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Growth and Morphological Changes in *Scenedesmus dimorphus* Induced by Substances Released from Grazers, *Daphnia magna* and *Moina macrocopa*

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Grazer-induced colony formation was examined using a green alga *Scenedesmus dimorphus* (Türpin) Kützing. Algae were cultured in a medium with or without filtered water taken from cultures of *Daphnia magna* Straus (300 ind./L) or *Moina macrocopa* Straus (500 ind./L). The exposure to zooplankton filtered water (ZFW) promoted colony formation in *S. dimorphus*, with the magnitude of this response being directly proportional to the relative volume of ZFW that was added to the culture medium. The number of cells/colony and mean particle biovolume of *S. dimorphus* increased between 24 and 72 hours after exposure to ZFW, most likely due to the influence of chemicals released from *D. magna* or *M. macrocopa*, and possibly as a defense mechanism against zooplankton grazing.

Key words: Morphological change, colony formation, *Scenedesmus dimorphus*, *Daphnia magna*, *Moina macrocopa*

INTRODUCTION

Some freshwater phytoplankton species are plastic in their morphology, physiological characteristics, genetic structure, and biochemical composition. These characteristics of planktonic algae vary considerably under different environmental conditions in aquatic ecosystems. Algae such as *Staurastrum* sometimes lose their spiny form, and large multi-filament 'flakes' of *Aphanizomenon* grows as single filament (Lynch, 1980). Various blue-green algae, including *Microcystis aeruginosa* Kützing, have potential for both colonial morphology and chemical toxicity. In nutrient-deficient systems, where algal growth rate is slow, these features of algae might be effective defense mechanisms against zooplankton grazing (Van Donk *et al*., 1997). Defenses such as large size, hardness of cell wall structures, mucus excretion, and the production of toxins must offer considerable advantages that overcome the negative aspects such as increased energy demand and greater sinking losses of cells with these features (Van Donk *et al*., 1998).

Recently, Bronmark and Hansson (2000) summarized the concept of defense mechanisms of prey being induced by chemical factors released by aquatic predators. 'Info-chemicals' (Dicke and Sabelis, 1988) convey information between two individuals, evoking a behavioral or physiological response in the receiver. This information transfer by chemicals in aquatic ecosystems has gained attention recently but has mainly focused on the response of zooplankton to the presence of fish and invertebrate predators (Larsson and Dodson, 1993). Relatively little information exists regarding grazer-mediated anti-herbivore responses in phytoplankton.

There is some evidence that chemicals released

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by zooplankton can induce colony formation in certain phytoplankton, such as *Scenedesmus*. This green alga is common in freshwater lakes and it can occur both as unicells and colonies when maintained in laboratory cultures (Trainor, 1992). Although numerous factors might affect the morphology of *Scenedesmus*, it has been documented that chemicals released from *Daphnia* can induce colony formation (Hessen and Van Donk, 1993; Lampert *et al*., 1994). Except *Daphnia*, it is unknown whether other crustacean zooplankton can induce such a change in their prey.

We examined the effect of chemicals released by *Daphnia magna* Straus and *Moina macrocopa* Straus on the morphology (cells/colony) and total population biovolume of *S. dimorphus* (Türpin) Kützing using controlled laboratory experiments. We also determined whether the extent of colony formation increases with amounts of zooplankton chemicals in the water.

MATERIALS AND METHODS

Scenedesmus dimorphus (Türpin) Kützing (NIES 119) was obtained from the culture collection of the National Institute for Environmental Studies (NIES) in Tsukuba, Japan (Watanabe *et al*., 2000). *S. dimorphus* was axenically cultured in C medium at 20°C and a 14 h light/10 h dark photoperiod (irradiance = 120 μmol m/s). The chemicals in cultured media (C media) for *S. dimorphus* (Türpin) Kützing 15 mg $Ca(NO_3)_2 \cdot 4H_2O$, 10 mg KNO_3, 5 mg β-Na_2 glycerophosphate · $5H_2O$, 4 mg $MgSO_4 \cdot 7H_2O$, 0.01 μg Vitamine B_{12}, 0.01 μg Biotin, 1 μg Thiamine HCl, 0.3 ml PIV metals, 40 mg Tris (hydroxymethyl) aminomathane and 99.7 ml distilled water.

