_2 \cdot 2H_2O$	36.8000 g
② $MgSO_4 \cdot 7H_2O$	37.0000 g
③ $NaHCO_3$	12.6000 g
④ $K_2HPO_4 \cdot 3H_2O$	11.4000 g
⑤ $NaNO_3$	85.0000 g
⑥ $Na_2O_3Si \cdot 5H_2O$	21.2000 g
⑦ Combined trace elements:	
- EDTA Na_2	4.3600 g
- $FeCl_3 \cdot 6H_2O$	3.1500 g
- $CuSO_4 \cdot 5H_2O$	0.0100 g
- $ZnSO_4 \cdot 7H_2O$	0.0220 g
- $CoCl_2 \cdot 6H_2O$	0.0100 g
- $MnCl_2 \cdot 4H_2O$	0.1800 g
- $Na_2MoO_4 \cdot 2H_2O$	0.0060 g
- H_3BO_3	1.0000 g
⑧ Vitamin solution:	
- Thiamine HCl	0.1000 g
- Biotin	0.0005 g
- Cyanocobalamin	0.0005 g
⑨ Buffer (add dry when making up medium): Per litre final medium	
- TES	0.1150 g
Medium	**per liter**
Stock solutions 1-8	1.0 mL each
Dry buffer 9	0.1150 g

*Combine stock solutions 1-8 and the dry buffer and make up to 1 liter with deionized water. Autoclave at 121°C for 15 minutes.

the original waters of Miryang dam and Sangsa dam reservoirs, and cultivated these under conditions of 15°C, 1,000 lux, and 12:12h (L:D cycle).

We evaluated the rapid toxicity of the freshwater red tide causing organisms against *Daphnia magna* and *Oryzias latipes*. For the experiment using *Daphnia magna* we used M4 culture medium (Table 4) for cultivating *Daphnia* in a 100 mL glass beaker, with a temperature maintenance device to maintain the water temperature at 20°C and an air pump to constantly inject air into the beaker to prevent the exhaustion of dissolved oxygen. We used an extraction net, designed in-laboratory, to extract the *D. magna*, and inserted it into the control group and the treatment group. This experiment was conducted on the control group and Sangsa dam reservoir netting samples (treatment group). We inserted 20 individuals of *D. magna* into both culture groups, and observed during the 48 hours at 12 time intervals. The experiment using *Oryzias latipes* was conducted in a 5 L water tank made of glass. We used the temperature maintenance device to keep the water temperature at 23°C and inserted air through the air pump. We selected *O. latipes* specimens that were under 3 cm to use in the experiment and placed 10 into each water tank. Using 5 water tanks we inserted 0, 500, 1,000, 3,000, and 5,000 cells/mL of *P. bipes* var. *occultatum* into each tank, and observed every 24 hours for 96 hours. After 24 hours after the experiment 1 individual of *O. latipes* died in the tank that we did not inject any *P. bipes* var. *occultatum* into, and after 48 hours 1 individual of *O. latipes* died in each of the tank into which we injected 0 cells/mL, 1,000 cells/mL, and 3,000 cells/mL of *P. bipes* var. *occultatum*.

Table 3. Composition of Carefoot Medium

Stocks	per 500 mL
① $MgSO_4 \cdot 7H_2O$	0.2450 g
② NaCl	0.8250 g
③ KH_2PO_4	1.1250 g
④ K_2HPO_4	0.4850 g
⑤ $CaCl_2$	0.8250 g
⑥ $NaNO_3$	12.5000 g
⑦ PIV metal solution	
- EDTA Na_2	0.7500 g
- $FeCl_3 \cdot 6H_2O$	0.0970 g
- $MnCl_2 \cdot 7H_2O$	0.0410 g
- $ZnCl_2$	0.0050 g
- $CoCl_2 \cdot 6H_2O$	0.0020 g
- $Na_2MoO_4 \cdot 2H_2O$	0.0049 g
⑧ Vitamin solution:	
- Thiamine HCl	5.0000 g
- Biotin	5.5000 g
- Cyanocobalamin	5.5000 g
Medium	**per 935 mL**
Stock solutions 1-6	10.0 mL
PIV metal solution	5.0 mL
Vitamin solution	0.1 mL

*Autoclave at 121°C for 15 minutes. Do not titrate for pH.

