Université de Montréal

Interactions écologiques et toxicologiques entre le zooplancton et le phytoplancton dans les lacs eutrophes

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Thèse présentée à la Faculté des études supérieures en vue de l'obtention du

Grade de docteur ès sciences (Ph.D.)

En sciences biologiques

Décembre 2001

Université de Montréal

Faculté des études supérieures

Cette thèse intitulée

Interactions écologiques et toxicologiques entre le zooplancton et le phytoplancton dans les lacs eutrophes

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Résumé en Français

La présente thèse traite d'une étude à plusieurs échelles spatio-temporelles des interactions entre le zooplancton et le phytoplancton dans les lacs eutrophes, dominés par les cyanobactéries coloniales et filamenteuses. L'objectif de cette étude était d'évaluer les effets de l'eutrophisation, suite aux activités anthropiques (i.e., déforestation), sur les communautés zooplanctoniques des lacs. Une approche expérimentale a été utilisée afin d'évaluer la réponse du zooplancton à l'augmentation de la biomasse des cyanobactéries coloniales et filamenteuses. Pour ce faire, la biomasse des cyanobactéries a été expérimentalement augmentée par l'ajout d'éléments nutritifs (P, N) dans le cadre d'une expérience *in situ* en enclos limniques. L'augmentation de la biomasse des algues non-ingérables, particulièrement dans les enclos peu profonds, a causé une diminution de la structure en taille et de la biomasse des cladocères. Les mêmes patrons ont été observés dans le cadre d'une expérience de déboisement des bassins versants des écosystèmes lacustres eutrophes de l'Alberta (Programme TROLS) où la structure en taille et la dominance des cladocères ont diminué en faveur des plus petits organismes (i.e., copépodes) à la suite de l'augmentation de la biomasse des cyanobactéries. L'examen par vidéo-cinématographie à une échelle fine des interactions entre les cladocères (i.e., *Daphnia*) et les cyanobactéries a révélé que les baisses observées dans la taille et la biomasse des cladocères lors des expériences en enclos pouvaient être reliées à la réduction des activités alimentaires des daphnies suite à l'augmentation du nombre des filaments et colonies de cyanobactéries. Les daphnies ont aussi montré une inhibition de leur activité alimentaire à la suite de l'exposition à la microcystine-LR. Cependant, nos résultats suggèrent que la cause principale de la réduction alimentaire dans le milieu naturel est la présence de colonies et filaments de cyanobactéries en

grand nombre dans l'environnement immédiat des cladocères. D'un autre côté, nos expériences ont révélé la présence d'une réponse phénotypique à l'augmentation des algues non-ingérables. En effet, la morphométrie et la structure des appendices de filtration ont montré des changements chez les cladocères qui ont été exposés à de fortes biomasses de cyanobactéries. Ceci suggère que les daphnies ont la capacité de répondre par des changements phénotypiques à l'augmentation des algues non-ingérables. Cependant, les implications de cette plasticité phénotypique au niveau des communautés à long terme reste à déterminer. Ainsi, l'examen détaillé des interactions cyanobactéries-zooplancton montrent que les communautés zooplanctoniques peuvent répondre à l'eutrophisation au niveau de l'individu, de la population et de la communauté en fonction de la durée de l'exposition. La compréhension de la nature des réponses des communautés biologiques aux perturbations environnementales comme l'eutrophisation peut ainsi améliorer notre connaissance des écosystèmes et aider à l'implantation de meilleurs outils de gestion durable.

Mots clés: Eutrophisation, phytoplancton, cyanobactéries, microcystines, zooplancton, cladocères, *Daphnia*, lacs.

Résumé en anglais

The present thesis is a multiscale study of the interactions between zooplankton and phytoplankton in eutrophic cyanobacteria-dominated lakes. The main objective of this study was to evaluate the effects of increasing trophy, following watershed disturbance (i.e, forest harvesting), on zooplankton community dynamics. An experimental approach, at several temporal and spatial scales, was used to evaluate the response of zooplankton communities to the increase in biomass of colonial and filamentous cyanobacteria species in lakes. Cyanobacterial blooms were experimentally induced in large enclosures by adding different amount of nutrients (N, P). The addition of nutrients produced different responses of the phytoplankton as a function of light climate, whereby cyanobacteria biomass increased in shallow enclosures but not in deep enclosures. The increase in the biomass of inedible algae produced a reduction in the size structure and the biomass of large zooplankters (i.e., daphnids). The same patterns were found in lakes which were subject to a major ecosystemic harvesting experiment (TROLS), where cladoceran size structure and dominance were reduced in favor of small zooplankters (i.e., copepods) after the increase in cyanobacteria biomass. An examination of the cyanobacteria-zooplankton interaction at fine scale, revealed that the decline in cladoceran size and biomass may be related to feeding inhibition caused by the increase in large colonial and filamentous cyanobacteria and other inedible algae. Although the response to the addition of purified microcystin-LR to the food of daphnids revealed the same inhibitory effects, our results point more toward feeding inhibition by colonies and filaments as a cause of the changes in the communities. On the other hand, the examination of the filtering appendages of daphnids exposed to large biomass of cyanobacteria biomass revealed an increase in the size and changes in the ultrastructure of those filtering

appendages. These results suggest that daphnids have the ability to respond phenotypically to the changes in their resources as an adaptative strategy. However, the implications of these phenotypic changes on the whole community, on the long term, are yet to be elucidated. The examination of cyanobacteria-zooplankton interactions revealed that the response of zooplankton communities to changes in their resources, from edible to inedible algal particles, can be displayed at the individual, population or community levels as a function of the duration of the perturbation. Understanding the nature of the response of biological communities (i.e., zooplankton) to perturbations such as eutrophication improve our knowledge of the ecosystems and help the implementation of better management strategies.

Key words: Eutrophication, phytoplankton, cyanobacteria, microcystins, zooplankton, cladoceran, *Daphnia*, lakes.

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Références bibliographiques

Cette thèse est dédiée à
mon cercle d'amour inconditionnel
mes parents Ahmed et Myriam
ma sœur Faïza
mes frères Tarik et Chakib el Mokhtar
et à ma conjointe
Karyne

Remerciements

Je tiens à présenter mes sincères remerciements à ma directrice de recherche, Bernadette Pinel-Alloul, pour son soutien et son support tout au long de la réalisation de cette thèse de doctorat. Ses conseils éclairés et sa disponibilité exemplaire m'ont grandement aidés durant chacune des étapes importantes de ce projet. Je tiens à souligner ses qualités humaines exceptionnelles qui ont rendu mon séjour dans son laboratoire des plus agréable. Je la remercie aussi pour m'avoir permis de participer à plusieurs rencontres scientifiques nationales et internationales qui ont grandement stimulé mon intérêt pour la recherche et m'ont permis d'exposer ma recherche à la grande communauté scientifique.

Je voudrais aussi remercier ma co-directrice, Ellie E. Prepas, pour avoir supporté mon travail de recherche lors de mon séjour à l'Université de l'Alberta et plus particulièrement à la station de biologie de Meanook. De nombreuses discussions avec elle, m'ont aidé à mener à bien mes expériences dans les lacs eutrophes de l'Alberta. Merci de m'avoir permis de participer à de nombreuses réunions durant lesquels j'ai compris l'importance de la multidisciplinarité en recherche scientifique.

Je tiens aussi à exprimer mes sincères remerciements aux membres de mon comité conseil, Antonella Cattaneo et Warwick Vincent, pour leurs conseils et leur soutien tout au long de ce projet.

Le directeur de l'institut Max-Planck de limnologie (Plön, Allemagne), Winfried Lampert, en m'invitant à un séjour de recherche m'a aidé à réaliser une bonne partie de cette recherche dans un cadre très stimulant sur le plan de la technologie de recherche mais aussi sur le plan humain. Je tiens à le remercier pour m'avoir permis d'interagir avec les membres de son institut.

Mes sincères remerciements vont aussi à Ginette Méthot et Emma Ivania Mangas pour leur participation et leur aide lors de l'analyse des échantillons de zooplancton pour les différents projets que j'ai menés. Je les remercie aussi pour leur amitié et leur bonne humeur que j'ai pu appréciées lors de nos repas quotidiens pendant plusieurs années. Ces moments, auxquels Bernadette participait aussi, vont me manquer.

Je remercie aussi tous les étudiants gradués et post-docs pour les nombreux échanges et discussions lors des cours gradués, séminaires et journal clubs. Je remercie particulièrement Carol Avois, Caroline Bouffard, Edenise

Garcia, Sébastien Lamontagne, Yves Couillard, Matthew Norton, Alain Patoine, et Olivier Perceval.

Je voudrais aussi remercier tous les membres du secrétariat de biologie et de la bibliothèque EPC-Biologie pour m'avoir aidé à de nombreuses occasions tout au long de mon séjour dans ce département. Les membres du service de références, Adèle Brodeur, Ginette Fafard et Marc Waller, m'ont toujours permis d'obtenir tous les outils bibliographiques dont j'avais besoin. Je tiens à les remercier pour un service des plus sympathique. Je tiens aussi à remercier Lucie Tittely, Nelly Banon, Louise Pelletier, Jean-Luc Verville et Hélène Lavigne qui m'ont toujours fourni un service exemplaire; soit pour mes nombreuses activités d'enseignements ou pour mes activités de recherche.

Je tiens particulièrement à remercier mes parents, Ahmed et Myriam, ma sœur Faïza, et mes frères Tarik et Chakib el Mokhtar pour leur soutien tout au long de mes études doctorales. Depuis le début de mes études, tous les membres de ma famille ont contribué au financement de mes études et n'ont jamais cessé de m'encourager durant ces nombreuses années. Je tiens aussi à remercier mes grands-parents Abdelkader et Halima, ainsi que mes oncles Driss, Miloudi, Larbi, Abdellah et Mohamed pour leur soutien.

Je tiens finalement à exprimer mon amour pour ma conjointe Karyne, avec qui je vis les plus beaux moments de ma vie. Je voudrais la remercier pour sa patience et sa compréhension durant les périodes les plus difficiles de mes études doctorales. Son talent pianistique m'a ouvert au merveilleux monde de la musique classique. Sa grande sensibilité et sa belle personnalité m'ont fait découvrir de nouveaux aspects de la vie. Je tiens aussi à remercier ma belle-mère, Christiane Godbout, qui nous a toujours soutenu, aidé et aimé, Karyne et moi. Merci aussi à ma famille québécoise formée de l'oncle et la tante de Karyne, Jean-Georges Major et Marie-Claire Fillion, ses cousins, Solange et Stéphane qui m'ont initié aux traditions du Québec lors d'inoubliables célébrations de Noël et ce depuis plusieurs années. Je tiens à les remercier pour l'accueil qu'ils m'ont réservé dès le premier Noël en leur compagnie.

Chapitre 1

Introduction générale

Les historiens ont rapporté que le troisième consul de Rome, *Lucius Cassius Longinius*, avait ordonné la construction d'une route pour permettre a son armée des mouvements rapides afin de décourager la dissidence rurale. La route fut construite sur le bassin versant d'un petit lac, *Lago di Monterosi*, qui ne tarda pas à développer d'importante biomasse algale à la suite de l'infiltration d'éléments nutritifs (Hutchinson 1970). Ce phénomène est connu sous le nom d'eutrophisation. On attribut ce terme aux premiers limnologistes qui l'avaient défini comme l'augmentation des substances nutritives, particulièrement le phosphore (P) et l'azote (N), dans les eaux du lac (Naumann 1931). L'eutrophisation, pour ainsi dire, n'est pas un phénomène nouveau. Plusieurs autres recherches rapportent le contexte historique de l'eutrophisation dans

plusieurs lacs européens à la fin du 19ième et début du 20ième siècle. Hastler (1947) rapporte plusieurs de ces cas historiques, dont notamment ceux des lacs *Windermere* (Angleterre), *Växjö* (Suède), *Ströms-Vattudal* (Finland), *Hallwilersee, Rotsee*, et *Zürichsee* (Suisse). Pour ce dernier, le lac *Zürichsee*, l'auteur rapporte plusieurs travaux limnologiques très détaillés, principalement de Minder (Minder 1918, 1926), documentant l'eutrophisation d'un des bassins du lac *Zürichsee* sous l'effet de l'enrichissement des eaux causé par les effluents domestiques d'une petite communauté non loin de la ville de Zürich. Minder (Minder 1926) était convaincu que l'eutrophisation du bassin lacustre (Untersee) était causée par l'augmentation des apports en P et N par les effluents domestiques.

Bien que l'eutrophisation ne soit pas un phénomène nouveau, ce n'est qu'au début des années 1960 que le problème s'est aggravé suite à l'augmentation démographique et l'intensification des activités industrielles et agricoles. L'arrivée sur le marché des fertilisants agricoles a donné au problème une envergure mondiale. Considérant les effets de l'eutrophisation sur le plan socio-économique, on a commencé alors à s'intéresser de façon plus systématique au phénomène et à ses causes immédiates. Le rôle des éléments nutritifs, principalement le P et N tel que soupçonné par les premiers limnologistes (Minder 1918, 1926, Naumann 1931), s'est confirmé suite à la

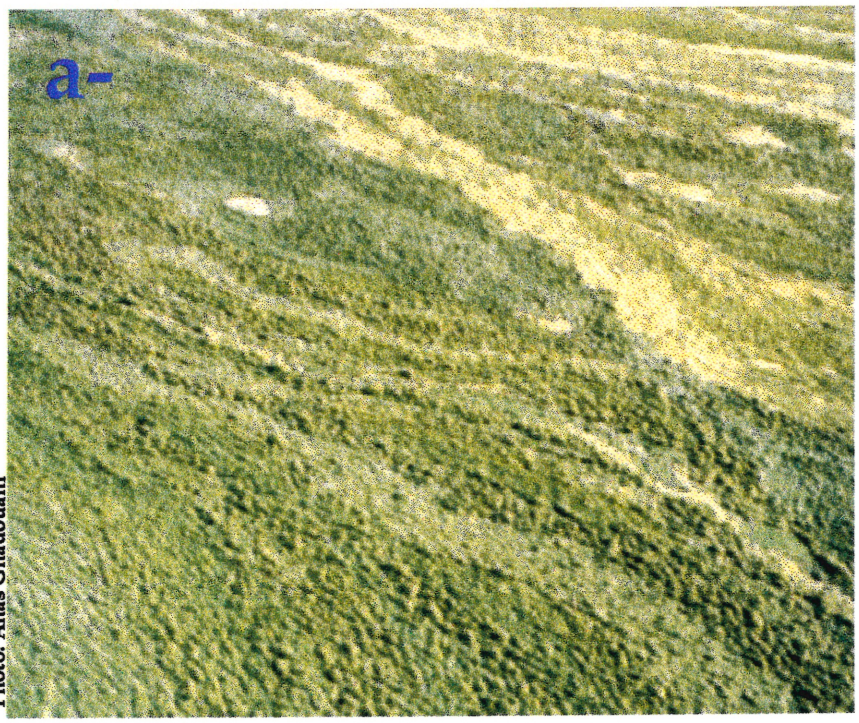
publication de l'article de Sakamoto (1966) et surtout du rapport de Vollenweider (1968). Le modèle proposé par Vollenweider (1968) a permis d'introduire la notion de prédiction de l'augmentation du niveau trophique sur la base de l'augmentation des charges de P. Ceci a été considéré comme une importante avancée en limnologie, considérant les implications de ce modèle à la fois dans la gestion des systèmes aquatiques mais aussi dans les études les plus fondamentales sur le rôle des éléments nutritifs. Depuis, ce modèle a été beaucoup étudié et raffiné ce qui a servi au développement de la plupart des outils de gestions modernes (Kalff 2002). Un peu plus tard, le rôle du phosphore comme facteur limitant de la productivité algale est démontré à l'aide des expériences de fertilisation réalisées dans la région expérimentale des lacs en Ontario, Canada (Experimental Lake Area) et dont l'impact a été décisif sur le plan politique (Schindler 1977).

Depuis, l'eutrophisation des systèmes aquatiques, incluant les lacs, réservoirs, eaux courantes, et plus récemment les zones côtières marines, continue à s'accroître. Particulièrement dans les systèmes d'eaux douces l'eutrophisation se manifeste souvent par l'augmentation de la dominance d'un groupe particulier d'algues appelé les cyanobactéries (anciennement algues bleues ou encore bleue-vertes). Les cyanobactéries ont la particularité de dominer de façon presque totale (> 90%) la biomasse du phytoplancton lors des

évènements spectaculaires de floraisons algales ou blooms qui sont souvent observés dans les lacs eutrophes. Les floraisons algales (blooms) sont le résultat d'un développement inhabituel d'importante biomasse algale qui s'accumule à la surface de l'eau (Wetzel 2001, Kalff 2002). Bien que les floraisons algales ne soient pas exclusives aux cyanobactéries, ces phénomènes sont souvent associés avec ce groupe d'algues et particulièrement les espèces coloniales et filamenteuses comme *Microcystis aeruginosa, Anabaena flos-aquae, Aphanizomenon flos-aquae* et *Oscilatoria sp.* Ces espèces sont de plus en plus étudiées à cause de leur présence massive lors des floraisons algales; elles contribuent de façon significative à la dégradation de la qualité récréative des systèmes aquatiques à cause de l'aspect visuel (photo 1) et des mauvaises odeurs produites par ces floraisons.

Un autre aspect qui retient l'attention des chercheurs est celui des substances toxiques souvent produites par les espèces de cyanobactéries lors des évènements de floraisons. En effet, ces espèces coloniales et filamenteuses sont capables de produire plusieurs formes des toxines appelées microcystines (Carmichael 1992). Ces toxines sont des peptides cycliques produits principalement par les espèces du groupe des cyanobactéries. On en dénombre plus d'une cinquantaine de formes différentes (Carmichael 1994, Reinehart et al. 1994). La plupart de ces cyanotoxines appartiennent à la famille des

Photo 1. a) Accumulation de couches épaisses de cyanobactéries en décomposition sur le bord du lac Steele (Alberta, Canada) pendant des évènements de floraisons de cyanobactéries pendant l'été 1996. b)- Floraisons de cyanobactéries dans la zone la plus profonde du lac Steele (Alberta, Canada) pendant les floraisons de cyanobactéries pendant l'été 1996. Les flèches montrent les 'flocons' mesurant plusieurs centimètres formés par les filaments de *Aphanizonenom flos-aquae*. (Photo: Anas Ghadouani).



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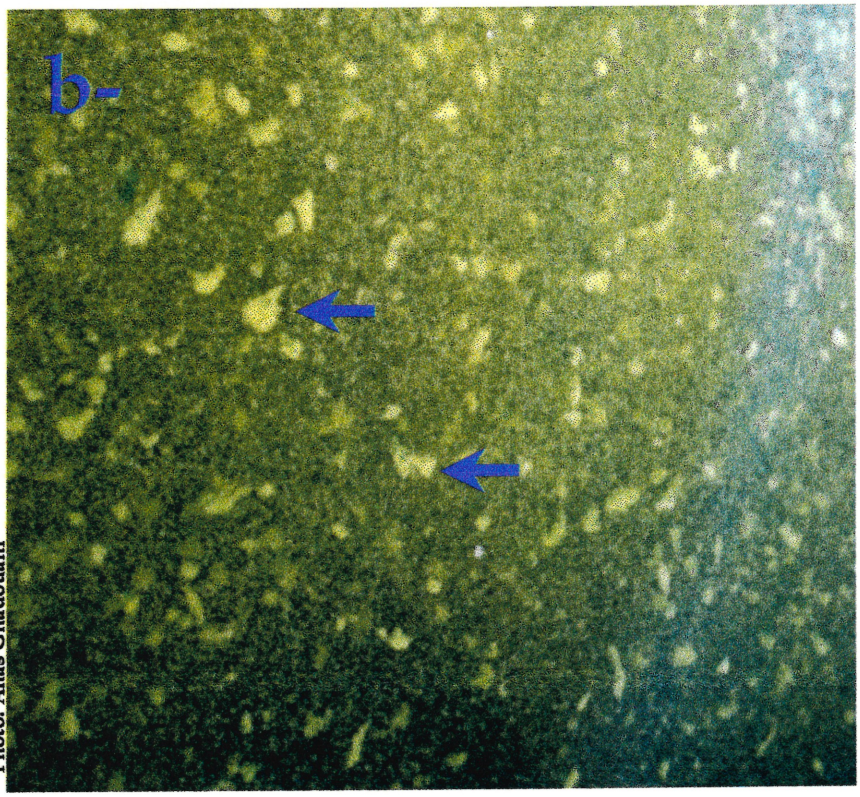


Photo: Anas Ghadouani

microcystines qui sont principalement hépatotoxiques, cependant, d'autres formes appartenant aux familles des anatoxines ou saxotoxines peuvent être neurotoxiques (Carmichael et al. 1988, Rapala et al. 1993, Codd 1995). Plusieurs études ont documenté des cas d'intoxication d'animaux à la suite de contacts avec les floraisons de cyanobactérie (Codd 1995, Christoffersen 1996). Cependant, en février 1996, la contamination de l'eau d'une clinique au Brésil par des toxines de cyanobactéries a causé la mort de 26 patients suite à de graves dommages au foie (Jochimsen et al. 1998). Cette tragédie a attiré l'attention des autorités sanitaires internationales et a conduit à l'établissement de normes par l'organisation mondiale de la santé. Il faut cependant noter que cette tragédie s'est produite à la suite de l'introduction accidentelle d'une quantité importante de microcystines dans les produits sanguins qui étaient administrés aux patients (Jochimsen et al. 1998). Santé Canada publie régulièrement des mises en garde à l'intention des utilisateurs de lacs en précisant que le contact normal avec les floraisons de cyanobactérie (baignade, douche etc..) ne causerait que des problèmes gastriques mineurs.

Considérant l'étendue du problème d'eutrophisation et des floraisons des cyanobactéries, des questions fondamentales sont posées à la limnologie moderne. Ces questions sont reliées à notre capacité de prédire les floraisons de cyanobactérie et leurs impacts sur les différents compartiments biologiques des

écosystèmes lacustres. En effet, notre connaissance actuelle des conditions favorables au développement des floraisons de cyanobactérie est très limitée. Tous les modèles actuels de prédiction des floraisons de cyanobactérie se basent sur une seule étude empirique qui stipule que les faibles ratios N:P sont favorables au développement des cyanobactéries (Smith 1983). En effet, la capacité de fixer l'azote atmosphérique chez certaines espèces de cyanobactéries leur donnerait un avantage de compétitif sur les autres groupes d'algues (Smith 1983, 1986). Bien que cette étude soit basée sur l'approche théorique de la compétition pour les ressources, de plus en plus utilisée en écologie (Tilman 1977), son pouvoir de prédiction reste limité en milieu naturel. Plusieurs études rapportent que les variations dans les apports en P prédisent mieux la prolifération des cyanobactéries que le ratio N:P (Trimbee and Prepas 1987, Downing et al. 2001). D'autres études, basées sur une combinaison de travaux expérimentaux et de modélisations mathématiques, suggèrent que les facteurs physiques comme la lumière et la stabilité de la colonne d'eau seraient des variables importantes pour la prolifération des cyanobactéries (Smith 1986, Huisman 1999, Huisman et al. 1999a, 1999b). Ainsi, il apparaît clairement qu'il existe une controverse sur les variables qui seraient à même de prédire les floraisons de cyanobactérie.

D'un autre côté, il est important de connaître les répercussions de l'augmentation des cyanobactéries sur les autres compartiments biotiques des lacs. L'interface phytoplancton-zooplancton joue un rôle capital dans le fonctionnement des réseaux trophiques (Fig. 1) et dans le transfert de l'énergie (Reynolds 1994). Cependant, il existe une importante controverse en écologie aquatique qui est très liée au phénomène de prolifération des cyanobactéries et peut être même causée par celui-ci. En effet, une des techniques de contrôle d'eutrophisation qui se base sur le principe de control descendant (Top-down) introduite pour la première fois sous le nom de biomanipulation par Shapiro et Wright (1984), stipule que l'introduction de poissons piscivores est effective pour réduire l'eutrophisation par des effets en cascades (Carpenter et al. 1985). On serait ainsi capable de favoriser le gros zooplancton et principalement *Daphnia* (Photo 2), en réduisant sa prédation par les poissons planctivores, qui à leur tour maintiendrait la biomasse algale à des niveaux bas par broutage (Carpenter et al. 1985). Bien qu'il existe des évidences expérimentales du succès de la biomanipulation dans certains petits lacs expérimentaux (Carpenter et al. 1985), l'élargissement de la pratique expérimentale de la biomanipulation à d'autres systèmes a connu un succès très limité.

Une des explications de l'incapacité des daphnies à contrôler les communautés phytoplanctoniques dans les lacs eutrophes serait la présence

Photo 2. *Daphnia pulicaria* organisme modèle utilisé lors des expériences au laboratoire (Chapitre 4) et aussi trouvé dans le lac Steele lors des expériences en enclos (Chapitre 2 et 3), ici photographiée en présence de colonies de *Microcystis aeruginosa* montré par les flèches. (Photo: Anas Ghadouani).

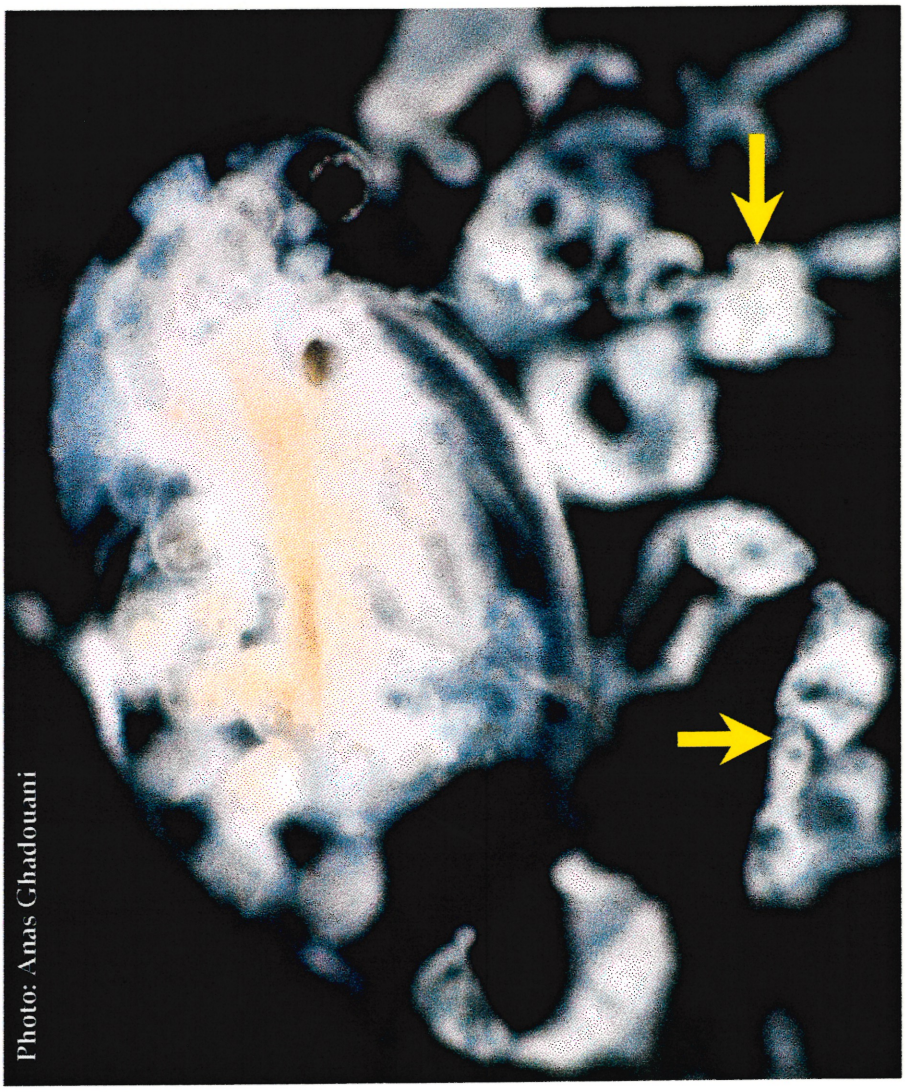
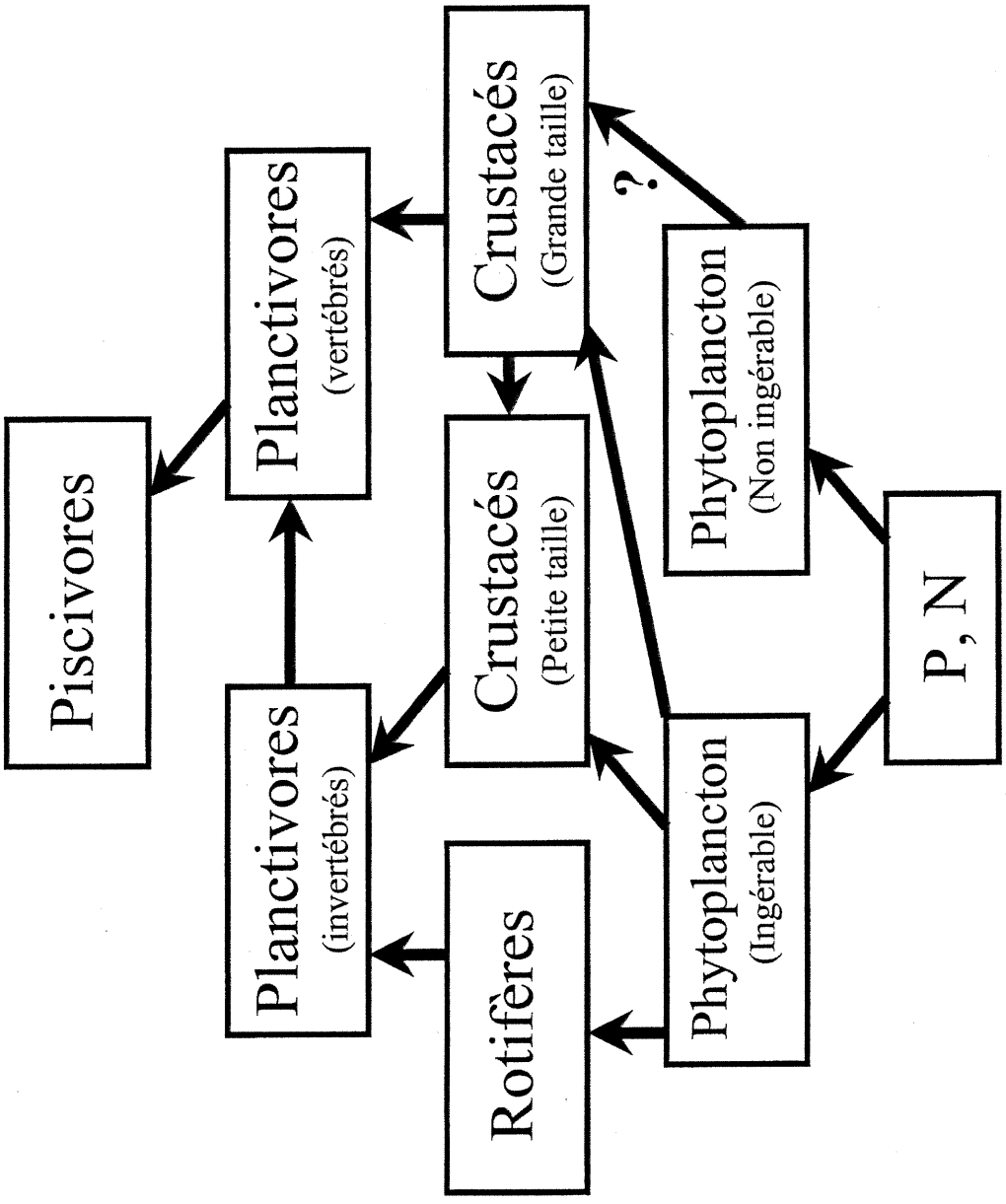


Photo: Anas Ghadouani

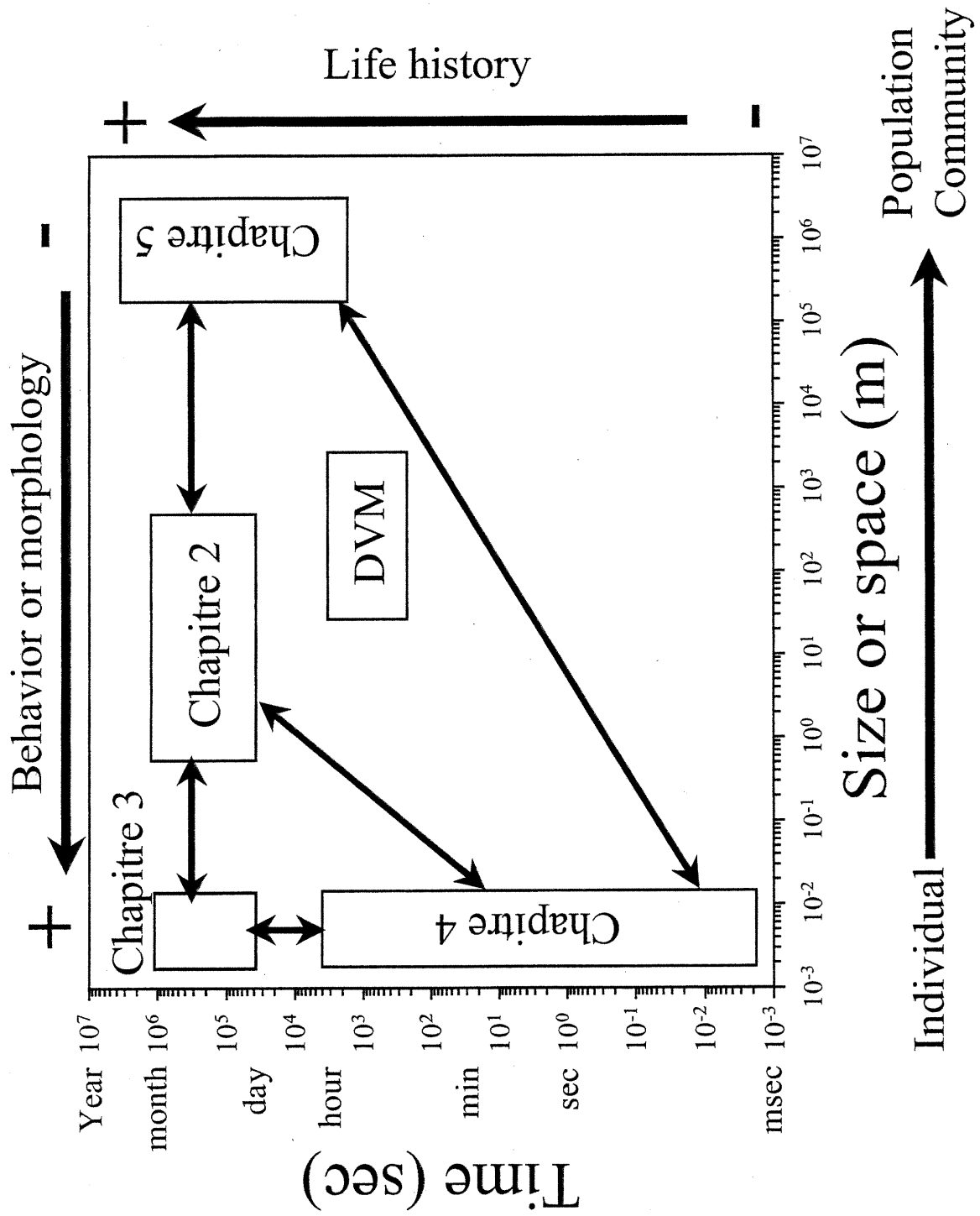
Fig. 1. Diagramme simplifié montrant les principales interactions trophiques dans un réseau trophique lacustre.


Piscivores
Planctivores (vertébrés)
Planctivores (invertébrés)
Crustacés (Grande taille)
Crustacés (Petite taille)
Rotifères
?
Phytoplancton (Non ingérable)
Phytoplancton (Ingérable)
P, N

d'importante biomasse de cyanobactéries coloniales et filamenteuses. En effet, plusieurs études en laboratoire ont suggéré que les brouteurs les plus efficaces en l'occurrence les daphnies n'assimilent presque pas ou pas du tout les cyanobactéries (Porter 1973, Porter and McDonough 1984, Fulton and Paerl 1987). Il apparaît que la nature coloniale et filamenteuse de ces algues (Webster and Peters 1978, DeMott et al. 2001) et/ou la présence de microcystines seraient à l'origine de ces inhibitions (Lampert 1981, 1982, Reinikainen et al. 1995, 1999, Rohrlack et al. 1999a, 1999b, 2001).

Le principal objectif de cette thèse est l'étude des effets de la prolifération des cyanobactéries sur les communautés zooplanctoniques dans les lacs eutrophes. La stratégie de recherche qui a été choisie est la combinaison d'expériences à plusieurs échelles d'observation afin d'examiner les interactions entre les cyanobactéries et le zooplancton à plusieurs niveaux d'organisation (Fig. 2). La durée de l'exposition aux cyanobactéries peut être reflétée par des changements de nature comportemental (heure), ou morphologique (mois) (Fig. 2). Si l'échelle spatiale est grande, les effets peuvent se ressentir au niveau de la population ou de la communauté (Fig. 2). Ainsi, à l'aide d'expériences *in situ* en enclos limniques, à une échelle spatio-temporelle intermédiaire (Chapitre 2), nous avons entrepris une expérience au cours de laquelle nous avons artificiellement augmenté la biomasse des cyanobactéries et autres algues non-

Fig. 2. Diagramme spatio-temporel théorique montrant les niveaux étudiés et la nature de la réponse des communautés zooplanctoniques à l'augmentation de la biomasse du phytoplancton non ingérable. L'emplacement de chacune des études présentées dans cette thèse est déterminé en fonction de l'échelle d'étude. L'emplacement du processus de migration vertical du zooplancton (DVM) est indiqué à titre comparatif (d'après Tollrian and Dodson 1999).


Life history
+
-
Behavior or morphology
+
-
Chapitre 5
Chapitre 2
Chapitre 3
DVM
Chapitre 4
Time (sec)
Year 10^7
month 10^6
day 10^5
hour 10^4
min 10^3
sec 10^2
10^1
10^0
10^{-1}
10^{-2}
msec 10^{-3}
Size or space (m)
10^{-3} 10^{-2} 10^{-1} 10^0 10^1 10^2 10^3 10^4 10^5 10^6 10^7
Individual
Population
Community

ingérables afin d'évaluer les effets de ces proliférations algales sur les communautés zooplanctoniques. Notre postulat de recherche était que les gros cladocères, principalement les daphnies, seraient négativement affectés par la présence d'importantes biomasses algales dominées par les cyanobactéries. Nous avons également examiné la réponse phénotypique des daphnies à la présence d'importante biomasse d'algues non-ingérables (chapitre 3). Il a été démontré que les daphnies seraient capables d'apporter des changements importants au niveau de la structure de leurs appendices de filtration en réponse à un changement dans les ressources alimentaires (Brendelberger and Geller 1985, Brendelberger 1991, Lampert 1994, Lampert and Brendelberger 1996, Repka et al. 1999). Ces changements pouvaient s'exprimer au niveau de l'individu (Fig. 2) à une échelle temporelle de l'ordre du mois. Dans le chapitre 4, nous avons examiné, dans le cadre d'expériences en laboratoire à une petite échelle spatio-temporelle (Fig. 2), les changements du comportement alimentaire des daphnies en présence de différentes formes de *Microcystis aeruginosa* et de concentrations de microcystin-LR. Ces expériences ont été réalisées lors d'un séjour à l'institut Max-Planck de limnologie (Plön, Allemagne), où un nouveau système vidéoscopique couplé à un analyseur d'image a été développé pour ce genre d'étude. Notre hypothèse principale stipulait que l'exposition des daphnies à des cyanobactéries de l'espèce *Microcystis aeruginosa* allait causer une inhibition de leur comportement

alimentaire. D'un autre côté, nous avons postulé que l'ajout de microcystin-LR purifié à une suspension d'algues ingérables (*Scenedesmus obliquus*) pouvait produire la même inhibition. Dans le chapitre 5, nous examinons les interactions entre les cyanobactéries et le zooplancton à l'échelle spatio-temporelle du lac-année dans le cadre d'une expérience écosystèmique qui impliquaient le suivi de quatre lacs pendant quatre ans. Nous avons alors postulé à la lumière des résultats obtenus en enclos (Chapitre 2), que l'augmentation du niveau trophique suite à la perturbation du bassin versant des lacs expérimentaux allait nous permettre de voir 1) une diminution de la taille des populations de cladocères, 2) de la biomasse des gros cladocères, et par conséquence de la réduction de la structure en taille, et 3) peu ou pas d'effet sur les copépodes et ainsi on assisterait au passage de la dominance des gros cladocères aux copépodes. La présence d'un gradient ascendant de la biomasse du phytoplancton et surtout des cyanobactéries dans les 4 lacs expérimentaux dans les deux dernières années de l'étude nous a permis de tester notre hypothèse principale dans des conditions écosystèmiques et donc de valider nos résultats expérimentaux en enclos et en laboratoire.

