Inhibitory effect of *Anabaena* sp. on *in situ* filtering rate of *Daphnia*

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We found that *in situ* filtering rates of *Daphnia* spp. measured in a lake containing *Anabaena* were significantly lower than those measured in a filament-free lake. Even after accounting for the depressing effects of high nannoplankton biomass concentration, filtering rates in the lake with *Anabaena* were 64% lower than those from the filament-free lake. We also found that filtering rates for *Daphnia pulex* in laboratory experiments were lower when *Anabaena* was present in experimental beakers than when *Chlorella* was present. When *Anabaena* was removed from Three Mile Lake water, filtering rates compared closely with predicted rates based on nannoplankton concentration and carapace length alone. Our analysis indicates that the presence of *Anabaena* filaments depresses *Daphnia* grazing rates in general, and that the filaments themselves are ingested at a lower rate than algae such as *Chlorella*.

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Les taux de filtration *in situ* de *Daphnia* spp. se sont avérés significativement plus faibles dans un lac qui contenait *Anabaena* que dans un lac qui n'en contenait pas. Même en tenant compte de la diminution encourue à cause de la présence d'une concentration élevée de biomasse nannoplanctonique, les taux de filtration sont restés de 64% plus faibles dans le lac qui contenait *Anabaena*. En laboratoire, les taux de filtration de *Daphnia pulex* se sont également avérés plus faibles en présence d'*Anabaena* qu'en présence de *Chlorella*. Après l'enlèvement d'*Anabaena* de l'eau du lac Three Mile, les taux de filtration ont atteint les valeurs prévues d'après la concentration de nannoplancton et la longueur de la carapace. Notre analyse démontre que la présence de filaments d'*Anabaena* réduit les taux de broutage de *Daphnia* en général et que les filaments eux-mêmes sont ingérés à un taux plus faible que les algues de type *Chlorella*.

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Introduction

Investigation of inhibitory effects of blue-green algal filaments on the grazing rates of *Daphnia* has rarely been carried out *in situ*; it has necessarily been restricted to experimentation under controlled laboratory conditions with cultured algae and animals (Burns 1968; Arnold 1971; Gliwicz and Siedlar 1980; Lampert 1981; Holm et al. 1983; Porter and McDonough 1984) or filaments isolated from lakes (Richman and Dodson 1983). Although field experiments are logistically more difficult to conduct, and less controllable, than laboratory experiments, field results are more realistic and can be applied directly without extrapolation.

The Haney (1971) technique was successfully applied *in situ* to study the grazing rates of a number of cladocerans in 10 lakes of south central Ontario that did not have *Anabaena* blooms during the summer (Chow-Fraser and Knoechel 1985). In this study, we also used the Haney method to measure *in situ* filtering rates of *Daphnia* spp. in Three Mile Lake, a lake in which *Anabaena* filaments occurred throughout the summer between July and September.

We used labelled *Chlorella* and *Scenedesmus* as in Chow-Fraser and Knoechel (1985) to study the effect of *Anabaena* in the environment on filtering rates of *Daphnia* in laboratory and field experiments. We also conducted parallel grazing experiments to examine the effect of using *Anabaena* as tracer on daphniid filtering rates. We thus show that the overall negative effect of *Anabaena* filaments results from both an interference on the grazing of edible algae as well as an apparent selection against filaments when feeding.

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Methods and materials

In situ *grazing experiments*

Three Mile and Gull lakes are large inland lakes located in south central Ontario. Three Mile Lake is relatively shallow compared with Gull Lake, and is more productive (Table 1).

Chlorella vulgaris (longest dimension 6–8 μm), *Scenedesmus ovalis* (unicellular form, longest dimension 4–6 μm), and *Anabaena* sp. (Carolina Biological Supply Co.) were labelled with ^{14}C. Vegetative cells of *Anabaena* were approximately 4 μm in diameter and ranged in length from 6 to 8 μm. Trichomes were approximately 80 to 120 μm in length.