RESULTS

The classification of the freshwater red tide-causing organism

We examined and classified the *Peridinium* species collected from Miryang dam and Sangsa dam reservoirs using SEM. The *Peridinium* cells that appeared in the two dam reservoirs were round to oval, their lengths ranged from 43 to 72 μm (avg. 54.2 μm) and width from 37 to 68 μm (avg. 47.8 μm), and were quite flat dorsoventrally. The surface of the plate was reticulated, and the cingu-

Table 4. Composition of M4-Medium

Stocks	per liter
① $CaCl_2 \cdot 2H_2O$	73.52000 g
② $MgSO_4 \cdot 7H_2O$	123.30000 g
③ KCl	5.80000 g
④ $NaHCO_3$	64.80000 g
⑤ Cation stock solution	
- $MnCl_2 \cdot 4H_2O$	3.56000 g
- LiCl	3.06000 g
- RbCl	0.71000 g
- $SrCl_2 \cdot 6H_2O$	1.52000 g
- $CuCl_2 \cdot 2H_2O$	0.16750 g
- $ZnCl_2$	0.13000 g
- $CoCl_2 \cdot 6H_2O$	0.10000 g
⑥ Anion stock solution	
- $NaNO_3$	0.54800 g
- H_3BO_3	5.71900 g
- NaBr	0.03200 g
- $Na_2MoO_4 \cdot 2H_2O$	0.12600 g
- Kl	0.00650 g
- Na_2SeO_3	0.00438 g
- NH_4VO_3	0.00115 g
⑦ Na_2SiO_3	21.47500 g
⑧ Iron/EDTA stock solution	
- EDTA Na_2	0.50000 g
- $FeSO_4 \cdot 7H_2O$	0.19910 g
⑨ Phosphate stock solution	
- KH_2PO_4	0.28600 g
- K_2HPO_4	0.38600 g
⑩ Vitamin solution:	
- Thiamine HCl	0.75000 g
- Biotin	0.01000 g
- Cyanocobalamin	0.00750 g
Medium	**per liter**
Stock solutions 1	40 mL
Stock solutions 2-4	10 mL
Stock solutions 5, 10	1 mL
Stock solutions 6, 9	5 mL
Stock solutions 7	2 mL
Stock solutions 8	50 mL

*Autoclave at 121°C for 15 minutes.

lum and sulcus that were laid vertically and horizontally across the cells respectively, could be seen quite clearly. The width of the cingulum was in the range of 3.0-3.5 μm, and at the copula with the sulcus, the right side was located about one cingulum width higher. The sulcus was laid out downwards starting from the epitheca and appeared to be wider as it went down the hypotheca. This species could be separated into the epitheca and hypotheca by the cingulum and a 4-7 μm long apical pore plate could be seen in the epitheca. The epitheca was made up of 1 apical pore plate (Po), 4 apical plates (4′), 3 intercalary plates (3a), and 7 precingular plates (7′′), and the hypotheca consisted of 4 postcingular plates (4′′′) and 2 antapical plates (2′′′′). By considering the morphological characteristics using the SEM examinations and the classification of Pestalozzi (1968) and Pascher (1990) methods, we established that the organism causing the freshwater red tides in Miryang dam and Sangsa dam reservoirs was *Peridinium bipes* var. *occultatum* (Fig. 2).

The ecological characteristics of freshwater red tide occurrences

The characteristics and water environmental factors of the freshwater red tide phenomena of Miryang dam and Sangsa dam reservoirs: In this research we investigated the characteristics of the freshwater red tides that occurred at Miryang dam and Sangsa dam reservoirs, and determined the relationship between freshwater red tides and environmental factors. The freshwater red tide that was observed in Miryang dam reservoir showed the characteristics of a temporary occurrence in the form of a 'belt' of algae in July and August of 2004 near the upper stream entry point of the dam reservoir, which subsequently disappeared. The phytoplankton standing crops at the occurrence point of the freshwater red tide was measured at 2,883 cells/mL, with the standing crops of *Peridinium bipes* var. *occultatum* were 2,363 cells/mL, accounting for 82% of the total amount of phytoplankton. The water temperature during the freshwater red tide was 26.1°C, DO concentration 9.8 mg/L, and conductivity 40 μs/cm. As for the levels of environmental factors from January to October of 2004, T-P was measured at a range of 0.003-0.021 mg/L, and T-N at 0.683-1.159 mg/L which surpasses the yearly rich nutrition levels. Also the levels of T-P and T-N were measured at their highest during September, when the freshwater red tide had disappeared. Lake Trophic Status Index (LTSI; Yang and Dickman 1993) analysis showed a minimum number of 0.84 in January and a maximum of 5.91 in July, and that the dam reservoir had a mesotrophic state every month except for July (Fig. 3).