Chapitre 2

Effects of experimentally induced cyanobacterial blooms on crustacean zooplankton communities

Effects of experimentally induced cyanobacterial blooms on crustacean zooplankton communities

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Accepted for publication in *Freshwater Biology*

Running title: Cyanobacteria effects on herbivores

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Acknowledgments

Funding for this research was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC) collaborative special projects grant and the University-Industry Cooperative Research and development grant, FCAR and the Québec Ministry of Education. Additional funding was provided by the following industrial and governmental partners: Alberta-Pacific Forest Industries, Alberta Environmental Protection, Alberta Economic Development and Tourism, Manning Diversified Forest Products, Employment Canada, Weyerhaeuser Canada, National Water Research Institute. We are grateful to M. Serediak, J. Burke, A. S. McNaught, E. Braun, K. Gibson, S. Manchour, A. Smart, and C. Wichuk for their help in the fieldwork and limnological analysis. G. Méthot and A. Mohr helped with zooplankton taxonomic analysis. Special thanks to S. Paquet for analyzing phytoplankton samples. We are grateful to Y. Prairie for advise on statistical analyses. We would like also to thank N. Mookerji, Y. Vadeboncoeur, and C. Ouimet for providing helpful comments on an earlier version of this paper. This research was based at the Meanook Biological Research Station of the University of Alberta and is a contribution of the Groupe de Recherche Interuniversitaire en Limnologie et en Environnement Aquatique (GRIL) of the Université de Montréal.

Abstract

Large *in situ* enclosures were used to study the effects of experimentally induced cyanobacterial blooms on zooplankton communities. A combination of N and P was added to shallow (2-m) and deep enclosures (5-m) with the goal of reducing the TN:TP ratio to a low level (~5:1) to favor cyanobacterial blooms. Cyanobacterial blooms occurred only in the shallow enclosures. In the shallow enclosures, particulate phosphorus (PP) was on average 35% higher in comparison to deep enclosures, suggesting that depth plays a key role in P uptake by algae. Phytoplankton communities in both deep and shallow enclosures were dominated by three cyanobacteria species Ap*hanizomenon flos-aquae, Anabaena flos-aquae,* and *Microcystis aeruginosa* which accounted for up to 70% of total phytoplankton biomass. However, the absolute biomass of the three species was much higher in shallow enclosures, especially *Aphanizomenon flos-aquae.* The three cyanobacteria species responded in contrasting ways to nutrient manipulation due to their different physiology. Standardized concentrations of hepatotoxin microcystin-LR increased as a result of nutrient manipulations by a factor of 4 in the treated enclosures. Increased biomass of inedible and toxin producing cyanobacteria was associated with a decline in *Daphnia pulicaria* biomass caused by a reduction in the number of individuals with a body length of >1 mm. Zooplankton biomass showed no sign of decline at moderate cyanobacteria biomass, but when cyanobacteria reached high biomass large cladocerans were reduced. Our results demonstrate that zooplankton communities can be negatively affected by cyanobacterial blooms and therefore the potential to use herbivory to reduce algal blooms in such eutrophic lakes appears limited.

Introduction

Ecologists have always been preoccupied by the processes that control primary producers (Hairston et al. 1960). This question has generated vigorous debates as to the magnitude of control by consumers and/or resources of primary producers (e.g., Carpenter and Kitchell 1992, DeMelo et al. 1992). Many attempts have, nevertheless, been made to model and test foodweb interactions (e.g., Brooks and Dodson 1965, Oksanen et al. 1981, Carpenter et al. 1985, McQueen et al. 1986, 1989). From the application of one of these theories (trophic cascade theory) to aquatic systems, biomanipulation emerged as a technology to reverse eutrophication (e.g., Power 1990, Reynolds 1994). Despite sustained effort to generalize the application of trophic cascade theory, the success of biomanipulation is still very low, especially in cyanobacteria dominated systems (e.g., Brett and Goldman 1996). The assumption in the application of biomanipulation to eutrophication control is that large zooplankton species filter-feed sufficiently on phytoplankton, such as cyanobacteria, that biomass of the latter could be reduced (Carpenter et al. 1985). However, the potential for zooplankton to reduce colonial and/or filamentous cyanobacterial biomass remains unknown. There is evidence, mainly based on laboratory experiments, for low assimilation of cyanobacteria species by large filter-feeding *Daphnia* (Lampert 1981, 1982, Fulton and Paerl 1987). There are at least three major aspects which may render cyanobacteria

unsuitable as food for zooplankton: 1) mechanical interference created by the large size and the shape of some cyanobacteria species (e.g., Webster and Peters 1978, DeMott et al. 2001), 2) chemical composition of these cyanobacteria that may reduce the efficiency of growth and the reproduction of some zooplankters (Brett and Müller-Navarra 1997, Kilham et al. 1997), and 3) the toxins contained in some cyanobacteria can be harmful to zooplankton (Lampert 1981, 1982, Haney et al. 1994, Reinikainen et al. 1995, 1999, Rohrlack et al. 2001). Other studies support an opposite view which states that zooplankton are capable of reducing undesirable cyanobacteria species by means on intensive grazing (Boon et al. 1994, Matveev et al. 1994).

Eutrophication of aquatic systems is a major water quality problem (Moss 1996). Phytoplankton communities in eutrophic and hypereutrophic systems are often dominated by potentially toxic colonial and filamentous cyanobacterial species, such as *Microcystis aeruginosa* and *Aphanizomenon flos-aquae*, that can be harmful not only to zooplankton but to humans as well (Jochimsen et al. 1998). Such cyanobacterial species usually dominate the phytoplankton communities in eutrophic lakes and produce excessive biomass, a phenomenon referred to as cyanobacterial blooms. As pointed out by DeMott (1999), there is a serious lack in experimental assessment of the effect of cyanobacteria blooms on zooplankton in natural systems; our knowledge of

cyanobacteria-zooplankton interactions is mainly based on results of laboratory experiments with *Daphnia* as a model (Burns 1968b, 1968a, Porter 1973, Lampert 1981, 1982, Porter and McDonough 1984). However, application of laboratory results can hardly be achieved without considering the complexity of natural communities (Haney 1987, DeMott 1999). To our knowledge, there has been no attempt to evaluate the effects of cyanobacteria blooms on zooplankton as a whole community in large scale *in situ* experiments.

The goal of this study was to examine cyanobacterial zooplankton interactions experimentally and to assess the effect of blooms on zooplankton communities in a natural system. Large *in situ* enclosures were used to achieve this goal. The first step consisted of artificially enhancing cyanobacteria biomass by the addition of phosphorus and nitrogen. Enclosures with different depths (deep and shallow) were used to test for the effect of depth and consequently light regime on the development of high cyanobacterial biomass or blooms (Huisman et al. 1999b) and possible implications for cyanobacteria-zooplankton interactions (Berthon and Brousse 1995). We anticipated that increased biomass of bloom forming colonial and filamentous cyanobacterial species (i.e., *Microcystis aeruginosa* and *Aphanizomenon flos-aquae*) would cause 1) a reduction in the size structure and the biomass of large filter-feeding cladocerans (i.e, *Daphnia*) and consequently in the whole zooplankton community; 2) less or no

effect on copepod and small cladoceran species; and 3) a shift in zooplankton community from dominance by large to small cladocerans and copepods.

Materials and Methods

Study site - This research was conducted in Steele Lake (54°39'N 113°46'W), a fairly large (6.6 km^2 surface area) eutrophic lake located in the northern boreal forest of Alberta, Canada. Steele Lake is shallow (3.2 m mean depth, 6.1 m maximum depth) enough to mix vertically during most of the summer. The drainage basin of Steele Lake is very large compared to that of many lakes on the boreal plain, representing 37 times the area of the lake (Mitchell and Prepas 1990). Large amounts of nutrients are transported to the lake from the naturally rich drainage basin, which causes frequent cyanobacterial blooms during the summer season (Trimbee and Prepas 1987). The blooms are usually dominated by species such as *Aphanizomenon spp.*, *Anabaena spp.* and *Microcystis spp.* which made this lake an ideal site for these experiments.

Experimental design - Two sets of cylindrical enclosures were placed at the deepest part of the lake in a sufficiently quiet area to minimize the effects of the wind on the enclosures, the sampling docks and the flotation devices. The enclosures were closed off from the bottom of the lake and were made of clear

thick woven polyethylene fitted to a flotation collar of ethafoam logs. The collars were ~50 cm above the surface of the water to keep enclosure and lake water separated. Attaching each three to one side of a dock ensured the stability of each set of six enclosures. The whole structure (enclosures + dock) was anchored at several spots to ensure maximum stability. Water from the lake was then pumped into each enclosure. The enclosures were covered by a gill net to keep fish from jumping into the enclosures. Prior to the beginning of the experiments the enclosures were left to settle for few days. Both sets of enclosures were 2.5-m diameter opening and 2-m deep with a volume of ~10 m^3 for the shallow enclosures and 5-m deep with a volume of ~25 m^3 for the deep enclosures.

Nutrient manipulations - Prior to manipulations, the enclosures were sampled to ensure that they had similar initial conditions. Preliminary analyses revealed that nutrient concentrations and algal biomass were practically identical in all the experimental units as they were all filled with the same lake water at the same day. To enhance phytoplankton biomass the enclosures were fertilized and each set of the enclosures (shallow and deep) received three treatments: no nutrient addition (control), nutrient and high nutrient additions with two enclosures per treatment for a total of twelve enclosures. A combination of phosphorus (P) as potassium phosphate monobasic (KH_2PO_4)

and nitrogen (N) as ammonium nitrate (NH_4NO_3) was added to the treated enclosures. In the nutrient addition treatment (NA) P concentration was increased by ~3 times in comparison to the control and N additions were three times P by weight. In high nutrient addition (HNA), P concentration was increased by ~7 times in comparison to the control (double in comparison to the NA treatment) and N additions were the same as P by weight. The concentrations of nutrients after NA and HNA treatments were comparable to those found in highly eutrophic Canadian prairie lakes (Trimbee and Prepas 1987) or other regions in the world (Smith 1986). Shallow polymictic lakes in Canadian prairies and elsewhere usually reach nutrient concentrations as high as those of the treated enclosures due to internal nutrient loading (Riley and Prepas 1984). All the treatments were performed only once and all the enclosures were treated on the same day.

Sampling and analytical methods - The experiments took place in summer 1996 and lasted for five weeks including the pretreatment period. Common zooplankton species would produce several generations in five weeks in rich systems at 20°C (Gillooly 2000). All the enclosures were sampled for a total of 7 times at 4 days interval, after a pretreatment period of 6 days. Prior to the beginning of the experiments, all the enclosures had the same initial conditions. Water temperature and dissolved oxygen concentration were

measured with YSI oxymeter. Light penetration was measured in enclosures and lake water during each visit to the site with a LiCor (model 1000) underwater quantum sensor. Water column integrated samples were collected for measurements of P and N as well as chlorophyll *a* (chl-*a*). The water samples were collected by weighted Tygon tubing fitted with a one-way foot valve especially designed for collecting vertically integrated water samples. Water samples were taken at several spots within each enclosure and mixed in a bucket, then sub-sampled for water quality analysis. The entire water column of the shallow enclosures (2-m), the deep enclosures (5-m) and the lake (5-m) were sampled. Water samples were maintained at 4°C in the field and laboratory prior to analysis. Another sub-sample was taken and preserved in Lugol for phytoplankton identification and enumeration. A plankton net of 53 μm mesh size was used to concentrate algal biomass which was later frozen for cyanotoxin quantification.

Total phosphorus (TP) and total dissolved phosphorus (TDP) concentrations were measured following the modified Menzel and Corwin (1965) potassium persulfate method (Prepas and Rigler 1982). Particulate phosphorus (PP) was calculated by subtracting TDP from TP and was expressed as percentage of TP. Total dissolved nitrogen (TDN) and particulate nitrogen (PN) in water samples were measured by a technicon analyzer according to

Solórzano (1969) and Stainton et al. (1977). Total nitrogen (TN) was calculated by adding TDN to PN. TN:TP is expressed by weight. Particulate carbon was measured according to the automated technique described in Stainton et al. (1977). The cold ethanol extraction method (24h extraction time) was used to spectrophotometrically estimate chl-*a* concentration in water samples (Bergmann and Peters 1980). Chl-a measurements were not corrected for phaeopigments because it was shown that these corrections were not necessary in such productive systems (Webb et al. 1992). Particulate carbon was also used as an index of phytoplankton biomass (Reynolds 1984) when chl-*a* biomass was not available due to the loss of a sample-date. Toxin concentrations were measured by HPLC with UV detection based on microcystin-LR standard following a modified Harada et al. (1988) method (Kenefick et al. 1992). Toxin concentrations are quantified in the total phytoplankton biomass collected including toxic and nontoxic species. Any difference in the phytoplankton community composition can introduce important bias in the measurement expressed in µg per mg dry mass of total phytoplankton. To avoid such bias, the measured amounts of toxins were standardized by dividing the concentration in each treated enclosure by its corresponding control. The concentrations in the lake were standardized relative to the deep control.

Phytoplankton sub-samples were settled in a sedimentation chamber and enumerated with an inverted microscope according to the Utermöhl method (Lund et al. 1958). Phytoplankton were identified to species level based on appropriate taxonomic references (e.g., Prescott 1951, Anagnostidis and Komarek 1988). Phytoplankton biomass was estimated from specific biovolume calculation based on simple geometric formula (Lewis 1976, Reynolds 1984). Phytoplankton biomass was expressed using mass rather than volume assuming that cells density, mainly composed of water (Reynolds 1984), is equal to one. Inedible phytoplankton was defined as the sum of biomass of all the species larger than 30 μm in size.

Zooplankton were sampled by means of a Wisconsin plankton net (53 μm mesh size, 29 cm diameter and 90 cm length). The opening of the net was lowered to the bottom of the enclosure so that the entire water column could be sampled. The samples were preserved in a buffered 4% sugar-formaldehyde solution and stored for later analysis (Haney and Hall 1973, Prepas 1978). A 5-mL sub-sample was taken with a large opening pipet, transferred to a Ward rotative cell and analyzed under a stereomicroscope (Ward 1955). The goal of this study was to evaluate the effect of increased cyanobacterial biomass on large planktonic herbivores and therefore only crustacean zooplankters were counted. Adult crustaceans were identified mostly up to species level. The

copepodite stages (C1-C5) were categorized to appropriate suborder (*Calanoida* or *Cyclopoida*), while nauplii were only enumerated. Identification of zooplanktonic crustacean taxa was done according to Brooks (1957), Edmondson (1959), Deevey and Deevey (1971), Brandlova et al. (1972), and Smith and Fernando (1978). The length of all organisms was measured with a calibrated micrometer ocular. Biomass of the measured organisms was estimated based on published length-dry mass zooplankton relationships (Malley et al. 1989).

Statistical analysis - Repeated-measures ANOVA was used to compare treated enclosures with their respective controls or shallow with deep enclosures. All the data was log-transformed prior to the analysis to give small weights to outliers knowing that these observations contribute greatly to the lack of normality of ecological data (Conover and Iman 1981). The power of repeated-measures ANOVA tends to decrease when the number of sampling dates or dependent variables (7 in this case) is higher than the number of replicates (2 in this case), especially when dealing with highly dynamic plankton communities (Peterman 1990, Potvin et al. 1990, von Ende 1993). Therefore, P values < 0.10 were considered to be statistically significant. All the statistical results are provided in summary tables. All the repeated-measures ANOVAs were performed on the full length of the experiment (7 dates) and on data from

the end of the experiment (last 3 dates). Performing the analysis on the last 3 sampling periods allowed us to increase the statistical power of our analysis and hence to better detect treatment and depth effects especially when the variability was high. In addition, the biological response is more likely to be observed at the end of the experiments. Time effect and its interaction with treatment were also tested in the same manner (full length and end of experiment). Hence, we consider ANOVA results for the end of the experiments (last 3 dates) as more indicative of treatment effect than tests done on the 7 sampling periods. This is particularly true because time effect was rarely significant when we consider only the end of the experiment; however, it was almost always significant when all 7 sampling periods were considered. Wilcoxon nonparametric test was used to test for difference in the size structure of *Daphnia pulicaria* reported in Fig. 9.

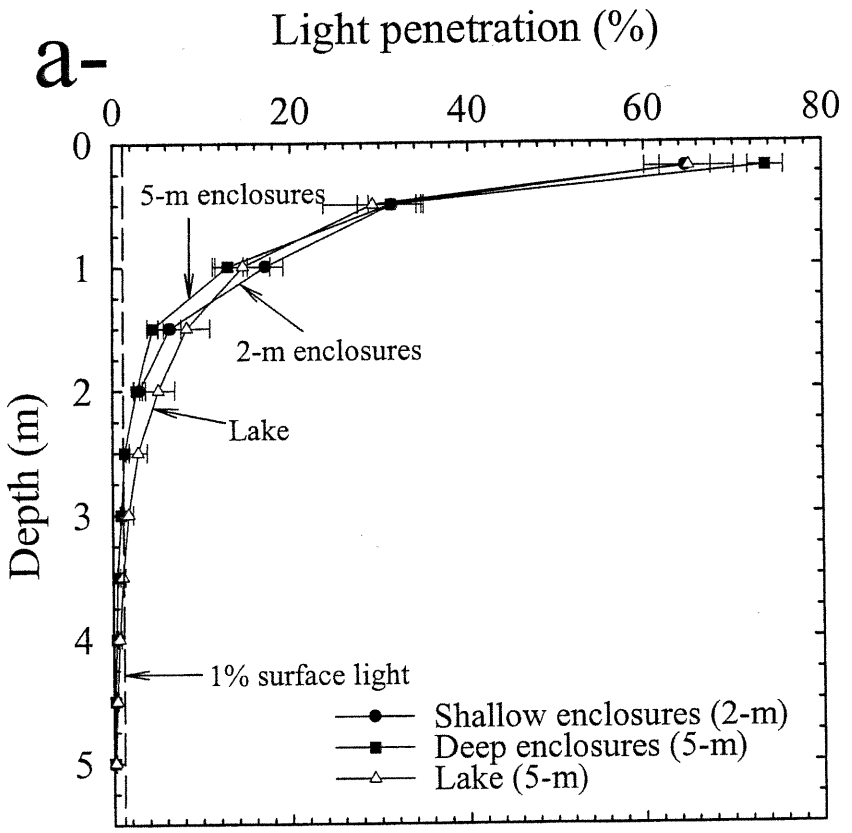
The relationships between crustacean zooplankton and phytoplankton appeared to have a nonlinear shape. Different nonlinear models were fitted to the data only to find out that, based on the statistical parameters, the 3-parameter Gaussian peak function and the quadratic function allowed better fit. Quadratic function was preferred over the peak function because of its common use among ecologists. The relationships were constructed based on a combined data set (shallow, deep enclosures and the lake); however, data from different experimental units are represented graphically by different

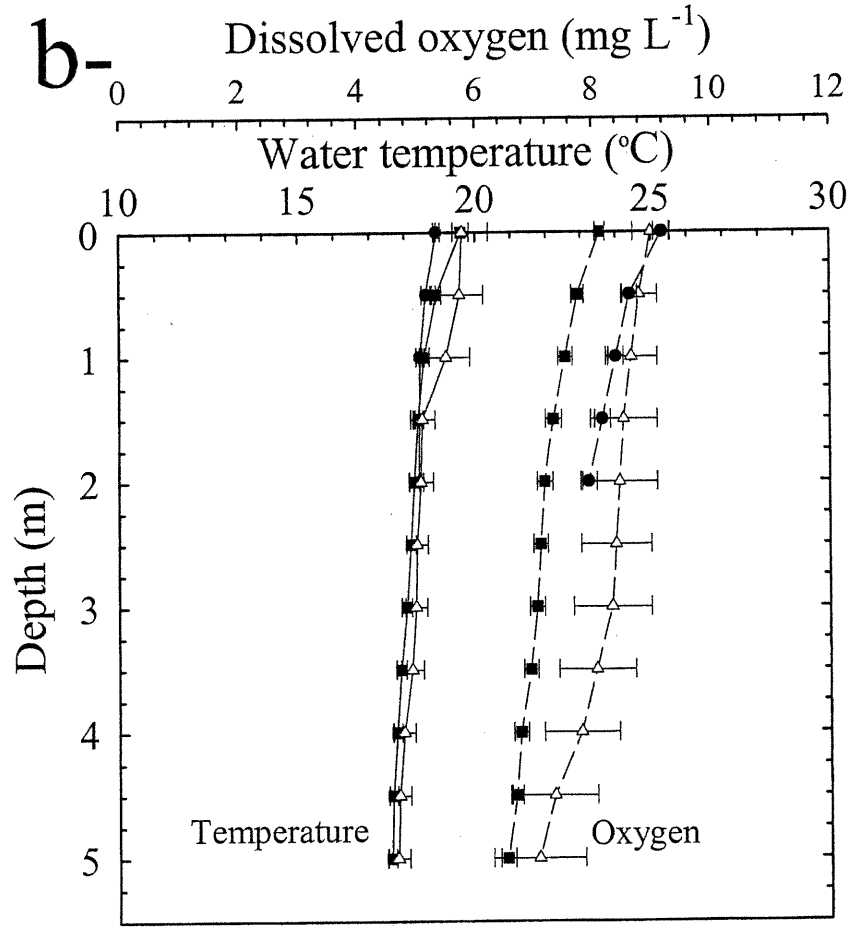
symbols. The data used to construct these relationships are collected over time in the same experimental units and hence are not truly independent (Hurlbert 1984). To evaluate the effect of time on these relationships, the degree of temporal autocorrelation was investigated by means of partial autocorrelogram (Legendre and Legendre 1998). This type of analysis is usually used to determine at what temporal scale data are sufficiently independent to be used as independent observations (Swihart and Slade 1985, Underwood 1997). The data used to construct these relationships were assumed to be independent after the autocorrelogram analysis showed no statistically significant temporal autocorrelation for all the possible time lags (Swihart and Slade 1985, 1986). Considering the short-term nature of these experiments in comparison to the whole summer season and the outcome of partial autocorrelogram analysis, we concluded that the time factor would have only a mild effect on the estimation of the regression parameters despite the lack of genuine replication. All statistical tests were performed with Systat version 8.0.

Results

Physical and chemical dynamics and phytoplankton response - Light conditions were similar in the lake, the deep and shallow enclosures; at least 2% of the incident light reached the first two meters (Fig. 1a). Less than 1% of incident light was available in the water strata below 2 and 3-m in the deep

Fig. 1. Depth profiles of a) light penetration (%), b) water temperature (°C) and dissolved oxygen (mg L^{-1}) in the lake, shallow and deep enclosures. Error bars are standard error of the mean based on data from the full length of the experiments (7 dates).


a-
Light penetration (%)
0
20
40
60
80
Depth (m)
0
1
2
3
4
5
5-m enclosures
2-m enclosures
Lake
1% surface light
Shallow enclosures (2-m)
Deep enclosures (5-m)
Lake (5-m)


b-
Dissolved oxygen (mg L-1)
0
2
4
6
8
10
12
Water temperature (°C)
10
15
20
25
30
Depth (m)
0
1
2
3
4
5
Temperature
Oxygen

enclosures and the lake, respectively (Fig. 1a). All the enclosures were well mixed and water temperature was similar in the entire water column below 0.5 m (Fig. 1b). The lake water was also mixed and showed only a weak thermal stratification in surface waters (Fig. 1b). In all the enclosures and the lake, the entire water column was well oxygenated (Fig. 1b). Water in the shallow enclosures and the lake was consistently more oxygenated in comparison to the deep enclosures (Fig. 1b).

The addition of P created a gradient in the concentration of TP (Fig. 2a). TP was increased by a factor of ~3 (NA) and ~7 (HNA) in comparison to the control in shallow and deep enclosures, respectively (Fig. 2a). A large proportion of the added P remained in the dissolved form (Fig 2b). Despite N additions only a slight increase was measurable in shallow but not in deep enclosures indicative of N loss (Fig. 2c). However, TN:TP ratio (by weight) corresponded to the desired set up, with a decreasing trend from control to highly fertilized enclosures. TN:TP ratio was decreased from as high as ~20:1 to less than 5:1 in the HNA treatment (Fig. 2d). The amount of P transformed from dissolved to particulate sestonic form, expressed as the percentage of PP, was negatively related to TP in both shallow and deep enclosures (Fig. 3). However, the percentage of PP was ~35% higher (ANCOVA, $P < 0.0001$) in shallow enclosures (Fig. 3). This observation is an indication that more phosphorus was

Fig. 2. Concentration of a) total phosphorus, b) total dissolved phosphorus, c) total nitrogen and d) total nitrogen to total phosphorus ratio (by weight) in the lake, shallow and deep enclosures. Error bars are standard error of the mean based on data from the full the length of the experiments (7 dates).

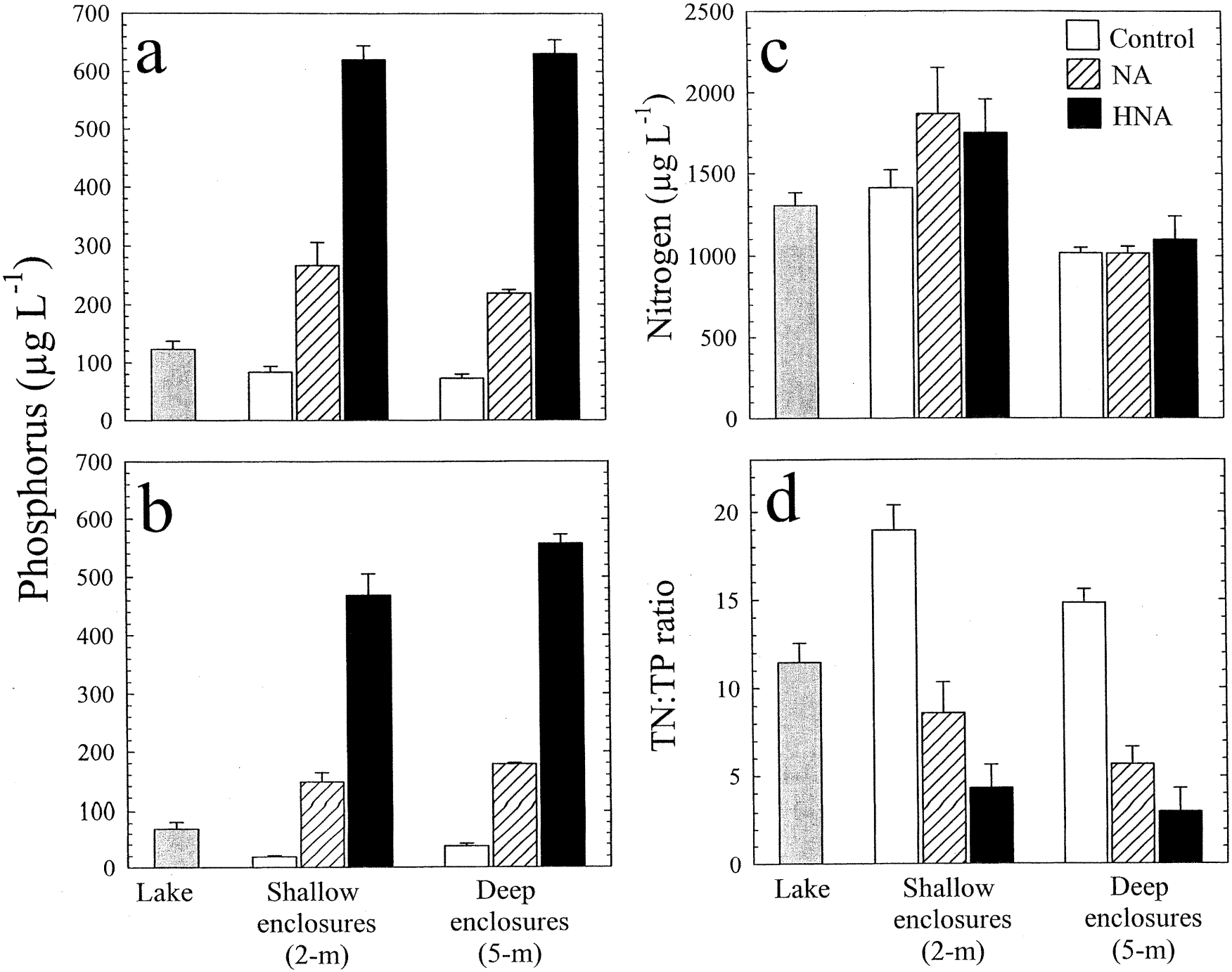
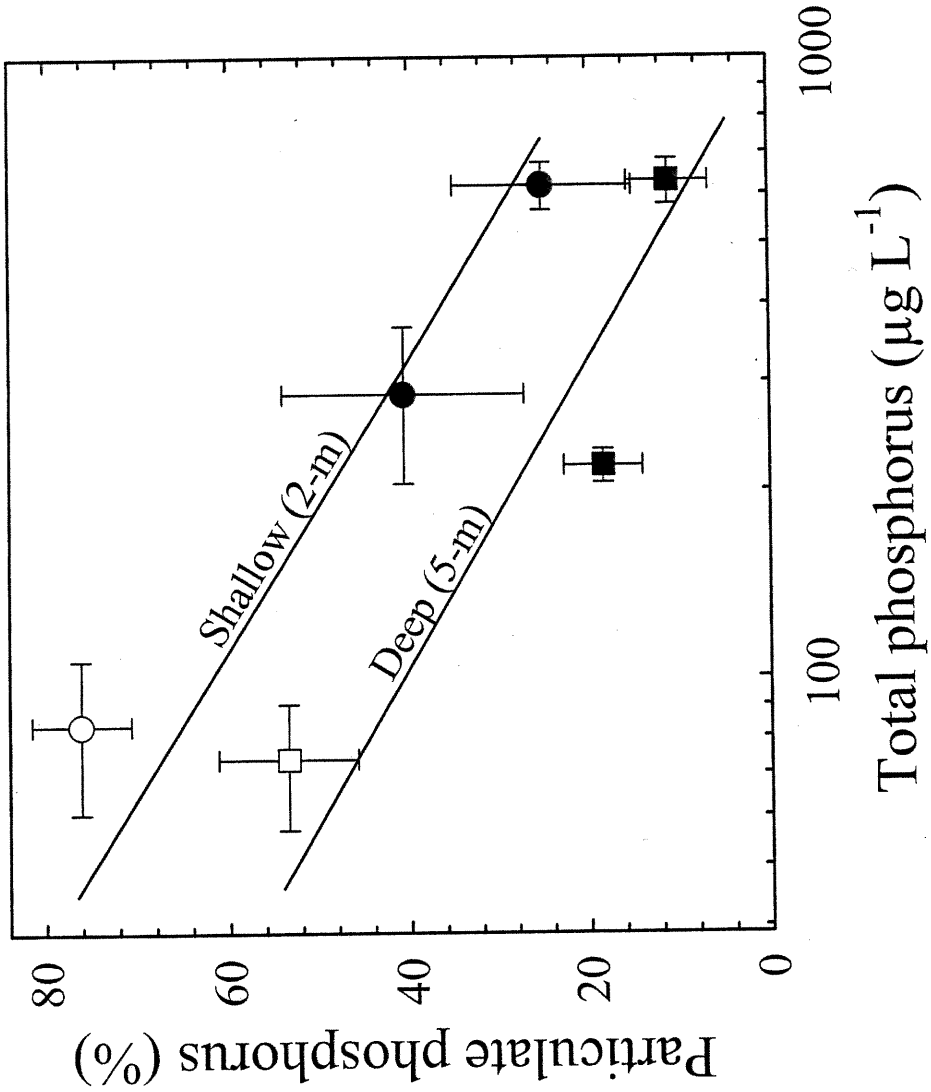

a
b
c
d
Phosphorus (µg L-1)
Nitrogen (µg L-1)
TN:TP ratio
Control
NA
HNA
Lake
Shallow enclosures (2-m)
Deep enclosures (5-m)

Table 1. Results of repeated-measures ANOVAs for comparison of phytoplankton and zooplankton in shallow and deep enclosures. Depth (df=1) and time (df=6 or 2) effects were tested for all the sampling dates (7) and only the last 3 dates. The interaction term has the same df as time (6 or 2). For chl-a the analyses were done for 6 dates only due to the lost of one sample-date.

	Shallow vs. deep enclosures			
	All 7 dates		Last 3 dates	
	F	*P*	*F*	*P*
a) Phytoplankton				
Chl-a				
Depth	17.8	0.002	55.7	<0.001
Time	37.4	<0.001	0.7	0.473
Depth X Time	9.1	<0.001	14.8	<0.001
Inedible fraction				
Depth	42.2	0.022	17.2	0.008
Time	2.7	0.069	0.8	0.441
Depth X Time	3.1	0.049	0.2	0.803
Anabaena flos-aquae				
Depth	0.6	0.510	17.4	0.008
Time	5.1	0.008	4.4	0.042
Depth X Time	1.4	0.278	0.3	0.723
Aphanizomenon flos-aquae				
Depth	65.9	<0.001	345.0	<0.001
Time	40.4	<0.001	3.9	0.056
Depth X Time	7.3	<0.001	9.5	0.005
Microcystis aeruginosa				
Depth	0.4	0.571	10.6	0.022
Time	4.0	0.004	1.9	0.192
Depth X Time	3.1	0.017	0.1	0.916
b) Zooplankton				
Daphnia pulicaria				
Depth	50.6	0.019	31.5	0.004
Time	4.5	0.012	0.3	0.751
Depth X Time	2.3	0.102	0.6	0.584
Cladocerans				
Depth	65.1	0.015	35.9	0.003
Time	5.5	0.005	0.4	0.714
Depth X Time	2.5	0.077	0.6	0.539
Copepods				
Depth	23.3	0.040	3.6	0.130
Time	7.2	0.002	5.9	0.026
Depth X Time	2.8	0.058	2.9	0.110
Cladoceran:copepod				
Depth	16.1	0.056	6.7	0.060
Time	7.6	0.001	3.6	0.075
Depth X Time	1.2	0.359	0.4	0.671
Total zooplankton				
Depth	84.9	0.011	43.2	0.002
Time	6.6	0.002	0.2	0.859
Depth X Time	3.1	0.044	1.1	0.407

Fig. 3. Relationship between particulate phosphorus (%) and total phosphorus concentrations in the shallow and deep enclosures. Linear regressions are fitted separately to shallow enclosures data represented in circles with bi-directional standard errors (open circles for control and solid circles for NA and HNA treatments) and deep enclosures data represented in squares with bi-directional standard errors (open squares for control and solid squares for NA and HNA). ANCOVA showed that shallow enclosures have significantly more particulate phosphorus per unit total phosphorus in comparison to deep enclosures (F = 36.8, P < 0.0001). Correlation coefficients were 0.63 and 0.67 in shallow and deep enclosures respectively, with P < 0.0001 in both cases. Data displayed are from the full length of the experiment (7 dates).


Particulate phosphorus (%)
0
20
40
60
80
100
1000
Total phosphorus (µg L-1)
Shallow (2-m)
Deep (5-m)

in the sestonic form, likely taken up by phytoplankton in the shallow enclosures (Fig. 3). This difference translated into a more pronounced response of chl-*a* to fertilization in the shallow enclosures (Fig. 4a). Algal biomass was significantly higher in the shallow enclosures in comparison to deep enclosures (Fig. 4a, Table 1). Chl-*a* biomass increased by a factor of ~2 in the fertilized (NA and HNA) shallow enclosures (Table 2), but only slightly in the deep enclosures (Fig. 4a, Table 2). There were no detectable differences in chl-*a* biomass between NA and HNA treatment in both shallow and deep enclosures (Fig. 4a). In the lake, chl-*a* biomass was comparable to the shallow controls (Fig. 4a). In all the enclosures and also in the lake, cyanobacteria were the most dominant group of phytoplankton and reached on average from ~50 to 70% of the total biomass (Fig. 4b). The fertilized shallow enclosures had higher biomass of inedible phytoplankton species; however, the treatment effect was statistically significant only for HNA treatment when considering the last 3 dates of the experiments (Fig. 4c, Table 2). Particulate carbon was strongly correlated with chl-*a* biomass and explained more than 80% of the variation in chl-*a* (Fig. 5). Phytoplankton biomass was then expressed as particulate carbon in further analyses (see methods).

Nutrient treatment to enclosed lake water induced contrasting responses in cyanobacteria at the species level (Fig. 6). The phytoplankton

Fig. 4. Biomass of a) chlorophyll *a* (µg L^{-1}), b) cyanobacteria (%), and c) inedible phytoplankton biomass (all species > 30 µm combined) in the lake, shallow and deep enclosures. Error bars are standard error of the mean based on data from the end of the experiment (last 3 dates).