Algae were prepared for ^{14}C-labelling with the forced uptake technique by first purging a 10-mL aliquot of log-phase algae of inorganic carbon (see Holtby and Knoechel (1981) for details). The solution was neutralized, and 10 μCi (1 μCi = 37 kBq) of $NaH^{14}CO_3$ was added. We calculated the final concentration in the grazing chamber to be 1000 cells·mL^{-1} for *Chlorella* or *Scenedesmus*, or 100 trichomes·mL^{-1} for *Anabaena*.

Amounts of *Chlorella* and *Scenedesmus* added to grazing chambers constituted less than 13% of natural nannoplankton concentration in Gull Lake and less than 10% of that in Three Mile Lake. Addition of *Anabaena* to Gull Lake was approximately 30% of natural nannoplankton biomass whereas that for Three Mile Lake was 15%. Biomass concentrations of tracer and natural filaments in Three Mile Lake were calculated to be 50 and 212 mg·m^{-3}, respectively.

All grazing experiments were conducted *in situ* at one station in the epilimnion of each lake (see Chow-Fraser and Knoechel (1985) for complete details of the grazing experiments). Ambient lake temperatures ranged from 20 to 22°C. Experiments were conducted once each in mid-July of 1981 and 1982 in Three Mile Lake, and once in August 1982 in Gull Lake.

Animals used in this study reflect the natural species mix of grazers in Three Mile and Gull lakes; *Daphnia* species in the lakes consisted of *D. galeata mendotae*, *D. pulex*, and *D. rosea*. Length measurements of *Daphnia* were taken from the base of the tail spine to the

TABLE 1. Description of study lakes

	Surface area (km^2)	Mean depth (m)	Chl *a* ($mg \cdot m^{-3}$)	Phytoplankton biomass concentration 0–10 μm*	10–30 μm*	Blue-green filaments
Three Mile Lake	7.9	3.5	7.5	56.5	125.7	211.9
Gull Lake	9.9	9.9	1.8	23.2	133.3	1.2

NOTE: Biomass concentrations of phytoplankton are in $mg \cdot m^{-3}$ (mass estimated from geometric solids presuming a specific gravity of 1). Chl *a* value is the mean summer value taken from Zimmerman et al. (1983) and Ontario Ministry of Natural Resources (Minden, Ontario, unpublished data).
*Longest linear dimension.

TABLE 2. Comparison of regression statistics for *in situ* experiments

	Algal tracer	*n*	Slope	SE	Intercept	SE	r^2	*P*
Three Mile Lake	*Chlorella*	157	1.22*a*	0.20	0.47*b*	0.04	0.20	0.0001
	Scenedesmus	115	1.65*a*	0.27	0.41*b*	0.04	0.26	0.0001
	Chlorella + *Scenedesmus*	272	1.30	0.15	0.43	0.03	0.22	0.0001
	Anabaena	11	1.80*a*	0.45	0.54*b*	0.10	0.44	0.0258
Gull Lake	*Scenedesmus*	10	2.01*a*	0.65	1.06*c*	0.08	0.65	0.0047
	Anabaena	11	2.13*a*	0.35	0.66*d*	0.04	0.77	0.0001

NOTE: The regression equations relate log filtering rate ($mL \cdot d^{-1}$) to carapace length (mm). Slope and intercept values followed by the same letter are statistically homogeneous ($P > 0.05$) according to an analysis of covariance. $P < 0.05$ (last column) indicates that the slope in that row is significantly different from zero.

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anteriormost point of the carapace, along the long axis of the body (referred to as "carapace length" in this study). Daily zooplankton filtering rate was calculated as in Chow-Fraser and Knoechel (1985).

Four-metre integrated phytoplankton samples were collected with a polyethylene hose (inner mouth diameter 1.5 cm; see Chow-Fraser and Knoechel (1985)) at the same station at which grazing experiments were conducted in each lake. Algae were identified and enumerated as described in Chow-Fraser and Knoechel (1985).