At Sangsa dam reservoir a freshwater red tide occurred continuously from April to July of 2004, over all areas of the dam reservoir. The standing crop of phytoplankton was 3,144 cells/mL, and that of *Peridinium bipes* var. *occultatum* was 3,012 cells/mL, comprising 96% of all the phytoplankton. The water temperature during the freshwater red tide was 18°C, DO concentration 10.8

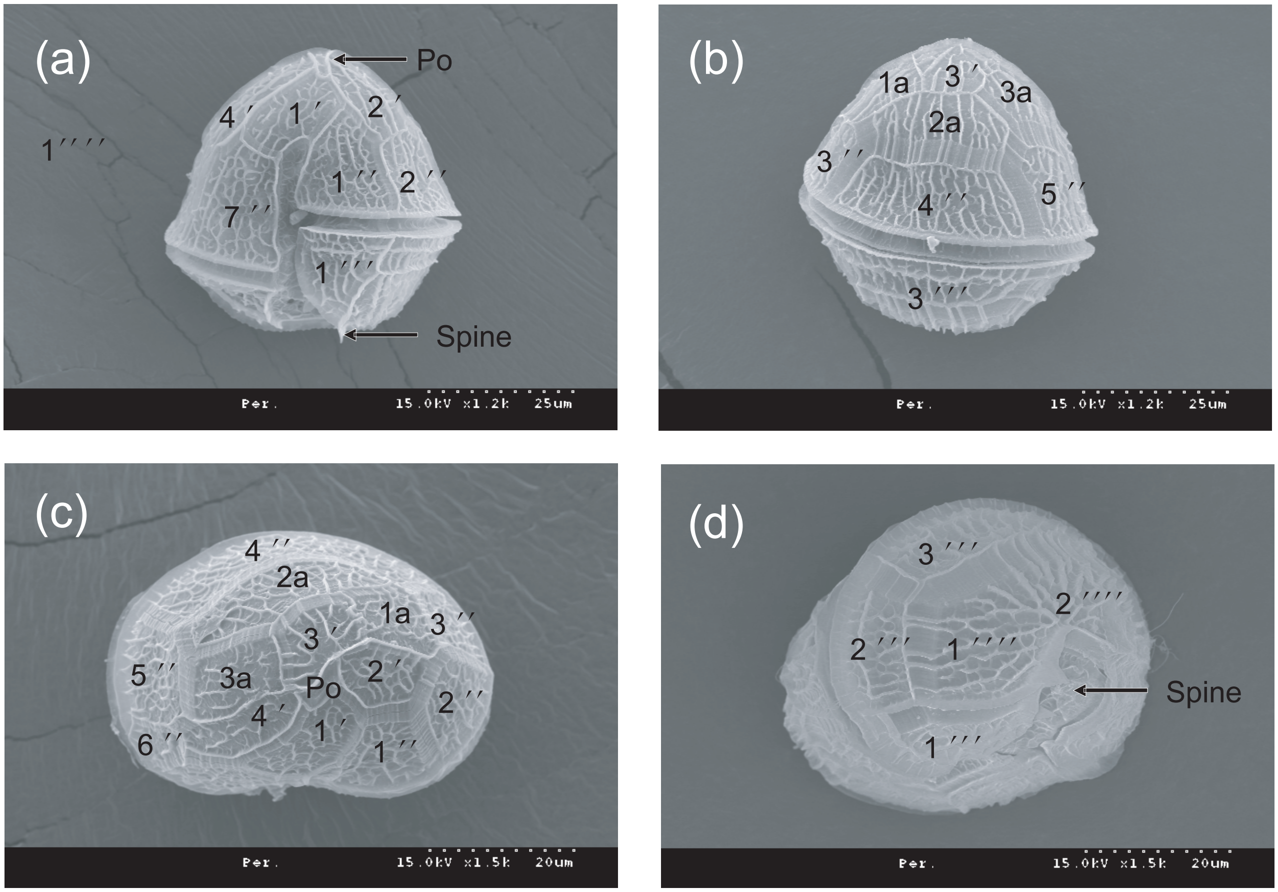

Fig. 2. SEM micrographs of *Peridinium bipes* var. *occultatum* collected from Miryang and Sangsa dam reservoirs: ventral view (a), dorsal view (b), apical view (c) and antapical view (d) (Po: apical pore).

mg/L, and conductivity 58 μs/cm. Analysis of the environmental factors from January to October of 2004 showed T-P at 0.012-0.058 mg/L and T-N at 0.654-1.744 mg/L, all surpassing the yearly eutrophic states. The LTSI analysis results also showed that the dam reservoir was in an eutrophic state, numbers being in a range of 5.81-12.84 (Fig. 3).