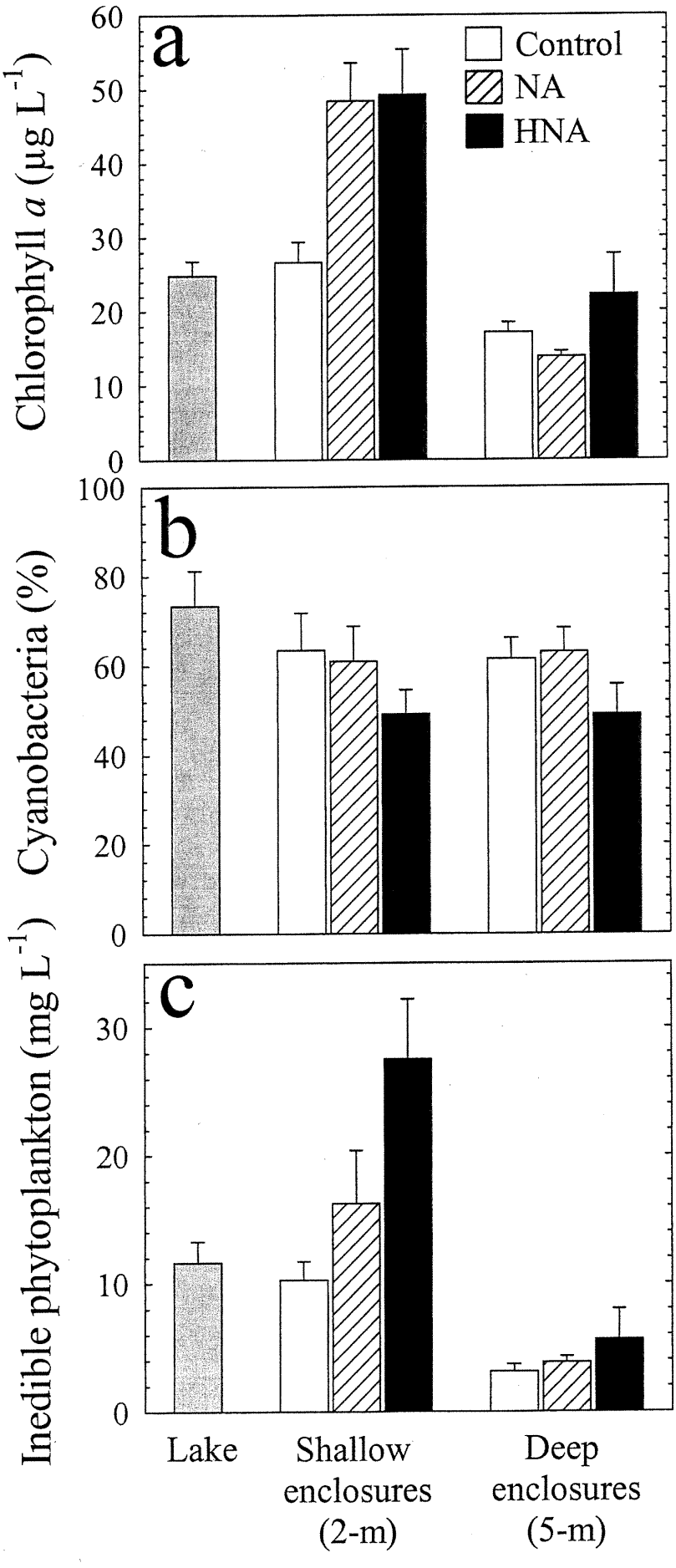
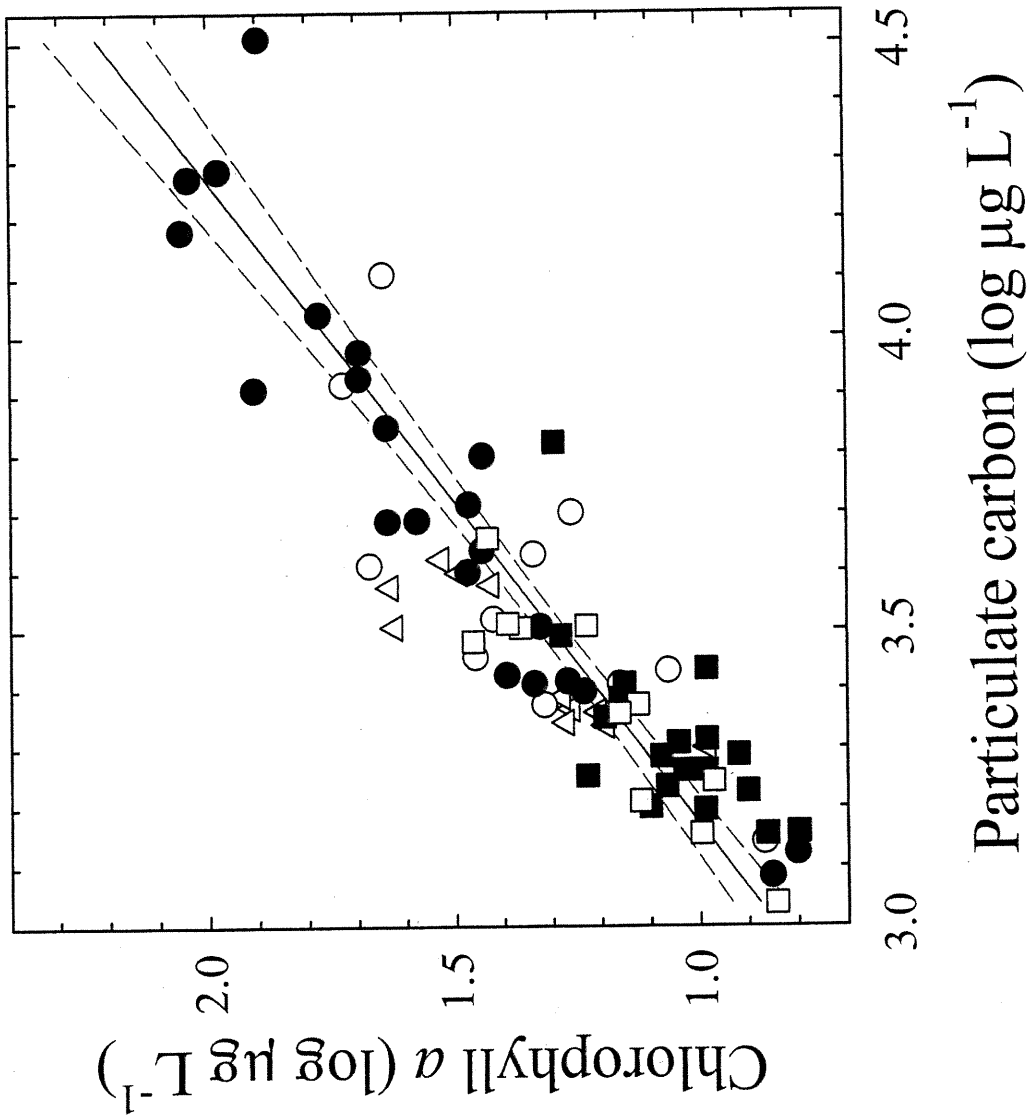

a
b
c
Control
NA
HNA
Chlorophyll *a* (µg L^{-1})
Cyanobacteria (%)
Inedible phytoplankton (mg L^{-1})
0
10
20
30
40
50
60
80
100
Lake
Shallow enclosures (2-m)
Deep enclosures (5-m)

Fig. 5. Relationship between chlorophyll a biomass (log μg L^{-1}) and particulate carbon (log μg L^{-1}). The solid line is the regression line fitted to shallow enclosures (control in open circles; NA and HNA treatments in solid circles), deep enclosures (control in open squares; NA and HNA treatments in solid squares), and lake data (in open triangles). The relationship is highly significant ($P < 0.0001$) with an r^2 of 0.82. The dashed lines represent upper and lower 95% confidence intervals of the regression line based on data from the full length of the experiment (7 dates).


Chlorophyll *a* (log µg L^{-1})
2.0
1.5
1.0
Particulate carbon (log µg L^{-1})
3.0
3.5
4.0
4.5

communities were dominated by three cyanobacterial species *Anabaena flos-aquae*, *Aphanizomenon flos-aquae* and *Microcystis aeruginosa*. *Anabaena* biomass was ~2 times higher in the control enclosures, the magnitude of this response was again greater in shallow compared to deep enclosures (Fig. 6a, Table 1). *Anabaena* biomass was 4 times higher in shallow than in deep enclosures at the end of the experiments (Fig. 6a, Table 1). *Anabaena* biomass displayed high variability especially in shallow control enclosures in comparison to the other enclosures and the lake (Fig. 6a). Therefore, this difference was not statistically detectable when each treatment was compared to the control (Table 2). In the deep enclosures, *Anabaena* biomass was lower in treated, but the difference was not statistically significant (Table 2). *Aphanizomenon* was not enhanced by nutrient addition and displayed on average the same biomass in all the enclosures (Fig 6b). There was no statistically significant difference in *Aphanizomenon* biomass when each treatment level was compared to its control (Table 2). In deep enclosures, *Aphanizomenon* developed a much lower (~4 times less) biomass in comparison to the shallow enclosures and the lake (Fig. 6b, Table 1). In contrast, *Microcystis* colonies increased in shallow enclosures by a factor of ~2 and ~2.5 in NA and HNA treatments, respectively (Fig. 6c). This difference was statistically detectable in NA treatment but not in HNA treatment (Fig. 6c, Table 2). During the bloom events, *Microcystis* biomass in treated enclosures reached more than 4 times the highest biomass measured in

Fig. 6. Biomass (µg L^{-1}) of the main dominant phytoplankton species a) *Anabaena flos-aquae,* b) *Aphanizomenon flos-aquae* and c) *Microcystis aeruginosa* in the lake and the different treatment levels in shallow and deep enclosures. Error bars are standard errors of the mean based on data from the end of the experiment (3 dates).

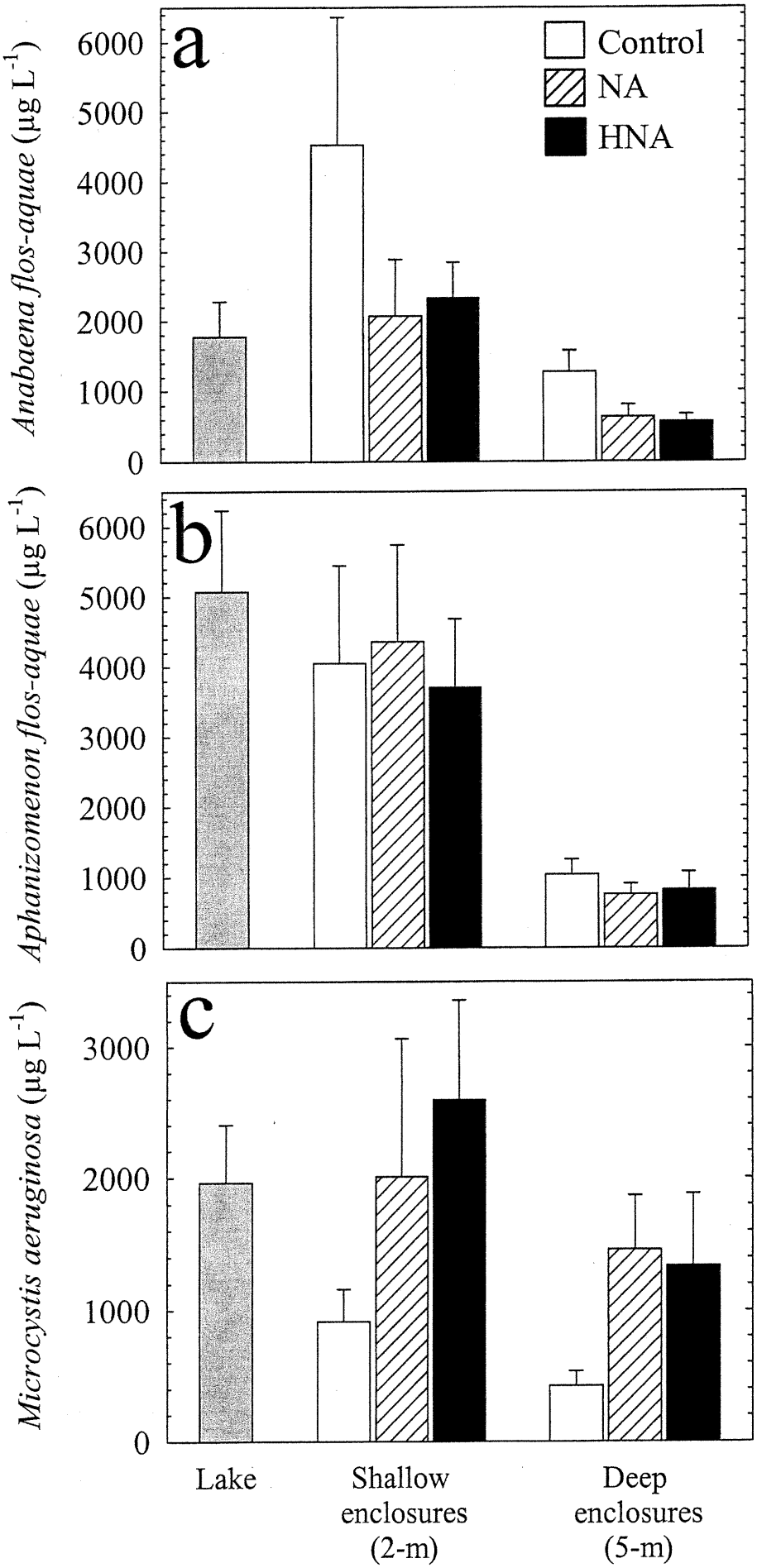

a
b
c
Control
NA
HNA
Anabaena flos-aquae (µg L^{-1})
Aphanizomenon flos-aquae (µg L^{-1})
Microcystis aeruginosa (µg L^{-1})
0
1000
2000
3000
4000
5000
6000
Lake
Shallow enclosures (2-m)
Deep enclosures (5-m)

Table 2. Results of repeated-measures ANOVAs for comparison of phytoplankton in treated (NA and HNA) and untreated enclosures. Treatment (df=1) and time (df=6 or 2) effects were tested for all the sampling dates (7) and only the last 3 dates. The interaction term has the same df as time (6 or 2). For chl-a the analyses were done for 6 dates only due to the lost of one sample.

	Shallow enclosures (2-m)								Deep enclosures (5-m)							
	Nutrient addition (NA)				High nutrient addition (HNA)				Nutrient addition (NA)				High nutrient addition (HNA)			
	All 7 dates		Last 3 dates		All 7 dates		Last 3 dates		All 7 dates		Last 3 dates		All 7 dates		Last 3 dates	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Chl-a																
Treatment	59.7	0.016	64.2	<0.001	31.1	0.030	162.0	<0.001	57.1	0.017	24.1	0.004	28.3	0.033	1.3	0.310
Time	51.7	<0.001	3.5	0.067	297	<0.001	5.9	0.019	166.0	<0.001	10.4	0.003	75.2	<0.001	11.5	0.002
Treatment X Time	6.6	0.005	2.7	0.112	40.3	<0.001	11.2	0.002	31.1	<0.001	91.9	<0.001	53.6	<0.001	7.5	0.010
Inedible fraction																
Treatment	0.7	0.562	0.1	0.937	1.8	0.408	28.4	0.037	0.1	0.856	0.3	0.688	91.8	0.066	8.7	0.207
Time	4.7	0.040	5.1	0.165	4.9	0.035	1.1	0.492	42.6	<0.001	9.9	0.091	2.4	0.146	0.1	0.939
Treatment X Time	1.0	0.487	0.3	0.749	0.7	0.649	0.7	0.575	5.4	0.030	32.6	0.029	3.4	0.078	1.2	0.455
Anabaena flos-aquae																
Treatment	0.6	0.565	1.3	0.454	0.9	0.517	0.2	0.739	1.6	0.420	0.1	0.886	38.8	0.101	4.1	0.293
Time	14.6	0.002	8.1	0.109	19.4	0.001	0.6	0.618	2.8	0.113	1.1	0.496	5.1	0.034	1.9	0.339
Treatment X Time	1.5	0.304	0.9	0.517	3.9	0.060	15.3	0.061	3.3	0.085	1.2	0.449	1.4	0.341	0.4	0.697
Aphanizomenon flos-aquae																
Treatment	0.5	0.505	2.6	0.352	9.5	0.199	4.4	0.281	42.7	0.096	0.2	0.706	1.1	0.480	0.2	0.715
Time	40.6	<0.001	21.4	0.044	107.0	<0.001	78.6	0.012	27.6	<0.001	5.7	0.147	6.1	0.022	5.7	0.148
Treatment X Time	0.7	0.652	0.8	0.549	1.1	0.461	1.5	0.405	0.9	0.524	0.01	0.961	1.2	0.422	0.9	0.514
Microcystis aeruginosa																
Treatment	1.4	0.447	671	0.024	3.5	0.312	8.8	0.206	5.8	0.250	8.1	0.215	11.1	0.185	3.7	0.304
Time	4.6	0.042	8.4	0.106	5.6	0.027	0.8	0.553	4.5	0.044	74.4	0.013	8.7	0.009	4.5	0.180
Treatment X Time	1.7	0.254	3.4	0.227	0.6	0.724	0.7	0.592	3.1	0.097	4.1	0.194	2.8	0.114	0.3	0.789

the controls. The response of *Microcystis* was similar in both shallow and deep enclosures; however, the magnitude was higher in shallow enclosures (Fig. 6c, Table 2). There was a proportional increase in cyanobacteria toxin microcystin-LR (MCLR) by a factor of ~4 in fertilized shallow enclosures (NA and HNA) relative to the control (Fig. 7). However, no increase in cyanotoxin concentrations was observed in the deep enclosures (Fig. 7).

The addition of nutrients created a trophic gradient typical of cyanobacterial dominated lakes. These conditions were achieved after the occurrence of cyanobacteria blooms in the treated enclosures, which translated into a large increase in phytoplankton biomass and especially in filamentous and colonial inedible species. These conditions were very appropriate for testing our principal hypothesis about the response of zooplankton community to a gradient in cyanobacteria colonial and filamentous species.

Effects on crustacean zooplankton - Zooplankton composition was similar in the lake and in all enclosures. The community was dominated mainly by cladocerans which contributed by ~60 to 90% of the total biomass (Table 3). *Daphnia pulicaria* was the dominant cladoceran species, while other smaller species such as *Chydorus sphaericus, Ceriodaphnia lacustris, Diaphanosoma brachyurum* and *Bosmina longirostris* represented only a small fraction of this

Fig. 7. Concentrations of microcystin-LR in the lake, shallow and deep enclosures. The concentrations are expressed relative to the controls by dividing the concentration in each treated enclosures by the corresponding control. The concentration in the lake was expressed relative to the controls of the deep enclosures. Error bars are standard error of the mean based on data from the end of the experiment (last 3 dates).

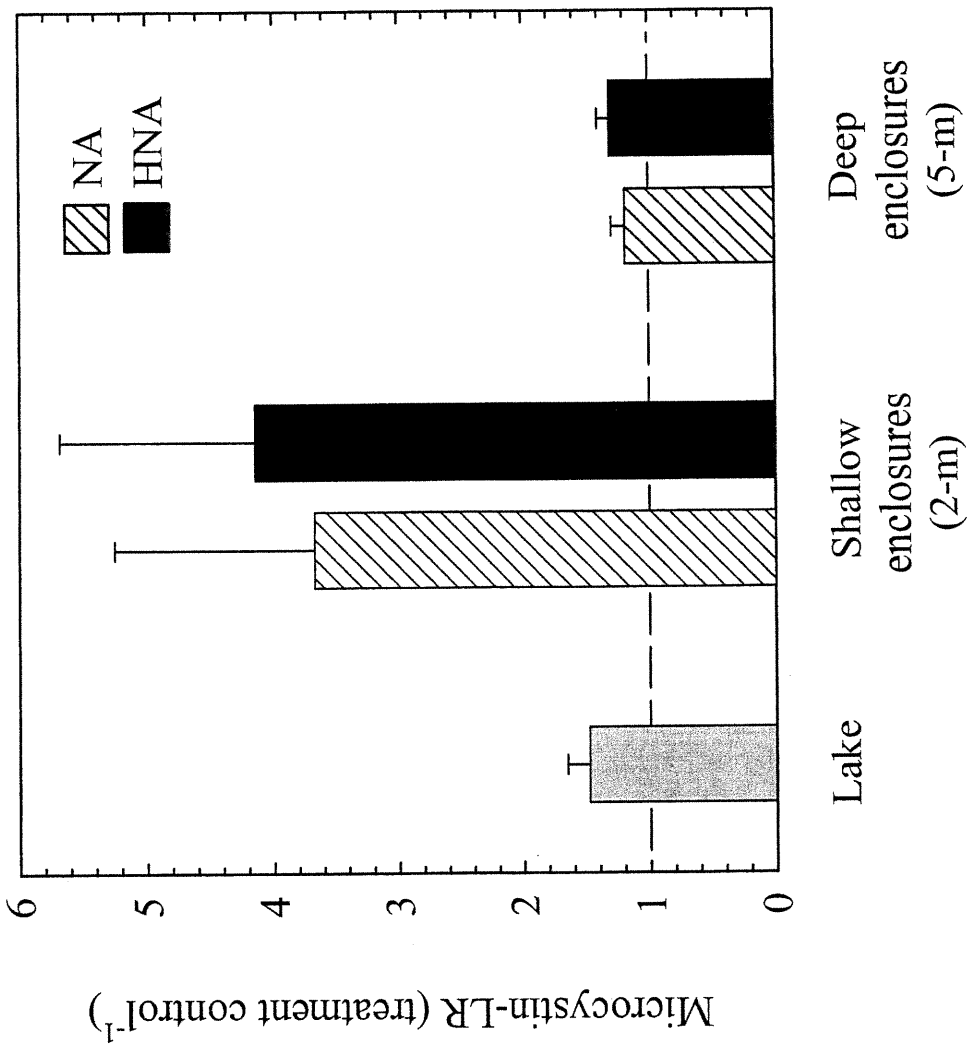

NA
HNA
Lake
Shallow enclosures (2-m)
Deep enclosures (5-m)
0
1
2
3
4
5
6
Microcystin-LR (treatment control^{-1})

Table 3. Zooplankton biomass (mg m^{-3}) in the lake and all treatment levels in shallow and deep enclosures. Biomass of major crustacean species of crustaceans is presented. All copepodite stages were included in one category (C1-C5). The numbers are means of the last 3 dates of the experiment ± one standard error. Control: enclosures with no nutrient addition, NA: enclosures treated with nutrient addition and HNA enclosures with high nutrient addition.

	Lake	Shallow enclosures (2-m)			Deep enclosures (5-m)		
		Control	NA	HNA	Control	NA	HNA
Cladocerans							
Daphnia pulicaria	129.3±42.8	191.9±28.3	167.7±41.4	92.7±36.2	38.7±7.2	56.3±17.5	65.8±10.4
Chydorus sphaericus	4.9±0.6	3.5±1.6	4.2±1.6	4.5±0.8	0.9±0.2	1.9±0.4	1.8±0.3
Ceriodaphnia lacustris	0.8±0.2	7.4±2.8	5.8±1.9	1.3±0.5	10.8±3.9	4.4±1.1	7.9±1.9
Diaphanosoma brachyurum	1.4±0.4	0.6±0.3	0.9±0.5	0.7±0.2	0.5±0.1	0.5±0.2	0.5±0.2
Bosmina longirostris	0.2±0.1	0.1±0.0	0.4±0.2	1.2±0.6	1.6±0.3	1.3±0.3	0.5±0.1
Total cladocerans	136.8±43.6	203.6±27.3	179.3±41.3	100.6±36.8	52.6±8.8	64.5±17.4	76.6±10.0
Copepod calanoids							
Skistodiaptomus oregonensis	10.8±3.9	4.4±1.2	10.1±2.5	12.0±3.8	5.3±0.7	3.7±0.5	3.5±0.9
Copepodites C1-C5	20.4±3.4	17.1±1.9	19.4±3.2	17.6±3.7	18.8±2.3	18.5±2.3	15.2±0.8
Total Calanoids	31.2±7.0	21.5±2.3	29.4±5.6	29.7±7.1	24.2±2.8	22.2±2.7	18.7±1.4
Copepod cyclopoids							
Diacyclops bicuspidatus thomasi	6.4±1.3	2.4±1.2	3.1±1.6	2.8±1.4	2.4±0.5	2.8±0.6	2.4±0.4
Copepodites C1-C5	3.4±0.9	4.5±1.1	6.8±3.2	5.5±1.0	3.4±0.3	2.0±0.5	1.6±0.3
Total cyclopoids	9.9±1.5	6.9±1.4	9.8±3.1	8.3±2.1	5.8±0.5	4.8±1.0	4.0±0.5
Total copepods	41.2±7.5	28.5±3.4	39.3±6.8	38.0±7.1	30.0±3.0	27.1±3.3	22.8±1.5
Total zooplankton	177.9±45.5	232.1±29.6	218.6±44.5	138.6±42.7	82.7±10.2	91.6±17.1	99.4±11.0

taxonomic group (Table 3). Calanoid copepods were the second dominant group after cladocerans and contributed ~9 to 29% to the total zooplankton biomass (Table 3). *Skistodiaptomus oregonensis* was the only calanoid species found. Cyclopoid copepods were represented by one species, *Diacyclops bicuspidatus thomasi*, and contributed only ~3 to 6% to total zooplankton biomass (Table 3).

In fertilized shallow enclosures, zooplankton biomass appeared to be inversely related to phytoplankton biomass. In general, higher zooplankton biomass was observed in the lake and the shallow enclosures in comparison to deep enclosures (Fig. 8, Table 1). This pattern can be explained by the limited phytoplankton biomass observed in deep enclosures following treatment. The inedible fraction (> 30 µm) of phytoplankton mainly dominated by large colonial and filamentous cyanobacteria species was higher in the treated shallow enclosures. These shallow enclosures had also low cladoceran biomass (Fig. 8a). However, the difference between the treated and the control was only significant for HNA treatment (Fig. 8, Table 4). Zooplankton communities were different in the treated shallow enclosures as shown by the low dominance of cladocerans over copepods in comparison to the shallow control (Fig. 8a,b). The dominance of cladocerans measured by the ratio of cladoceran to copepod biomass, was on average 40% and 70% lower in NA and HNA treatments,

Fig. 8. Biomass of a) cladocerans, b) copepods, c) ratio of cladoceran to copepod and d) total zooplankton in the lake, shallow and deep enclosures. Error bars are standard error of the mean based on data from the end of the experiment (last 3 dates).

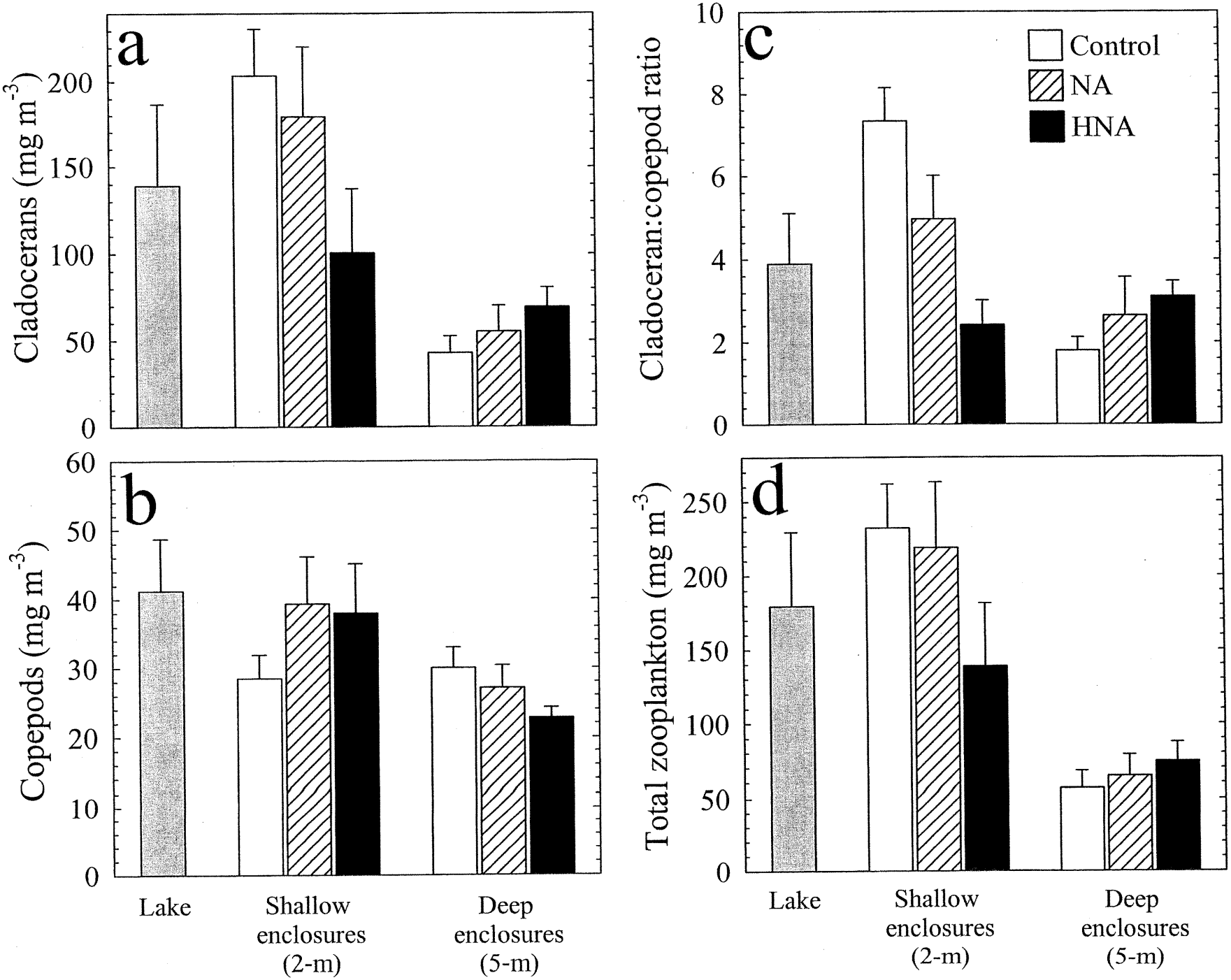

a
Cladocerans (mg m-3)
0
50
100
150
200
b
Copepods (mg m-3)
0
10
20
30
40
50
60
c
Cladoceran:copepod ratio
0
2
4
6
8
10
Control
NA
HNA
d
Total zooplankton (mg m-3)
0
50
100
150
200
250
Lake
Shallow enclosures (2-m)
Deep enclosures (5-m)

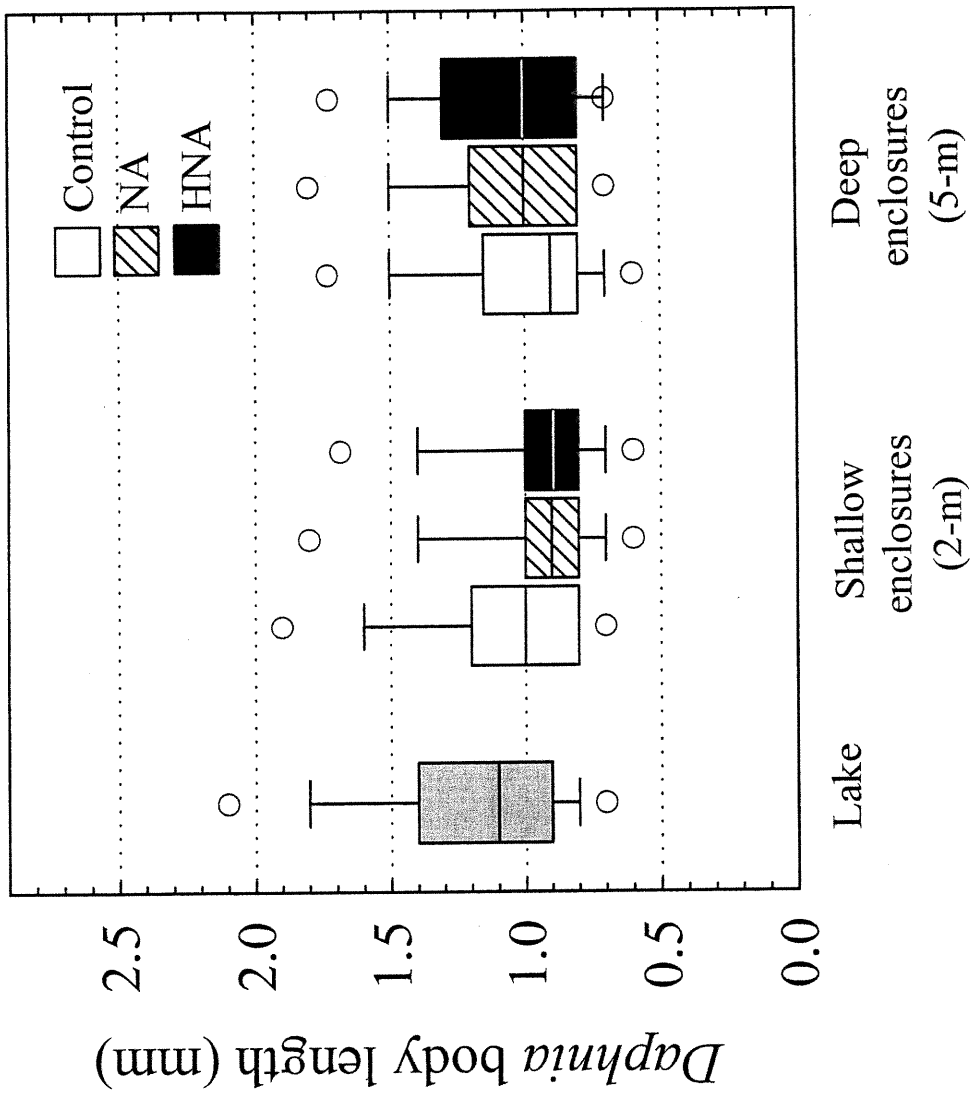
Table 4. Results of repeated-measures ANOVAs for comparison of zooplankton in treated (NA and HNA) and untreated enclosures. Treatment (df=1) and time (df=6 or 2) effects were tested for all the sampling dates (7) and only the last 3 dates. The interaction term has the same df as time (6 or 2).

	Shallow enclosures (2-m)								Deep enclosures (5-m)							
	Nutrient addition (NA)				High nutrient addition (HNA)				Nutrient addition (NA)				High nutrient addition (HNA)			
	All 7 dates		Last 3 dates		All 7 dates		Last 3 dates		All 7 dates		Last 3 dates		All 7 dates		Last 3 dates	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Daphnia pulicaria																
Treatment	0.3	0.641	0.8	0.535	12.5	0.175	263.0	0.012	24.7	0.126	0.7	0.554	141.0	0.016	0.2	0.713
Time	15.4	0.002	0.2	0.856	2.3	0.163	0.3	0.676	29.8	<0.001	6.4	0.133	79.1	<0.001	11.7	0.078
Treatment X Time	0.7	0.681	1.4	0.407	2.1	0.186	0.8	0.695	0.9	0.537	0.1	0.925	0.5	0.721	0.3	0.768
Cladocerans																
Treatment	0.1	0.797	0.7	0.552	8.4	0.210	193.0	0.014	13.7	0.167	0.6	0.596	81.3	0.070	0.2	0.751
Time	11.9	0.004	0.2	0.816	2.5	0.137	0.3	0.738	37.1	<0.001	7.3	0.119	49.5	<0.001	10.3	0.088
Treatment X Time	1.2	0.391	1.8	0.347	2.3	0.157	0.4	0.724	2.9	0.110	0.1	0.897	1.0	0.498	0.3	0.757
Copepods																
Treatment	2.5	0.356	0.3	0.672	0.1	0.910	0.5	0.594	0.1	0.766	84.8	0.068	0.2	0.740	8.9	0.205
Time	19.3	0.001	2.6	0.274	22.5	<0.001	2.6	0.278	10.4	0.005	2.1	0.323	13.6	0.002	6.7	0.128
Treatment X Time	2.4	0.152	1.0	0.492	8.4	0.010	12.1	0.075	0.7	0.635	0.2	0.821	1.1	0.450	0.2	0.867
Cladoceran:copepod																
Treatment	0.4	0.634	9.7	0.197	18.3	0.145	21.5	0.135	51.2	0.088	4.1	0.292	15.9	0.156	3.3	0.316
Time	4.0	0.056	0.4	0.687	0.7	0.625	0.1	0.885	7.4	0.013	2.8	0.259	61.9	<0.001	4.1	0.194
Treatment X Time	0.9	0.517	1.2	0.440	2.2	0.183	0.1	0.982	1.7	0.257	0.1	0.970	2.4	0.150	3.6	0.215
Total zooplankton																
Treatment	1.5	0.434	0.3	0.692	3.7	0.303	74.9	0.073	175.0	0.004	0.1	0.967	64.7	0.078	0.1	0.978
Time	15.3	0.002	0.5	0.667	4.3	0.047	0.6	0.632	61.3	<0.001	8.6	0.103	32.3	<0.001	9.9	0.091
Treatment X Time	1.7	0.265	1.8	0.351	3.4	0.081	0.8	0.547	0.9	0.532	0.5	0.643	0.5	0.785	0.1	0.901

respectively, in comparison to the control (Fig. 8c). Although the difference was not statistically significant (Table 4), low cladoceran to copepod ratio may be an indication that *Daphnia pulicaria* was negatively affected by the occurrence of high biomass of bloom forming cyanobacteria species, whereas copepod species were less or not affected (Fig. 8b). The low biomass of *Daphnia pulicaria* translated into a low biomass of total zooplankton in NA and HNA treatments (Fig. 8d). The difference in total zooplankton biomass was not always statistically detectable probably due to the compensation by copepods of the low cladoceran biomass (Fig. 8d, Table 4). Total zooplankton biomass in HNA treatment was ~40% lower than in the control (Fig. 8d, Table 4). The lower amplitude in the response of phytoplankton to the treatment in deep enclosures seems to have affected zooplankton which had only a limited biomass in comparison to the shallow enclosures (Fig. 8d, Table 1). In general, no statistically significant differences were observed when the two-treatment levels (NA and HNA) of the deep enclosures were compared to the control (Table 4).

Daphnia pulicaria body length was smaller in NA and HNA treatments in shallow enclosures where a major fraction (~75%) of the population measured less than 1mm in comparison to the control (Fig. 9). This may indicate that *Daphnia* populations experienced higher mortality of individuals with body length larger than 1mm after the occurrence of the

Fig. 9. *Daphnia pulicaria* body length boxplots (mm) for the lake, shallow and deep enclosures. The number of *Daphnia* individuals measured varied between ~300 and 600. Open circles represent upper and lower 95% confidence intervals based on data from the end of the experiment (last 3 dates). Wilcoxon nonparametric test revealed statistically significant difference between all the treatment (NA and HNA) and their respective control in shallow and deep enclosures (Wilcoxon, $P < 0.0001$).


Control
NA
HNA
2.5
2.0
1.5
1.0
0.5
0.0
Daphnia body length (mm)
Lake
Shallow enclosures (2-m)
Deep enclosures (5-m)

blooms as a result of the treatment. In contrast, the body length of *Daphnia* populations in the lake and the deep enclosures was centered around 1mm, with half of the population over 1mm and the other half below this size (Fig. 9).

The effect of cyanobacteria blooms on the *Daphnia pulicaria*, cladocerans and total zooplankton biomass was evaluated by relating zooplankton variables to particulate carbon (Fig. 10). A quadratic model was used to describe the nonlinear relationships observed between zooplankton and phytoplankton (Fig. 10, Table 5). Zooplankton variables were strongly related to particulate carbon ($r = 0.65$ to 0.71, $P < 0.0001$). These relationships displayed two distinct phases, a first one where zooplankton seem to be positively influenced by increasing algal biomass and a second one, mainly driven by data from NA and HNA treatments in shallow enclosures, showing a decline in zooplankton biomass when phytoplankton biomass reached very high level (Fig. 10). This model suggests that zooplankton and phytoplankton are related in a nonlinear fashion in eutrophic cyanobacteria dominated systems. In all the three relationships, the quadratic term is highly significant suggesting that the second phase of the nonlinear relationships is an actual decline over increasing phytoplankton biomass and not only a level out (Fig. 10, Table 5).

Fig. 10. Relationships between a) *Daphnia pulicaria* biomass ($r = 0.65$, $P < 0.0001$), b) cladocerans biomass ($r = 0.67$, $P < 0.0001$), and c) total zooplankton biomass ($r = 0.71$, $P < 0.0001$) and particulate carbon. Data are represented by circles for shallow enclosures (controls in open circles and treatments in solid circles), by squares for deep enclosures (controls in open squares and treatments in solid squares), and by open triangle for the lake. The solid curve represents a quadratic regression fitted to the combined data. The dashed lines are upper and lower 95% confidence intervals, based on data from the full length of the experiment (7 dates).