Laboratory experiments

We conducted four laboratory experiments to evaluate the effect of different tracer species on grazing rate measurements under controlled conditions. In these experiments we used *D. pulex*, captured from Three Mile Lake, that had been maintained in culture for 2 months. Approximately 35 animals, ranging in size from 0.7 to 2.5 mm were placed overnight in 1 L of 0.45 μm filtered lake water before the grazing experiments.

We placed unlabelled *Anabaena* in two beakers and unlabelled *Chlorella* in two others. *Daphnia* were acclimated in experimental beakers for 1 h. We then added ^{14}C-*Chlorella* to one of the *Chlorella* beakers and to one of the *Anabaena* beakers. Similarly ^{14}C-*Anabaena* was added to the other *Chlorella* and *Anabaena* beakers. Both species of labelled and unlabelled algae were added in equal biomass concentrations to each experimental beaker so that the final concentration in each approximated the equivalent of 2×10^4 *Chlorella* $cells \cdot mL^{-1}$. We assumed that one *Chlorella* cell has a biovolume of 200 μm^3 (a sphere with an approximate diameter of 7 μm) and that one *Anabaena* filament has 100 cells each of equivalent spherical diameter to a *Chlorella* cell. Animals grazed for 15 min and were processed as in the field grazing experiments.

We conducted a laboratory experiment to test directly for the adverse effect of filaments by removing *Anabaena* from Three Mile Lake water before grazing. *Daphnia g. mendotae*, *D. pulex*, and *D. dubia* were collected with a vertical townet (130-μm mesh) from the epilimnion of Three Mile Lake and transported in filtered lake water (64-μm mesh Nitex screen) to a field laboratory. *Anabaena* were removed by filtering lake water serially through Nitex screens (from 130 to 53 μm); subsequently we examined the water microscopically to ensure that most of the filaments had been removed. This procedure did not apparently affect the concentration of nannoplankton in the treated water since the amount of algae <30 μm in the treated and untreated water was 255 and 264 $mg \cdot m^{-3}$, respectively.

Twenty-five *Daphnia* of different species and sizes (chosen to include a range in size from 0.70 to 2.00 mm) were added to 1 L of this filtered water; only animals judged to be actively swimming were chosen for the experiment. The temperature of "filtered" lake water was similar to that of ambient lake water (22°C). Animals were acclimated for approximately 1 h in the beaker before grazing. Labelled *Scenedesmus* (approximately 20 $mg \cdot m^{-3}$) was then added to the container and animals were allowed to feed for 15 min. Other details of the grazing experiment were as described for *in situ* experiments.

Statistical analyses

Chow-Fraser and Knoechel (1985) found no statistical differences among filtering rates of several species of *Daphnia*. Therefore, species were combined in this study to increase sample sizes for regression analyses. Data from 13 *Chlorella* experiments, 10 *Scenedesmus* experiments, and 2 *Anabaena* experiments from Three Mile Lake were combined for respective regression analyses. Data from two each of *Anabaena* and *Scenedesmus* experiments from Gull Lake were likewise combined for regression analyses.

Least-squares regression equations relating filtering rate to carapace length (Sokal and Rohlf 1981) were computed for both field and laboratory data. We then used an analysis of covariance to determine statistical similarity among slopes and intercepts for the regression equations (using a procedure in Statistical Analysis Systems, Ray 1982).

Results

Since filtering rate (FR) of *Daphnia* varies with body length (*L*) (McMahon and Rigler 1965; Burns and Rigler 1967), we present our *in situ* grazing rates as a function of carapace length (Table 2). Regardless of the tracer used in Three Mile Lake and Gull Lake, slopes of the regression equations were statistically homogeneous, even though numerically they ranged from 1.22 to 2.01. The intercepts, however, were more variable.