Cultivation of the freshwater red tide-causing organism: In this research we hoped to examine the environmental factors that affect the growth of the freshwater red tide organisms in culture. After 15 days of cultivation the MWC culture medium and Carefoot culture medium showed similar growth rates with existing levels of 38 cells/mL and 40 cells/mL, respectively, whereas the DM culture medium showed relatively low growth levels with existing cell levels of only 22 cells/mL (Fig. 4). In order to understand what caused this difference in growth rate we examined the constitution of each culture medium. MWC culture medium and Carefoot culture medium contained microelements such as PIV metals and combined trace elements, which were not found in the DM culture medium. This research alone was not be enough to make definite conclusions, but it suggested that microelements such as those mentioned above affect the growth of *P. bipes* var. *occultatum*.

We then examined the growth rate of *Peridinium bipes* var. *occultatum* by controlling the construction of the Carefoot culture medium, which showed the highest growth rate amongst culture media. For culture medium constitution we used the methods put forth by KOWACO (1997). Also, in order to examine the effects of microelements we used the standard solutions (Ca, K, Mg, Mn and Si; Kanto chemical co., Japan) to control the amount of elements included in the Carefoot culture medium (Table 5). We cultivated cells for 15 days under the same conditions as stated previously and found that, using Chl-a as a proxy for growth, the cultures to which we added P+EDTA, NO_3^-, and vitamins showed Chl-a concentrations of 3.424 μg/L, 3.404 μg/L and 3.630 μg/L, respectively, which means that the growth rates increased by more than two-fold compared to the 1.498 μg/L in the untreated culture medium. As for the microelements, the culture to which we added Mn showed the highest growth rate, with the Chl-a concen-

