Hydrobiologia **294**: 99–103, 1994.

Possible toxic effects on *Daphnia* resulting from the green alga *Scenedesmus obliquus*

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Received 22 November 1993; accepted 24 November 1993

Key words: Scenedesmus, toxic, culture, *Daphnia*

Abstract

We cultured individuals of two *Daphnia* species and their hybrid on two different algae, *Scenedesmus obliquus* and *Chlamydomonas globosa*, in different concentrations. Our results suggest that culture conditions of *S. obliquus* can be such that the algal cells become toxic to *Daphnia*

Introduction

Many studies have shown that cyanobacteria (blue-green algae) are relatively poor food items for cladocerans. Ahlgren *et al.* (1990) reported that the nutritional value of cyanobacteria is relatively poor compared with green algae. Moreover, as a result of the filamentous nature of many cyanobacteria the handling time of these species is higher than the handling time of unicellular organisms (Hartmann & Kunkel, 1991). Furthermore, filamentous cyanobacteria are known to physically interfere with uptake by cladocerans of smaller algal cells (Gliwicz & Lampert, 1990). Also, some species of cyanobacteria (e.g. strains of *Microcystis*, *Anabaena*, *Gomphosphaeria* and *Oscillatoria*) actually increase mortality in zooplankton by producing toxins (de Bernardi & Giussani, 1990; Klapes, 1990; Sivonen *et al.*, 1990; Rothhaupt, 1991; DeMott *et al.*, 1991; Forsyth *et al.*, 1992). In contrast to the vast knowledge about toxicity of cyanobacteria very little is known about toxicity of green algae (Ryther, 1954). In general, field and laboratory studies have shown that green algae are very suitable as food for cladoceran zooplankton, and therefore used universally in culture experiments (Vijverberg, 1989). Especially *Scenedesmus*, *Chlamydomonas* and *Rhodomonas* are considered appropriate food for cladocerans (Vijverberg, 1989), although the suitability as food varies between different cladoceran species. Ahlgren *et al.* (1990) reported that *Chlamydomonas sp.* was a high quality food for *Daphnia longispina*. *Eubosmina longispina*, however, showed no positive population growth on this food organism (see also Taub & Dollar, 1968; Infante & Litt, 1985; Lundstedt & Brett, 1991). In this study we present some evidence of the possible toxicity of *Scenedesmus obliquus* to *Daphnia* species. Both *S. obliquus* and other *Scenedesmus* species are very commonly used as food for zooplankton in culture experiments. (e.g. Kryutchkova & Sládeček, 1969; Vijverberg, 1976; 1989; Duncan *et al.*, 1985; Bohrer & Lampert, 1988; Elendt & Bias, 1990; Ahlgren *et al.*, 1990; Urabe, 1991), and are generally considered as high quality food organisms, although Vijverberg (1989) discussed the problem of sedimentation of the cells under static conditions at high food concentrations.

Material and methods

Scenedesmus obliquus and *Chlamydomonas globosa* were cultured axenically in a two litre continuous culture system, using a medium derived from de Haan *et al.* (1982) (Table 1). Light conditions consisted of 16 hours of illumination and 8 hours of darkness. Temperature of the algal culture vessels was approximately 20 °C. Sterile air was added to the culture vessels, using membrane pumps, with the CO_2 in the air as the main carbon source for the algae. *Ca* 200 ml of algal suspension of both *S. obliquus* and *C. globosa* were harvested

Table 1. Medium used to culture *Chlamydomonas globosa* and *Scenedesmus obliquus*. Concentrations are given in mg l^{-1}.

Compound	Concentration
KNO_3	250.00
$(NH_4)_2HPO_4$	15.00
$NaH_2PO_4.H_2O$	115.00
KH_2PO_4	10.00
$MgSO_4.7H_2O$	125.00
$CaCl_2.2H_2O$	5.00
Na_2EDTA	5.00
$FeNH_4(SO_4)_2.12H_2O$	4.30
$NaHCO_3$	7.50
H_3BO_3	0.038
$MnSO_4.H_2O$	0.106
$ZnSO_4.7H_2O$	0.179
$CuSO_4.5H_2O$	0.002
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.008
$Co(NO_3)_2.6H_2O$	0.025
$Na_3VO_4.14H_2O$	0.012

daily from the 'surplus' bottles, centrifuged twice for 20 minutes at 3000 r.p.m., and rinsed with distilled water. The algae were resuspended in 0.45 μm filtered lake water from Tjeukemeer, a shallow eutrophic lake in the northern part of the Netherlands (Beattie *et al.*, 1979). Using a haemocytometer, a minimum of 500 cells were counted to determine the concentration.