Intercepts for Three Mile Lake regression equations were statistically homogeneous ($P > 0.05$), whether *Chlorella* or *Scenedesmus* was used as the tracer. The intercept of the Gull

TABLE 3. Comparison of regression statistics for laboratory experiments

Expt. No.	Algal species in beaker	Tracer species	n	Slope	SE	Intercept	SE	r^2	P
1	*Chlorella* only	*Chlorella*	35	1.19*a*	0.240	0.70*b*	0.058	0.45	0.0001
2	*Chlorella* + *Anabaena*	*Anabaena*	34	0.86*a*	0.425	0.31*c*	0.070	0.11	0.0500
3	*Chlorella* + *Anabaena*	*Chlorella*	32	1.49*a*	0.361	0.46*d*	0.096	0.34	0.0002
4	*Anabaena* only	*Anabaena*	35	1.18*a*	0.350	0.19*e*	0.100	0.26	0.0002

NOTE: The regression equations relate log filtering rate ($mL \cdot d^{-1}$) to carapace length (mm). Slope and intercept values followed by the same letter are statistically homogeneous ($P > 0.05$) according to an analysis of covariance. $P < 0.05$ (last column) indicates that the slope in that row is significantly different from zero.

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Lake – *Scenedesmus* equation, however, was statistically higher than that for the Three Mile Lake – *Scenedesmus* equation ($P < 0.01$), indicating that a 1-mm Gull Lake animal would filter 11.5 $mL \cdot d^{-1}$ compared with 2.6 $mL \cdot d^{-1}$ for a Three Mile Lake animal.

One reason for the lower intercept for Three Mile Lake relative to Gull Lake may be the higher nannoplankton biomass in Three Mile Lake since filtering rate varies inversely with nannoplankton concentration (Geller 1975; Chisolm et al. 1975). Accounting for high nannoplankton concentrations by using Chow-Fraser and Knoechel's (1985) empirical relationship between filtering rate, carapace length, and nannoplankton concentration (Eq. 3 in their study) yields a mean filtering rate of 7.5 $mL \cdot d^{-1}$ for a 1-mm animal in Three Mile Lake. This is still higher than the 2.7 $mL \cdot d^{-1}$ (using combined equation, Table 2) typical of the Three Mile Lake animals, and it is our contention that this 60% reduction in filtering rate is due to high *Anabaena* concentrations in Three Mile Lake.

To test this directly, we removed filaments from Three Mile Lake water before conducting a laboratory grazing experiment. We used freshly caught *Daphnia* from Three Mile Lake to make the experiments more realistic. Regression of filtering rate against carapace length for this experiment yielded the following regression equation:

$$[1] \quad \log FR = 2.11\ (\pm 0.809) \log L + 0.82\ (\pm 0.190)$$

($r^2 = 0.20$; $P = 0.02$; numbers in parentheses are standard errors). This slope was statistically homogeneous with those in Table 2 for Three Mile Lake ($P = 0.25$, analysis of covariance). The higher intercept, however, means that a 1.0-mm *Daphnia* would filter 6.6 $mL \cdot d^{-1}$, a value more consistent with the 7.5 $mL \cdot d^{-1}$ predicted above for filament-free water with the same nannoplankton concentration, but still 144% higher than the 2.7 $mL \cdot d^{-1}$ typical of Three Mile Lake *Daphnia*. This provides further evidence that filaments in Three Mile Lake are responsible for reduced *in situ* daphniid grazing rates.

We next conducted a series of field experiments to study the effect of using *Anabaena* as tracer since we wished to determine whether or not filaments could be ingested at the same rate as *Chlorella* or *Scenedesmus*. In Three Mile Lake, intercepts of filtering rate regressions measured with ^{14}C-labelled *Anabaena* were statistically similar to those measured with labelled *Scenedesmus* and *Chlorella* (Table 2); however, in Gull Lake the *Anabaena* intercept was statistically lower than that determined with labelled *Scenedesmus*. Since these results were discrepant, we conducted four laboratory experiments to further examine the effect of using *Anabaena* as tracer under controlled experimental conditions (Table 3).