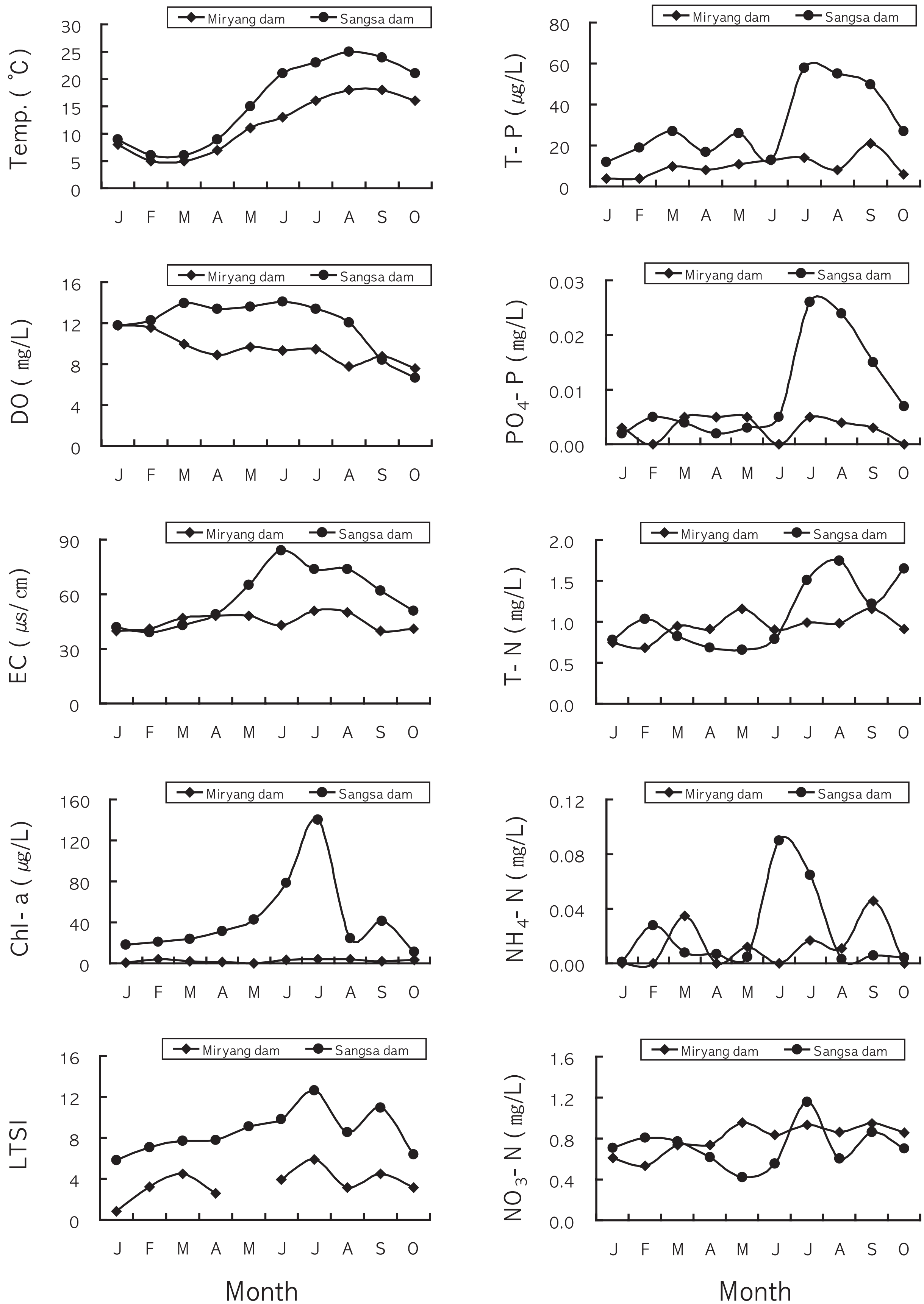

Fig. 3. The changes of environmental factors in Miryang and Sangsa dam reservoirs from January to Ocotber in 2004.

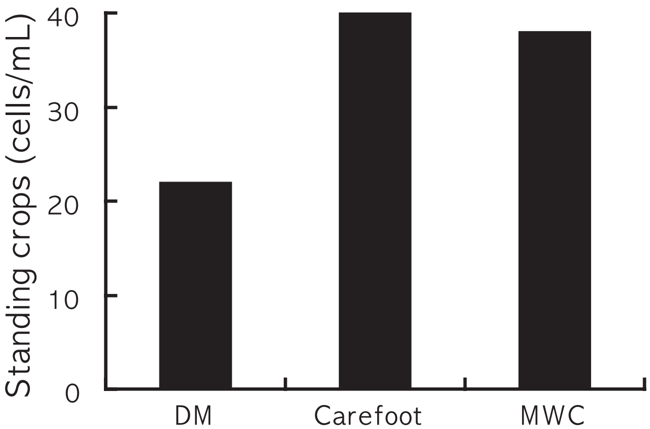

Fig. 4. Growth rates of *Peridinium bipes* var. *occultatum* of each media for 15 days.

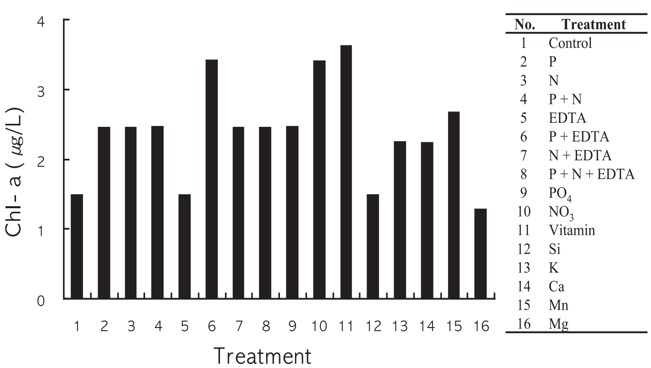

Fig. 5. Growth rates of *Peridinium bipes* var. *occultatum* of each treatment for 15 days.

tration of 2.672 μg/L (Fig. 5). Throughout this experiment we discovered that P+EDTA, NO_3^-, vitamins and Mn were environmental factors that affected the growth of *P. bipes* var. *occultatum*.

The rapid toxicity assessment of freshwater red tides through bioassays

We conducted experiments on the test organisms *Daphnia magna* and *Oryzias latipes* to assess the rapid toxicity of freshwater red tides.

The result of the experiment using *Daphnia magna*, after 48 hours, all of the *D. magna* injected into the natural group and processed group were still alive (Fig. 6).