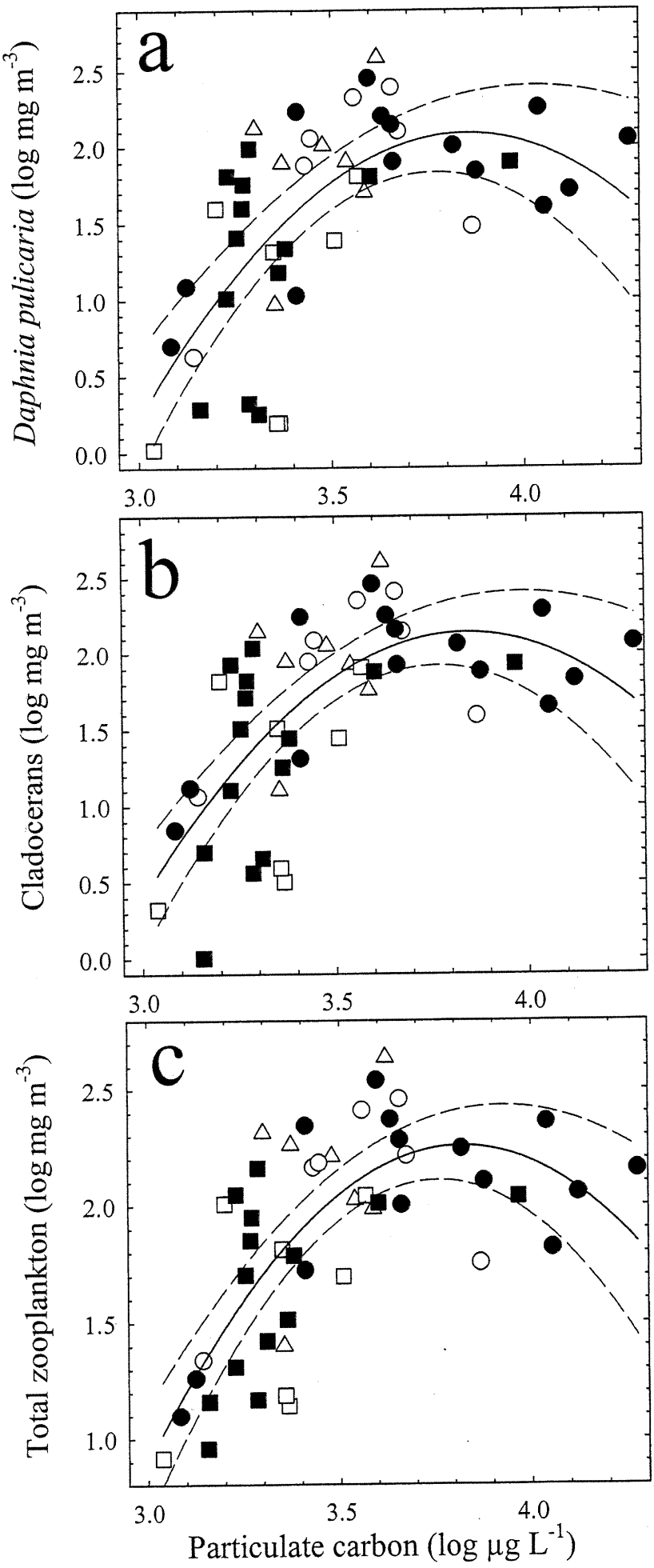

a
b
c
Daphnia pulicaria (log mg m^{-3})
Cladocerans (log mg m^{-3})
Total zooplankton (log mg m^{-3})
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Particulate carbon (log µg L^{-1})

Table 5. Summary of analysis of variance and associated tests for quadratic regressions used to describe relationships between zooplankton variables (*Daphnia pulicaria*, cladocerans, and total zooplankton biomass) and particulate carbon as a measure of phytoplankton biomass.

Source	df	SS	MS	*F*	*t*	R	*P*
Daphnia pulicaria							
Regression	2	9.15	4.5	16.7		0.65	<0.0001
Residual	46	12.3	0.3				
Intercept					-3.6		0.0008
Linear coefficient					3.5		0.0010
Quadratic coefficient					-3.3		0.0018
Cladocerans							
Regression	2	8.3	4.2	19.1		0.67	<0.0001
Residual	46	10.0	0.21				
Intercept					-3.8		0.0003
Linear coefficient					3.8		0.0004
Quadratic coefficient					-3.6		0.0008
Total zooplankton							
Regression	2	4.9	2.5	23.1		0.71	<0.0001
Residual	46	4.9	0.1				
Intercept					-4.4		<0.0001
Linear coefficient					4.4		<0.0001
Quadratic coefficient					-4.2		0.0001

Discussion

The effect of cyanobacterial blooms on zooplankton communities is an important topic from both the scientific and lake management perspective. Our study is an experimental test of the interactions between cyanobacteria and zooplankton at a larger scale than that of the laboratory. Our experiments confirmed that high biomass of filamentous and colonial potentially toxic cyanobacteria produced during bloom events can be harmful to zooplankton communities. To our knowledge this is the first large-scale experimental evidence of such a negative impact on zooplankton. Several studies have previously reported large midsummer declines of zooplankton biomass in eutrophic lakes as a result of a decline in the large *Daphnia* concomitantly with the occurrence of cyanobacterial blooms (e.g., Wright 1965, Clark and Carter 1974, Threlkeld 1979, Ghadouani et al. 1998). However, there is no consensus as to the cause of this phenomenon that was explained by changes in physical conditions (i.e., water temperature), invertebrate or fish predation pressure and only rarely by changes in food conditions and precisely cyanobacteria dominance (Threlkeld 1979). Our results are consistent with the late hypothesis and suggest that the exposition to high biomass of cyanobacteria, even for short-term, can cause *Daphnia* population to decline and consequently cause a decline in the total zooplankton biomass. Our study also examines cyanobacteria response to nutrient addition in different depths considering that a better

understanding of cyanobacteria dynamics could lead to better predictions of the response of zooplankton communities to cyanobacterial blooms.

Cyanobacteria dynamics - In this study, the use of shallow and deep enclosures with the same experimental conditions was not originally aimed to test the role of depth in initiating cyanobacteria blooms, but rather the possible changes in migratory behavior of zooplankton in the presence of cyanobacteria as shown in laboratory experiments (Berthon and Brousse 1995). Zooplankton may be able to avoid direct contact with floating cyanobacteria by choosing to stay in an appropriate depth. However, it appeared, during the first week of the experiments, that the difference in enclosure depths was responsible for a contrasting response of phytoplankton communities to fertilization by N and P. Our results indicated that in the same nutrient conditions, shallow enclosures produced higher biomass of cyanobacteria than deep enclosures. These results are consistent with recent mathematical models and laboratory experiments which identify, among others, incident light intensity and mixing depth as possible triggers for cyanobacterial blooms (Huisman 1999, 1999a, Huisman et al. 1999b, 1999c). The possible explanation for the absence of blooms in our deep enclosures could be a combination of light limitation and greater sinking rate of cyanobacterial filaments and colonies. As it is clear from the light penetration profiles in the deep enclosures, less than 1% of incident light was

available to phytoplankton communities below 2.5-m. The difference between deep and shallow enclosures was much more pronounced if we consider the *Aphanizomenon flos-aquae* response. This strong difference could be explained by higher mixing in shallow enclosures, which is consistent with model predictions (Huisman et al. 1999c) as well as field observations (Jacobsen and Simonsen 1993, Jacobsen 1994) that this species develop higher biomass in well-mixed shallow lakes.

It is also clear from our results that the three dominant species (*Anabaena flos-aquae, Aphanizomenon flos-aquae,* and *Microcystis aeruginosa*) which formed up to 70% of phytoplankton total biomass, responded in different fashions to the fertilization. However, the pattern of response of these species to fertilization was similar in both shallow and deep enclosures, even if the amplitude was smaller in deep systems. This may suggest that the differences observed are mainly due to difference of physiological requirements of each of the three blooming species. For example, *Aphanizomenon flos-aquae* have been described as a better competitor than other species in case of pulse nutrient addition (this experiment, Suttle et al. 1987, De Nobel et al. 1997), was much more abundant than *Anabaena flos-aquae.* Whereas, *Anabaena* biomass was higher in unfertilized enclosures possibly due to its higher N fixation activity in low nitrogen condition in comparison to *Aphanizomenon* (De Nobel et al. 1997).

Aphanizomenon and *Anabaena* are both nitrogen fixers however their conditions for bloom formation are different. In contrast, *Microcystis* biomass was positively enhanced by fertilization in an opposite manner to *Anabaena*. Hence, it seems that *Microcystis* blooms may likely occur in very low TN:TP conditions (below ~5:1), whereas *Anabaena* blooms may be favored by higher TN:TP ratio (~20:1). On the other hand, the growth conditions favorable for *Aphanizomenon* appeared not to be related to TN:TP ratio. Recent physiological studies suggested that *Aphanizomenon* can be a superior competitor over *Anabaena*; however, the physiological requirement of *Aphanizomenon* in terms of nutrient conditions are yet to be elucidated (De Nobel et al. 1997). There has been extensive effort to try to predict cyanobacterial blooms based on N:P ratio since Smith paper (1983); however, our results suggest that other variables such as nutrient concentration not only ratio (Trimbee and Prepas 1987), or light can be as important (e.g., Smith 1986, Huisman et al. 1999b). More investigation is also needed for better understanding the physiology and the ecology of common cyanobacteria species found during bloom events (e.g., De Nobel et al. 1997).

Zooplankton responses - Our experiments demonstrated that zooplankton and phytoplankton biomass were strongly related; and that zooplankton can respond quickly to changes in phytoplankton biomass and species composition. By increasing the biomass of colonial and filamentous

cyanobacteria species, we were able to reduce the size structure and the biomass of large cladocerans in few weeks. These results suggest that phytoplankton-zooplankton relationships are not linear as argued by previous empirical studies (McCauley and Kalff 1981). In eutrophic lakes, phytoplankton-zooplankton relationships seem to be curvilinear rather than linear. During the first phase of moderate dominance by cyanobacteria, zooplankton may not be negatively affected, probably due to the presence of enough edible particles. However, as soon as cyanobacteria reach high biomass, zooplankton become more negatively affected. It is still not completely clear that the observed effects are entirely due to toxin production alone, the lack of edible phytoplankton could be an alternative explanation. The observed results can be also explained by a combination of the interference of colonies and filaments with other edible phytoplankton (Webster and Peters 1978, DeMott et al. 2001) and the increase in cyanotoxins produced by *Microcystis* cells (Lampert 1981, 1982, Reinikainen et al. 1995, 1999). It is clear from these results that large *Daphnia* were less abundant where high biomass of inedible phytoplankton (mainly cyanobacteria) were observed (shallow fertilized enclosures). Our interpretation of the loss of large *Daphnia* (> 1mm) is that large animal experienced high mortality because of the reduction in their filtering capacity in high biomass of filamentous and colonial cyanobacteria (Webster and Peters 1978, DeMott et al. 2001). Indeed, our results suggest that the negative effects reflected by zooplankton

communities may be caused by a possible collapse in *Daphnia pulicaria* which was the major contributor to total zooplankton biomass. Large *Daphnia* (> 1mm) seemed to be more vulnerable to cyanobacteria bloom events (DeMott et al. 2001). Previous studies found a similar loss of large *Daphnia pulicaria* (> 1mm) after the occurrence of blooms of filamentous cyanobacteria (Threlkeld 1979). It is well established that *Daphnia* are a key player in lake foodwebs due to their ability to filter a large biomass of phytoplankton and reduce algal biomass (e.g., Lampert et al. 1986). However, the ability of *Daphnia* to reduce algal biomass can be significantly compromised in the presence of large colonial and filamentous cyanobacteria toxic species (Lampert 1981, 1982, Haney 1987). The occurrence of these particular cyanobacteria have long been neglected and treated as an exception in several attempt for oversimplification of aquatic foodweb interactions (Carpenter and Kitchell 1992, DeMelo et al. 1992). However, several lines of evidence indicate that lake response to biomanipulation of top predators may be constrained at the interface phytoplankton-zooplankton (e.g., Moss et al. 1991, MacKay and Elser 1998). Foodwebs can display complex and especially non-linear patterns which are quite difficult to simplify and hence to model if all major interactions are not considered (see discussion by Polis and Strong 1996).

Implications for phytoplankton-zooplankton interactions – Eutrophication is not a new environmental problem; however, there is evidence that aquatic systems are experiencing more severe cyanobacterial blooms for example, due to increasing nutrient input and higher water temperatures (Moss 1996). The occurrence of cyanobacterial blooms is also believed to be more frequent as summers get warmer as predicted by global warming trends in temperate regions. Cyanobacteria blooms in particular and toxic algal blooms in general are not restricted to fresh waters; coastal low salinity regions developed large algal blooms of *Nodularia spp.* and *Aphanizomenon spp.*, during the last decade (Sellner 1997). As the problem of toxic algal blooms invades aquatic systems worldwide, there is an urgent need for ecologists to understand better the implications of such events on aquatic foodwebs. Our results illustrate the serious limitation for the application of trophic cascade theory, for example, in the reduction of eutrophication by biomanipulation in cyanobacteria dominated lakes. We have shown that *Daphnia* can be negatively affected by cyanobacterial blooms. The crucial link in the energy transfer from lower to higher trophic levels is the extraordinary filtering efficiency of *Daphnia* in lakes (Carpenter et al. 1985, Brett and Goldman 1996). We believe that a better understanding of the processes controlling the occurrence of cyanobacteria blooms as well as the dynamic and nonlinear relationship between phytoplankton and zooplankton in

eutrophic systems can lead to successful reduction of eutrophication and associated toxic algal problems.

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Chapitre 3

Phenotypic plasticity in *Daphnia pulicaria* as an adaptation to high biomass of colonial and filamentous cyanobacteria: Experimental evidence

Phenotypic plasticity in *Daphnia pulicaria* as an adaptation to high biomass of colonial and filamentous cyanobacteria: Experimental evidence

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Journal of Plankton Research (In press)

Abstract

We investigated the ability of water flea *Daphnia* to adapt the size and structure of its filtering apparatus as a response to experimentally increased biomass of inedible filamentous and colonial cyanobacteria in a large *in situ* enclosure experiment. Predator-induced phenotypic plasticity in water flea *Daphnia* have been extensively documented but only a small number of studies have focused on morphological changes induced by food quantity and quality. Here we show that *Daphnia* responds to increased biomass of inedible phytoplankton by enlarging the area and the mesh size of its filtering apparatus. These observations suggest that *Daphnia* responds to increased concentrations of inedible particles in the same fashion as they do in a very low food environment. In our study, daphnids exposed to high biomass of inedible algae, in fertilized enclosures, had significantly larger (12 to 15%) filter-screens attached to their 3rd and 4th limb in comparison to daphnids exposed to low biomass of inedible algae (ANCOVA, $P < 0.001$). The mesh size also increased in the same conditions. These results suggest that daphnids used their phenotypic plasticity to respond to changes in their food quality and quantity. By using this strategy, daphnids can maximize their food uptake and hence compensate for the scarcity of suitable food encountered in very oligotrophic conditions or even in eutrophic conditions when phytoplankton communities are dominated by large inedible species.

Key words: *Phenotypic plasticity; filter-screens; Daphnia pulicaria; cyanobacteria; inedible phytoplankton.*

Introduction

Zooplankton, like all living organisms, exhibit a large variety of responses to changes in their habitat (Schlichting, 1989; Schlichting and Pigliucci, 1998). To benefit from adaptive advantages, these organisms occasionally display major changes in their behaviour, life cycle or morphology (Dodson, 1989; 1990). In the presence of planktivorous fish or invertebrate predators, they can migrate vertically or horizontally to avoid predation (Haney, 1988; Ohman, 1990; Bollens and Frost, 1991; Pijanowska, 1993; Dodson, *et al.*, 1997). Some freshwater cladocerans such as water flea *Daphnia*, have been shown to reduce their body size in presence of large size selective predators such as fish (Stibor, 1992), and do just the opposite in the presence of small size selective predators such as phantom midge *Chaoborus* (Stibor and Lüning, 1994). In lakes with planktivorous fish, the antipredator morphological plasticity is a result of a rather complex mechanism used by *Daphnia* to reduce egg size and produce neonates which mature at small size (Lampert, 1993). To moderate the effects of predation, cladocerans also use morphological changes such as helmet enlargement (Tollrian, 1993; Tollrian, 1994), or neckteeth formation (Tollrian, 1993; Tollrian, 1995). The antipredator defences disappear rapidly in the absence of predators or their chemical cues because this defence mechanism has high energetic cost (Lampert, 1993; Tollrian and Dodson, 1999). In recent

decades, ecologists have focused on predator inducible defences such as diel vertical migration (Neill, 1992; De Meester, *et al.*, 1995), changes in life history, and body shape and size (Lampert, 1993; Stibor and Lampert, 1993) but less on the response of these organisms to major changes in their food quality and quantity.

Zooplankton also use phenotypic plasticity to adapt to seasonal variations in bottom up processes in terms of food quality and quantity (Lampert, 1994; Repka, *et al.*, 1999). Laboratory studies have clearly shown that a number of *Daphnia* species are able to adapt to low food conditions by changing the size and structure of their filter-screens on their 3rd and 4th limbs (Geller and Müller, 1981; Koza and Korínek, 1985; Pop, 1991; Stuchlík, 1991; Lampert, 1994). This mechanism is used by *Daphnia* to optimize food gathering with moderate energy cost and hence compensate for occasional low food conditions in lakes (Lampert, 1994; Lampert and Brendelberger, 1996). Cladocerans with large filter-screens have higher filtering rate than animals with smaller ones (Egloff and Palmer, 1971; Stuchlík, 1991). On the other hand, cladoceran filter-screens affect food selection, and could explain some zooplankton seasonal succession patterns in lakes (Geller and Müller, 1981). For example, species with coarser filter mesh tend to have lower retention efficiency

for small particles (Brendelberger, 1991). Depending on the food environment, daphnids are capable of adjusting the area and/or mesh size of their filter-screens (Brendelberger and Geller, 1985). The phenotypic plasticity in *Daphnia* can take place in a relatively short time (one or two generations) in natural populations as well as in monoclonal laboratory populations (Pop, 1991).

Previous studies of *Daphnia* phenotypic plasticity focused primarily on food quantity as a trigger for changes in filter screens (Lampert, 1994; Lampert and Brendelberger, 1996). Here we report the results of an *in situ* enclosure experiment in which we investigated the ability of daphnids to adapt to high biomass of large filamentous and colonial algae by changing particular morphological traits of their filtering apparatus. We anticipated that *Daphnia* populations exposed to high biomasses of colonial and filamentous cyanobacteria or large inedible algae would 1) enlarge their filter screen area to compensate for the relative scarcity of small edible phytoplankton cells; and 2) adjust their mesh size to be able to feed on smaller food particles, including bacteria, more efficiently. It has been suggested that eutrophic conditions are favourable for cladocerans with finer mesh size which are capable of filtering small particles including bacteria (Geller and Müller, 1981; Brendelberger, 1991). We investigated these questions by comparing morphometric measurements of

filter screens (filter screen area, setular width, and mesh size) of daphnids which were exposed to contrasting phytoplankton populations with low and high biomass of colonial and filamentous cyanobacteria in large *in situ* enclosures. To our knowledge, there has been no large scale experimental testing of phenotypic plasticity in daphnids as a response to changes in food quality and quantity. One of the aims of this study was to evaluate whether or not daphnids display, in natural environments, phenotypic variations in their filter-screens such as those observed in the laboratory under controlled conditions.

Methods

Study site

The study was conducted in Steele Lake, (54°39'N 113°46'W), a relatively large (6.6 km^2 surface area) eutrophic lake in the northern boreal forest of Alberta, Canada. Steele Lake is shallow (3.2 m mean depth, 6.1 m maximum depth) enough to mix vertically during most of the summer. The drainage basin of Steele Lake is very large compared to that of many lakes on the boreal plain, representing 37 times the area of the lake (Mitchell and Prepas, 1990). Large amounts of nutrients are transported to the lake from the naturally rich drainage basin, which causes frequent cyanobacterial blooms during summer (Trimbee and Prepas, 1987). The blooms are usually dominated by

species such as *Aphanizomenon spp.*, *Anabaena spp.* and *Microcystis spp.* which make this lake an ideal site for these experiments.

Experimental set up and nutrient manipulations

The experiments were performed in six large cylindrical enclosures which were placed at the deepest part of the lake in a sufficiently quiet area to minimize the effect of the wind. The enclosures were closed off from the bottom of the lake and were made of clear thick woven polyethylene fitted to a flotation collar of ethafoam logs. The collars were ~50 cm above the surface of the water to keep enclosure and lake water from mixing. The stability of each of the six enclosures was ensured by attaching each three enclosures to one side of a floating dock. The whole structure (enclosures + dock) was anchored at several points to ensure maximum stability. Water from the lake was pumped into each enclosure. The filled enclosures were then covered by a gill net to keep fish from jumping into the enclosures. The enclosures had 2.5-m diameter openings, were 2-m deep and contained ~10 m^3 of water each.

To enhance phytoplankton biomass we fertilized four of the six enclosures by adding a combination of phosphorus (P) and nitrogen (N) to four enclosures and left the remaining two as controls (no nutrient additions). We

added P as potassium phosphate monobasic (KH_2PO_4) and N as ammonium nitrate (NH_4NO_3). We intended to create an exponential gradient in nutrients in comparison to the concentrations measured at the beginning of the experiments. Hence, we increased P concentration in the first two treated enclosures by ~3 fold, and hereafter refer to this treatment as nutrient addition (NA). In the last two enclosures, P concentrations were increased roughly by ~7 fold in comparison with the control and were referred to as high nutrient addition (HNA). N was added in the proportions of 3:1 and 1:1 relative to P by weight in NA and HNA treatments, respectively. The nutrients were added as single pulse at the beginning of the experiments and all the treated enclosures were fertilized at the same time.

Sampling and analytical methods

The experiments took place in summer 1996 and lasted for five weeks including the pretreatment period of six days. Enclosures were sampled every fourth day. Water temperature, dissolved oxygen concentration and light penetration were measured in the enclosures and the lake during each visit to the site. Water samples for chemical analyses and chlorophyll *a* (chl-*a*) estimation were collected with an integrated sampler made of tygon tubing. Water samples were taken and preserved in Lugol solution for phytoplankton

identification and enumeration. P concentrations were determined following the modified Menzel and Corwin (1965) potassium persulfate method (Prepas and Rigler, 1982). The cold ethanol extraction (24h) method was used to spectrophotometrically estimate chl-*a* concentration in water samples (Bergmann and Peters, 1980). Preserved sub-samples were settled in a sedimentation chamber and identified with an inverted microscope according to the Utermöhl method (Lund, *et al.*, 1958). Phytoplankton communities were regrouped here in three classes: cyanobacteria including all colonial and filamentous species, large diatoms considered as inedible for daphnids based on their large size (> 30 µm), and all other species considered as edible due to their small size (< 30 µm).

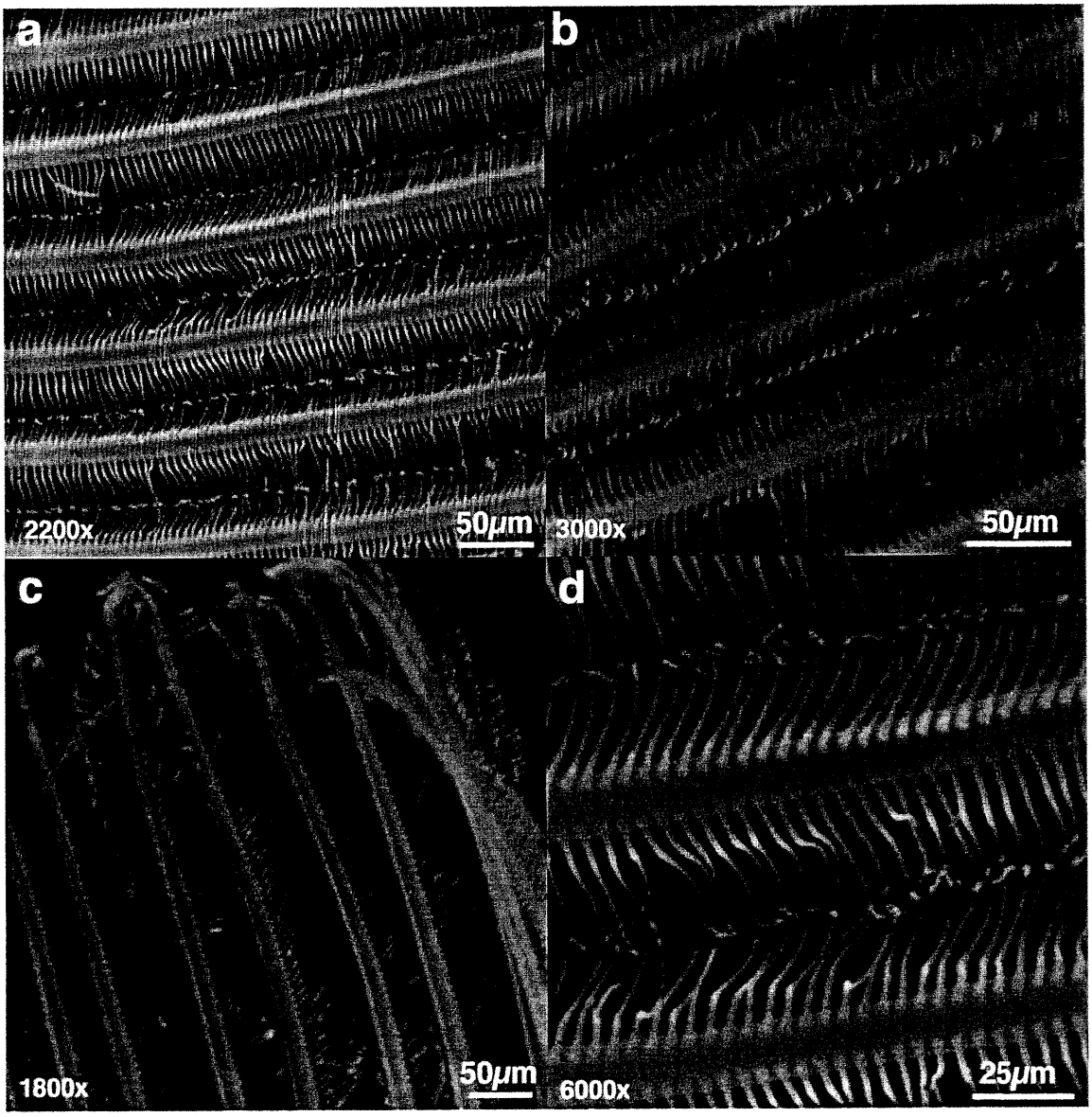
Daphnia filter-screen measurements

Zooplankton were sampled by means of a Wisconsin plankton net of 53 µm mesh size, 29 cm diameter and 90 cm length. The samples were preserved in a buffered 4% sugar-formaldehyde solution (Haney and Hall, 1973; Prepas, 1978). *Daphnia pulicaria* individuals ranging from ~0.6 to ~3.0 mm in body length were picked out from preserved zooplankton samples. The animals used in this study all came from zooplankton samples taken at the end of the experiments to ensure that they were exposed to the different environments for sufficiently long periods. Between 30 and 40 daphnids belonging to the

dominant cladoceran species, *Daphnia pulicaria*, were taken from each treatment. Each individual was measured and dissected under a stereomicroscope to take out separately the pair of filter-screens attached to the 3rd and 4th limbs. The filters were identified and spread out on a microscopic slide. The projected filter areas, *sensu* Brendelberger and Geller (Brendelberger and Geller, 1985; Koza and Korínek, 1985), were plotted on paper with a stereomicroscope equipped with a drawing tube. The plots of the filters were scanned and the area determined precisely by computerized planimetry with image analysis software (SigmaScanpro 5.0).

Fine morphometric measurements of the filter-screens such as intersetal and intersetular distances were made from scanning electronic microscope (SEM) photos (Plate 1). Screen-filters of large animals of the same size (3 mm) were selected from the different enclosures and prepared for SEM observations after critical point drying and metal coating (Brendelberger and Geller, 1985; Bozzola and Russell, 1992). Photographs were taken from each group of filter-screens, scanned and specific measurements made. Between 150 and 300 measurements of intersetal (Plate 1a,b) and intersetular (Plate 1d) distances were made for each group of filter-screens (Control, NA, HNA) to ensure an accurate comparison.

Plate 1. Scanning electronic microscope photos showing the fine structure of *Daphnia pulicaria* 3rd and 4th limb filter-screen fine structure at different magnifications ranging from 1800 to 6000X. The first order structures called setae support second order structures called setules in a bird plume-like fashion (c and d). Each setula appeared on the photo (d) to be hooked at the end to the next setula which is attached to the next seta (Photo by Anas Ghadouani).


a
2200x
50μm
b
3000x
50μm
c
1800x
50μm
d
6000x
25μm

Data analyses

Filter-screen areas of all the measured daphnids were plotted against their body length. Differences between the control and the treatments were tested with ANCOVA after all the data were log-transformed. Before the ANCOVA was calculated, we tested the assumption of homogeneity of slopes by making sure there was no significant interaction between the covariate (*Daphnia* size) and the treatment (control, NA and HNA). Since preliminary comparison of the area of filter-screens of daphnids from NA and HNA treatments showed no significant difference, the two groups were pooled together. Further comparisons were made between control and treatment as one group (NA + HNA). Cases with high absolute studentized residual were considered outliers and removed from the analysis (Velleman and Welsch, 1981). Hence, we were able to reduce the effects of outliers on the outcome of ANCOVA analysis. However, all data are shown on the figures including the outliers. ANCOVA were used to test the differences in filter-screen area of daphnids of all lengths (from 0.6 to ~3.0 mm), as well as small and large daphnids separately. The separation of the two groups was decided arbitrarily as 1.7 mm based on visual examination of the relationships. A one-way ANOVA was used to test for possible changes in the intersetal and intersetular distances after exposure to high cyanobacteria biomass in NA and HNA

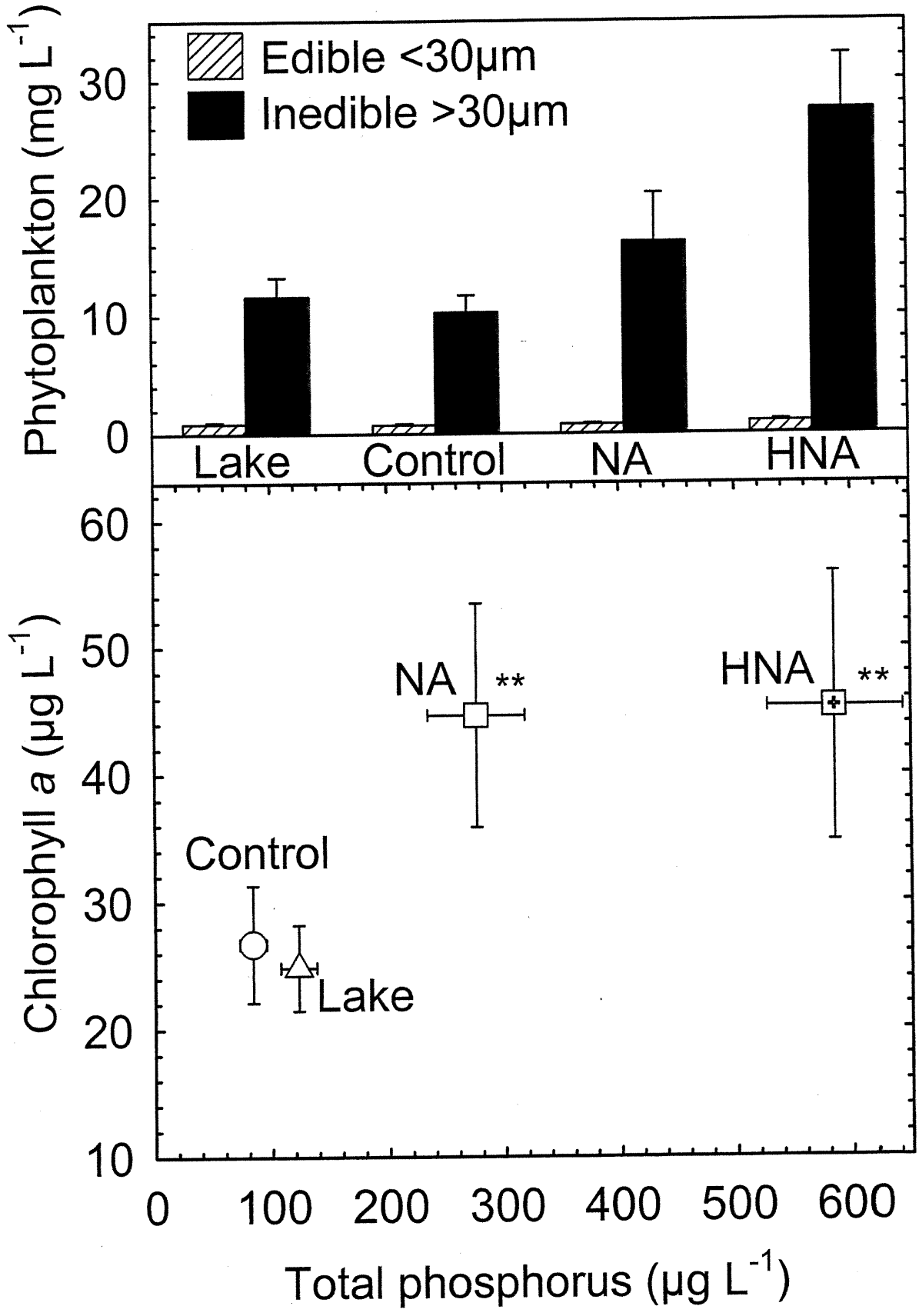
enclosures in comparison with the control. All the data presented are from the six enclosures as well as the lake; however, the statistical tests were only used to compare the treatments (NA and HNA) with the control. We consider the lake to be different from the control, since the conditions of the lake were not the same as the control, and any comparison with the treatment would not be relevant and conclusive. However, we show the data from the lake in all tables and figures for indicative purpose only. All statistical analyses were performed with Systat version 8.0.

Results

Phytoplankton response

The addition of nutrients (N, P) was responsible for doubling the biomass of phytoplankton as estimated by chl-*a* biomass (Figure 1). The increase in chl-*a* biomass was caused by cyanobacteria blooms which occurred in the fertilized enclosures (NA and HNA) after the addition of nutrients. During the bloom events, chl-*a* reached peaks as high as 94 and 112 $\mu g\ L^{-1}$ in NA and HNA treatments, respectively. Phytoplankton communities were dominated by large colonial and filamentous cyanobacteria species (*Microcystis aeruginosa, Aphanizomenon flos-aquae and Anabaena flos-aquae*) as well as large diatoms. These two groups which are considered inedible food for *Daphnia* accounted for an average of 80 to 90 % of the total phytoplankton biomass

Fig. 1. Chlorophyll a biomass (µg L^{-1}) as a function of total phosphorus (µg L^{-1}) in the lake, control, NA and HNA enclosures. Data are presented as mean of each group and bidirectional horizontal and vertical error bars (one standard error of the mean). Phytoplankton are classified in two fractions: edible fraction including all the unicellular species of size below 30µm; represented in clear bars and inedible fraction including all the species of size higher than 30 µm; represented in dark bars. One standard error of the mean is presented on each bar. ** $P < 0.01$ (ANOVA comparison of control vs. NA and HNA treatments).


Edible <30µm
Inedible >30µm
Phytoplankton (mg L-1)
30
20
10
0
Lake
Control
NA
HNA
60
50
40
30
20
10
Chlorophyll a (µg L-1)
NA
**
HNA
**
Control
Lake
0
100
200
300
400
500
600
Total phosphorus (µg L-1)

leaving only a small fraction of edible phytoplankton for the daphnids (Figure 1). The absolute biomass of the inedible fraction of phytoplankton was higher in the fertilized enclosures in comparison with the control. The biomass of large inedible filamentous and colonial phytoplankton species was ~2 to 3 times higher in NA and HNA enclosures, respectively (Figure 1). These conditions allowed us to expose daphnid populations to an increasing gradient of dominance by large inedible phytoplankton species in conditions closer to natural conditions than common laboratory experiments with one or two species.

Changes in *Daphnia* filter-screen area

Daphnia total filter-screen area (mm^2) increased with body length in all the treatments; however, the increase was more rapid in *Daphnia* which were exposed to higher biomass of inedible cyanobacteria and diatoms (Figure 2). ANCOVA revealed that *Daphnia* total filter-screen area was significantly larger in hypereutrophic systems (NA + HNA) in comparison with moderately eutrophic systems (control) (Table I, $P = 0.008$). As the daphnids grew larger the difference between the two groups became clear (Figure 2). The difference in screen-filter area was not statistically significant for daphnids smaller than 1.7 mm in body length (Table I, $P = 0.922$). When larger daphnids (>1.7 mm) were

Fig. 2. Total filter-screen area (mm^2) of *Daphnia pulicaria* from the lake (cross symbols), control enclosures (open circles) and NA and HNA treatment enclosures pooled together (solid circles), as a function of body length (top of the head to spine base). Outliers are shown on the graphs but not included in the ANCOVA analyses.

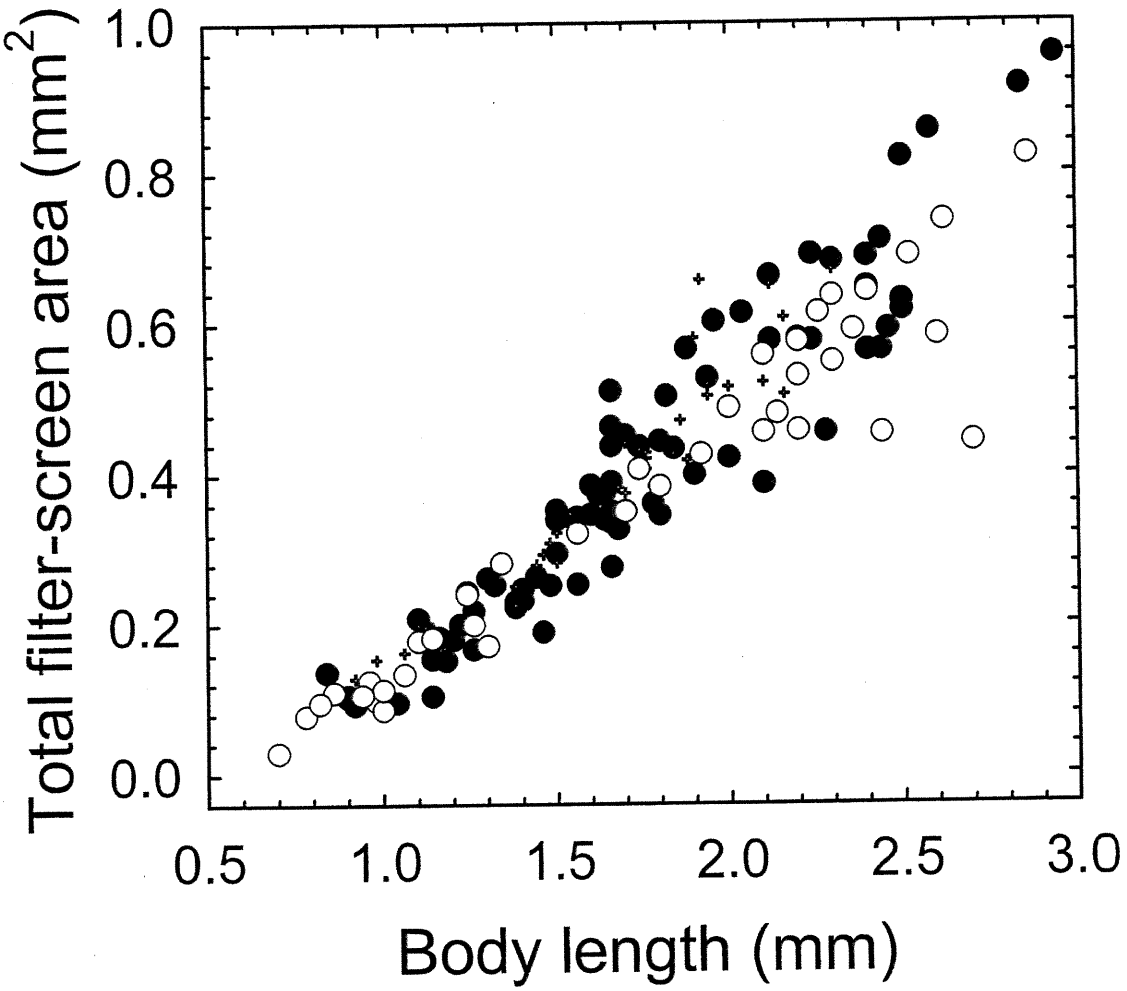

Total filter-screen area (mm^2)
1.0
0.8
0.6
0.4
0.2
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Body length (mm)

Table I. Analysis of covariance (ANCOVA) of a) total filter-screen area, b) 3rd limb's filter area and c) 4th limb's filter-screen area for all measured daphnids, small (< 1.7 mm) and large ones (>1.7 mm). Treatment represented control or NA + HNA pooled together and were coded 1 and 0, respectively. Daphnia body length was the covariate. The interactions between the two variables was used to test for homogeneity of slope which is an assumption for ANCOVA analysis.