Experimental treatment did not affect the slopes of the filtering rate – length regression equations (Table 3, $P = 0.10$). However, in identical environments with respect to unlabelled algae, intercepts were statistically lower when labelled *Anabaena* was used than when labelled *Chlorella* was used (expt. 1 compared with expt. 2; expt. 3 compared with expt. 4), indicating that *Daphnia* handle and ingest *Anabaena* filaments at lower rates than *Chlorella*. The intercept for expt. 1 was significantly higher than that for expt. 3; filtering rates in the absence of *Anabaena* filaments in lake water are 74% higher. Thus filaments interfered with the grazing of edible algae such as *Chlorella*, and this is entirely consistent with the 58% increase in filtering rate in our filament-removal experiment (Eq. 1). These results substantiate the observations of others that blue-green filaments cause a mechanical inhibition of filtering rates (Burns 1968; Gliwicz and Siedlar 1980; Porter and McDonough 1984).

The difference in intercepts between expts. 2 and 3 is further evidence that filaments were processed at lower rates than were *Chlorella*, given identical environments with respect to algal concentration and composition. Filtering rates measured in expt. 3 reflect the mechanical inhibition of the rate of grazing on other algae caused by *Anabaena* whereas those measured in expt. 2 reflect the grazing rate using *Anabaena* as tracer. As would be expected, the intercept for expt. 4 is lowest overall since it reflects the combined effect of mechanical inhibition as well as the lower grazing rate when using *Anabaena* as tracer.

Discussion

Although slopes of filtering rate – length regressions for *Daphnia* were the same in Three Mile and Gull lakes, intercepts were consistently lower in Three Mile Lake. This indicates that, for a given-sized *Daphnia*, *in situ* filtering rates are lower in Three Mile Lake than in Gull Lake. We have provided four lines of evidence that the presence of *Anabaena* filaments is the cause of this inhibition. First, *in situ* grazing rates measured with labelled *Scenedesmus* in Three Mile Lake were statistically lower than those measured with the same tracer species in Gull Lake (Table 2). Second, *in situ* grazing rates in Gull Lake were lower when *Anabaena* was used as tracer than when *Scenedesmus* was used. Third, we found that when *Anabaena* were removed from Three Mile Lake water, filtering rates increased by 144%. Fourth, we found that filtering rates of *D. pulex* in laboratory experiments were lower in the presence of *Anabaena*. The only evidence not consistent with this explanation is the observation that *in situ* Three Mile Lake filtering rates were not lower when *Anabaena* was used as a tracer (Table 2); we have no explanation for this.

The presence of *Anabaena* in lake water appeared to have a twofold effect on the grazing rate of *Daphnia*. It interfered with the rate of filtering edible algae such as *Chlorella*. In addition, grazing rates measured with labelled *Anabaena* were lower than

those measured with labelled *Chlorella*, and we suggest that *Anabaena* was being processed at a lower rate than edible algae.

The role of *Anabaena* in the diet of *Daphnia* may be greater than is presently considered since we found measurable, albeit, lower grazing rates in all of our experiments. This contrasts with the observations of Sorokin et al. (1965) and Porter (1975) that *Anabaena* was absent from the gut contents of *Daphnia*, but is consistent with Schindler's (1971) observation that *Daphnia* assimilated up to 50% of ingested *Anabaena*. Ingestion of *Anabaena* was probably not by chance since *Daphnia* can discriminate against unicells of *Anabaena* on the basis of taste (Porter and Orcutt 1980).

Future studies involving high-speed cinematography should greatly enhance our understanding of how the presence of *Anabaena* causes a reduction in the rate of filtering edible algae, e.g., whether it is a toxic or a mechanical inhibition. Further investigation should be undertaken in other lakes so that a complete picture of inhibition in the field can be constructed.

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ARNOLD, D. E. 1971. Ingestion, assimilation, survival and reproduction by *Daphnia pulex* fed seven species of blue-green algae. Limnol. Oceanogr. **16**: 906–921.

BURNS, C. W. 1968. Direct observations of mechanisms regulating feeding behavior of *Daphnia* in lakewater. Int. Rev. Gesamten. Hydrobiol. **53**: 83–100.