The result of the experiment using *Oryzias latipes*, after 72 hours, 1 individual died in each of the tank into which we injected 1,000 cells/mL and 5,000 cells/mL, and 2 individuals died in the tank into which we injected 3,000 cells/mL. At the end of the experiment, 2 individuals of *O. latipes* died in the tanks into which we injected 0 and 1,000 cells/mL of *P. bipes* var. *occultatum*, leaving 8 individuals alive in each tank. 1 individual died in the tank with 500 cells/mL, leaving 9 individuals surviving. In the tank injected with 3,000 cells/mL of *P. bipes* var. *occultatum* a total of 3 individual of *O. latipes* died, this being the largest number of *O. latipes* deaths in this experiment (Fig. 7). The relation between the death of the *O. latipes* and the *P. bipes* var. *occultatum* standing crops did not appeared in this test.

Through these rapid toxicity experiments conducted using *Daphnia magna* and *Oryzias latipes*, we were able to establish that the *P. bipes* var. *occultatum* that caused freshwater red tides to appear in Miryang dam and Sangsa dam reservoirs caused no rapid toxicity.

Table 5. Compositions of modified Carefoot media

No.	Treatment	Remark
1	Control	Control
2	Control + 0.05 mg/L P as K_2HPO_4	P
3	Control + 1.00 mg/L N as $NaNO_3$	N
4	Control + 0.05 mg/L P + 1.00 mg/L N	P + N
5	Control + 1.00 mg/L Na_2EDTA	EDTA
6	Control + 0.05 mg/L P + 1.00 mg/L Na_2EDTA	P+EDTA
7	Control + 1.00 mg/L N + 1.00 mg/L Na2EDTA	N+EDTA
8	Control + 0.05 mg/L P + 1.00 mg/L N + 1.00 mg/L Na_2EDTA	P+N+EDTA
9	Control + 4.75 mg/L PO_4 as PO_4 standard solution	PO_4
10	Control + 62 mg/L NO_3 as NO_3 standard solution	NO_3
11	Control + 1 mg/L vitamins as vitamin solution	Vitamins
12	Control + 2.8 mg/L Si as Si standard solution	Si
13	Control + 1.95 mg/L K as K standard solution	K
14	Control + 10.01 mg/L Ca as Ca standard solution	Ca
15	Control + 0.05 mg/L Mn as Mn standard solution	Mn
16	Control + 3.609 mg/L Mg as Mg standard solution	Mg

DISCUSSION

In this research we have defined the morphological characteristics of the *Peridinium* which caused freshwater red tides in Miryang dam and Sangsa dam reservoirs from April to July of 2004, and classified this organism. The *Peridinium* that caused freshwater red tides in the two dam reservoirs were round to oval shaped, with sizes in the range of 43-72 μm length and 37-68 μm width. The width of the cingulum was 3.0-3.5 μm, and at the copula with the sulcus, the right side was located about one cingulum width higher. The sulcus started at

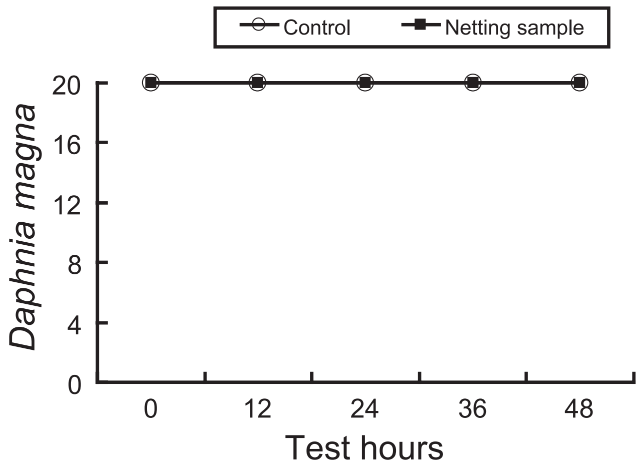

Fig. 6. Evaluation of rapid toxicity of *Peridinium bipes* var. *occultatum* to *Daphnia magna*.