The *Daphnia* clones used for the experiments were caught in Tjeukemeer, transferred to the laboratory, and adapted to a 1:1 mixture of *S. obliquus* and *C. globosa*, with an algal carbon content of 1 mg C l^{-1}. Since survival of *D. cucullata* was low on this mixture, animals taken from the field were first reared on 30 μm filtered lake water, to produce a high number of newborns. These newborn were then reared on the algal mixture. The animals used in the experiments were the grandchildren of those caught from the lake. The effect of food concentration and food type on the mortality of *Daphnia* was tested with four food levels (0.04, 0.13, 0.25, and 2.5 mg C l^{-1} and three food combinations (100% *C. globosa* (CHLAM), 100% *S. obliquus* (SCENE), and a 1:1 mixture (CH-SC)). Since cells of *S. obliquus* are slightly smaller than *C. globosa* cells the number of cells per ml was ca. 10% higher for *S. obliquus*, resulting in equal carbon contents of the media. The experiments were carried out for *Daphnia galeata*, *D. cucullata* and for the hybrid between the two, *D. galeata x cucullata* (Spaak & Hoekstra, 1993). In total forty newborn per food type-concentration were used. Newborn were harvested twice a day, and put individually in 100 ml test tubes, with medium which was changed daily. The animals were removed when they reached their fourth adult instar. The experiments were carried out at 17.5 °C, with a light-dark cycle of 16 hours light, 8 hours dark. Mortality of the animals in the experiments was calculated as the percentage that died per day. Standard errors of these mortality values in the different series were computed using a Jackknifing method (Meyer *et al.*, 1986). The significance of the interactions between food type and food concentration was analysed using the Jackknife pseudo-values in an analysis of variance.

The experiments with the different species were carried out sequentially: starting with *D. galeata*, next *D. galeata x cucullata*, and finally *D. cucullata*. Each experiment lasted about a month. During the experiments the growing conditions of the algae apparently did not change: visual inspection of the cells showed no change in size or morphology, also algal concentrations in the cultures were relatively constant.

Results and discussion

In our experiments we found a distinct difference between the survival of the different species (Fig. 1). Mortality in the first experiment, with *D. galeata*, was low (Fig. 1a) (CHLAM: 0.9% d^{-1}, CH-SC 0.2% d^{-1}, SCENE 0.5% d^{-1}). The analysis of variance with the Jackknife pseudo-values revealed a non significant interaction, but a significant food type and concentration effect. Mortality was lowest on the CH-SC media (see also Boersma & Vijverberg, in prep. a). The significant effect of food level was caused by a somewhat higher mortality at the highest food level. The amount of mortality found for *D. galeata* corresponds well to the mortality of 1–2% d^{-1}, reported by Vijverberg (1989), for 'well-designed' cultures. Overall mortality for *D. galeata x cucullata* was higher (Fig. 1b). We observed a significant food type effect, and a significant interaction term. Mortality was highest on the SCENE media (9.3% d^{-1}), lower on the mixture (3.0% d^{-1}), and lowest on the CHLAM series (1.4% d^{-1}). No significant relationship was found between mortality and the food concentration. Mortality was highest in *D. cucullata*, with a significant food type, concentration and interaction effect (Fig. 1c). Mortality averaged 4.4% d^{-1} in the pure CHLAM cultures,