BURNS, C. W., and F. H. RIGLER. 1967. Comparison of filtering rates of *Daphnia rosea* in lakewater and in suspension of yeast. Limnol. Oceanogr. **12**: 492–502.

CHISOLM, S. W., R. G. STROSS, and P. A. NOBBS. 1975. Environmental and intrinsic control of filtering and feeding rates in Arctic *Daphnia*. J. Fish. Res. Board Can. **32**: 219–226.

CHOW-FRASER, P., and R. KNOECHEL. 1985. Factors regulating in situ filtering rates of Cladocera. Can. J. Fish. Aquat. Sci. **42**: 567–576.

GELLER, W. 1975. Food ingestion of *Daphnia pulex* as a function of food concentration, temperature, animal's body length and hunger. Arch. Hydrobiol. Suppl. **48**: 47–107.

GLIWICZ, Z. M., and E. SIEDLAR. 1980. Food size limitation and algae interfering with food collection in *Daphnia*. Arch. Hydrobiol. **88**: 155–177.

HANEY, J. F. 1971. An *in situ* method for the measurement of zooplankton grazing rates. Limnol. Oceanogr. **16**: 970–977.

HOLM, N. P., G. C. GANF, and J. SHAPIRO. 1983. Feeding and assimilation rates of *Daphnia pulex* fed *Aphanizomenon flos-aquae*. Limnol. Oceanogr. **28**: 677–687.

HOLTBY, B., and R. KNOECHEL. 1981. Zooplankton filtering rates: error due to loss of radioisotopic label in chemically preserved samples. Limnol. Oceanogr. **26**: 774–779.

LAMPERT, W. 1981. Inhibitory and toxic effects of blue-green algae on *Daphnia*. Int. Rev. Gesamten. Hydrobiol. **66**: 285–298.

MCMAHON, J. W., and F. H. RIGLER. 1965. Feeding rates of *Daphnia magna* Straus in different food labelled with radioactive phosphorus. Limnol. Oceanogr. **10**: 105–113.

PORTER, K. G. 1975. Variable gut passage of gelatinous green algae ingested by *Daphnia*. Verh. Int. Ver. Theor. Angew. Limnol. **19**: 2840–2850.

PORTER, K. G., and R. MCDONOUGH. 1984. The energetic cost of response to blue-green filaments by cladocerans. Limnol. Oceanogr. **29**: 365–369.

PORTER, K. G., and J. D. ORCUTT, JR. 1980. Nutritional adequacy, manageability, and toxicity as factors that determine the food quality of blue-green algae of *Daphnia*. Am. Soc. Limnol. Oceanogr. Spec. Symp. **3**: 268–281.

RAY, A. A. (*Editor*). 1982. Statistical Analysis System user's guide: statistics. SAS Institute, Inc., Cary, NC.

RICHMAN, S., and S. I. DODSON. 1983. The effect of food quality on feeding and respiration by *Daphnia* and *Diaptomus*. Limnol. Oceanogr. **28**: 948–956.

SCHINDLER, J. E. 1971. Food quality and zooplankton nutrition. J. Anim. Ecol. **40**: 589–595.

SOKAL, R. R., and F. J. ROHLF. 1981. Biometry. 2nd ed. W. H. Freeman and Co., San Francisco, CA.

SOROKIN, JU. I., A. V. MONAKOV, E. D. MORDUKHAI-BOLTOVSKAYA, E. A. TSIKHON-LUKANINA, and R. A. RODOVA. 1965. Application of C^{14} method for studies of trophic role of blue-green algae. (In Russian.) *In* Ecology and physiology of blue-green algae. Akad. Nauk SSSR, Moscow. pp. 235–239.

ZIMMERMAN, A., K. M. NOBLE, M. A. GATES, and J. E. PALOHEIMO. 1983. Physicochemical typologies of south-central Ontario lakes. Can. J. Fish. Aquat. Sci. **40**: 1788–1803.

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