	All daphnids			Large daphnids (>1.7 mm)			Small daphnids (<1.7 mm)		
Source	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
a) Total filter-screen area									
Treatment	1	7.3	0.008	1	6.0	0.017	1	<0.1	0.922
Body length	1	1277.0	<0.001	1	99.9	<0.001	1	319.0	<0.001
Treatment X body length	1	3.3	0.778	1	0.9	0.342	1	1.4	0.234
Error	116			53			60		
b)- 3rd limb's filter-screen area									
Treatment	1	10.1	0.002	1	10.9	0.001	1	0.7	0.404
Body length	1	2572.9	<0.001	1	178.5	<0.001	1	729.8	<0.001
Treatment X body length	1	0.5	0.467	1	2.1	0.153	1	0.5	0.461
Error	237			109			124		
c)- 4th limb's filter-screen area									
Treatment	1	18.3	<0.001	1	9.9	0.002	1	1.9	0.169
Body length	1	1695.3	<0.001	1	153.1	<0.001	1	370.3	<0.001
Treatment X body length	1	4.3	0.038	1	1.3	0.256	1	7.9	0.006
Error	235			109			123		

compared, the difference in total filter-screen area was statistically significant (Table I, $P = 0.017$).

We also compared separately the areas of each filter-screen attached to the 3rd and 4th limb of *Daphnia*. The area of the 3rd filter was generally two times larger than the 4th one as shown by 4th:3rd area ratio (Table II). The ratio was always very stable around 0.55 to 0.58 with a standard error of less than 2% (Table II). The daphnids exposed to high biomass of inedible algae appeared to have a higher ratio than the control; however, the difference was only marginally statistically significant, especially considering the high number of observations (Table II, $P = 0.0424$). Filter-screen area of the 3rd limb was significantly larger in daphnids from the fertilized enclosures in comparison with the control (Figure 3a). The difference between the two groups was statistically significant (Table I, $P = 0.002$). As the daphnids from the treated enclosures grew, their 3rd limb filter-screens area became larger (Fig 3a). The difference was statistically significant for large daphnids (Table I, $P = 0.001$), but not for small ones (Table I, $P = 0.404$). The same results were observed for 4th limb filter-screens area. Again, daphnids from the treated enclosures had larger filter-screens in comparison with the control (Figure 3b). The difference was statistically significant for all daphnids (Table I, $P < 0.001$), large daphnids

Fig. 3. Filter-screen area of the a) 3rd (top panel) and b) 4th limb (bottom panel) of *Daphnia pulicaria* from the lake (cross symbols), control enclosures (open circles) and NA and HNA treatment enclosures pooled together (solid circles), as a function of body length (top of the head to spine base). Outliers are shown on the graphs but not included in the ANCOVA analyses.

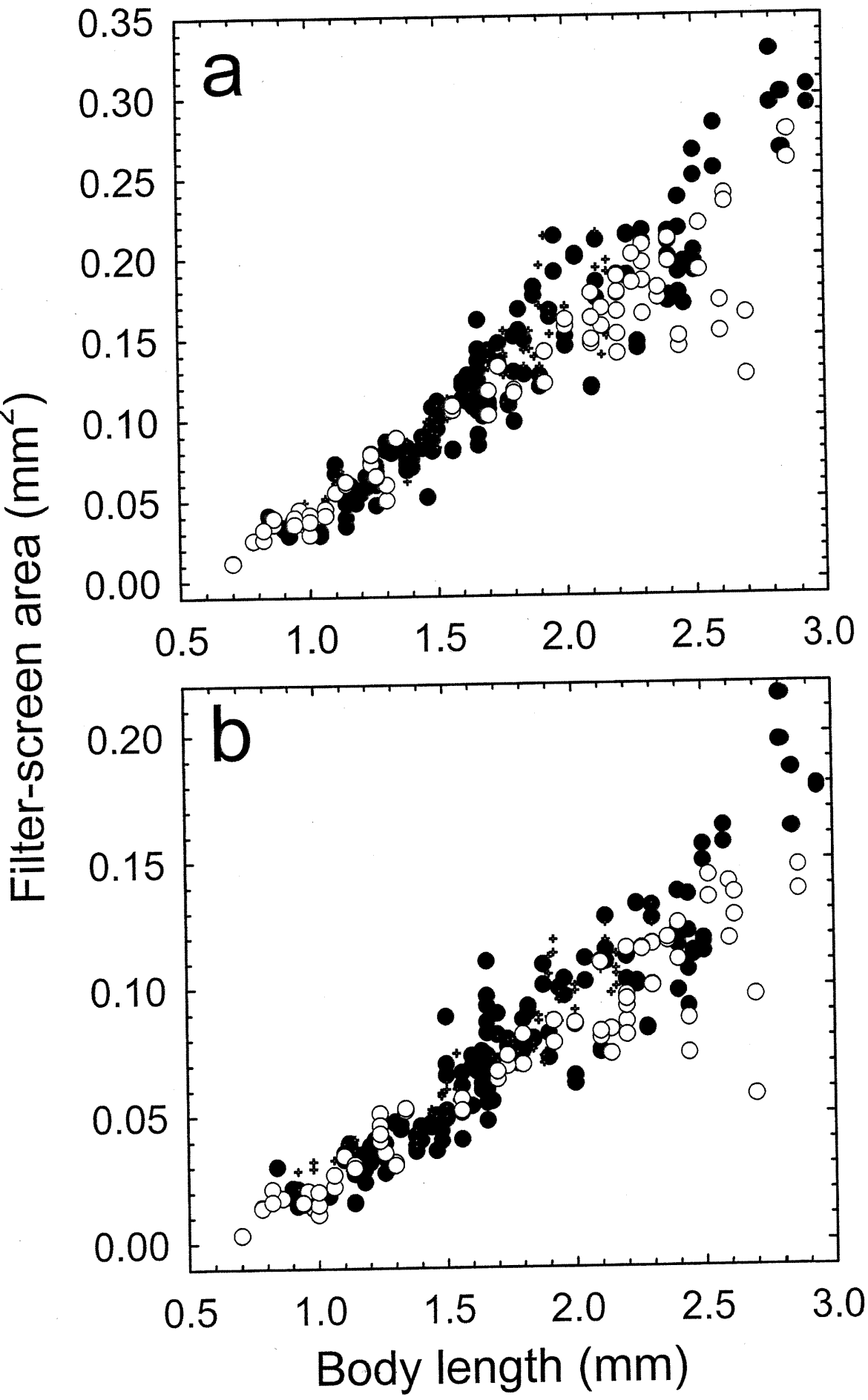

a
b
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.5
1.0
1.5
2.0
2.5
3.0
Filter-screen area (mm^2)
Body length (mm)

Table II. Averages (± 1 standard error of the mean) of ratio of filter-screen area (FSA) of the 4th to the 3rd limb, setular density, intersetal and intersetular distances as well as analysis of variance (ANOVA) results of the comparison between control and NA+HNA treatments pooled together.

	Lake	Control	NA+HNA	*P*
Variables	Mean ± 1 SE	Mean ± 1 SE	Mean ± 1 SE	Control vs NA+HNA
4th FSA : 3rd FSA	0.58 ± 0.01	0.55 ± 0.01	0.58 ± 0.01	0.0424
Setular density (# per µm of setae)	0.16 ± 0.00	0.18 ± 0.00	0.16 ± 0.00	0.1254
Intersetal distance (µm)	91.83 ± 2.11	86.25 ± 1.40	82.66 ± 0.83	0.0189
Intersetular distance (µm)	5.52 ± 0.07	6.16 ± 0.10	6.49 ± 0.09	0.0221

(Table I, P = 0.002), but not for small ones (Table I, P = 0.169). Hence, the observed increase in the area of the filter-screens of daphnids exposed to high cyanobacteria concentrations and large inedible diatoms was a result of a proportional increase in each of the two pairs of filter-screens of the 3rd and 4th limbs in large daphnids. The 3rd and 4th limb filter-screen areas of daphnids in the fertilized enclosures were 12 and 15% larger than the control, respectively (Figure 4). ANOVA indicated that the difference was statistically significant for 3rd (P = 0.002) and 4th limbs ($P < 0.001$).

Changes in filter-screen structure

In all the populations, the number of setulae per unit of setae did not increase as filter-screen area increased. The number of setulae attached to setae was stable around 16-18 per 100 µm for all the lake, control and treatment populations (Figure 5). There was no detectable difference between control and treatment in setular density (Table II, P = 0.125). Intersetal distance, appears to have decreased by 5% from an average of 86 to 82 µm in control and treatment populations, respectively (Figure 6). The change was statistically significant (Table II, P = 0.019). In contrast, intersetular distance showed a 5% increase from 6.2 µm in the control to 6.5 µm in the treatment (Figure 7). The increase was statistically significant (Table II, P = 0.022).

Fig. 4. 3rd and 4th limb filter-screen area (FSA) : body length ratio of *Daphnia pulicaria* from control and treatment enclosures NA and HNA pooled together. The average increase (%) of each FSA relative to the body length is shown. Bars represent means + one standard error of the mean. ANOVA probabilities are shown on the graph (** $P < 0.01$; *** $P < 0.001$).

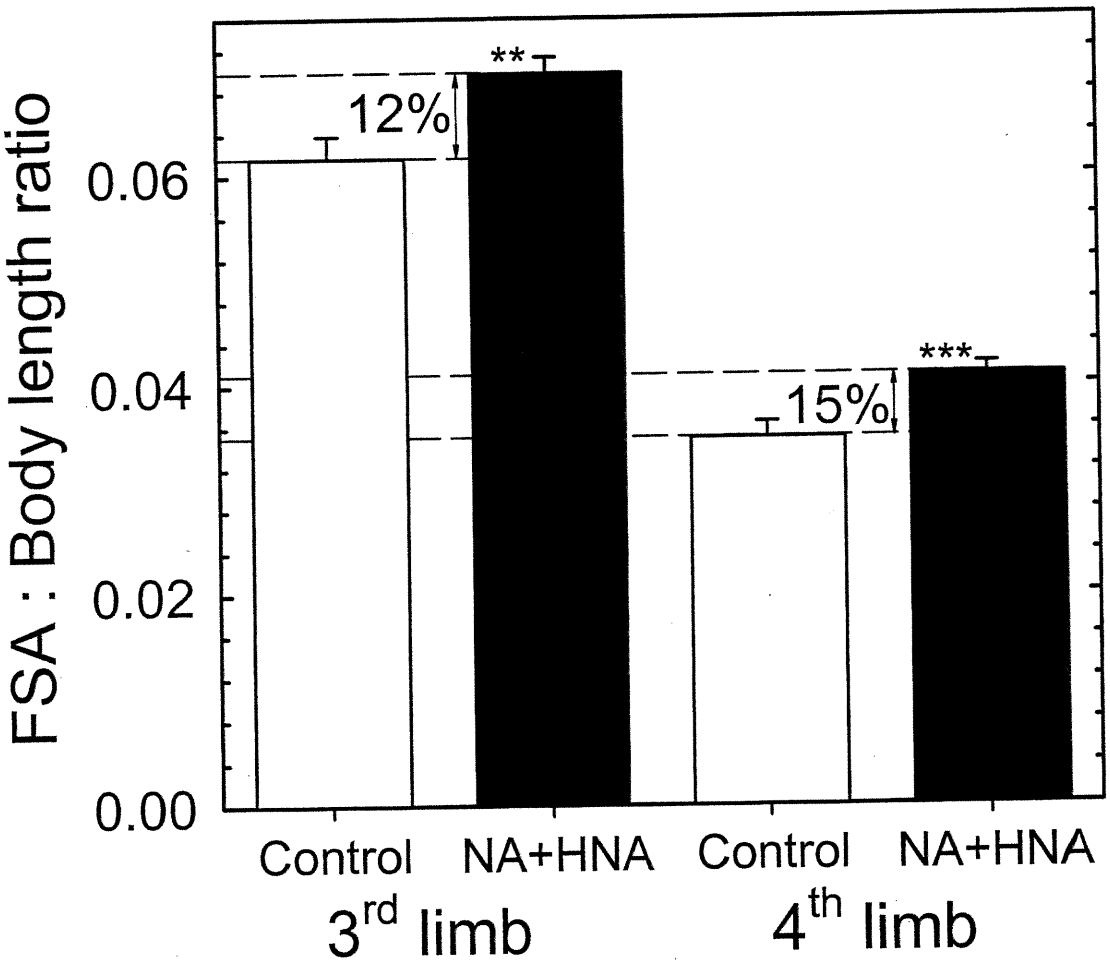

FSA : Body length ratio
0.06
0.04
0.02
0.00
**
12%
***
15%
Control
NA+HNA
Control
NA+HNA
3rd limb
4th limb

Fig. 5. Setular density expressed in number per μm of seta for *Daphnia pulicaria* from the lake, control and NA and HNA enclosures pooled together. Bars represent means + one standard error of the mean.

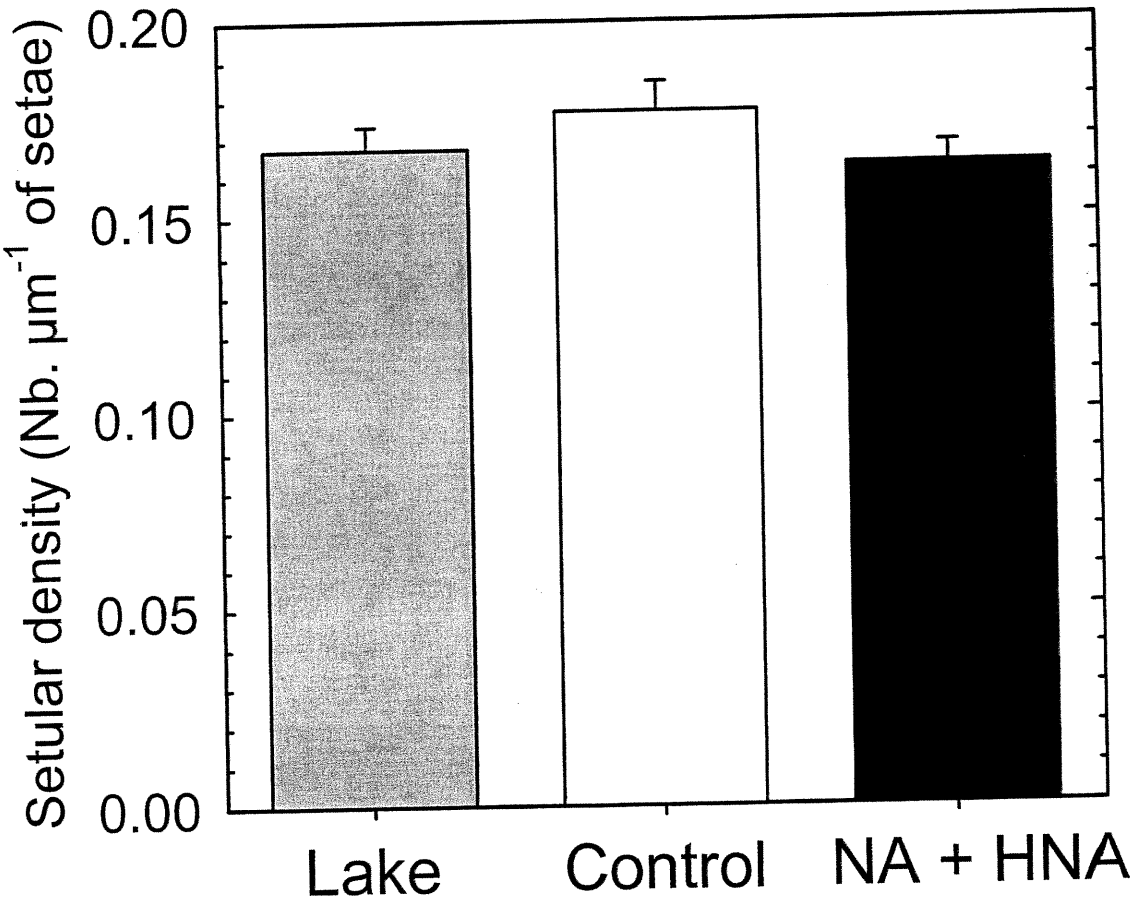

Setular density (Nb. µm-1 of setae)
0.20
0.15
0.10
0.05
0.00
Lake
Control
NA + HNA

Fig. 6. Intersetal distance (μm) for *Daphnia pulicaria* from the lake, control and NA and HNA enclosures pooled together. Bars represent means + one standard error of the mean.

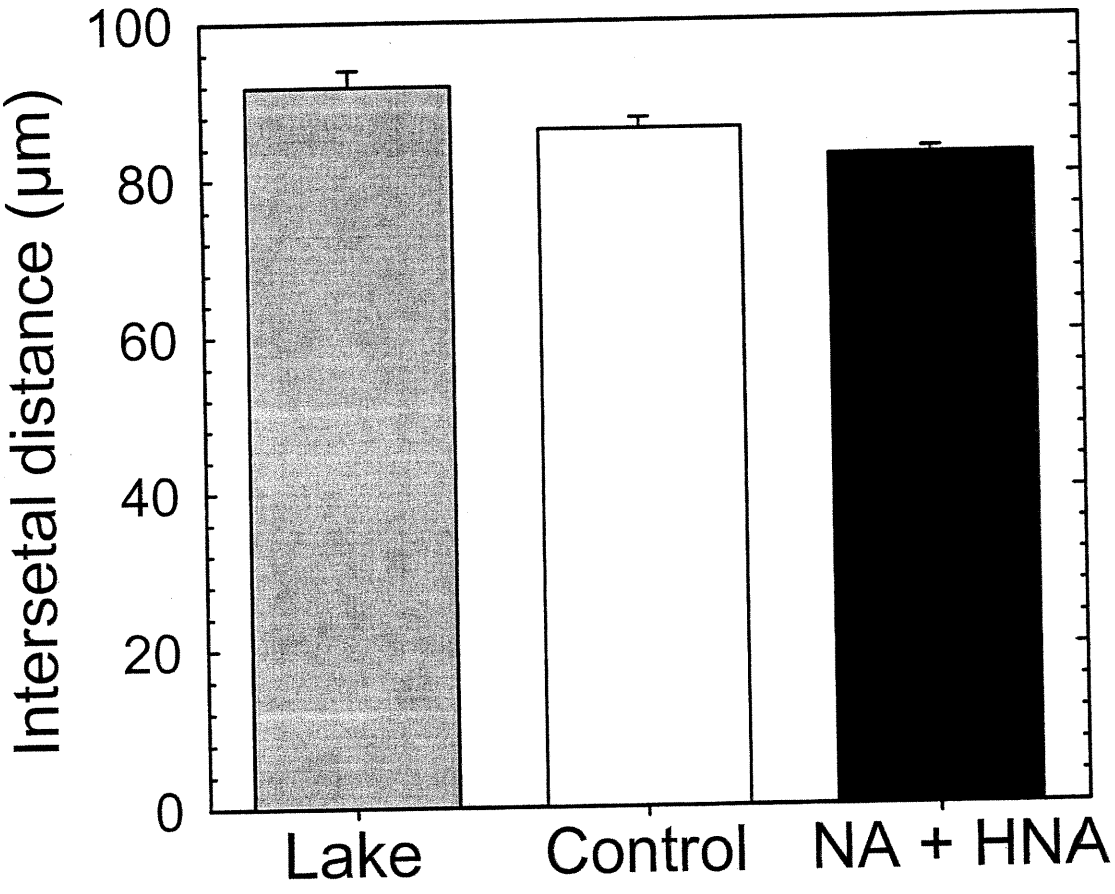
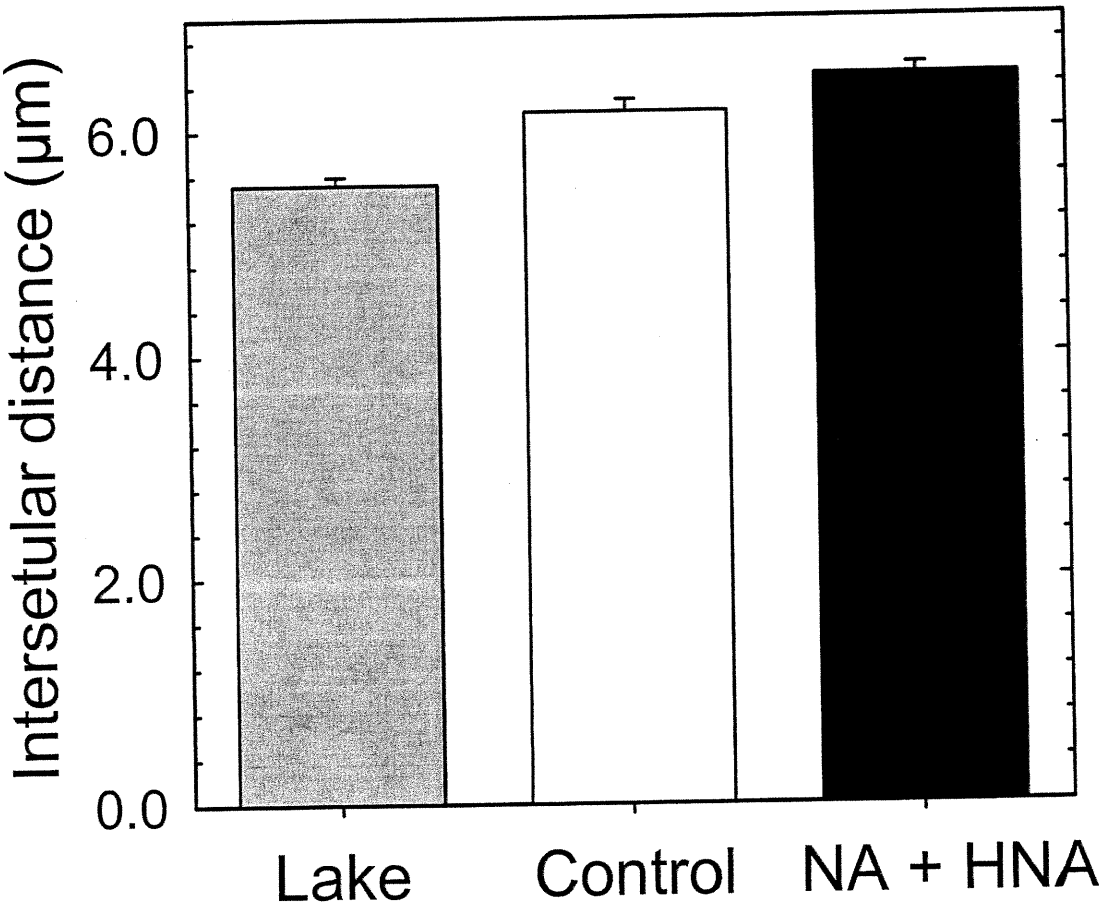

Intersetal distance (µm)
100
80
60
40
20
0
Lake
Control
NA + HNA

Fig. 7. Intersetular distance (μm) for *Daphnia pulicaria* from the lake, control and NA and HNA enclosures pooled together. Bars represent means + one standard error of the mean.


Intersetular distance (µm)
6.0
4.0
2.0
0.0
Lake
Control
NA + HNA

Discussion

The phenomena of enlarging the area of filter-screens observed in daphnids and other cladocerans have been interpreted as an adaptive strategy in low food conditions (Koza and Korínek, 1985; Pop, 1991). More recently, Lampert (1994) demonstrated that several species of *Daphnia* grown in the laboratory in very low food conditions were able to enlarge the area of their filter-screen from 19 to 83% relative to the same clones grown in high food conditions. Here we show that *Daphnia pulicaria* can enlarge their filter-screen area in hypereutrophic conditions with high phytoplankton biomass. These conditions are usually referred to as rich when one considers chl-a concentrations as sole indicator of resource availability without looking at the phytoplankton species composition. In our experimental conditions, nutrient additions enhanced the biomass of large inedible colonial and filamentous species of cyanobacteria and large diatoms, leaving daphnids with only a small fraction of potentially edible particles. A high biomass of large colonial and filamentous cyanobacteria interferes with other algae and renders the gathering of suitable particles difficult for daphnids (Webster and Peters, 1978; Fulton and Paerl, 1987; DeMott, *et al.*, 2001). Our results suggest that daphnids use the same adaptive response to increase the efficiency of food uptake in very low

food conditions or in environment dominated by large inedible phytoplankton. Experimental studies and mathematical modelling have suggested that an enlarged filtering area is a profitable strategy that allows the animals to increase their filtering rate without spending more energy (Lampert and Brendelberger, 1996).

Our results clearly show that both 3rd and 4th limb's area increased in the hypereutrophic systems by 12 and 15%, respectively (NA and HNA). In fact, the area of the filter-screens of the 4th limb is much smaller than the 3rd one and any change may be difficult to detect as previously reported (Pop, 1991). We demonstrate that the increase in the total filter-screen area is a contribution of both 3rd and 4th filter screens. Pioneering studies by Koza and Kořínek (1985) have shown that *Daphnia pulicaria* displayed the largest changes in the size of the filtering area in comparison with other species. In our experiments, *D. pulicaria* did not show a large change in comparison to species used by Lampert (1994) and which displayed between 19 and 89% increase of their filtering area in very low food conditions. However, our results are comparable to the changes observed in *D. pulicaria* and *D. magna* by Lampert and Brendelberger (1996). One possible explanation for the difference in response of different species and/or clones of the same species may be attributable of diverse

ecological strategies used by particular species and clones in their respective habitat (Koza and Korínek, 1985; Lampert, 1994). A possible alternative explanation may be that daphnids enlarge their filter-screen as continuous function of food condition and in this case they may display larger plasticity in highly contrasted conditions. We believe that our *in situ* experimental conditions did not differ to the same degree as the one used in previous laboratory studies, where the low food condition controls were 25 folds lower than the high food conditions (Lampert, 1994). It might be interesting to test whether or not the magnitude of the changes in the filtering area is actually a function of the food scarcity. The answer to this question may have important implications for the understanding of the evolution of daphnids. Hence, the plasticity of the filtering apparatus of daphnids may fall in the category of reaction norms such as the predatory induced cyclomorphic changes observed in several species of water flea *Daphnia* (Dodson, 1989; Stearns, 1989; Tollrian and Dodson, 1999). Earlier field studies have reported smaller filtering areas for the same *Daphnia* species grown at high food concentration in laboratory; this difference was explained by the fact that the seston concentration was much higher in the field than in the high food treatments used in the laboratory experiments (Lampert, 1994). These observations support the hypothesis that the magnitude of the plasticity of the filtering apparatus is a continuous

function of the food environment which supports the reaction norm scenario. However, further experimental testing is needed before one can make such a conclusion.

Our morphometric measurements show an increase of intersetular distance (or mesh size) with the increase in filtering area. This finding is consistent with the results of previous studies (Brendelberger and Geller, 1985; Lampert and Brendelberger, 1996); however, these authors reported a much smaller mesh size for *Daphnia pulicaria* in comparison with our results. This discrepancy is probably due to the different feeding history and habitat of the daphnids. Geller and Müller (1981) proposed a scenario which could explain the highly variable mesh size they observed in their survey of a number of cladoceran species and which could also explain the highly variable mesh size reported in the literature. Their idea was that the seasonal succession of cladoceran species is controlled by the size of the seston available. In environments dominated by small particles, species with finer mesh size would dominate, while those species with coarser mesh size would dominate in large-particle environments. Based on this interpretation, one can speculate that species such as *Daphnia pulicaria*, with a high ability for filtering apparatus plasticity, could adjust their mesh size to maximize their food uptake in

changing environment over the course of a season, for example. The consideration of such mechanisms, after further testing, in our interpretation of food webs would certainly lead to a better understanding of the interactions between zooplankton and their habitat.

Based on previous studies of the phenotypic plasticity of cladocerans and on our own observations, we can conclude that there is consistent evidence that aquatic organisms like daphnids gain benefits from allocating energy to adapt their body size, shape or behaviour to their environmental conditions. Daphnid reactions to environmental changes have been shown to be very rapid and usually take place within a single generation (Pop, 1991; Stibor, 1992; Tollrian, 1993; Lampert, 1994; Stibor and Lüning, 1994; Lampert and Brendelberger, 1996). In aquatic environments, phenotypic plasticity have been described for several different organisms and appeared to be a common strategy used by fish (Day, *et al.*, 1994), cladocerans (Dodson, 1989), rotifers (Gilbert, 1966), and even in some phytoplankton species such as *Scenedesmus* (Hessen and Van Donk, 1993; Lampert, *et al.*, 1994; Wiltshire and Lampert, 1999), to reduce the impact of predators on their communities. In the case of *Daphnia* filtering apparatus adaptation it is actually the predator which adapts to the quantity and quality of the prey. While not as extensively studied as the predator

induced changes, this phenomenon is of fundamental ecological and evolutionary significance. In an elegantly designed study, Hairston et al. (1999) showed, by hatching individuals from ancient dormant ephippia, that *Daphnia pulicaria* that lived in the early 1960 was less resistant to cyanobacteria than the late 1990 one. They have interpreted their results as evidence for a rapid evolution induced by the changes in the environment as Lake Constance became more eutrophic and more dominated by cyanobacteria species. However, the mechanisms which are responsible for this rapid evolution are not well known. One possible explanation could be that genotypes that have the ability to cope with cyanobacteria-dominated environments have been selected (Hairston, *et al.*, 1999). Another alternative could be that genotypes with the ability to change the size and structure of their filtering apparatus have been favoured by the increasing eutrophication. Hence, it is clear that phenotypic evolution needs special attention and further studies because of the potential implications in theoretical ecology and evolution.

Acknowledgments

This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) collaborative special projects grant and University-Industry Cooperative Research and development grant, FCAR and

the Québec Ministry of Education. Additional funds were provided by the Terrestrial Riparian Organisms Lakes and Streams project (TROLS) and the Groupe de Recherche Interuniversitaire en Limnologie et en Environnement Aquatique (GRIL) of the Université de Montréal. We are grateful to G. Lazure-Demers and M. Hugues for their help in laboratory as well as J. Burke, A. S. McNaught, E. Braun, K. Gibson, S. Manchour, A. Smart, and C. Wichuk for field assistance. We warmly thank S. Paquet for analysing phytoplankton samples from this experiments. Our special thanks are extended to L. Pellettier for kindly providing her technical expertise in scanning electronic microscopy for our study and to J.-L. Verville for assistance with photographic processing.

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Chapitre 4

Effects of *Microcystis aeruginosa* and purified microcystin-LR on the feeding behavior of *Daphnia pulicaria*

Effects of *Microcystis aeruginosa* and purified microcystin-LR on the feeding behavior of *Daphnia pulicaria*

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Running title: *Microcystis* effects on *Daphnia*

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Acknowledgements

This study was conducted at the Max Planck Institute for Limnology, Plön, Germany. The authors would like to thank Yvonne Harder for kindly taking care of the algal cultures used in these experiments. AG would like to thank all the researchers and staff of the MPI for their help and the stimulating research environment. This research was supported locally by the MPI and by traveling funds from BPA NSERC-industry collaborative TROLS project.

Abstract

The effects of *Microcystis aeruginosa* single cells, colonies and toxins on the feeding behavior of *Daphnia pulicaria* were investigated in three independent experiments. The animals were fed a mixture of *Scenedesmus* supplemented by increasing proportions (0, 20, 50, 80 and 100%) of *Microcystis* or concentrations (0, 50, 500 and 5000 ng mL^{-1}) of purified microcystin-LR. The changes in their feeding behavior, as indicated by the appendage beat, mandibular and labrum movement rate, were evaluated using a new direct observation method based on a coupling of video recording and computerized image analysis. *Daphnia* responded in contrasted manner to the presence of single cells or colonies. In the case of the single cells, a more marked response was observed on the mandibular movement rate (MMR) than on appendage beat rate (ABR) suggesting that *Daphnia* have the ability to discriminate between *Microcystis* and *Scenedesmus*. The colonies produced a typical feeding interference response by which the animals increased their labral rejection rate and showed starvation signs. The labral rejection rate clearly increased in the presence of unicellular and colonial *Microcystis*. In both cases, the changes in MMR and ABR were unlikely to be caused by intoxication because of the reversibility of those behaviors. In contrast, the addition of purified microcystin-LR at the concentration of 5000 ng mL^{-1} produced a non-reversible decline of ABR and MMR. This study provides insights on the mechanism of feeding inhibition observed in *Daphnia* when exposed to different forms of colonial or filamentous toxin producing cyanobacteria.

Introduction

Massive growth of cyanobacteria, also called blooms, represents a serious threat to aquatic ecosystems. Besides the fact that cyanobacterial blooms contribute to the degradation of water quality and recreational value of lakes and reservoirs, cyanobacteria species produce a large variety of potent cyanotoxins (Carmichael 1992). During the last decade, several forms of cyanobacterial secondary metabolites, mainly microcystins, have been identified as potent hepatotoxic or neurotoxic for a wide range of organisms including humans (Dawson 1998, Jochimsen et al. 1998). Several cases of severe intoxication caused by cyanotoxins have since been reported worldwide (Carmichael et al. 1988, Codd 1995, Jochimsen et al. 1998). The phenomenon of toxic algal blooms is not limited to freshwaters, it is also a largely recognized problem in shallow costal marine environments; however, in marine systems dinoflagelates are the main cause of algal blooms (Horner et al. 1997, Zingone and Enevoldsen 2000, Walsh and Steidinger 2001). Hence, it is critical to increase our knowledge about the ecological and toxicological implications of these harmful algal blooms in our water bodies (Codd 1995, Christoffersen 1996).

The first order effects of cyanobacterial blooms may be experienced by zooplankton communities which constitute the closest link to phytoplankton

communities in aquatic systems (Haney 1987, Lampert 1987). While several studies have reported that mechanical feeding interference (Webster and Peters 1978, DeMott et al. 2001), low nutritional quality (Brett and Müller-Navarra 1997, DeMott and Müller-Navarra 1997, Kilham et al. 1997), and/or toxin production (Haney et al. 1994, Reinikainen et al. 1995, 1999) of cyanobacteria make them unsuitable food for zooplankton, other studies support an opposite view which states that zooplankton are capable of reducing undesirable cyanobacteria species by means of intensive grazing (Boon et al. 1994, Matveev et al. 1994). The ability of large filter-feeders, such as *Daphnia*, to be able to survive and control cyanobacterial blooms is critical to the success of biomanipulation in eutrophic systems (Brett and Goldman 1996). However, controversy still surrounds the success of biomanipulation in cyanobacteria dominated systems (Benndorf and Henning 1989, Benndorf 1990, Carpenter and Kitchell 1992, DeMelo et al. 1992, MacKay and Elser 1998).

Although several studies have recognized the changes in feeding behavior of daphnids after exposure to cyanobacteria cell or colonies and their toxins (Haney et al. 1995, Rohrlack et al. 1999b, 2001) the mechanisms behind the inhibitory effects are yet to be understood. For example, it is not clear yet whether the change in feeding behavior observed in *Daphnia* when exposed to cyanobacteria is attributable to their endocell toxic content (Lampert 1981a,

Rohrlack et al. 1999b, 2001), some other substances released by the cyanobacterial cells (Jungmann 1992), or simply the size and the shape of some cyanobacterial colonies and/or filaments (Webster and Peters 1978, DeMott et al. 2001). It is well established that cladocerans unlike copepods have poor abilities to discriminate food on the basis of taste (Kerfoot and Kirk 1991); however, there have been some recent evidence that daphnids feeding can be inhibited by chemical substances released by cyanobacteria (Haney et al. 1995).

The present study aims to evaluate the effects of the addition of *Microcystis aeruginosa* (unicellular and colonial) and purified microcystin-LR on *Daphnia pulicaria* feeding behavior in three separate experiments. We used the filtering appendage beat rate (ABR), mandibular movement rate (MMR), and labral rejection as indicators of feeding behavior of *Daphnia*. While these rates do not represent a direct measurement of food utilization, they were shown to be good indicators of feeding behavior in several cladoceran species. Pioneering studies by McMahon and Rigler (1963), have shown that ABR was a good measure of pumping rate and any decrease of ABR at a given food level means a decrease in the amount of particle gathered in *Daphnia* food groove. Whereas, MMR constitutes a good indicator of food ingestion and could be used as an indirect measure of feeding rate of daphnids (McMahon and Rigler 1963, Burns 1968). This decrease in feeding rate was shown to be accompanied by an

increase in labral and postabdominal rejection rate as the animals tried to remove the excess or large food particles (Burns 1968). In the present study we evaluate and compare the changes in feeding behavior of *Daphnia pulicaria* when exposed to 1) unicellular *Microcystis aeruginosa,* 2) colonies from the same species and 3) purified microcystin-LR. To examine whether feeding inhibition changes with increasing proportion of *Microcystis* cells or colonies, we tested the response of daphnids in 0, 20, 50, 80 and 100% *Microcystis* biomass relative to *Scenedesmus obliquus.* We also tested the feeding behavior changes of daphnids in four different concentrations of microcystin-LR (0, 50, 500 and 5000 ng mL^{-1}).