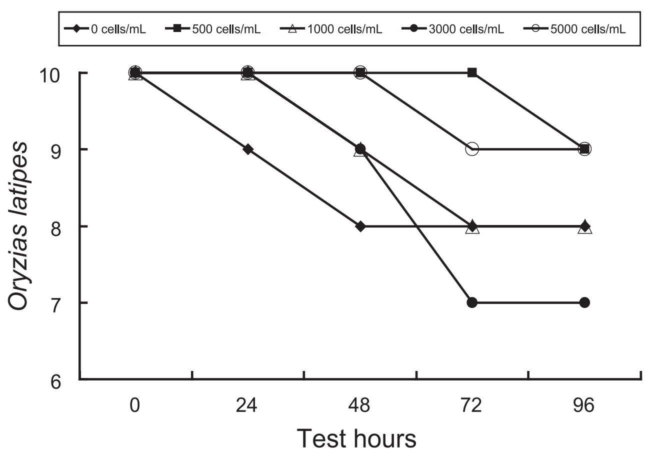

Fig. 7. Evaluation of rapid toxicity of *Peridinium bipes* var. *occultatum* to *Oryzias latipes*.

the one third point of the epitheca, lay out in a hypotheca, and appeared wider as it got closer to the hypotheca. The plate arrangements of this organism were Po, 4′, 3a, 7′′, 4′′′, 2′′′′. A horn was smaller than that of the *P. bipes* could be seen in the hypotheca. In classifying *Peridinium*, Pascher (1990) first divided them into Cleistoperidinium and Poroperidinium, depending on their possession of Po. The Poroperidinium that had Po were then divided into the 5 sections of bipes, gutwinskii, lomnickii, allorgei and umbonatum according to the arrangement of the plate. The specimen discovered in our investigation possessed Po, the plate arrangement of the epitheca was symmetrical, and the la plate and 4′′ plate did not meet, making it belong to the bipes section. Pestalozzi (1968) divided the *P. bipes* group into *P. bipes*, *P. bipes* var. *occultatum*, and *P. bipes* var. *tabulatum* according to the antapical horn structure found at the hypotheca. The *P. bipes* has a large and definite horn structure whereas *P. bipes* var. *occultatum* has a much smaller horn structure compared to *P. bipes*, and *P. bipes* var. *tabulatum* has no horn structure at all. The *Peridinium* species that appeared in Miryang dam and Sangsa dam reservoirs turned out to be *P. bipes* var. *occultatum*, with very small horn structures. This organism is the same as the organism that caused freshwater red tides in the Togyo dam and Chuam dam reservoirs, Korea (Ki *et al.* 2005b).

Generally Dinophyceae can be found in sea water or in brackish waters, and freshwater Dinophyceae appears in many different environments including rivers and ponds, greatly polluted areas and swamps (Pascher 1990). According to Fukuju *et al.* (1998), reservoirs where freshwater red tides occur are valley type reservoirs in a temperate climate with average temperatures of 12-18°C that have stored water for 5 years or more, and have oligomesotrophic states. Also, Yamada *et al.* (1998) reported that freshwater red tides appear at the point where the upper stream of the dam reservoir enters, because red tide causing organisms collect at these points. The freshwater red tides of the two dam reservoirs shown in our investigation are quite contrary to these existing theories in terms of the time and place of occurrence, trophic states, and other factors. The freshwater red tide that occurred in Miryang dam reservoir appeared and disappeared between July and August of 2004 near the entry point of the upper stream of the dam reservoir, and the Lake trophic state was mesotrophic. The freshwater red tide that occurred in Sangsa dam reservoir lasted from April to July of 2004 and occurred all over the dam reservoir, unlike the red tide of Miryang dam reservoir. The trophic states of the dam reservoir also appeared to be eutrophic states throughout the year. Red tides like the one that appeared in Sangsa dam reservoir are very exceptional, and there have been no former research on such a red tide in Korea or elsewhere. However, we found that both dam reservoirs showed a sharp increase in the concentration of T-P and T-N when the red tides disappeared, and therefore special efforts must be put in to maintain the water quality after red tides disappear. Unlike cyanobacterial blooms, freshwater red tides do not occur at fixed times for a long time, but they usually appear temporarily and then disappear, as in the case of Miryang dam reservoir. Therefore, in order to determine the features of freshwater red tide occurrences, it appears necessary that diverse and precise investigations on factors such as the reservoir characteristics, river valley, water quality, and the cysts of the

Table 6. Environmental facotrs for growth of dinoflagellates

Reference	Environmental factors
Anderson and Wall (1978) Pfiester and Anderson (1987) Stosch (1969)	Temperature, DO, nitrogen and light intensity
Brettum (1996)	NO_3
Bruno and McLaughlin (1977) Holt and Pfiester (1981)	Inorganic nutrients and Vitamin B_{12}
Höll (1928)	Temperature, pH, DO, organic matter, nitrogen, phosphorus, calcium, chlorine and light intensity
Horne and Goldman (1994)	Nutrients and vitamins
Pfiester (1975, 1976, 1977) Champman and Pfiester (1995)	Nitrogen
Present Study	Phosphorus, NO_3^-, vitamins and trace elements

bottom of the dam reservoir must be carried out from the beginning to the end of the red tide, and even when red tides are not present. Somia *et al.* (1994) reported that freshwater red tide occurrences were not controlled just by the concentration level of nutritious salts such as T-N and T-P. Therefore, we need take the parts that could not be explained by investigating the items that cause phytoplankton blooms and revise the items of investigation when researching freshwater red tides.