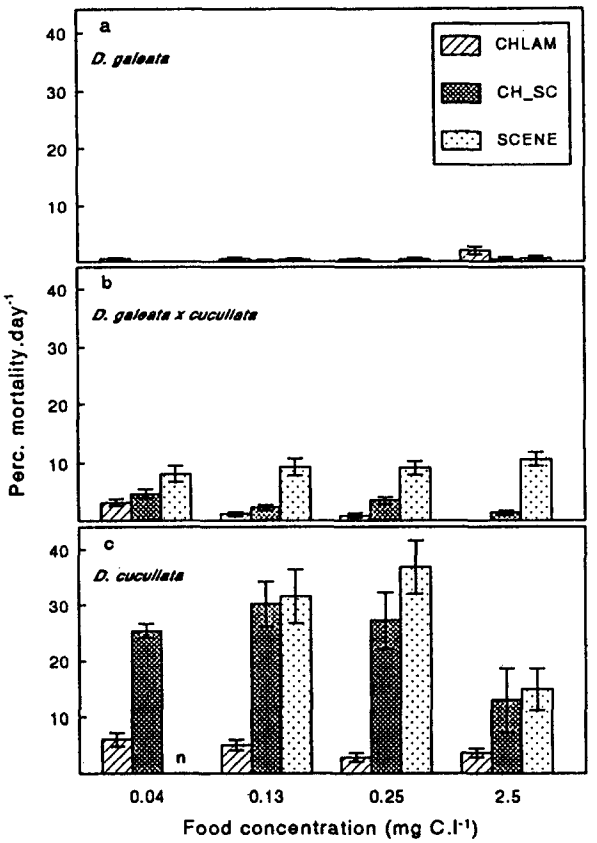

Fig. 1. Mortality (% d^{-1}) of *D. galeata* (a), *D. galeata x cucullata* (b), and *D. cucullata* (c) at different concentrations of *C. globosa* (CHLAM), *S. obliquus* (SCENE), and a 1:1 mixture of the two species (CH-SC). Error bars indicate standard errors (*n* = not available).

24.0% d^{-1}, in the CH-SC media and 27.7 % d^{-1} in the SCENE cultures.

The basis of our *Daphnia* culture medium was 0.45 μm filtered Tjeukemeer water, which might have changed during the experiments. However, it is very unlikely that the Tjeukemeer water is the cause for the high mortality, because the two algal species were suspended in the same water. Since it is known that high concentrations of nutrients may affect longevity in cladoceran zooplankton (Taub & Dollar, 1968) the algal culture medium was removed. Moreover, the composition of the culture medium for the algae was constant, and both algal species were treated exactly in the same way in all experiments. Therefore, we conclude that the cause for the increased mortality should be found within the *S. obliquus* cells.

It is unlikely, that the large differences in mortality between the three 'species' were caused by a different reaction to the *S. obliquus* cells. Although *D. cucullata* individuals seem to be more vulnerable to experimental conditions (Weider & Wolf, 1991) it is not likely that this is the cause for the higher mortality. To test this, we performed a second experiment with *D. galeata* during the *D. cucullata* experiment. *D. galeata* individuals tested with 0.25 mg C l^{-1} of *S. obliquus* showed a level of mortality comparable with the mortality found for *D. cucullata* at that time, *i.e.* 25% d^{-1}. From this we conclude that although algal culture conditions apparently did not change during the experiments, with temperature, light conditions and dilution rates of the flow-through cultures constant, the quality of the algae as food for daphnids did.

We propose three hypotheses for the high mortality of *Daphnia* individuals on *Scenedesmus* based media: First, the growth conditions of the algae could have changed in such a way that the nutritional value of the *S. obliquus* cells became very low. It has been demonstrated that nutritional values of algae vary with the growth condition of the algae. Ryther (1954) showed that individuals of *Daphnia magna* grew and survived better if fed on actively growing cells, compared with senescent cells, although he attributed this effect to the production of the antibiotic chlorellin. Mitchell *et al.* (1992) showed the same difference between log-phase cells of *Chlamydomonas reinhardtii* and nitrogen- or phosphorus-limited cells of the same species. However, if the nutritional value of *S. obliquus* cells were very low, one would expect a decrease in mortality with an increase in food concentration in the pure *S. obliquus* series, unless clearance rates of the animals were already maximal at the lowest food level, resulting in a constant ingestion. This, however, is not very likely, since growth on the lowest food concentration of *C. globosa* was much lower than growth on higher levels (Boersma & Vijverberg, in prep. b). Moreover, in the case of very low nutritive values of *S. obliquus* we would find a difference in mortality between the SCENE and the CH-SC series, which was not the case in the *D. cucullata* experiment. It is known that *Scenedesmus* cells may change their morphology as a result of the presence of *Daphnia*, leading to a decrease in digestibility of the algal cells for daphnids (Hessen & van Donk, 1993). However, as the media were refreshed daily this change is unlikely to have been important.