Materials and methods

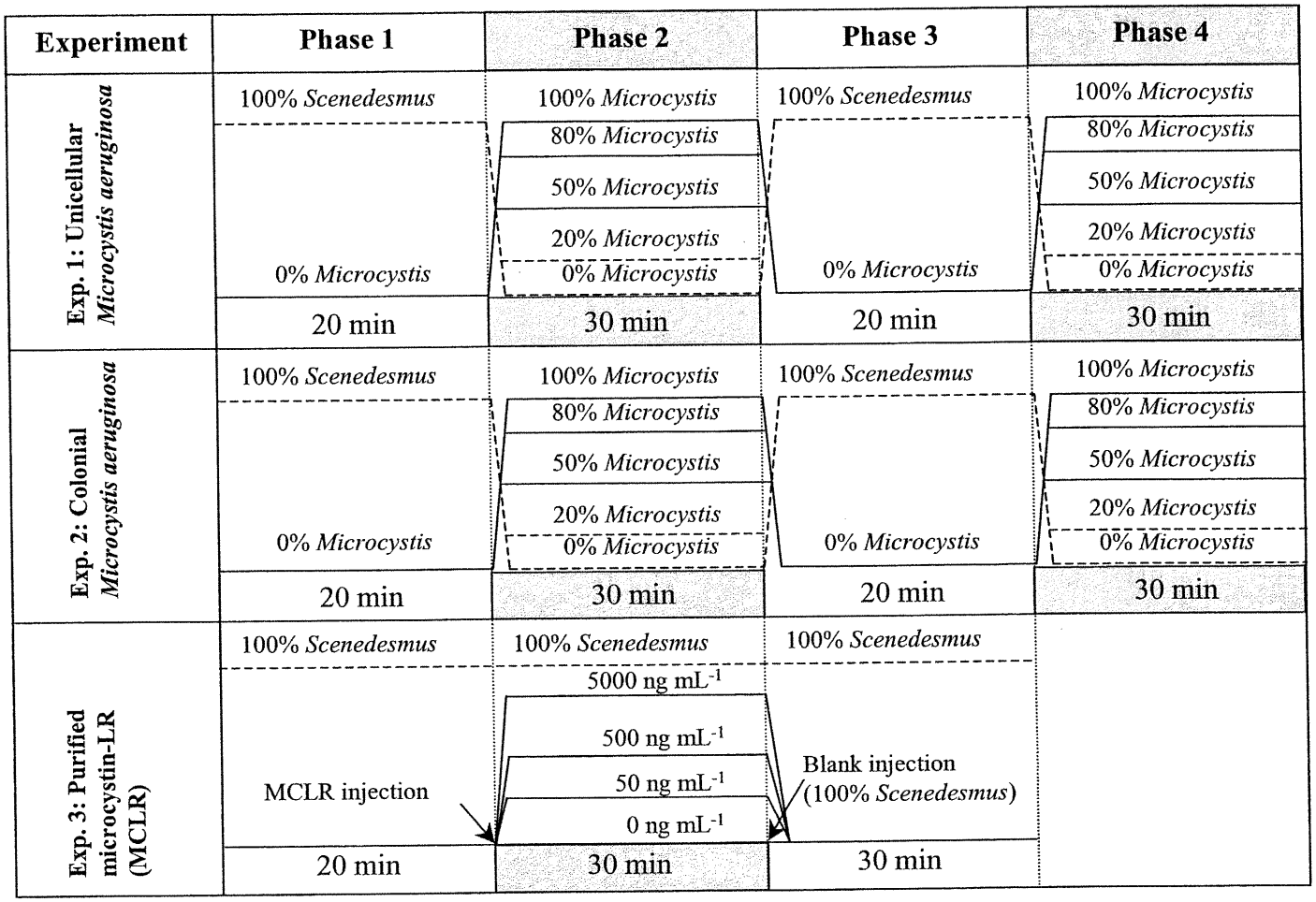
Experimental design

The present experiments were designed to evaluate the response of *Daphnia* to the addition of unicellular and colonial *Microcystis aeruginosa* as well as purified microcystin-LR to their regular food. This study was composed of three independent experiments (Fig. 1). The behavior of the animals was recorded during four phases in the experiments 1 and 2, and three phases in experiment 3. Phase 1 and 3 in experiment 1 and 2 represented the control phases where the animals were fed pure suspensions of *Scenedesmus obliquus* (Fig. 1). During phase 2 and 4, the animals were exposed to a mixture of *Microcystis* and *Scenedesmus* in different proportions according to the

experimental design (Fig. 1). Experiment 3 had only three phases, where the animals were exposed all the time to *Scenedesmus* and experienced the addition of four different concentrations (0, 50, 500 and 5000 ng mL^{-1}) of purified microcystin-LR at the beginning of phase 2 for about 30 min (Fig. 1). For this experiment, the flow-through rate was reduced to allow an exposition time of about 30 min before flushing the chamber with a blank injection (Fig. 1). *Scenedesmus* suspensions mixed with purified microcystin-LR were added injected into the observation chamber by a syringe because only a small quantity of the mix could be prepared due to the high cost of purified microcystins. However, our objective for these experiments was achieved since we intended to expose *Daphnia* to small concentrations of microcystin-LR for a short period of time (few minutes). It was shown that high concentrations of microcystin could be lethal to daphnids (DeMott et al. 1991, Jungmann 1992, Jungmann and Benndorf 1994). The highest concentration of purified microcystin-LR used in the present experiments was 5 to 6 times lower than the LC_{50} for *Daphnia pulicaria* generally reported in the literature (DeMott et al. 1991, Jungmann 1992, Jungmann and Benndorf 1994).

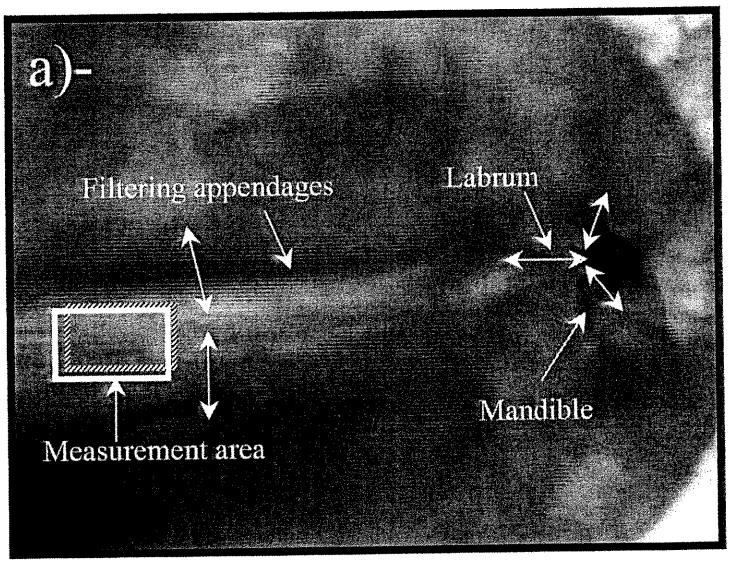
This study used the same methodology for the direct observation of *Daphnia* feeding behavior as the one developed and tested in earlier studies (McMahon and Rigler 1963, Burns 1968). During the observation, the animals

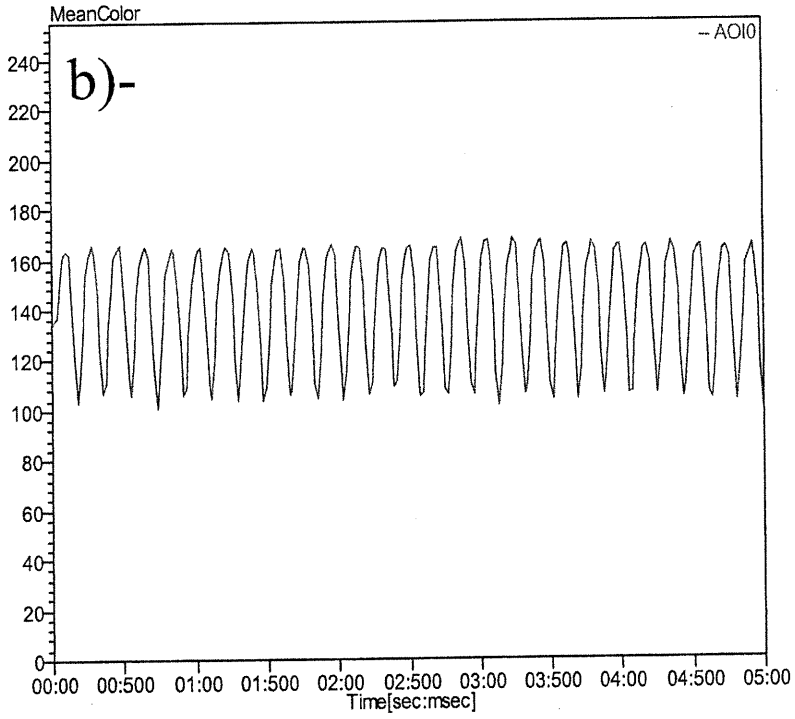
Fig. 1. Illustration of the experimental setup for the three experiments showing the conditions and durations of each experimental phase. Phase 1 represented a pre-treatment or control phase where the animals were observed in their original conditions with only *Scenedesmus obliquus* as food. At the end of phase 1, the conditions were changed by adding a proportion (0, 20, 50, 80 or 100%) of unicellular or colonial *Microcystis aeruginosa* in experiments 1 and 2, respectively. Phase 3 represented a second control phase with identical conditions as in phase 1. In phase 4, the conditions were switched back to treatment conditions identical to phase 2. Experiment 3 was conducted in the same manner except that there were only 3 phases. In this experiment, the animals were always fed the *Scenedesmus* suspension supplemented with different amounts (0, 50, 500 or 5000 ng mL^{-1}) of purified microcystin-LR during the treatment phase 2. During phase 3, the conditions were switched back to the control conditions as in phase 1.


Experiment
Phase 1
Phase 2
Phase 3
Phase 4
Exp. 1: Unicellular Microcystis aeruginosa
100% Scenedesmus
0% Microcystis
100% Microcystis
80% Microcystis
50% Microcystis
20% Microcystis
0% Microcystis
100% Scenedesmus
0% Microcystis
100% Microcystis
80% Microcystis
50% Microcystis
20% Microcystis
0% Microcystis
20 min
30 min
20 min
30 min
Exp. 2: Colonial Microcystis aeruginosa
100% Scenedesmus
0% Microcystis
100% Microcystis
80% Microcystis
50% Microcystis
20% Microcystis
0% Microcystis
100% Scenedesmus
0% Microcystis
100% Microcystis
80% Microcystis
50% Microcystis
20% Microcystis
0% Microcystis
20 min
30 min
20 min
30 min
Exp. 3: Purified microcystin-LR (MCLR)
100% Scenedesmus
MCLR injection
100% Scenedesmus
5000 ng mL-1
500 ng mL-1
50 ng mL-1
0 ng mL-1
100% Scenedesmus
Blank injection
(100% Scenedesmus)
20 min
30 min
30 min

were maintained in a newly designed observation chamber (Plath 1998) similar to those used in previous studies (Haney et al. 1995, Lampert and Brendelberger 1996). The animals were maintained in a 10 mL flow-through chamber alimented from a reservoir containing algal suspensions at a rate of ~ 13 mL min^{-1} at a controlled temperature of 20°C. At this rate, it was possible to rapidly change food mixture without disturbing the animals (Plath 1998). The suspension fed to the chamber could be changed easily by switching from the control-*Scenedesmus* to the treatment-*Microcystis* according to the experimental design (Fig. 1). The animals were maintained in upright position and could be viewed by an infrared-lighted video camera and a 4x macro lens. Prior to their installation in the chamber, the animals were affixed along their dorsal carapace margin to cover slip with a drop of Vaseline. The movements of the animals were then recorded on videotapes for a continuous period of 100 min divided into 4 phases for experiments 1 and 2, and 80 min divided into only 3 phases for experiment 3 (Fig. 1). All the animals were allowed a 30 min acclimatizing period prior to the beginning of the video recording (Burns 1968). The position of the camera allowed a ventral carapace opening view of the daphnids (Fig 2a). The determination of the appendage beat rate (ABR) was made with a fully automated method using an image analysis system (Medealab, Germany). The recorded tapes were viewed with a VCR connected to a computer equipped with a video image acquisition technology. The image analysis software was set

Fig. 2. An example of a) image used for the calculation of *Daphnia pulicaria* appendage beat rate (ABR), mandibular movement rate and labral rejection rate and b) computerized calculation of ABR based on the change in light intensity produced by the movement of the filtering appendages inside a carefully positioned measurement area as shown by double arrows. The direction of *Daphnia* mandibular movements and the labral rejections are also shown by double arrows in panel a.


a)-
Filtering appendages
Labrum
Mandible
Measurement area


MeanColor
AOI0
b)-
240
220
200
180
160
140
120
100
80
60
40
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0
00:00
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02:500
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Time[sec:msec]

to take a 5 sec measurement of light intensity every 90 sec within a measurement area positioned adequately to detect the movements of the filtering appendages (Fig. 2a). The software produced a precise count of the light intensity peaks over the 5 sec measurement time, which corresponded to the frequency of filtering appendage movement or ABR (Fig. 2b). The mandibular movements and labral rejections were not counted with the automated method because of their less regular nature in comparison to ABR. We simply counted them manually by viewing the recorded tapes. The time taken by the animals to produce 10 mandibular movements was recorded at a 90 sec interval and frequency calculated by simply dividing 10 by the time in seconds. Total labral rejections were counted in each experimental phase and the rate expressed as number per minute. Postabdominal rejections were not counted in this study as commonly done in similar studies (Haney et al. 1995), because in several occasions we noted that those movements took place out of the recorded area. However, we have noted that the labral (upper lip) movement occurred always before a postabdominal rejection. Hence in this study we use the labral rejections as an indicator of feeding interference in the mouth region. These rejections always occurred when the animal needed to clear the mouth region of large food particles (Burns 1968).

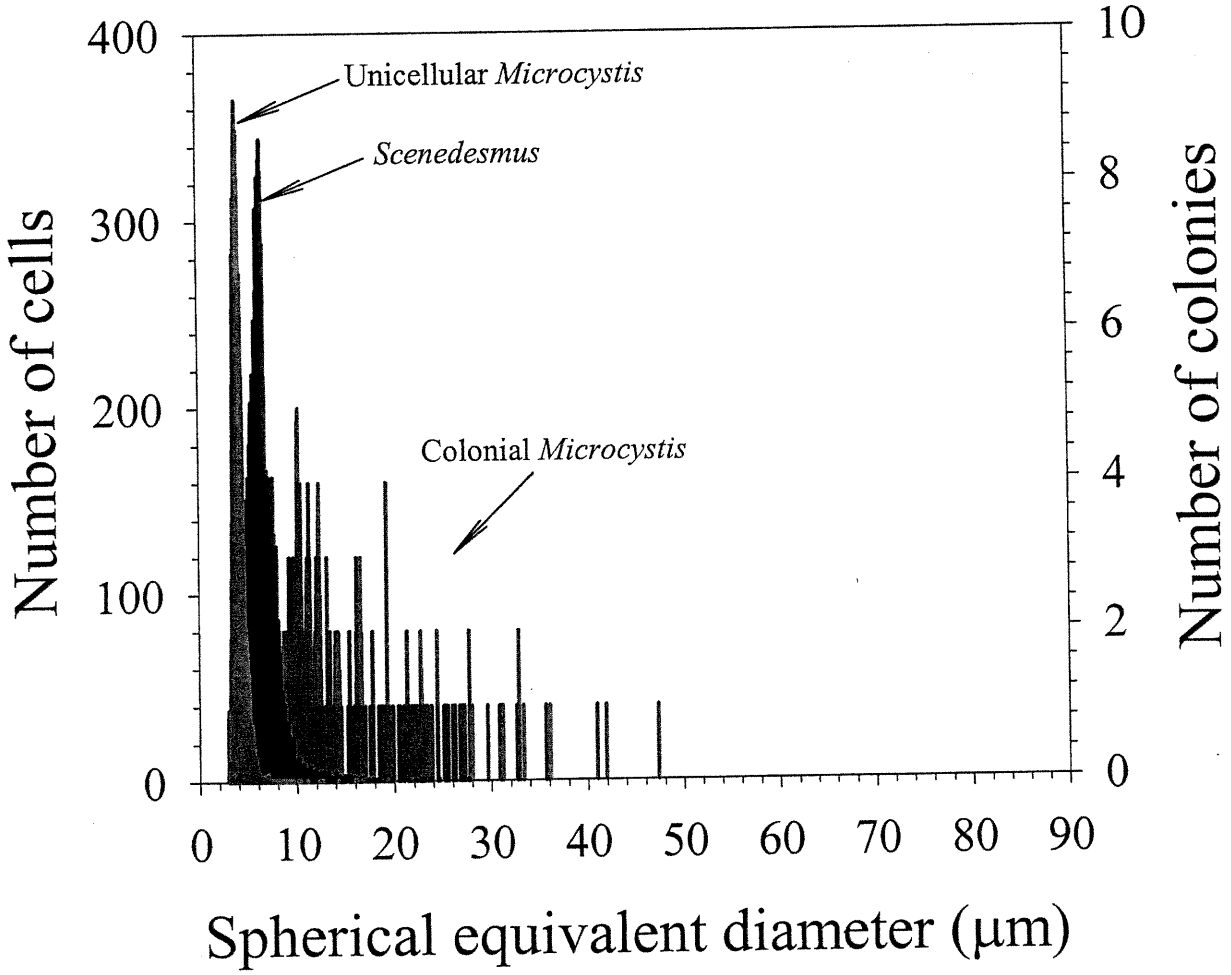
Daphnia and algal cultures

All the daphnids used in the present study belonged to a *Daphnia pulicaria* clone maintained in the laboratory for years in 20°C under constant dim light at the daphnids culture collection of the Max Planck Institute for Limnology, Plön, Germany. The animals used in the experiments were all descendants of one mother and were cultured in a flow-through culture system at a controlled temperature of 20°C (Lampert et al. 1988). This system is very efficient in maintaining the same food condition for all the experimental animals as long as they were needed for the experiments. The daphnids used in these experiment were fed a suspension of *Scenedesmus obliquus* equivalent to 0.5 mg C L^{-1}. The size of the animals used ranged from 2.3 to 2.8 mm in body length (top of the head to the base of tailspine). Only large animals were used to avoid any possible allometric changes in the feeding behavior of the animals and the difference between the smallest and the largest animal used in these experiments was as small as 0.5 mm. However, it was shown that body length is unlikely to affect the ABR of daphnids (Plath 1998).

Scenedesmus suspensions used in the flow-through system and the control phases (i.e., phase 1 and 3) of the experiments were prepared daily by adding a volume of a fresh culture to 0.45-µm filtered lake water to make an equivalent 0.5 mg C L^{-1} based on a previously established calibration curve

between carbon content of *Scenedesmus* and light absorbance at 800 nm. *Scenedesmus* was cultured in chemostat in a WC medium (Guillard and Lorenzen 1972). Two different strains of *Microcystis aeruginosa* were used in these experiments. A strain which grows only in single cells and which was maintained in the laboratory for years (Lampert 1981b), and a newly isolated strain (UTCC436) capable of growing in colonies in laboratory conditions. Both strains were cultured in a BG-11 medium in low cold light conditions (Moss 1972). The proportion of *Microcystis* to be added to *Scenedesmus* suspension was calculated to achieve the same total biovolume as a pure suspension of *Scenedesmus* of 0.5 mg C L^{-1}. The biovolume of algal suspensions (*Scenedesmus* and *Microcystis*) was measured using a Casy I particle counter. Based on the biovolume calculation different proportions of *Microcystis* were added to *Scenedesmus* to achieve 0, 20, 50, 80 and 100% of *Microcystis* relative biovolume. The total biovolume of the mixed *Scenedesmus-Microcystis* suspension was always maintained equivalent to the biovolume of a pure suspension of *Scenedesmus* of 0.5 mg C L^{-1}. Hence, the animals were always exposed to the same biovolume of algal suspension, either pure *Scenedesmus* (i.e., phases 1 and 3) or a mixture of *Scenedesmus* and *Microcystis* (i.e., phases 2 and 4). The spherical equivalent diameter of *Scenedesmus* and unicellular *Microcystis* was ~5 and ~7 µm in average, respectively (Fig. 3). The size of the colonies of *Microcystis* ranged from 10 to up to 50 µm in diameter (Fig. 3). All the

Fig. 3. An example of size spectra based on spherical equivalent diameter of *Scenedesmus obliquus,* unicellular and colonial *Microcystis aeruginosa* strains used in the present experiments.


400
300
200
100
0
Number of cells
10
8
6
4
2
0
Number of colonies
Unicellular *Microcystis*
Scenedesmus
Colonial *Microcystis*
0 10 20 30 40 50 60 70 80 90
Spherical equivalent diameter (µm)

Microcystis strains used in these experiments contained a large variety of microcystins (i.e, LR, YR, RR), but microcystin-LR was the dominant form (e.g., Krishnamurthy et al. 1986, Carmichael 1992, Codd 1995). The purified dried microcystin-LR was diluted in small amount of deionized water (1 mL) and added to *Scenedesmus* suspensions to achieve the desired concentration of 0, 50, 500 and 5000 ng mL^{-1}.

Statistical analyses

Repeated-measures ANOVA was used to test for significant differences between the different experimental phases. Only the central 20 min of the 30 min treatment phases (i.e, phase 2 and 4) were used in the comparison. For each experimental condition (i.e., 0, 20, 50, 80 and 100%), the comparisons were based on the observation of 5 independent animals (replicates). Repeated-measures ANOVA was used to test for the effect of the treatment and also the treatment X time interaction. The interaction term gives insights as to the nature of the effect produced by the treatment over time. In our experiments, this information was as important as the treatment effect, because it allowed to test whether the feeding behavior changed during the treatment phase or not (Potvin et al. 1990, von Ende 1993). Nonparametric Wilcoxon test was used to compare the labral rejection rates in the treatment and the control phases. Given the limited number of animals (n= 4 to 5) used in each experimental condition,

the rates of both treatment phases (2 and 4) and control phases (1 and 3) were pooled to perform the statistical test for the experiments with unicellular and colonial *Microcystis*. In the experiment with microcystin-LR, there was one treatment phase (2) and hence only the rates of the control period were pooled and compared to the treatment phase (2). Labral rejection rate data were log-transformed prior to performing Wilcoxon tests. All the statistical analyses were performed with Systat 8.0.

Results

The addition of unicellular *Microcystis* to *Daphnia* food produced only a slight decline in ABR in comparison to the stable behavior exhibited by the animals fed a pure suspension of *Scenedesmus* (Fig. 4). The daphnids, in treatment 20 to 100% *Microcystis*, exhibited a pattern of response showing a slight decline during phase 2 in comparison to phase 1, a recovery during phase 3 and again a slight decline in phase 4 especially at high proportions of *Microcystis* (Fig 4c,d,e). These changes were not strong enough to be statistically significant (Table 1). However, daphnid's food ingestion as measured by MMR declined after the addition of unicellular *Microcystis* (Fig. 5). The first exposure to *Microcystis* cells (phase 2) caused a statistically significant decline in MMR (Table 2). In all the cases except the 50 % *Microcystis* treatment, the animals recovered the same MMR in phase 3 which was not statistically significant from

Fig. 4. *Daphnia pulicaria* appendage beat rate (ABR) as a function of time in the experiment with unicellular *Microcystis aeruginosa* in the following proportion to *Scenedesmus*: a) 0%, b)- 20%, c) 50%, d) 80% and e) 100%. ABR is shown as mean of 5 animals ± one standard error of the mean. Vertical dashed lines represent the delimitation between the different experimental phases 1 to 4. Horizontal dotted lines represent the mean of the pre-treatment phase 1.

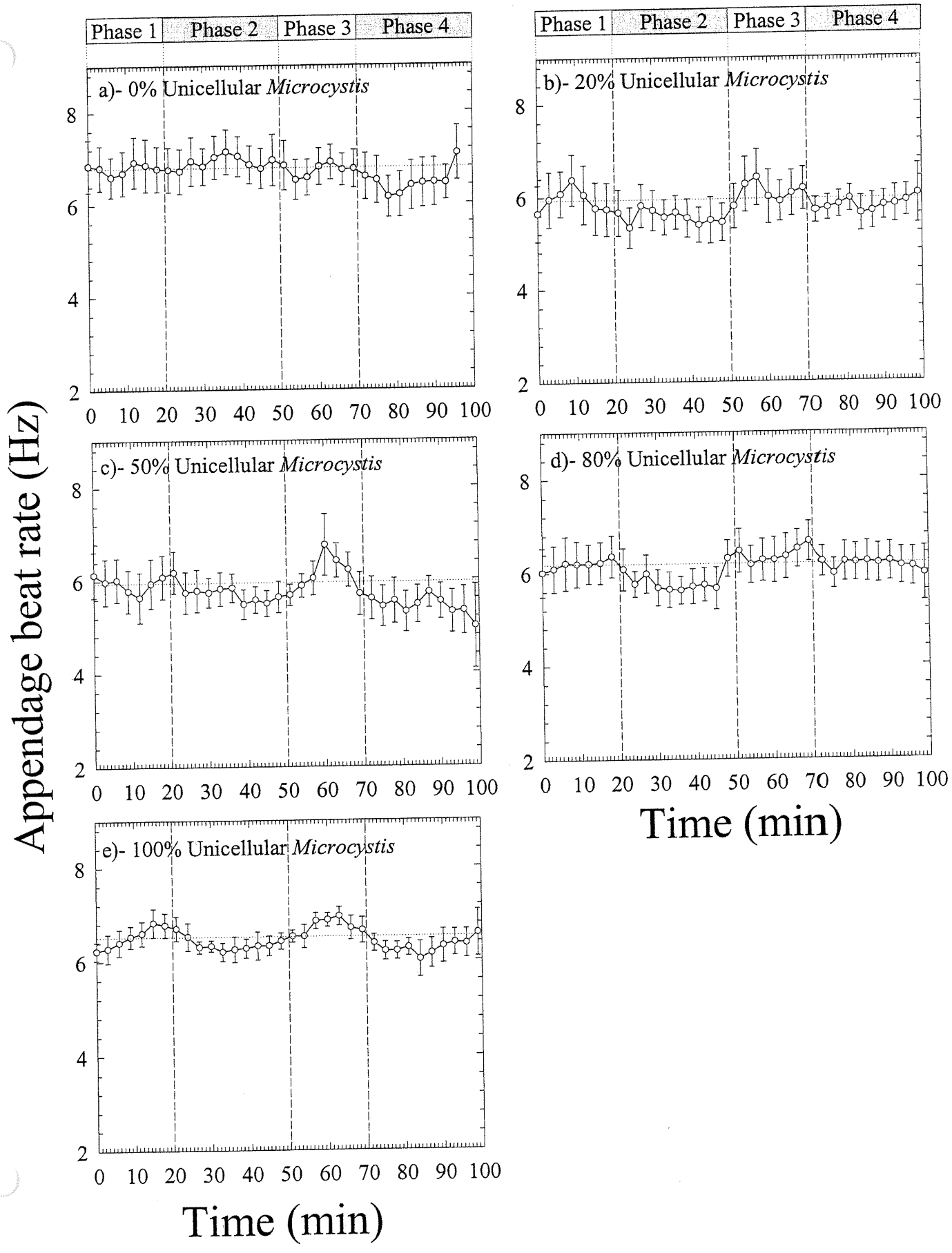
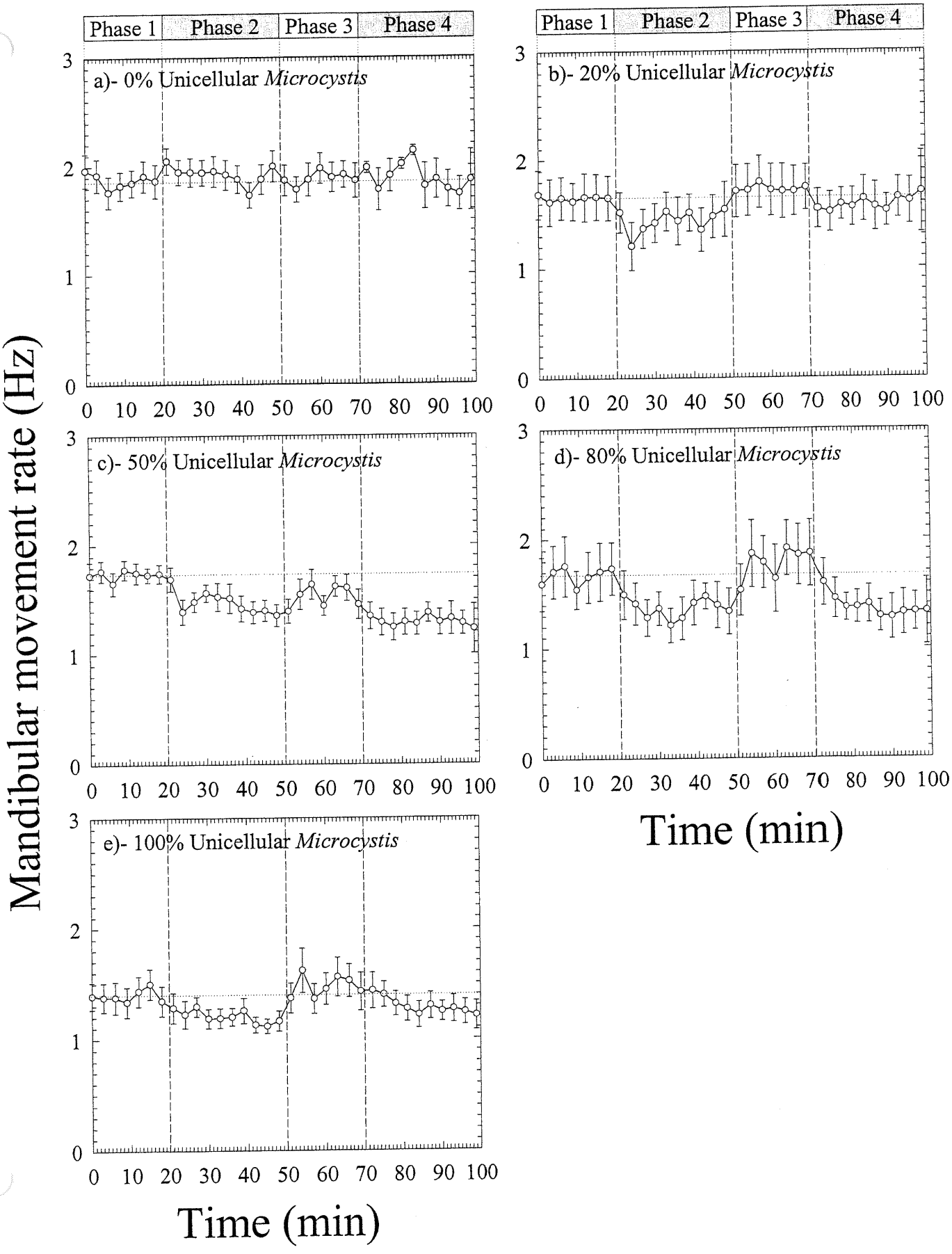

Phase 1
Phase 2
Phase 3
Phase 4
a)- 0% Unicellular *Microcystis*
b)- 20% Unicellular *Microcystis*
c)- 50% Unicellular *Microcystis*
d)- 80% Unicellular *Microcystis*
e)- 100% Unicellular *Microcystis*
Appendage beat rate (Hz)
Time (min)
Time (min)

Fig. 5. *Daphnia pulicaria* mandibular movement rate (MMR) as a function of time in the experiment with unicellular *Microcystis aeruginosa* in the following proportion to *Scenedesmus*: a) 0%, b)- 20%, c) 50%, d) 80% and e) 100%. MMR is shown as mean of 5 animals ± one standard error of the mean. Vertical dashed lines represent the delimitation between the different experimental phases 1 to 4. Horizontal dotted lines represent the mean of the pre-treatment phase 1.


Phase 1
Phase 2
Phase 3
Phase 4
a)- 0% Unicellular *Microcystis*
b)- 20% Unicellular *Microcystis*
c)- 50% Unicellular *Microcystis*
d)- 80% Unicellular *Microcystis*
e)- 100% Unicellular *Microcystis*
Mandibular movement rate (Hz)
Time (min)
Time (min)

phase 1 (Fig. 5, Table 2). After the second exposure to *Microcystis* during phase 4, the daphnids exhibited a reduction of their feeding rate consistent with the response observed in phase 2 (Fig. 5). The comparison of MMR showed a statistically significant decline in MMR from phase 3 to 4 for 50, 80 and 100% *Microcystis* treatments (Table 2).

The exposure of daphnids to colonies of *Microcystis* seems to have affected their ABR in a different manner in comparison to the effects observed with unicellular *Microcystis* (Fig. 6). In the treatments 20, 50 and 80% *Microcystis*, the first exposure to colonies produced a sharp decline of ABR in the first minutes immediately followed by an increase during the remaining duration of phase 2 (Fig. 6b,c,d). The changes were statistically significant only for 80% *Microcystis* treatment (Fig. 6, Table 1). It is important to note that unlike the experiment with unicellular *Microcystis*, ABR seems to change rapidly with time (Fig. 6). However, this observation was supported by the statistical analyses only for 80% *Microcystis* treatment where the interaction terms (treatment X time) were highly significant for treatment phases 2 and 4 in comparison to their respective controls (Table 1). During phase 3 of the same treatment (80%), the animals decreased their ABR when they were given the control treatment, but as soon as they were exposed again to colonies they rapidly increased their ABR (Fig. 6d). Surprisingly, the patterns of changes

Fig. 6. *Daphnia pulicaria* appendage beat rate (ABR) as a function of time in the experiment with colonial *Microcystis aeruginosa* in the following proportion to *Scenedesmus*: a) 0%, b)- 20%, c) 50%, d) 80% and e) 100%. ABR is shown as mean of 5 animals ± one standard error of the mean. Vertical dashed lines represent the delimitation between the different experimental phases 1 to 4. Horizontal dotted line represents the mean of the pre-treatment phase 1.

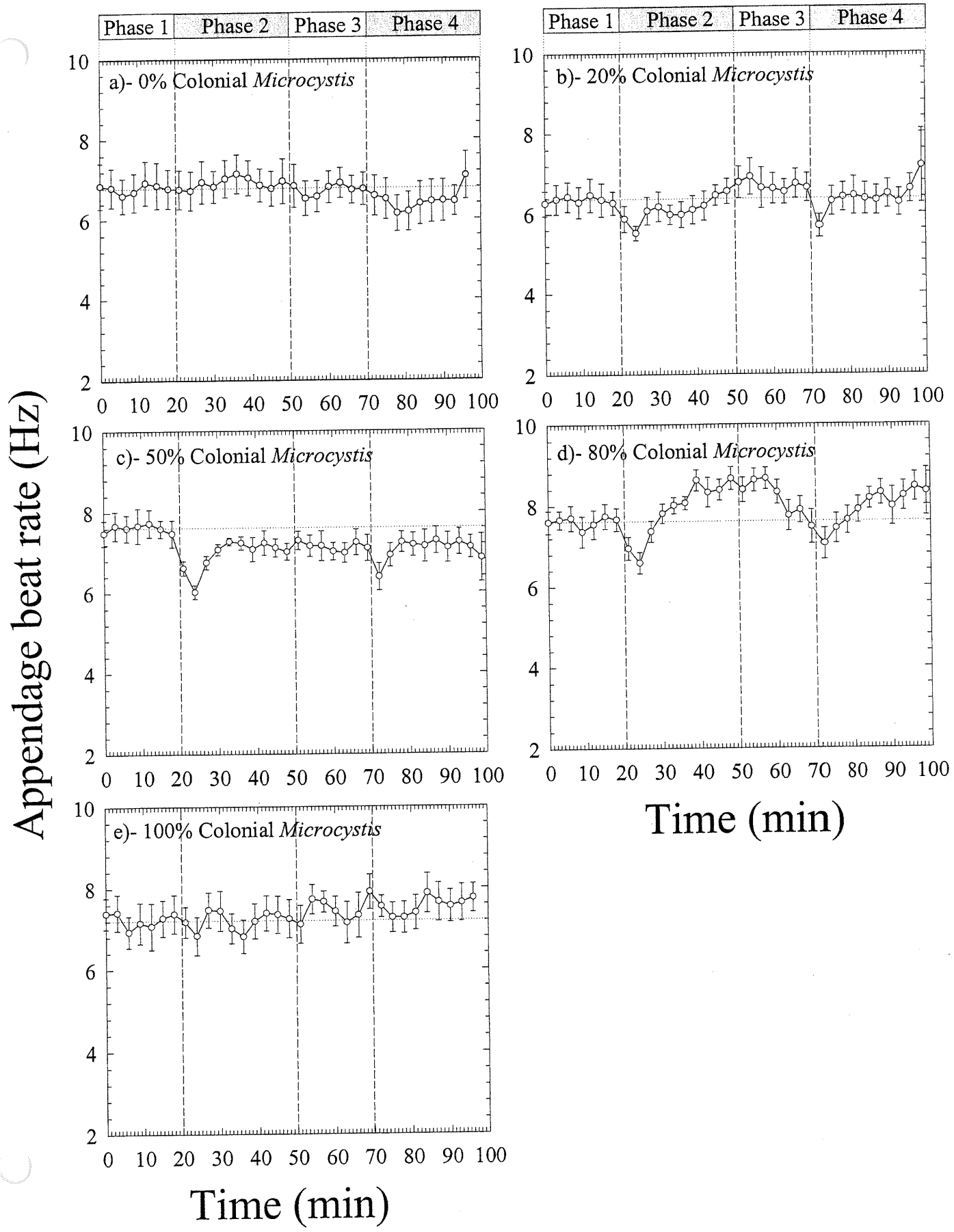

Phase 1
Phase 2
Phase 3
Phase 4
a)- 0% Colonial *Microcystis*
b)- 20% Colonial *Microcystis*
c)- 50% Colonial *Microcystis*
d)- 80% Colonial *Microcystis*
e)- 100% Colonial *Microcystis*
Appendage beat rate (Hz)
Time (min)
Time (min)

Table 1. Results of repeated-measures ANOVAs for comparison of *Daphnia pulicaria* appendage beat rate (ABR) in different phases of the experiments with different proportions (0, 20, 50, 80 and 100%) of unicellular and colonial *Microcystis aeruginosa* added to *Scenedesmus obliquus*.

		Unicellular *Microcystis*				Colonial *Microcystis*			
		Phase 1 vs. 2	Phase 1 vs. 3	Phase 1 vs. 4	Phase 3 vs. 4	Phase 1 vs. 2	Phase 1 vs. 3	Phase 1 vs. 4	Phase 3 vs. 4
	df	*P*	*P*	*P*	*P*	*P*	*P*		*P*
0% *Microcystis*									
Treatment	1	0.1511	0.8005	0.2087	0.1008	0.1511	0.8005	0.2087	0.1008
Time	6	0.7678	0.4613	0.0093	0.1235	0.7678	0.4613	0.0093	0.1235
Time X Treatment	6	0.6574	0.9647	0.2489	0.0915	0.6574	0.9647	0.2489	0.0915
20% *Microcystis*									
Treatment	1	0.1161	0.4719	0.5790	0.2024	0.2547	0.0118	0.8048	0.0657
Time	6	0.0203	0.0003	0.6098	0.1062	0.4144	0.8327	0.7949	0.5633
Time X Treatment	6	0.1222	0.0941	0.0013	0.1220	0.3260	0.4578	0.3608	0.2230
50% *Microcystis*									
Treatment	1	0.4158	0.6507	0.2845	0.0685	0.1015	0.0797	0.0238	0.9933
Time	6	0.0184	0.6171	0.5103	0.0305	0.3034	0.9272	0.9563	0.8322
Time X Treatment	6	0.3502	0.0017	0.0572	0.2422	0.6978	0.3144	0.8646	0.8763
80% *Microcystis*									
Treatment	1	0.0861	0.3552	0.9775	0.3350	0.0025	0.0066	0.0225	0.5589
Time	6	0.8865	0.2423	0.8772	0.7865	0.0001	0.0003	0.0385	0.0020
Time X Treatment	6	0.1315	0.8568	0.2045	0.4219	0.0045	<0.0001	0.0401	<0.0001
100% *Microcystis*									
Treatment	1	0.1250	0.3722	0.5707	0.1424	0.9676	0.3248	0.3529	0.6091
Time	6	0.0982	0.0232	0.0105	0.6505	0.0995	0.0631	0.8828	0.0077
Time X Treatment	6	0.0537	0.1002	0.3188	0.1508	0.8861	0.2857	0.1821	0.4700

observed in the ABR of daphnids in all treatments from 20 to 80% were inexistent in 100% *Microcystis* treatment (Fig. 6e).

Daphnia exposure to *Microcystis* colonies seem to have produced more effects on their ABR than MMR. A slight decline in MMR was observed in treatment 20 to 80% at the beginning of phase 2, but the animals quickly recovered their previous MMR during the remaining duration of the experiment (Fig. 7). The only treatment where the animals reduced significantly their MMR and consequently their ingestion rate is the 100% colonial *Microcystis* treatment (Fig. 7e) for which the ABR remained unchanged (Fig 6e). The reduction in the ingestion rate was highly statistically significant (Table 2). The changes were clearly visible in phase 2; however, the animals recovered quickly their previous MMR as soon as they were fed again *Scenedesmus* suspensions in phase 3 (Fig 7e). The response of *Daphnia* during phase 4 was again a consistent decline similar to the response observed in phase 2 (Fig. 7e).