Exponentially growing algae were used in the experiments. Non-egg bearing adults of *D. magna* (mean length ± SD, 3.38 ± 0.58 mm; mean height ± SD, 2.05 ± 0.30 mm) and *M. macrocopa* (mean length ± SD, 1.68 ± 0.35 mm; mean height ± SD, 0.68 ± 0.16 mm) were obtained from stock cultures maintained for > 10 y under constant laboratory conditions in the NIES (Table 1). To obtain water with chemicals from *Daphnia* or *Moina*, 300 individuals of matured adult *D. magna* (before reproduction) or 500 individual of matured adult *M. macrocopa* (before reproduction) were reared in a 1.0 L suspension of *S. acutus* (10^3 cells/ml, biovolume 1,005 μm^3/ml) in C medium without trace elements at 20°C. After 24 h of grazing, each medium was filtered through a 0.1 μm membrane to remove zooplankton (*Daphnia* or *Moina*), algal cells, bacteria, and other particulates (Lürling *et al*., 1997; Lürling and Van Donk, 2000).

Table 1. Length, height, dry weight and filtration rate of *Daphnia magna* and *Moina macrocopa* as measured in three experiments.

	Unit	*Daphnia magna*	*Moina macrocopa*	n
Length	mm	3.38 ± 0.58	1.68 ± 0.35	30
Height	mm	2.05 ± 0.30	0.68 ± 0.16	30
Dry weight	mg	0.43 ± 0.11	0.09 ± 0.03	25
Filtration rate	ml/ind./h.	0.83 ± 0.09	0.18 ± 0.03	15

We first determined the growth rates of *S. dimorphus* in media with or without zooplankton filtered water (ZFW). Exponentially growing *S. dimorphus* (1.8×10^5 cells/ml, biovolume 373 μm^3/ml) was inoculated into 150 ml C media containing *D. magna* or *M. macrocopa* filtered water (containing 0, 4, 8, 16 and 25% volume of *D. magna* or *M. macrocopa* filtered water, n = 3). Both alga was incubated for one month at 20°C under the photoperiod and irradiance conditions described above. Algal numeric densities (cells/ml) in all treatments were counted every 2 to 3 d with a haemocytometer under a fluorescence microscope (Olympus BH-2, Japan).

The timing of colony formation by *S. dimorphus* after exposure to ZFW for 14 d was also examined. The 8 d old *D. magna* and 4 d old *M. macrocopa* were used for this experiment. Exponentially growing *S. dimorphus* was placed into 150 ml of C media with filtered water from the cultures of *D. magna* (0, 8 and 16%) or *M. macrocopa* (0, 8 and 16%). The algae were incubated for 2 weeks in the same condition as previously described. The number of cells per colony was determined at 0, 3, 8 and 14 d after addition of the ZFW. Samples were fixed with Lugol's solution and then counted (at least 500 unicells or colonies) using an inverted microscope (Nikon 114, Japan) at 500 × magnification.

Changes in number of cells per colony and total biovolume (μm^3/ml) were examined during 72 hours of incubation. The same aged zooplankton was used to obtain the ZFW. Exponentially growing *S. dimorphus* was put in C medium with ZFW from *Daphnia* (0 and 25%) or *Moina* (0 and

25%). The algae were incubated for 3 days in the same condition as described above. The number of cells per colony and mean particle volume was determined after 0, 2, 24, 48, 72 hours. Cell volumes (length and width, μm^3) were measured by counting at least 600 unicells or colonies in a sub-sample preserved in Lugol's solution with a Nikon light microscope at 1,000 × magnification.