We tried to discover the environmental factors that affect the occurrence of freshwater red tides through cultivation. It was reported that Dinophyceae such as *Peridinium* is affected by environmental factors such as water temperature, nutritious salts of the nitrogen group, and DO, and is especially greatly affected by nitrogen (Stosch 1969; Anderson and Wall 1978; Pfiester and Anderson 1987). Brettum (1996) also reported that *Peridinium* was affected by NO_3^-. It is reported that *P. cinctum*, *P. gatunense*, and *P. willei* are organisms greatly affected by nitrogen under cultivation conditions (Pfiester 1975, 1976, 1977; Champman and Pfiester 1995). Höll (1928) reported water temperature, organic matter concentrations, nitrogen, phosphorous, calcium, chlorine, intensity of illumination and dissolved oxygen concentration as environmental factors that affect the growth of Dinophyceae. It has been reported that water temperature by itself does not affect the growth of *Peridinium*, however, and is only important when with other environmental factors (Park and Hayashi 1992). The intensity of illumination and DO has been reported as an environmental factor that affects the daily perpendicular range of Dinophyceae (Pascher 1990; Wu and Chou 1998). Horne and Goldman (1994) reported that in Clear Lakes the rise in water temperature heats up the lower levels which leads to the discharge of growth elements such as nutrient salts and vitamin B_{12} (Bruno and McLaughlin, 1977; Holt and Pfiester, 1981), which in turn cause freshwater red tides (Table 6). In our investigation we conducted cultivation twice under conditions of 15°C, 1,000 lux, and 12:12h (L:D cycle). Through the 1st cultivation experiment we found that the PIV metals and the microelements of combined trace elements included in the Carefoot culture medium and MWC culture medium affected the growth of *P. bipes* var. *occultatum*. Such results correspond with the results of research conducted by Somia *et al.* (1994), who reported that freshwater red tide occurrence is not simply controlled by the concentration of nutritious salts such as T-N, T-P, but other factors as well. This result suggests and this shows that investigation on microelements will be required in future research on freshwater red tide occurrences. In our second cultivation in which we took the Carefoot culture medium, which showed the greatest growth rate in the 1st experiment, and investigated the growth rate of *P. bipes* var. *occultatum* according to culture medium constitution, we discovered that P + EDTA, NO_3^-, vitamins, and Mn were environmental factors that affected the growth of *P. bipes* var. *occultatum*. These results show that P, NO_3^-, and vitamins affect the occurrence of freshwater red tides and are consistent with the results of many former studies (Table 6). Of the many microelements, Mn appeared to be the one with the greatest influence. Also, the results of our 1st cultivation experiment showed *P. bipes* var. occulatatum growth rates of 0.06-0.07/day which is slightly lower than the growth rate reported by Nishibori *et al.* (1991) which was 0.10-0.15/day, similar to the results reported by Yamada *et al.* (1998) of 0.06-0.13/day.

It was reported that there are about 2,000 species of Dinophyceae on Earth (Taylor 1990), and of these species about 30 are deemed to induce toxicity (Falconer 1993). The first studies on the toxicity of Dinophyceae were conducted on marine dinoflagellates (Sommer and Meyer 1937). Dinoflagellates that induce toxicity are *Alexandrium acatenella*, *A. catenella*, *A. monilata*, *A. tamarensis*, *Exuviaella mariaelebouriae*, *Karenia brevis*, *Gymnodinium veneficum*, *Lingulodinium polyedrum* and others, and they have been reported to secrete nervous toxins and liver toxins (Kadis and Ciegler 1971). Of the *Peridinium* group, *P. polonicum* has been reported as toxic (Nozawa 1968; Nakajima *et al.* 1981). In this research we conducted experiments using *Daphnia magna* and *Oryzias latipes* to assess the rapid toxicity of the freshwater dinoflagellates species *P. bipes* var. *occultatum*. However, we discovered that the *P. bipes* var. *occultatum* that was found in Miryang dam and Sangsa dam reservoirs presented no short-term toxicity to the test organisms.

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