The addition of microcystin-LR at low concentrations (50 and 500 ng mL^{-1}) did not produce a detectable response in *Daphnia* feeding behavior (Fig. 8b,c). The behavior of *Daphnia,* at 50 and 500 ng, remained similar to the controls (Fig. 8 a,b,c). At 50 and 500 ng, the comparison of *Daphnia* ABR during the treatment phase 2 with phase 1 and phase 3 showed no statistically

Fig. 7. *Daphnia pulicaria* mandibular movement rate (MMR) as a function of time in the experiment with colonial *Microcystis aeruginosa* in the following proportion to *Scenedesmus*: a) 0%, b)- 20%, c) 50%, d) 80% and e) 100%. MMR is shown as mean of 5 animals ± one standard error of the mean. Vertical dashed lines represent the delimitation between the different experimental phases 1 to 4. Horizontal dotted lines represent the mean of the pre-treatment phase 1.

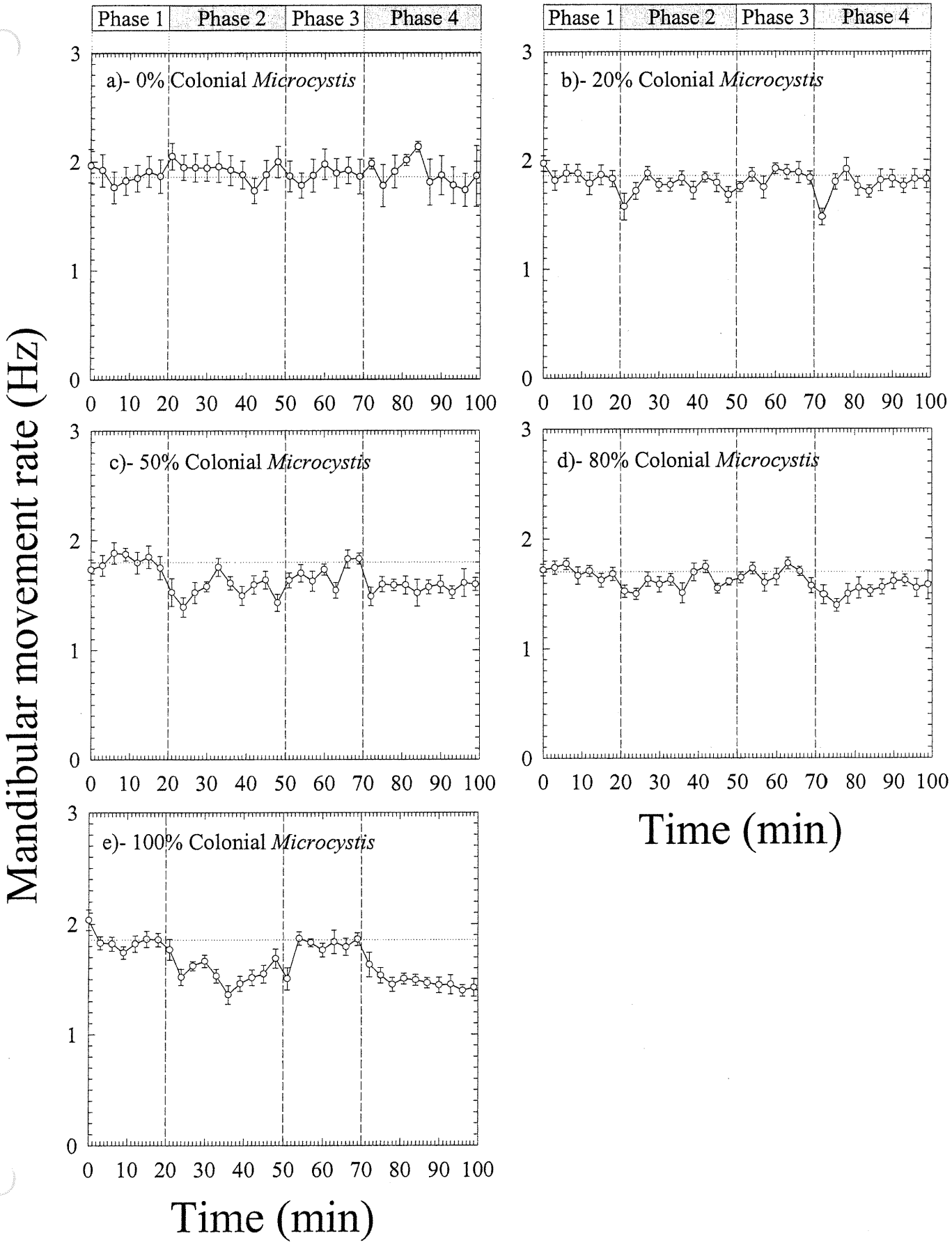

Phase 1
Phase 2
Phase 3
Phase 4
a)- 0% Colonial *Microcystis*
b)- 20% Colonial *Microcystis*
c)- 50% Colonial *Microcystis*
d)- 80% Colonial *Microcystis*
e)- 100% Colonial *Microcystis*
Mandibular movement rate (Hz)
Time (min)
0 10 20 30 40 50 60 70 80 90 100
0 1 2 3

Fig. 8. *Daphnia pulicaria* appendage beating rate (ABR) as a function of time in the experiment with purified microcystin-LR added to *Scenedesmus* in the following concentration: a) 0, b)- 50 ng, c) 500 ng, d) 5000 ng mL^{-1}. ABR is shown as mean of 5 animals ± one standard error of the mean. Vertical dashed lines represent the delimitation between the different measurement phases 1 to 3. Horizontal dotted lines represent the mean of the pre-treatment phase 1.

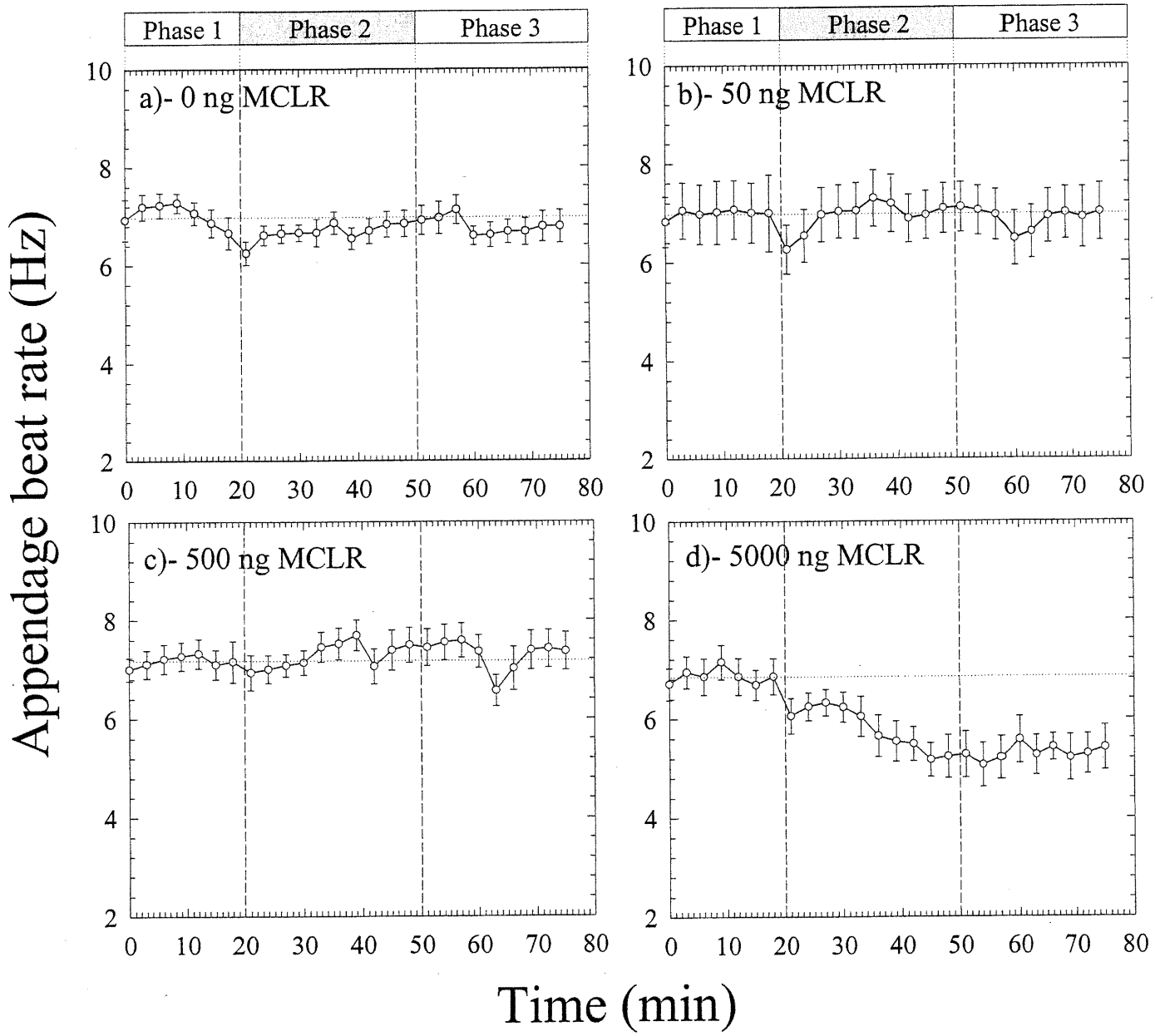

Phase 1
Phase 2
Phase 3
a)- 0 ng MCLR
b)- 50 ng MCLR
c)- 500 ng MCLR
d)- 5000 ng MCLR
Appendage beat rate (Hz)
Time (min)
2
4
6
8
10
0
10
20
30
40
50
60
70
80

Table 2. Results of repeated-measures ANOVAs for comparison of *Daphnia pulicaria* mandibular movement rate (MMR) in different phases of the experiments with different proportions (0, 20, 50, 80 and 100%) of unicellular and colonial *Microcystis aeruginosa* added to *Scenedesmus obliquus*.

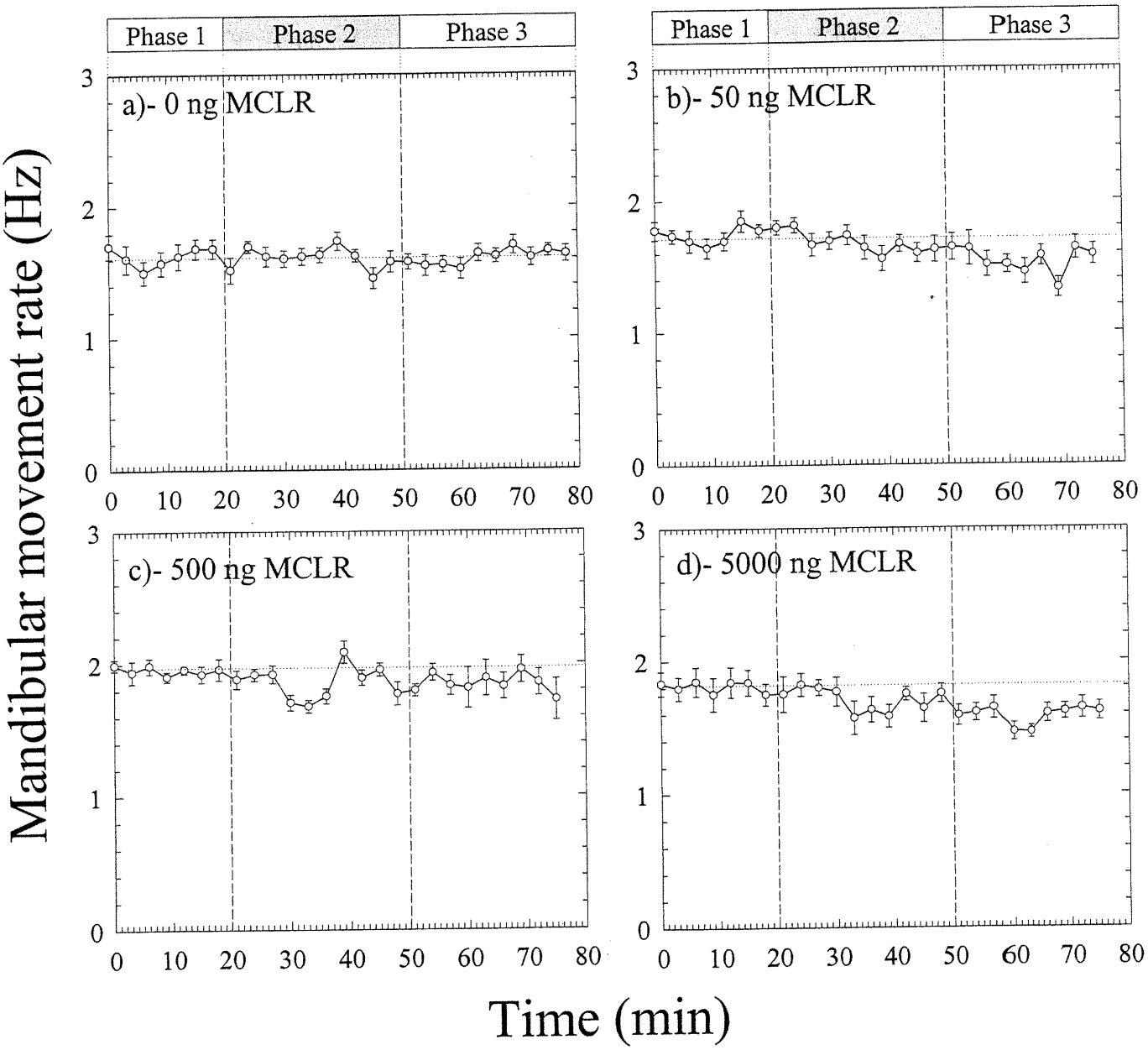
		Unicellular *Microcystis*				Colonial *Microcystis*			
		Phase 1 vs. 2	Phase 1 vs. 3	Phase 1 vs. 4	Phase 3 vs. 4	Phase 1 vs. 2	Phase 1 vs. 3	Phase 1 vs. 4	Phase 3 vs. 4
	df	*P*	*P*	*P*	*P*	*P*	*P*	*P*	*P*
0% *Microcystis*									
Treatment	1	0.6290	0.8097	0.9105	0.3225	0.6290	0.8097	0.9105	0.3225
Time	6	0.3302	0.6514	0.1620	0.9465	0.3302	0.6514	0.1620	0.9465
Time X Treatment	6	0.1363	0.1945	0.4504	0.4723	0.1363	0.1945	0.4504	0.4723
20% *Microcystis*									
Treatment	1	0.0025	0.1871	0.2297	0.0960	0.0893	0.8094	0.1574	0.5435
Time	6	0.6518	0.9870	0.8798	0.7005	0.2743	0.8786	0.1805	0.4229
Time X Treatment	6	0.7265	0.9193	0.9752	0.9854	0.8079	0.2899	0.7871	0.2115
50% *Microcystis*									
Treatment	1	0.0006	0.0123	0.0038	0.0083	0.1237	0.6721	0.1226	0.1129
Time	6	0.4685	0.5639	0.0287	0.0500	0.4237	0.4675	0.9975	0.0621
Time X Treatment	6	0.4344	0.1001	0.8846	0.3050	0.9621	0.2081	0.2009	0.2981
80% *Microcystis*									
Treatment	1	0.0117	0.2425	0.0293	0.0044	0.1235	0.3321	0.0212	0.0464
Time	6	0.1129	0.0179	0.2882	0.1801	0.2787	0.3279	0.9519	0.2616
Time X Treatment	6	0.5480	0.5858	0.6324	0.1313	0.1836	0.3328	0.4632	0.8857
100% *Microcystis*									
Treatment	1	0.0033	0.3129	0.0275	0.0148	<0.0001	0.1007	<0.0001	<0.0001
Time	6	0.3439	0.2753	0.7954	0.4918	0.0013	0.9483	0.1466	0.1512
Time X Treatment	6	0.1866	0.4880	0.6282	0.1611	0.4365	0.0006	0.2317	0.1854

Table 3. Results of repeated-measures ANOVAs for comparison of *Daphnia pulicaria* appendage beat rate (ABR) and mandibular movement rate (MMR) in different phases of the experiments with different amounts of purified microcystin-LR (0, 50, 500 and 5000 ng mL^{-1}) added to *Scenedesmus obliquus* suspensions.

		Appendage beat rate			Mandibular movement rate		
		Phase 1 vs. 2	Phase 2 vs. 3	Phase 1 vs. 3	Phase 1 vs. 2	Phase 2 vs. 3	Phase 1 vs. 3
	df	*P*	*P*	*P*	*P*	*P*	*P*
0 ng Microcystin-LR							
Treatment	1	0.1309	0.0782	0.3019	0.8945	0.9697	0.9048
Time	6	0.0650	0.1703	0.2630	0.0655	0.0272	0.2025
Time X Treatment	6	0.0801	0.0757	0.0583	0.0114	0.1354	0.0357
50 ng Microcystin-LR							
Treatment	1	0.8348	0.3267	0.6724	0.1882	0.0024	0.0002
Time	6	0.5514	0.0546	0.1171	0.0418	<0.0001	0.0219
Time X Treatment	6	0.3725	0.1798	0.2855	0.0466	0.4969	0.1177
500 ng Microcystin-LR							
Treatment	1	0.2617	0.4970	0.6796	0.1969	0.7733	0.5793
Time	6	0.0239	0.0057	0.0634	0.0003	0.0001	0.0058
Time X Treatment	6	0.4267	<0.0001	0.0002	<0.0001	0.1478	0.2906
5000 ng Microcystin-LR							
Treatment	1	0.0057	0.0015	0.0043	0.0486	0.0059	0.0020
Time	6	0.0599	0.0007	0.6125	0.2400	0.0026	0.2202
Time X Treatment	6	0.0386	<0.0001	0.4351	0.0908	0.1298	0.4381

significant difference (Table 3). However, the daphnids exposed to 5000 ng mL^{-1} showed a quick and sharp decline in their ABR as soon as the exposure started in phase 2 (Fig. 8d). The daphnids did not recover their previous ABR after the complete removal of the toxins from the observation chamber in phase 3 and their ABR was maintained ~30% lower in comparison to phase 1 (Fig. 8d). At 5000 ng, the comparison of phase 2 with phase 1 and 3 showed statistically highly significant differences (Table 3). In contrast, the animals showed some indication of low ingestion rate as MMR declined slightly for 50 and 500 ng additions, in comparison to no addition of microcystin (Fig. 9a,b,c). The decline in MMR was visible during phase 3 at 50 ng as shown by the statistically significant difference between phase 3 and the other two phases (Fig. 9b, Table 3). At 500 ng, there was a trend of decline in MMR during the first 9 min during phase 2; however, the animals recovered their previous MMR for the remaining duration of the experiments (Fig. 9c). This behavior was supported by the statistical analyses as the interaction term (Time X treatment) was highly significant when phase 1 and 2 were compared (Table 3). During phase 3, MMR remained slightly lower than the average of phase 1; however, this difference was not statistically significant (Fig. 9c, Table 3). The addition of 5000 ng per mL^{-1} produced a clear and statistically significant reduction in MMR during phase 2 and 3 in comparison to phase 1 (Fig. 9d, Table 3). It is important to note that the reduction in MMR occurred only 10 min after the addition of the toxins,

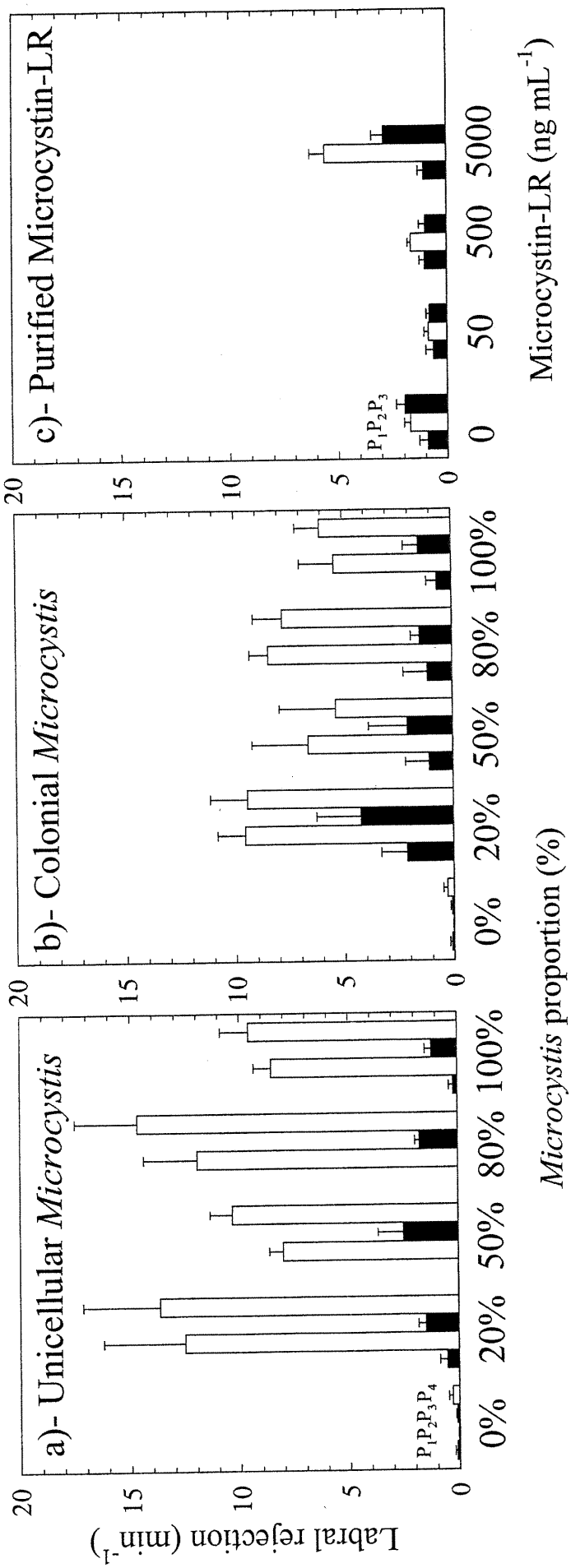
Fig. 9. *Daphnia pulicaria* mandibular movement rate (MMR) as a function of time in the experiment with purified microcystin-LR added to *Scenedesmus* in the following concentration: a) 0, b)- 50, c) 500, d) 5000 ng mL^{-1}. MMR is shown as mean of 5 animals ± one standard error of the mean. Vertical dashed lines represent the delimitation between the different measurement phases 1 to 3. Horizontal dotted lines represent the mean of the pre-treatment phase 1.


Phase 1
Phase 2
Phase 3
a)- 0 ng MCLR
b)- 50 ng MCLR
c)- 500 ng MCLR
d)- 5000 ng MCLR
Mandibular movement rate (Hz)
Time (min)
0
1
2
3
0 10 20 30 40 50 60 70 80

whereas the ABR was reduced immediately after the addition of the toxins (Fig 8d and 9d).

In the experiments with unicellular and colonial *Microcystis*, the daphnids responded to all the treatments from 20 to 100% by increasing the rate of their labral rejection significantly (Fig. 10a, b). In the absence of any *Microcystis* (cells or colonies) labral rejection rates were always below 1 min^{-1} in all the experimental phases (Wilcoxon, $P > 0.05$), but as soon as the animals were exposed to the smallest proportion (20%) there was an important increase reaching up to 15 min^{-1} (Fig. 10). The difference in labral rejection rate between the treatment and the control phases was statistically significant for all the experimental conditions from 20 to 100% with unicellular and colonial *Microcystis* (Wilcoxon, $P < 0.05$). It is important to note that the increase in labral rejection rates was not proportional to the amount of *Microcystis* added (Fig. 10a, b). The addition of microcystin-LR produced an increase in the rate of labral rejection only for the highest concentration used, as observed for the ABR and MMR (Fig. 10c). The difference between the treatment phase and the control phases was statistically significant only for highest (5000 ng) microcystin addition (Wilcoxon, $P < 0.05$). At 5000 ng the animals increased their labral rejection rate by a factor of 5 in comparison to the control phase where no toxins were present. However, this increase was not as high as in the experiments with

Fig. 10. *Daphnia pulicaria* labral rejection (min^{-1}) exhibited by animals exposed to a) 0, 20, 50, 80 or 100% of unicellular *Microcystis aeruginosa* to *Scenedesmus;* b) 0, 20, 50, 80 or 100% of colonial *Microcystis aeruginosa* to *Scenedesmus* and c) 0, 50, 500 and 5000 ng mL^{-1} of purified microcystin-LR added to *Scenedesmus*. Bars represent mean of labral rejection rate of 5 animals during phases 1 to 4 for the experiments with unicellular and colonial *Microcystis aeruginosa* and phases 1 to 3 for the experiment with purified microcystin-LR. Error bars represent one standard error of the mean. Solid bars represent phase 1 and 3 and open bars represent phase 2 and 4, respectively. Phases 1 to 4 = P_1 to P_4.


a)- Unicellular *Microcystis*
b)- Colonial *Microcystis*
c)- Purified Microcystin-LR
$P_1P_2P_3P_4$
$P_1P_2P_3$
Labral rejection (min^{-1})
Microcystis proportion (%)
Microcystin-LR ($ng\ mL^{-1}$)
0% 20% 50% 80% 100%
0 50 500 5000

unicellular or colonial *Microcystis* were the rate increase reached at times more than 20 times that of the control phase (Fig. 10a, b).

Discussion

The present experiments revealed unexpectedly contrasted patterns of feeding inhibition in *Daphnia pulicaria* in the presence of single cells, colonies of *Microcystis aeruginosa* or purified microcystin-LR in their food. The daphnids when presented a food mixture containing unicellular *Microcystis* did not reduce their ABR significantly; however, their food ingestion was reduced very quickly. These observations indicate that the animals were able to detect the presence of a small proportion of *Microcystis* cells (i.e., 20%) in their food suspension and to reduce their food ingestion. Since the *Microcystis* and *Scenedesmus* cell have comparable size and shape, it is unlikely that daphnids could detect *Microcystis* cells based on their size or shape. Our findings suggest that *Daphnia pulicaria* may have the ability to discriminate between food particles based on their 'taste', which contrast with the well established theory stating that cladocerans in general have poor 'tasting' abilities (Kerfoot and Kirk 1991). The rapid reduction in MMR and not in ABR suggest also that the toxic content of the cell may not be the reason for the change in *Daphnia* feeding behavior. A more plausible explanation of this inhibition would be the presence of a perceptible and hence 'tastable' factor on the surface of the cell as suggested by previous

studies (Lampert 1982, Henning et al. 1991). The inhibition of the food ingestion provoked by *Microcystis* single cells was easily reversible since the animals regain their previous MMR in phase 3 weakening the likelihood of the microcystin poisoning hypotheses. This conclusion is in agreement with previous studies which found no relationship between the amount of toxins in *Microcystis* cells and their inhibitory effect on *Daphnia* (Jungmann et al. 1991, Rohrlack et al. 1999a, 1999b, 2001). The presence of *Microcystis* single cells caused an important increase in the rate of labral rejection confirming the feeding inhibition demonstrated by the decrease in MMR. The increase in labral rejection was easily reversible suggesting that the animals were not intoxicated by the presence of the single cells.

In contrast with unicellular *Microcystis*, the presence of colonies in *Daphnia* food suspension produced a more complex pattern of feeding inhibition. The ABR decreased in contact with colonies in the very first minutes of treatment phase 2 and 4 and increased to reach sometimes even higher ABR in comparison to the control phases. At the same time, the animals increased their labral rejection substantially. This observation suggest that increase in the rejection rate prevented the animals from gathering food and as the food gathering decreases the animals were trying to compensate by increasing their ABR. The non-reduction in MMR at 20 to 80% of colonial *Microcystis* may be an

indication that the animals were maintaining their ingestion rate high to compensate for their hunger state. In pure colonial *Microcystis* suspensions, the animals did not change their ABR but rather reduced significantly their MMR which is in contrast with their response at 20 to 80%. One possible explanation could be that the animals didn't detect anymore the presence of edible *Scenedesmus* cells and hence reduced their ingestion rates as they where not gathering any food because of the increased labral rejection. These results suggest that the presence of large colonies (>30 μm) produced a typical feeding interference reaction in *Daphnia*. The high frequency of labral rejection prevented the animals from gathering food particle. To compensate for the lack of food, the animals tried to increase their ABR to higher rates and to maintain their MMR stable. This could be clearly an indication of a state of hunger. The reversibility of these changes again points towards a feeding interference rather than intoxication reaction of *Daphnia*. Previous studies by Webster and Peters (1978) and more recently by DeMott et al. (2001) described similar sensitivity in *Daphnia* exposed to cyanobacteria filaments.

While there was no clear response of *Daphnia* to the addition of small amount of microcystins (50 to 500), large concentration of 5000 ng mL^{-1} caused a non-reversible decrease in ABR, MMR and a stimulation of labral rejection. These observations suggest that *Daphnia* can respond to toxins presence by

modifying their feeding behavior. The decrease in ABR and MMR occurred only 10 min after the beginning of phase 2 suggesting that the changes is clearly an intoxication reaction. It is important to note that the highest concentration used in this experiments is very low in comparison to LC_{50} published in the literature (DeMott et al. 1991, Jungmann 1992, Jungmann and Benndorf 1994). These findings suggest that *Daphnia* can respond to relatively low concentrations of cyanotoxins in comparison to lethal concentration. However, the mechanisms involved in the low dose intoxication of *Daphnia* remain to be elucidated. Some scenarios have been proposed by recent studies pointing mainly to a possible inhibition by direct contact of the motor nerves controlling the feeding appendages with purified and dissolved cyanotoxins (Haney et al. 1995). However, the pathways of such intoxication mechanisms are not yet known.

In summary, our results demonstrated that *Daphnia pulicaria* reacted in different ways to the additions of *Microcystis* single cell, colonies or secondary metabolites; however, in the three cases the results is a decline in feeding rates. This study provided new insights as to the mechanisms of feeding inhibition produced by the different forms of *Microcystis* which could be extended to filamentous and other colonial cyanobacteria. It is important to note that while a specific concentration of purified toxin produced a decline in the feeding

behavior of *Daphnia,* the same response was also produced by the presence of a small number of colonies or single cells. These observations suggest that the mechanical interference produced by cyanobacterial colonies or filaments may be as damageable for the survival and the establishment of large filter-feeder communities as their toxin content.

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Chapitre 5

Relationships between zooplankton and cyanobacteria along increasing cyanobacterial biomass gradient in boreal Alberta lakes

Relationships between zooplankton and cyanobacteria along increasing cyanobacterial biomass gradient in boreal Alberta lakes

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To be submitted to *Canadian Journal of Fisheries and Aquatic Sciences*

Running title: Zooplankton-cyanobacteria interactions in lakes

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Abstract

Changes in phytoplankton and zooplankton communities were studied in four lakes for four years during a large scale experimental study as part of the TROLS research program. The main objective of this research program was to evaluate the effects of forest harvesting on terrestrial and aquatic systems in a before-after manipulation study. After forest harvesting, algal biomass and especially cyanobacterial biomass increased in some of the experimental lakes, up to an order of magnitude. Although the response of algal communities was not proportional to intensity of forest harvesting, this study provided a unique opportunity for studying the effects of an increasing gradient in cyanobacterial biomass on the zooplankton communities. Cladoceran size structure and biomass were negatively correlated with lake trophy, as was cladoceran to copepod ratio, suggesting that large cladocerans were inhibited by the increase in cyanobacterial biomass. Thus the zooplankton to phytoplankton ratio tends to decline with trophy because of increased biomass of inedible filamentous and colonial cyanobacterial species. Concentrations of the hepatotoxin, microcystin-LR, also increased with increasing trophy, however, we could not relate the occurrence of high concentrations of this toxin with the loss of large cladocerans. Our results towards feeding inhibition, by filamentous and colonial cyanobacteria, as a cause for the decline in large cladoceran biomass.

Introduction

Understanding the interactions between zooplankton and phytoplankton in aquatic systems is fundamental. Classical concepts in ecology such as biomanipulation (Shapiro and Wright 1984), trophic cascade (Carpenter et al. 1985), or bottom-up : top-down foodweb control (McQueen et al. 1986) are all based on efficient energy transfer through trophic links. Some of these concepts have been used in attempts to control algal biomass in eutrophic systems with limited success due to insufficient control of algal biomass by grazers (MacKay and Elser 1998). One of the hypotheses that have been put forward to explain an interruption in the zooplankton-phytoplankton link was the dominance of inedible colonial and filamentous cyanobacterial species in phytoplankton communities (MacKay and Elser 1998). Despite evidence of the negative impact of some cyanobacterial species on large filter-feeding cladocerans such as *Daphnia* (Lampert 1982), several studies support the idea that zooplankton can control phytoplankton in eutrophic conditions (Boon et al. 1994; Brett and Goldman 1996; MacKay and Elser 1998). This dialectic has long been the center of a vigorous debate about the success of biomanipulation in eutrophic, cyanobacterial dominated systems (Carpenter and Kitchell 1992; DeMelo et al. 1992).

Widely accepted empirical models predict that zooplankton biomass is positively related to phytoplankton biomass in lakes (McCauley and Kalff 1981). In contrast, plankton succession models predict that as inedible phytoplankton biomass increases, zooplankton usually decline starting from the mid-summer season in temperate lakes (Sommer et al. 1986). This decline in total zooplankton biomass is usually attributed to the loss of large cladocerans (Sommer et al. 1986). Although the mechanism of this mid-summer decline of large cladocerans is not elucidated, it could be explained by a shift in phytoplankton communities from small edible to large inedible phytoplankton, whereas it had been previously ascribed exclusively to predation (Threlkeld 1979; Benndorf et al. 2001). Many laboratory studies have shown that cladocerans (i.e., *Daphnia*) are negatively affected by cyanobacterial species (Lampert 1982; Haney et al. 1994; Reinikainen et al. 1995; Reinikainen et al. 1999); however, there is no clear indication that cyanobacterial abundance controls zooplankton in field conditions. Despite the trend towards more cyanobacteria which accompanies eutrophication, there are surprisingly few large-scale field based experiments. This void makes it difficult to apply the information gained from laboratory studies or small-scale experiments to natural conditions on cyanobacteria-zooplankton dynamics.

In the present study, we examined cyanobacteria-zooplankton interactions along a gradient of trophy in four cyanobacteria dominated lakes during four years. The four lakes were part of a large-scale ecosystem experiment whereby their watersheds received different intensities of forest harvesting after two years of pretreatment studies. Although phosphorus concentration and algal biomass increases were not proportional to buffer strip width or percentage of harvested watershed, we were able to obtain a clear gradient of algal biomass in the 16 lake-years (four lakes X 4 years). Based on previous observation in similar lakes (Ghadouani et al. 1998) and large enclosure studies (this thesis, chapter 2), we anticipated that increasing cyanobacterial biomass and their secondary metabolites (e.g., microcystins) along a positive gradient would result in: 1) a change of size structure from large to small zooplankters, 2) a consequent decline in large cladoceran biomass and 3) less or no effect on copepods. These changes would induce a shift in the zooplankton community from dominance by large cladocerans to smaller copepods.

Materials and methods

Study site

The study lakes are part of the terrestrial and riparian organisms lakes and streams (TROLS) research program which was created to evaluate the

effects of forest harvesting and buffer strips on terrestrial and aquatic systems (Prepas et al. 2001). The experimental lakes used in this study are all headwater lakes and are located in the South Pelican Hills (SPH) in the southern portion of Alberta's boreal mixedwood ecoregion. The experimental treatment consisted, after agreement with forest companies, of harvesting the forest up to a buffer strip around the lakes of 800, 200, 100 and 20-m in SPH800, 200, 100 and 20 lakes, respectively. The study started in 1995 and lasted for four years. The forest harvesting took place in the experimental regions from October 1996 to February 1997. Hence, we had a four years study with two pretreatment and two post-treatment years. The percent cut of the watersheds was 6, 10, 22 and 28% in SPH800, 100, 200 and 20, respectively (Prepas et al. 2001). After forest harvesting, nutrient input to the lakes increased and resulted in important increases in total phytoplankton and cyanobacteria biomass (Prepas et al. 2001). Although the response of the lakes to increased nutrients input was not proportional to their buffer strips or the percentage cut of their watershed, we had a gradient ranging from low to high biomass of cyanobacteria in our 16 lake-years (4 Lakes X 4 years).

Sampling and analyses

The lakes were sampled on a biweekly basis during the open water season between May and September from 1995 to 1998. On each sampling date,

routine limnological measurements including light penetration, water temperature and dissolved oxygen were taken at the central part of the lakes. Integrated water samples of the euphotic zone (to 1% of incident light) were taken with a weighted Tygon tubing fitted with a one way foot valve for various chemical analyses. Chlorophyll *a* biomass was estimated following a cold ethanol extraction method (Bergmann and Peters 1980). Lugol preserved phytoplankton samples were analyzed and phytoplankton species identified and counted with an inverted microscope according to the Utermöhl method (Lund et al. 1958). Microcystin-LR concentrations were estimated with a high-performance liquid chromatography method after extraction of lyophilized algal biomass (Zurawell et al. 1999). Microcystin-LR concentrations were then expressed in µg per g of lyophilized algal biomass or dry mass. More details on chemical and plankton methods can be found elsewhere (Prepas et al. 2001).

Zooplankton samples were collected in the pelagic zone near the deepest point of each lake with a 53-µm mesh size cantilevering net with a squared opening (Filion et al. 1993). Vertical net hauls over the entire water column were performed at each sampling site. Zooplankton were preserved in 4% buffered formaldehyde solution after being narcotized in the field with carbonated water. In the laboratory, 5-mL subsamples were taken from the concentrated samples with a large opening pipet and analyzed with a

stereomicroscope in a Ward cell (Ward 1955). Crustacean zooplankters were identified to the species level and their body length was measured. The length of each individual was converted to dry mass by applying published length-dry mass relationships (Malley et al. 1989).

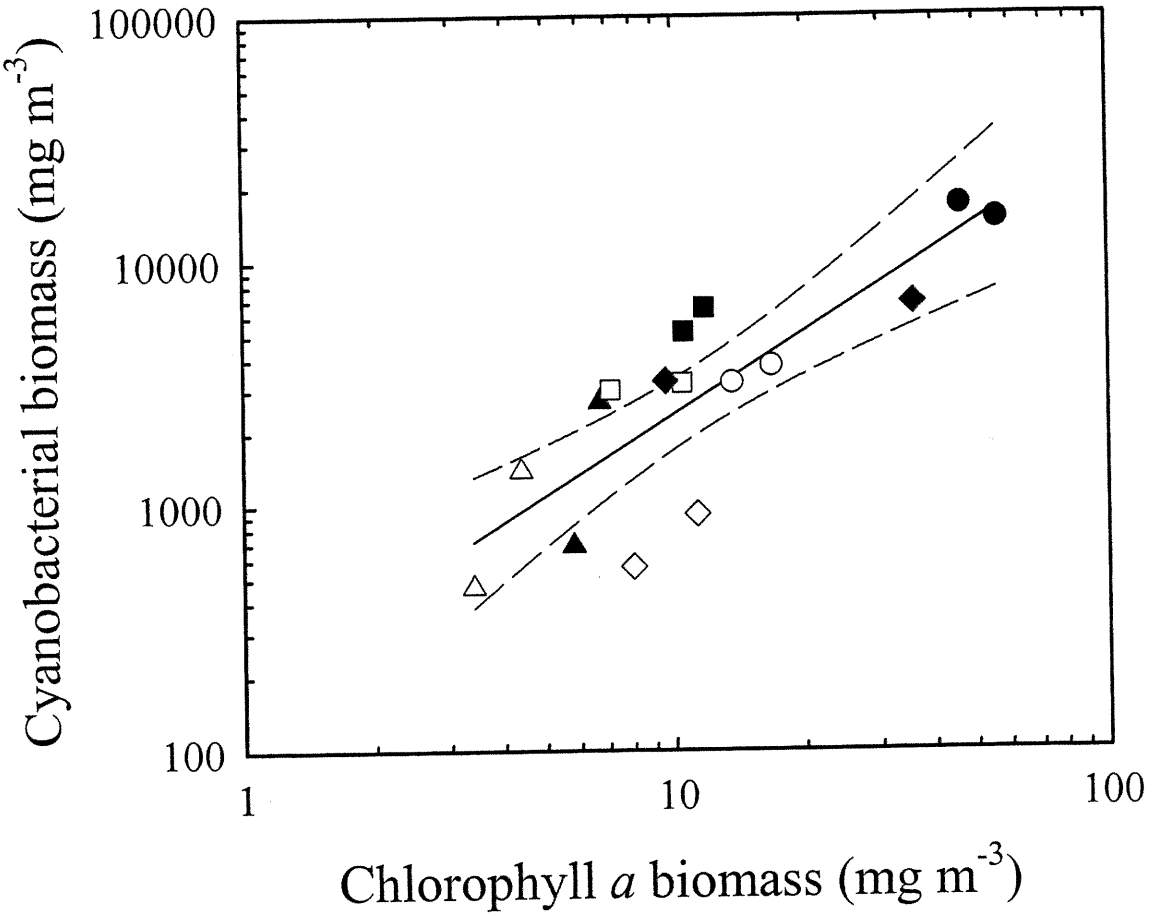
Statistical analyses

Relationships between zooplankton and cyanobacteria were tested by simple regression analysis and associated ANOVAs. Since data collected over a season are not truly independent, all data were average for each year to avoid temporal autocorrelation problems (Hurlbert 1984). Each data point shown in the relationships is a seasonal average for a study year (1995 to 1998). All data used in statistical analyses were log-transformed. Upper and lower 95% confidence intervals of the regression lines are shown.