RESULTS

1. Growth rates

The growth rates (based on Log (cells/ml)) of *S. dimorphus* were different among all populations cultured in the media with and without filtered water from *Daphnia* or *Moina* (Fig. 1). In differing volumes of ZFW (4, 8, 16, 25%), we rarely observed a difference in total biovolume of algae. However, growth rate (based on Log (cells/ml)) among control and treatments was clearly different, with the highest growth rates occurring in the control treatment. An exponential phase of all populations was observed from the 3rd to 7th day of growth. Growth rates decreased after the 7th day in all media with ZFW, while growth rates in the media without ZFW did not decrease until after the 12th day. After the 20th day, *Scenedesmus* cultures with both of ZFW from *Daphnia* and *Moina* reached carrying capacity, showing maximum yield. There were different carrying capacities for algae with and without ZFW after day 20 (ANOVA, $p < 0.01$). When various volume of ZFW (4, 8, 16, 25%) was applied to the *S. dimorphus* culture, an increase of in biovolume occurred that was directly proportional to the % ZFW added.

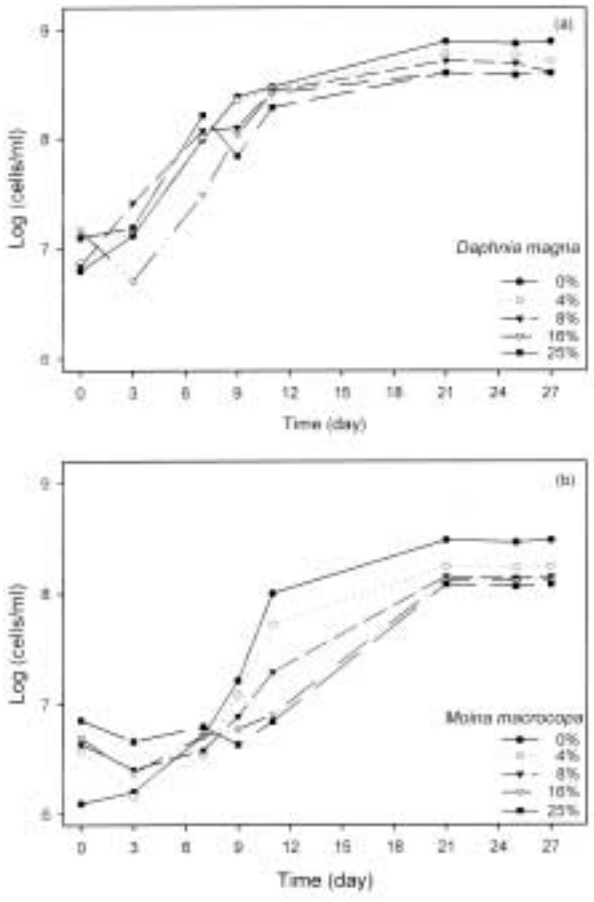

Fig. 1. Growth of *Scenedesmus dimorphus* in autoclaved C medium containing zooplankton filtered water (0, 4, 8, 16, 25% of *Daphnia magna* (a) and *Moina macrocopa* (b) filtered water volume per total media volume) during the experimental period (27 days). Each symbol represents mean of three samples.

2. Colony formation and mean particle biovolume

The number of cells per colony of *S. dimorphus* increased in all media with ZFW in the 14 d experiment while no change was observed in the control (Fig. 2). Colony formation was most prominent on the 3rd day in the treatment containing either 16% *Daphnia*-ZFW (7.5 cells/colony on average) or 16% *Moina*-ZFW (7.4 cells/colony on average), although one replicate of the control had 5.2 cells/colony. Afterwards, colony formation in the ZFW treatments decreased, and by day 14 it did not differ from the control (5.0 cells/colony in control; 5.7 cells/colony in 16% *Daphnia* ZFW; 5.5 cells/colony in 16% *Moina* ZFW). Colony induction by *D. magna*-ZFW was stronger than for ZFW from *M. macrocopa*. In both *Daphnia* and *Moina* treatments, however, colony formation and cells/colony at 16% ZFW were higher than at 8% ZFW.