Secondly, a few reports exist about the effect of pH on the survivorship of cladocerans. O'Brien & deNoyelles (1972) reported that a small increase in pH caused a drastic increase in mortality in *Ceriodaphnia*

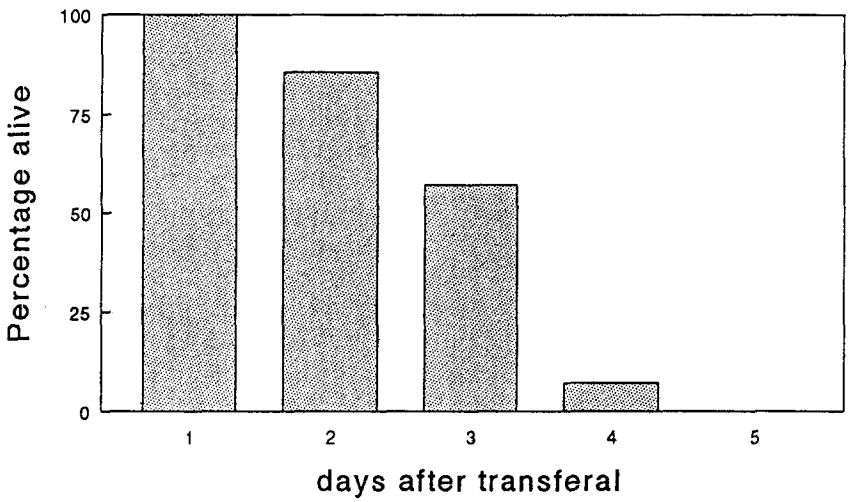

Fig. 2. Survival of *D. galeata x cucullata* taken from the highest level of the CHLAM medium, transferred to the highest concentration of the SCENE medium (2.5 mg C l^{-1}).

reticulata, i.e. a change in pH from 10.8 to 11.2 radically reduced survivorship, with relatively constant mortality at lower pH. Jeppesen *et al.* (1990) also found that a high pH under natural circumstances may suppress reproduction and survival of filter-feeding zooplankton. Consequently, the effect we observed could have been caused by an increase in pH, as result of very fast growing algae in the culture vessels (see also Walter, 1969) combined with the already relatively high pH of natural Tjeukemeer water (summer average around pH 9). However, if the effect found was caused by an effect of the growing *Scenedesmus* cells on the pH, one would expect that the effect found would increase with increasing densities of *S. obliquus* cells, which is not the case. Moreover, it is unlikely that the difference in growth rates between *C. globosa* and *S. obliquus* would be so large that the pH effect could only be observed in the *Scenedesmus* based media.

It seems that the most plausible explanation is that the cells of *S. obliquus* actually became poisonous for the daphnids. This is supported by the observation that large, well fed, individuals of the hybrid, which were reared on the highest concentration of *C. globosa*, and then transferred to *S. obliquus*, stopped reproducing immediately. Almost all animals died within four days after the transferal (Fig. 2), resulting in a mortality of *ca* 25% d^{-1}, which is even higher than the mortality of the animals actually cultured on the *Scenedesmus* media. This survival time is too short to be explained purely by the inedibility of the *S. obliquus* cells, and resulting starvation (Tessier *et al.*, 1983; Elendt, 1989), and hence apparently the cells contained or produced a substance deleterious for the daphnids.

We conclude that, although many workers showed that *S. obliquus* is an appropriate food item for cladoceran zooplankton, there may be circumstances in which it is not, even leading to toxicity of this algal species for *Daphnia*. This finding could explain the many conflicting results in determining the adequacy of different algal species as food for cladocerans.

Acknowledgements

The authors thank Aafje Landman, Theo Frank, and Diane Tischer for their assistance in the laboratory, and Ramesh Gulati, Onno van Tongeren, Karen Wiltshire, and Joop Ringelberg for their comments on the manuscript. These investigations were supported by the Foundation for Biological Research (BION), which is subsidized by the Netherlands Organization for Scientific Research (NWO).

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