The number of cells per colony of *S. dimorphus* increased significantly in the both *Daphnia* and *Moina* ZFW treatment between 24 and 72 h (Fig. 3) (*Daphnia*: ANOVA, $p < 0.01$; *Moina*: ANOVA, $p < 0.01$). In the 25% *Daphnia*-ZFW treatment, the increase in colony formation was slow during the first 2 hours, but more rapid up to 72 h (4.99 $\pm$0.02 cells/colony at 0 h, 5.68$\pm$0.10 at 2 h, and

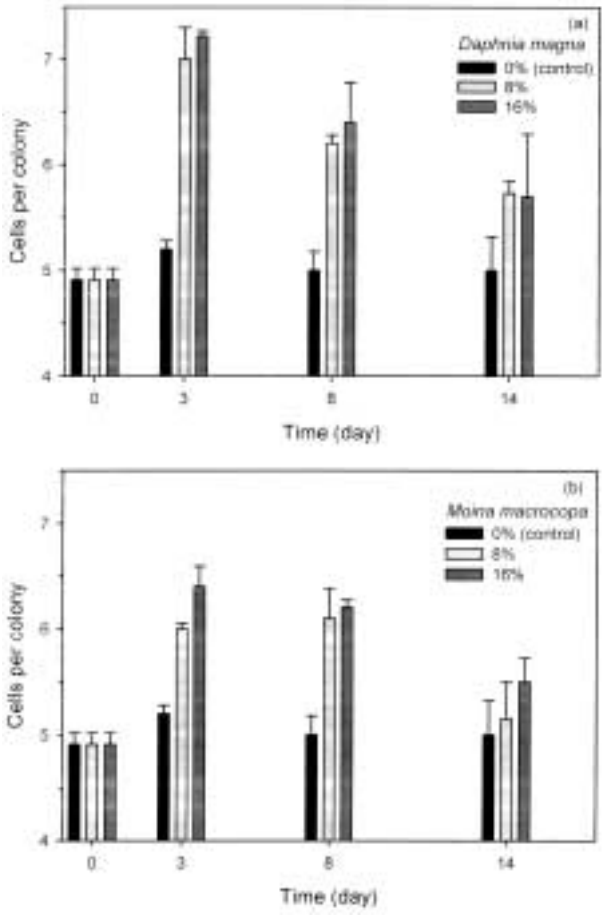

Fig. 2. Number of cells per each colony of *S. dimorphus* dosed by 0, 8 and 16% *D. magna* (a) or *M. macrocopa* (b) filtered water (volume %) during the 14-day experiment. Vertical bars show SD (n = 3).

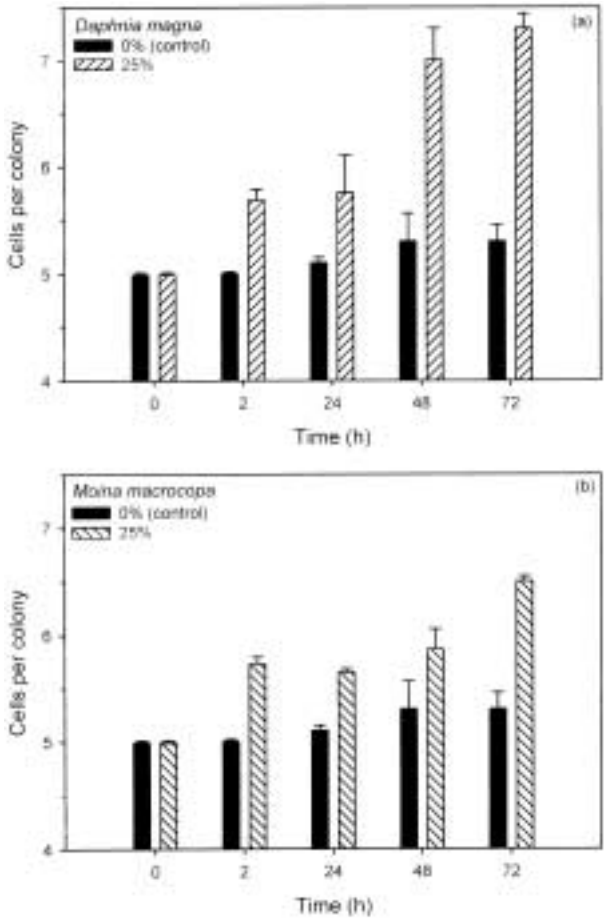

Fig. 3. Number of cells per each colony of *S. dimorphus* dosed by 0 and 25% *D. magna* (a) or *M. macrocopa* (b) filtered water (volume %) during the 72 hr experiment. Vertical bars show SD (n = 3).

7.30±0.14 at 72 h). In the *Moina* treatment a small increase in colony formation was observed at 2 h, and then this was maintained until 72 h (5.73±0.07 cells/colony at 2 h, 5.64±0.04 at 24 h, and 6.51±0.05 at 72 h).

Increases in biovolume were observed in both ZFW treatments during the 72 hr experiment (Fig. 4). The response was stronger in the *D. magna* treatment than the *M. macrocopa* treatment. There were sharp increases in *S. dimorphus* biovolume in the first 24 h and then a gradual increase until 72 h (7,124 μm^3/ml at 0 h, 10,023 μm^3/ml at 24 h, and 11,648 μm^3/ml at 72 hr). A similar pattern of change in mean biovolume was observed between 0 and 48 h in the *M. macrocopa* treatment (7,124 μm^3/ml at 0 h, 9,120 μm^3/ml at 24 h, and 9,295 μm^3/ml at 48 h). Biovolume decreased up to the final sampling at 72 h. Morphological changes of *S. dimorphus* were observed in both ZFW treatments, but increases in cells/colony and biovolume were more pronounced in the *D. magna* treatment.

DISCUSSION

The results indicate that chemicals released by both *D. magna* and *M. macrocopa* can cause morphological changes (colony formation) in the common green alga *S. dimorphus*. At this time the nature of the chemical signals is unknown. Until now, it was understood that algal colony formation could be induced by zooplankton, but results were limited to studies with *Daphnia*. This phenomenon may be more widespread among crustaceans, as indicated by our finding that colony

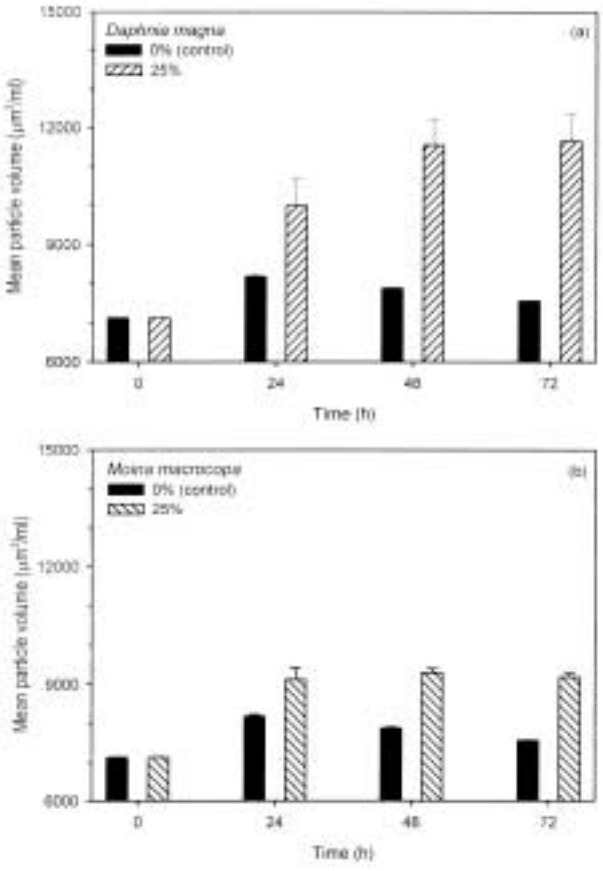

Fig. 4. Mean particle volume (μm^3/ml) of *S. dirmorphus* dosed by 0 and 25% *D. magna* (a) or *M. macrocopa* (b) filtered water (volume %) during the 72 hr experiment. Vertical bars show SD (n = 3).

formation was stimulated by water from a culture of the littoral cladoceran *Moina*. As indicated, the new results are consistent with those demonstrated in previous studies with *Scenedesmus* and *Daphnia* (Hessen and Van Donk, 1993; Lampert *et al*., 1994). Additional research is needed, considering other species of cladocerans, and perhaps also copepods, to elucidate how common this phytoplankton growth stimulus by zooplankton actually is.

The origin of zooplankton chemical signals remains unsolved, but it might be related to a food–grazer interaction and involve the animals' digestive system. Colony formation in phytoplankton appears to be absent when grazers are not fed or when they consume inert polystyrene beads before ZFW is collected (Lürling, 1998; Van Donk *et al*., 1998). We noticed that using matured adult zooplankton increased the effects of chemicals in ZFW rather than young zooplankton (data not shown). This may indicate that some physiological processing in their gut does not fully develop until several days of growth.

With increased colony formation in *Scenedesmus* there was not a decrease in biovolume, but population numeric densities were lower. It is of interest that adding ZFW stimulated a defensive structure (colonies) but did not suppress biovolume. Hessen and Van Donk (1993) and Lampert *et al*. (1994) observed that colony formation did not result in reduced population growth in *Scenedesmus*. Similar results have been reported for *S. acutus* when cultured under varying nutrient conditions (Sterner *et al*., 1993; Sterner and Smith, 1993) and with or without ZFW (Lampert *et al*., 1994). In future research it will be of interest to reconcile what degree of morphological defense in phytoplankton results in energy expenditures sufficient to reduce both population growth (cells/ml) and total biovolume in a consistent manner.

Colony formation by planktonic algae may be a common defense strategy against abiotic factors such as temperature and salinity (Trainor, 1992; Wasmund, 1992) and biotic factors such as the pressure of grazers, and biological toxin production in aquatic ecosystems. Understanding how chemical signals between zooplankton and their prey affect morphology and physiology of phytoplankton may be a key to fully understanding bioenergetics in the pelagic ecosystem.

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＜국문적요＞

동물플랑크톤 *Daphnia magna*와 *Moina macrocopa*에서 유도된 분비물질에 의한 *Scenedesmus dimorphus*의 형태변화

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상위포식자에 의해 유도되는 *Scenedesmus dimorphus* (Türpin) Kützing의 군체형성에 관한 실험을 수행하였다. 동물플랑크톤 *Daphnia magna* (300 ind./L)와 *Moina macrocopa* (500 ind./L) 배양한 후, 0.1 μm (millipore)로 여과하여 얻어낸 용액 (ZFW)을 *S. dimorphus*가 1.8 (10^5 cells/ml까지 자란 처리군에 첨가하여, ZFW를 넣지않은 대조군과 비교하였다. 대조군에 비해 두 동물플랑크톤 처리군에서 *S. dimorphus*의 군체형성의 유도가 뚜렷이 관찰되었다. 이 현상은 *M. macrocopa* 보다는 *D. magna*처리군에서 뚜렷하게 관찰되었으며, 첨가해준 ZFW의 양이 증가할수록 particle당 세포수도 비례적으로 증가하는 것으로 나타났다. ZFW처리군에서 1군체당 세포수(cells/colony)와 평균체적(mean particle biovolume)은 24~72시간 사이에 급격히 증가하는 것으로 관찰되었다. *S. dimorphus*의 군체형성현상은 동물플랑크톤 *D. magna*와 *M. macrocopa*의 분비물에서 유도되는 화학물질의 영향으로 보이며, 이와 같은 형태변화현상은 동물플랑크톤의 포식에 대한 방어기작으로 작용하게 될 것으로 사료된다.