Results and discussion

In the four experimental lakes, cyanobacterial biomass was strongly and positively correlated ($r = 0.82$, $P < 0.001$) with chlorophyll biomass (Fig. 1). This relationship was mainly driven by SPH100 and SPH800 where cyanobacterial biomass increased by almost an order of magnitude after the watershed disturbance in 1997 and 1998 (Fig. 1). Although variation was quite high in some lakes, chlorophyll biomass appeared to be a very good predictor of

Fig. 1. Relationship between cyanobacteria biomass and chlorophyll biomass (r = 0.82, $P < 0.001$) in the four experimental lakes from 1995 to 1998. SPH 20, 100, 200 and 800 are represented by triangles, circles, squares and diamonds, respectively. Pretreatment (1995 and 1996) data are represented in open symbols, while post-treatment (1997 and 1998) data are represented in solid symbols. Regression line is a solid line, while upper and lower 95% confidence intervals are shown in dashed lines.


100000
10000
1000
100
1
10
100
Cyanobacterial biomass (mg m-3)
Chlorophyll a biomass (mg m-3)

cyanobacterial biomass (Fig. 1). Algal communities in SPH region lakes were mainly dominated (> 90%) by filamentous cyanobacterial species such as *Aphanizomenon spp.* and *Anabaena spp.* as well as colonial *Microcystis spp.* (Prepas et al. 2001). This species association is typical of eutrophic lakes in the Boreal plain (Ghadouani et al. 1998; Zhang et al. 2001). The response of some lakes to watershed disturbance (SPH100 and SPH800) created a good opportunity to test our hypothesis about the changes in zooplankton communities as a response to increased cyanobacterial biomass.

An examination of zooplankton communities along a gradient of increased lake trophy indicated that cladocerans tend to be smaller as lakes become more eutrophic (Fig. 2). Lakes with relatively low cyanobacterial biomass tend to have large cladocerans (> 1mm), especially daphnids, whereas lakes with high cyanobacteria biomass have only smaller cladocerans (< 1mm) (Fig. 2). At low cyanobacterial biomass, more than 50% of the cladocerans or daphnids were > 1mm in body length (Fig. 2). Except in SPH200 Lake in 1997, more than 50% of the cladocerans in lakes with high cyanobacterial biomass were < 1mm in body length (Fig. 2). Cladoceran and *Daphnia* median size were negatively correlated (r = -0.71, $P < 0.01$) to cyanobacterial biomass (Fig. 3). The changes in the size structure caused a decline in cladoceran biomass as lake trophy increased. Cladoceran biomass was negatively correlated (r = 0.55, $P <$

Fig. 2. Cladoceran (top panel) and *Daphnia spp.* (middle panel) size structure represented in boxplots as a function of cyanobacterial biomass gradient starting from low to high biomass in the four experimental lakes: SPH 20, 100, 200 and 800 for 4 years (1995 to 1998). Each boxplot summarizes the size of cladoceran or *Daphnia* populations in one year and contains from 114 to 670 data point. Planktivorous fish density is shown (CPUE). Number between brackets are planktivorous fish densities for post-treatment years. Cyanobacterial biomass is shown as seasonal average for each of the study years (1995 to 1998) in the four experimental lakes. Error bars are standard errors of the mean based on seasonal data.

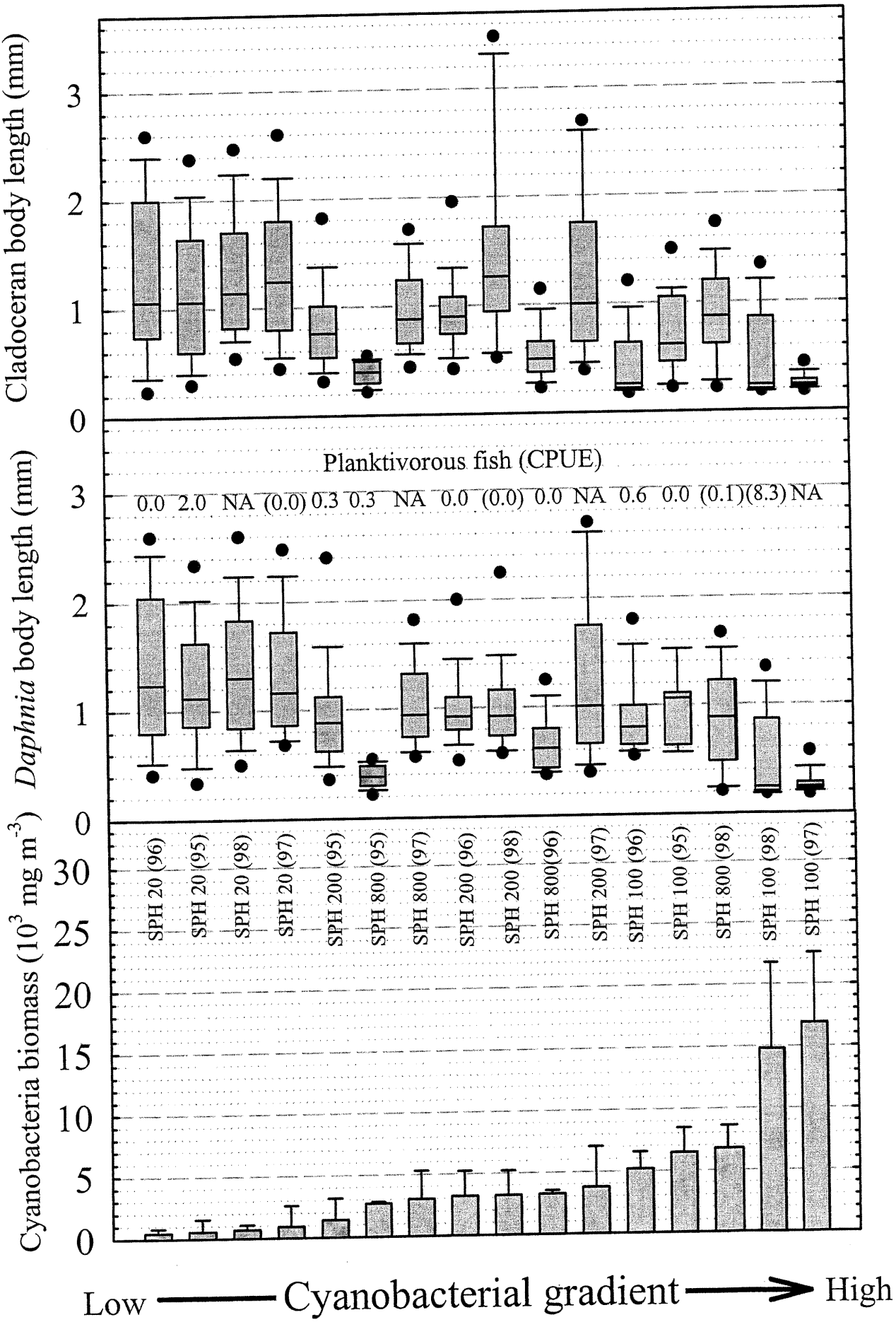

Cladoceran body length (mm)
3
2
1
0
Planktivorous fish (CPUE)
0.0 2.0 NA (0.0) 0.3 0.3 NA 0.0 (0.0) 0.0 NA 0.6 0.0 (0.1) (8.3) NA
Daphnia body length (mm)
3
2
1
0
Cyanobacteria biomass (10^3 mg m^{-3})
30
25
20
15
10
5
0
SPH 20 (96)
SPH 20 (95)
SPH 20 (98)
SPH 20 (97)
SPH 200 (95)
SPH 800 (95)
SPH 800 (97)
SPH 200 (96)
SPH 200 (98)
SPH 800(96)
SPH 200 (97)
SPH 100 (96)
SPH 100 (95)
SPH 800 (98)
SPH 100 (98)
SPH 100 (97)
Low
Cyanobacterial gradient
High

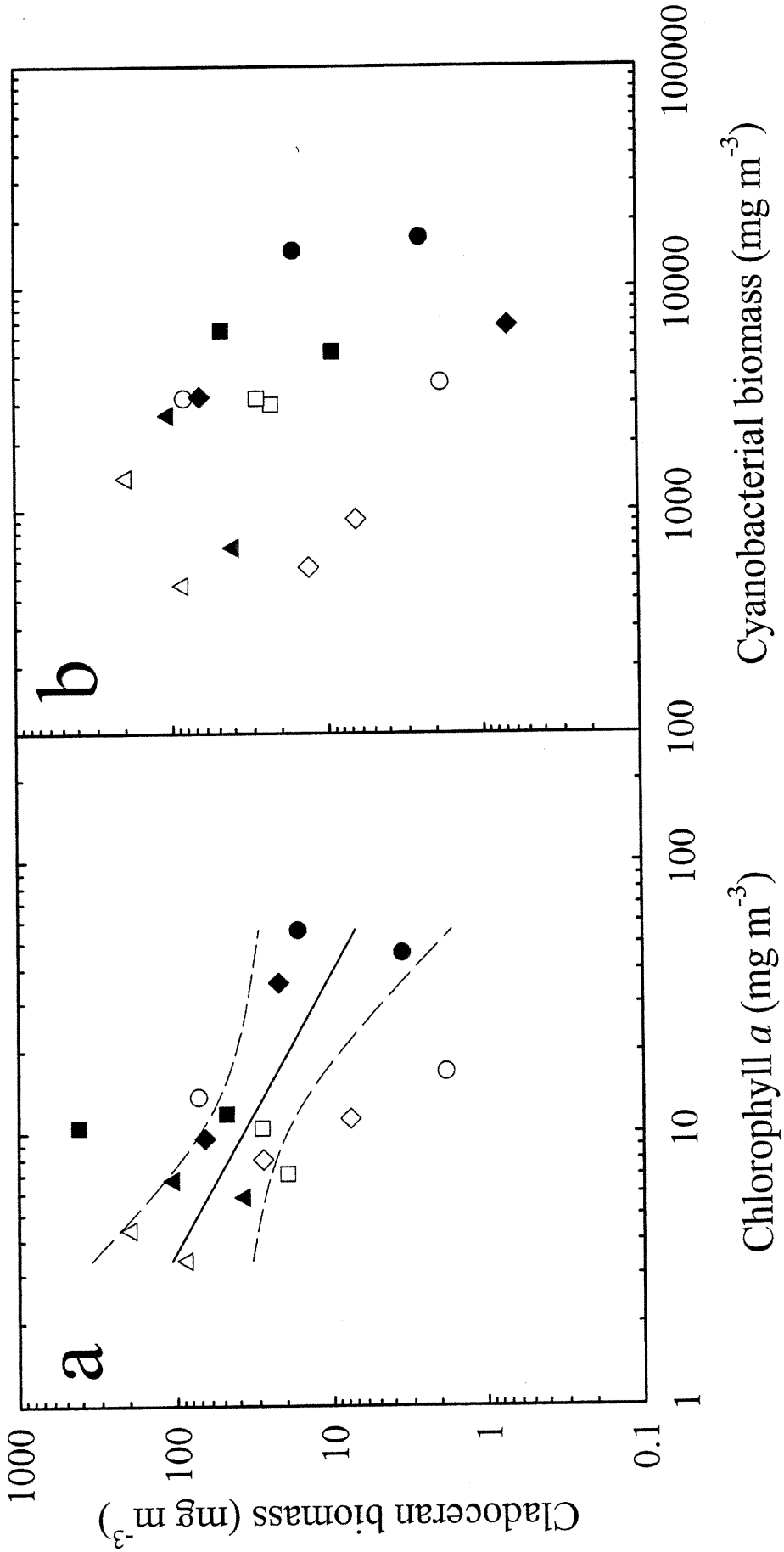
Fig. 3. Relationship between a) cladoceran median size (r = -0.71, $P < 0.01$), b) *Daphnia* median size (r = -0.71, $P < 0.01$) and cyanobacterial biomass or b) cyanobacterial biomass. SPH 20, 100, 200 and 800 are represented by triangles, circles, squares and diamonds, respectively. Pretreatment (1995 and 1996) data are represented by open symbols, while post-treatment (1997 and 1998) data are represented by solid symbols. Regression lines are solid lines, while upper and lower 95% confidence intervals are dashed lines.

a
b
Cladoceran median size (mm)
Daphnia median size (mm)
Cyanobacterial biomass (mg m^{-3})
0.1
1
100
1000
10000
100000

0.03) with chlorophyll biomass (Fig. 4a). Although it was not statistically significant, the same negative trend was noted between cladoceran biomass and cyanobacterial biomass (Fig. 4b). The combined decline in cladoceran size and biomass in high cyanobacterial environment may be explained by the feeding inhibition experienced by larger zooplankters in these specific conditions. Earlier laboratory experiments have shown that inhibition of zooplankton by cyanobacterial filaments may be size-dependent. Hence, smaller cladocerans may have a survival advantage since they can feed between cyanobacterial filaments, while larger cladocerans such as *Daphnia* may be severely inhibited (Webster and Peters 1978; DeMott et al. 2001). Our studies in enclosures or in the laboratory, suggest that large cladocerans are subject to feeding inhibition when they were exposed to colonial or filamentous cyanobacteria (this thesis chapter 2 and 4). Pioneer studies showed that the presence of cyanobacterial filaments may cause daphnids to reject the total content of their food groove (Burns 1968) and to reduce their filtering rate (McMahon and Rigler 1963). It was long speculated that these changes in feeding behavior may ultimately cause high mortality by starvation; however, there is no clear evidence that these predictions are true at the community level in natural systems.

Copepod biomass was neither (r = 0.35, P = 0.19) correlated with chlorophyll biomass (Fig. 5a), nor with cyanobacteria biomass (r = 0.13, P =

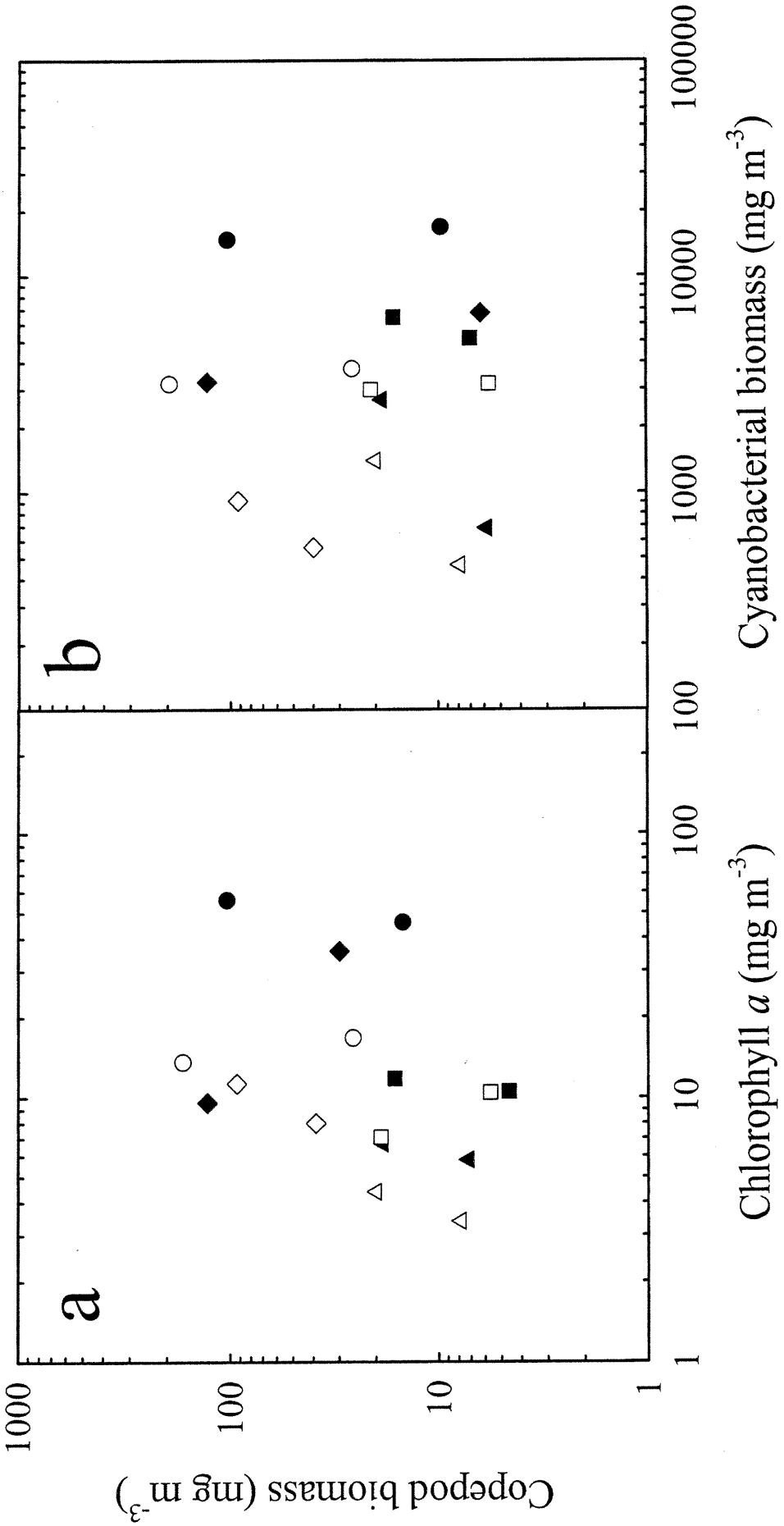
Fig. 4. Relationship between cladoceran biomass and a) chlorophyll biomass (r = -0.55, $P < 0.03$) or b) cyanobacterial biomass (r = -0.25, P = 0.35). SPH 20, 100, 200 and 800 are represented by triangles, circles, squares and diamonds, respectively. Pretreatment (1995 and 1996) data are represented by open symbols, while post-treatment (1997 and 1998) data are represented by solid symbols. Regression lines are solid lines, while upper and lower 95% confidence intervals are dashed lines.


a
b
Cladoceran biomass (mg m^{-3})
1000
100
10
1
0.1
Chlorophyll *a* (mg m^{-3})
1
10
100
Cyanobacterial biomass (mg m^{-3})
100
1000
10000
100000

0.64). This is an indication that copepods are not as affected by the presence of high biomass of cyanobacteria as cladocerans are. Copepods are not usually inhibited by cyanobacteria because of their "tasting" abilities whereby they can choose between edible and non-edible phytoplankton particles (Schaffner et al. 1994). This observation is in agreement with the results of our enclosure studies reported in chapter 2.

The strongest relationship was found between cladoceran:copepod ratio, used here as an indication of cladoceran dominance, and chlorophyll biomass (r = 0.67, P = 0.004). This negative relationship suggests that the cladoceran dominance over copepods became weaker as the lakes became more productive (Fig. 6a). No correlation was directly found between the same ratio and cyanobacteria biomass (r = 0.32, P = 0.21, Fig. 5b). This pattern may be a confirmation of the decline of cladocerans with increasing trophy in these experimental lakes. These observations are in contrast with the size-efficiency hypothesis which predicts a competitive advantage for large zooplankton in food collection over smaller ones (Brooks and Dodson 1965; Dodson 1974; Gliwicz 1990; Gliwicz and Lampert 1990). The dominance of large cladocerans can be possible in conditions of low predation pressure and when phytoplankton are dominated by edible particle; however, it is completely the opposite in cyanobacterial dominated lakes as shown by the clear negative

Fig. 5. Relationship between copepod biomass and a) chlorophyll biomass (r = 0.35, P = 0.19) or b) cyanobacteria biomass (r = 0.13, P = 0.64). SPH 20, 100, 200 and 800 are represented by triangles, circles, squares and diamonds, respectively. Pretreatment (1995 and 1996) data are represented in open symbols, while post-treatment (1997 and 1998) data are represented in solid symbols. Regression lines are solid lines, while upper and lower 95% confidence intervals are dashed lines.


a
b
1000
100
10
1
1
10
100
100
1000
10000
100000
Copepod biomass (mg m-3)
Chlorophyll a (mg m-3)
Cyanobacterial biomass (mg m-3)

relationship between cladoceran to copepod ratio and lake trophy (Fig. 6). Several studies have previously reported midsummer declines of zooplankton biomass in eutrophic lakes as a result of a decline in the large *Daphnia* concomitantly with the occurrence of cyanobacterial blooms (Wright 1965; Clark and Carter 1974; Threlkeld 1979; Ghadouani et al. 1998; Benndorf et al. 2001). However, these midsummer declines were more attributed, directly or indirectly, to predation than to changes in food quality or feeding inhibition by cyanobacteria (e.g., Benndorf et al. 2001). In the present study, the examination of planktivory variables showed no likelihood of cladocerans being controlled by small planktivorous fish (Fig. 2), as there was no relationship between the abundance of plankltivorous fish and zooplankton dynamics (Prepas et al. 2001). Furthermore, there has been no report of increased predation following forest harvesting in similar studies (St-Onge and Magnan 2000). On the contrary, the authors of this study reported a decrease in small fish abundance in lakes with harvested watersheds (St-Onge and Magnan 2000). There was no relationship between total zooplankton and chlorophyll biomass or cyanobacteria biomass (Fig. 7). The absence of relationship with zooplankton could be explained by the compensation of cladoceran biomass loss by a small increase in copepod biomass with increasing lake trophy.

Fig. 6. Relationship between cladoceran to copepod biomass ratio and a) chlorophyll biomass (r = -0.67, $P < 0.01$) or b) cyanobacteria biomass (r = -0.32, P = 0.21). SPH 20, 100, 200 and 800 are represented by triangles, circles, squares and diamonds, respectively. Pretreatment (1995 and 1996) data are represented in open symbols, while post-treatment (1997 and 1998) data are represented in solid symbols. Regression lines are in solid lines, while upper and lower 95% confidence intervals are dashed lines.

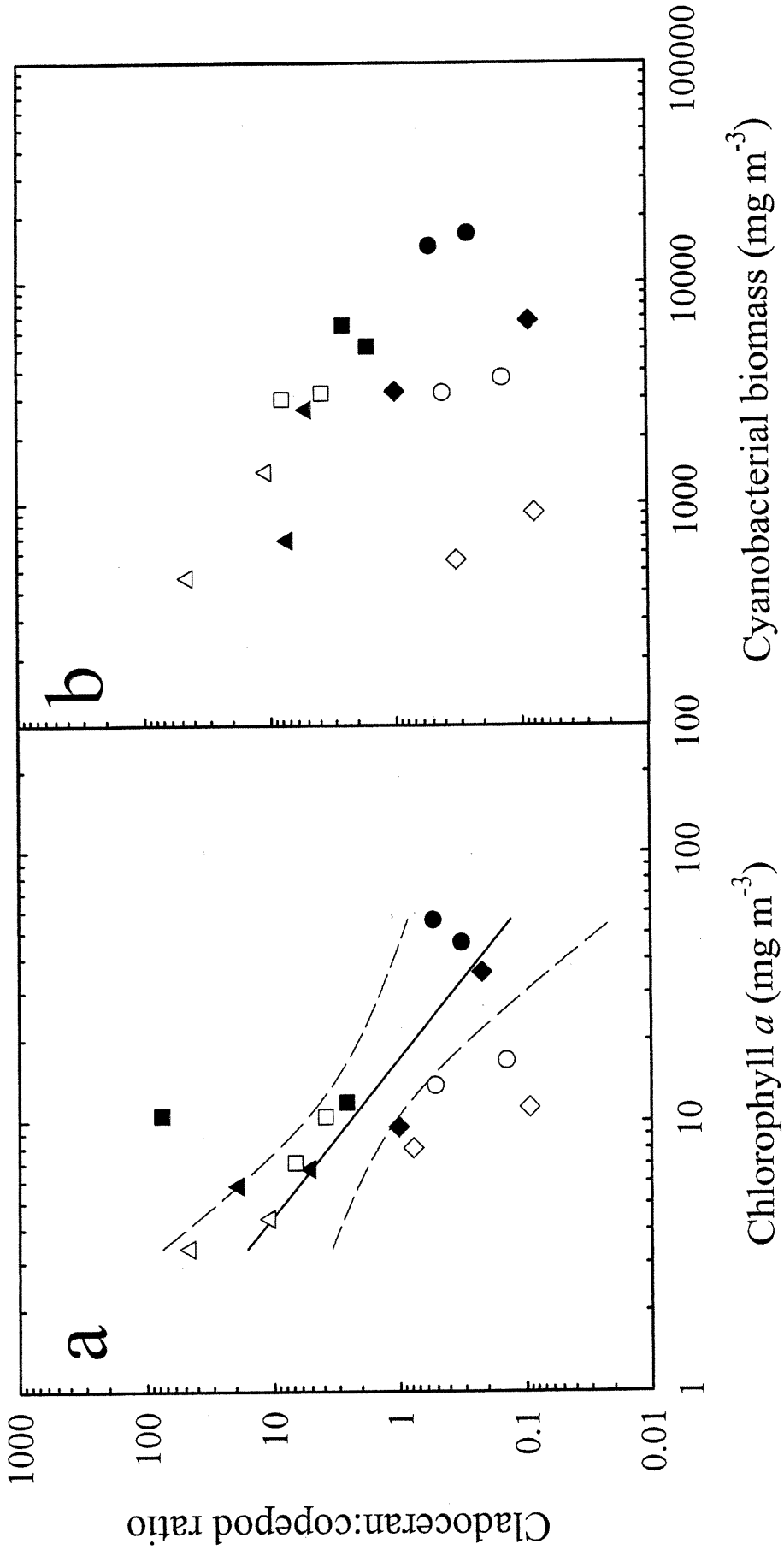
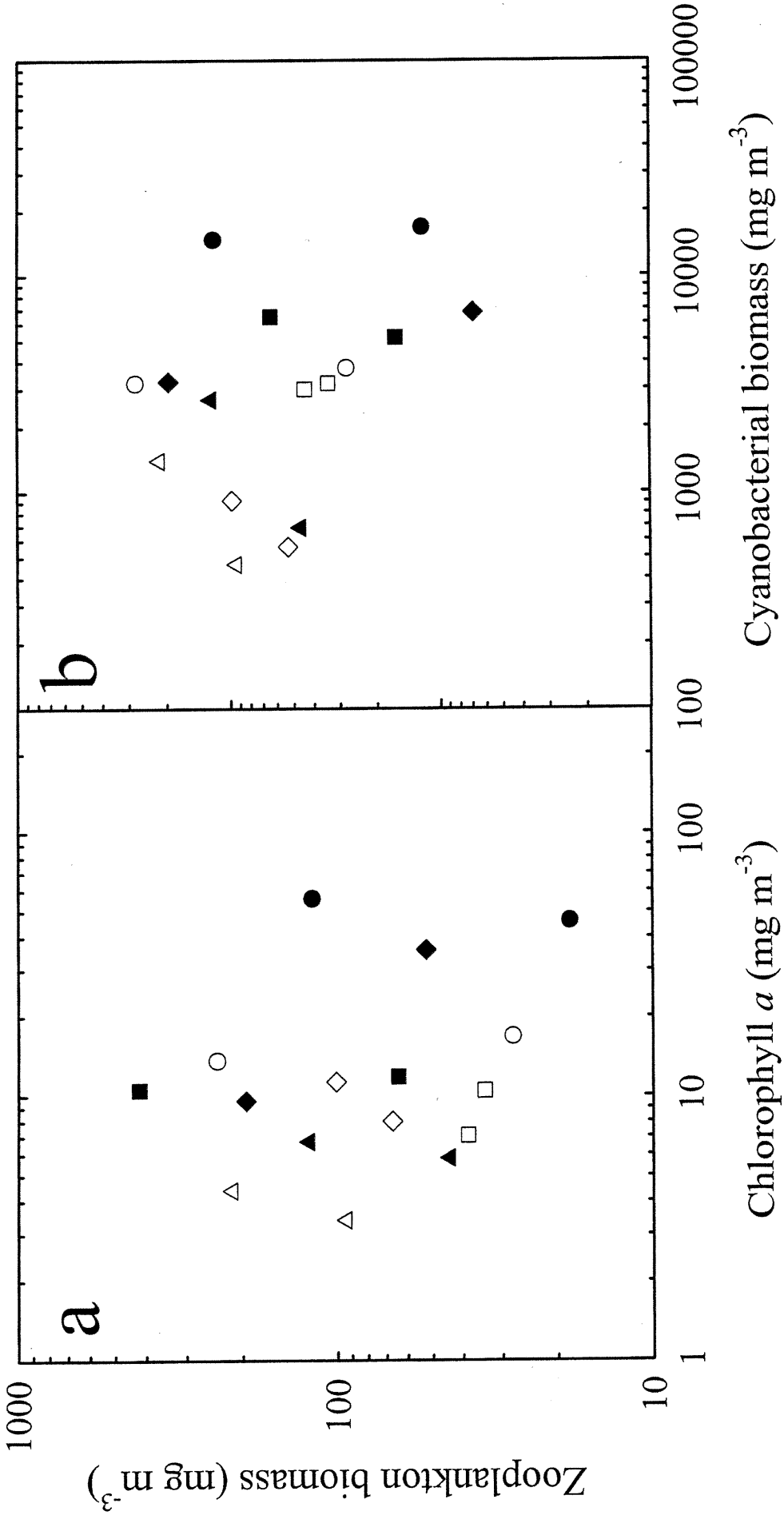
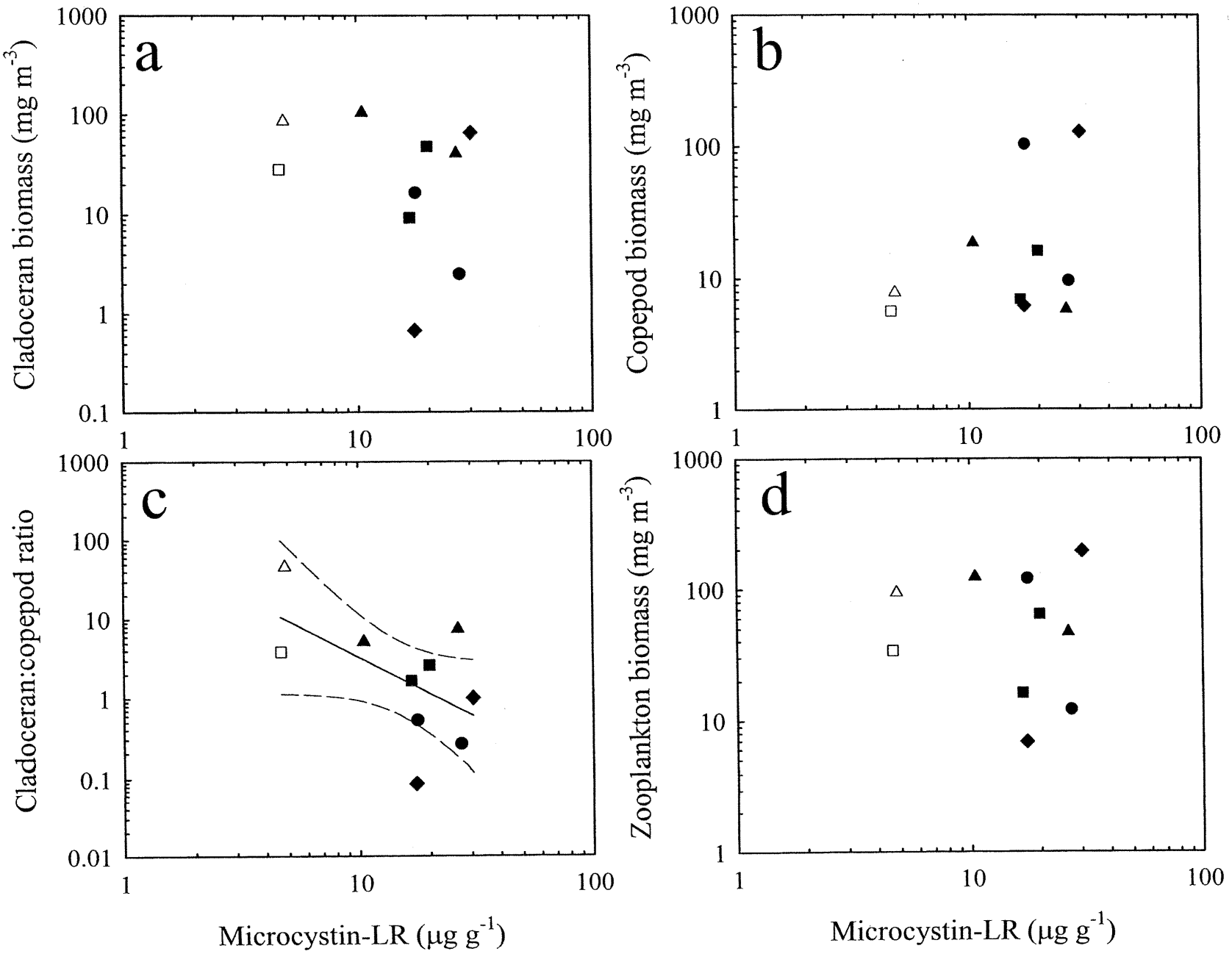

a
b
Cladoceran:copepod ratio
1000
100
10
1
0.1
0.01
Chlorophyll a (mg m-3)
1
10
100
Cyanobacterial biomass (mg m-3)
100
1000
10000
100000

Fig. 7. Relationship between total zooplankton biomass and a) chlorophyll biomass (r = -0.28, P = 0.29) or b) cyanobacteria biomass (r = -0.13, P = 0.62) in the four experimental lakes of SPH region. SPH 20, 100, 200 and 800 lakes are represented by triangles, circles, squares and diamonds, respectively. Pretreatment years (1995 and 1996) data are represented in open symbols, while post-treatment years (1997 and 1998) data are represented in solid symbols. Simple regression lines are shown in solid lines while upper and lower 95% confidence intervals are shown in dashed lines.


a
b
Zooplankton biomass (mg m^{-3})
1000
100
10
1
10
100
Chlorophyll *a* (mg m^{-3})
100
1000
10000
100000
Cyanobacterial biomass (mg m^{-3})

When zooplankton variables were correlated with microcystin-LR concentrations, similar trends to those with chlorophyll or cyanobacteria biomass were found, however the relationships were weaker (Fig. 8). The only significant relationship ($r = 0.63$, $P = 0.04$) was found between cladoceran to copepod ratio and microcystin-LR concentrations (Fig. 8c). The relationship between cladoceran, copepods or total zooplankton and microcystin-LR showed the same trend as with other trophic variable; however, none of them was statistically significant (Fig. 8 a, c, d). The absence of 1995 microcystin-LR data combined with the absence of detectable amount of toxins in a couple of lakes-years, reduced significantly the data point which make it difficult to test for the link between the increase in cyanotoxin and the decline in cladocerans. However, our laboratory experimental results on the feeding behavior of *Daphnia* in the presence of purified toxins (Chapter 4) combined with observations from the literature suggest that the effect of toxins may be less harmful to cladocerans (i.e., *Daphnia*) than feeding inhibition produced by the presence of high biomass of colonial and filamentous cyanobacterial species. In our experiments (Chapter 4), we observed some indication of inhibition by adding 5000 ng mL^{-1} to the food of *Daphnia pulicaria*. Several studies have reported that high amount of purified microcystin, in comparison to those found in natural lakes, are needed to cause daphnids mortality; as shown by the high LC_{50} (DeMott et al. 1991; Jungmann 1992; Jungmann and Benndorf 1994).

Fig. 8. Relationship between a) cladocerans (r = -0.31, P = 0.38), b) copepods (r = 0.37, P = 0.28) c) cladoceran:copepod ratio (r = -0.63, P = 0.0490) d) total zooplankton biomass (r = -0.09, P = 0.79) and microcystin-LR (in µg per g of algal dry mass) concentrations in the four experimental lakes of SPH regions. SPH 20, 100, 200 and 800 lakes are represented by triangles, circles, squares and diamonds, respectively. Pretreatment year (1996) data are represented in open symbols, while post-treatement years (1997 and 1998) data are represented in solid symbols. Microcystin-LR data were not available for 1995 study year. Simple regression lines are shown in solid lines, while upper and lower 95% confidence intervals are shown in dashed lines.


a
b
c
d
Cladoceran biomass (mg m-3)
Copepod biomass (mg m-3)
Cladoceran:copepod ratio
Zooplankton biomass (mg m-3)
Microcystin-LR (µg g-1)
Microcystin-LR (µg g-1)
1000
100
10
1
0.1
0.01
1
10
100

however, there is evidence that aquatic systems are experiencing more severe and more frequent algal blooms (Moss 1996). As zooplankton communities constitute a crucial link for energy transfer through the foodweb, it is important to better understand how large cladocerans are more affected then other zooplankters by increased cyanobacterial biomass in lakes.

Acknowledgments

Funding for this research was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC) collaborative special projects grant and University-Industry Cooperative Research and development grant, FCAR and the Québec Ministry of Education. Additional funding was provided by the following industrial and governmental partners: Alberta-Pacific Forest Industries, Alberta Environmental Protection, Alberta Economic Development and Tourism, Manning Diversified Forest Products, Employment Canada, Weyerhaeuser Canada, National Water Research Institute. We are grateful to Emma Ivania Mangas for providing her excellent expertise in zooplankton identification and analysis. Sharon Reedyk, M. Serediak, N. Scott, J. Burke, K. Gibson, S. Manchour, A. Smart, and C. Wichuk for their help in the fieldwork and limnological analysis. Special thanks to S. Paquet for analyzing phytoplankton samples. This research was based at the Meanook Biological Research Station of the University of Alberta and various TROLS field camps.

In natural conditions, cyanotoxins are contained in the cell and only released after senescence or destruction of the cyanobacterial cells and hence it is unlikely that animals could be exposed to high concentrations of cyanotoxins, high enough to cause severe inhibition or mortality. Our experimental data, suggest that *Daphnia* may be more severely affected by the presence of high concentration of *Microcystis* colonies than by the presence of low concentration of microcystin-LR (chapter 4).

In summary, our results suggest that cladocerans are negatively affected by the occurrence of high biomass of cyanobacteria. To our knowledge, this is the first large scale experiment which provide a confirmation of negative effects of cyanobacteria on cladocerans obtained from in laboratory experiments (McMahon and Rigler 1963; Burns 1968; Lampert 1982), or large scale enclosure experiments (This thesis chapter 2). Hence, it appeared that the application of biomanipulation as a management practice may not provide the desirable effects if the efficient filter-feeders (i.e., *Daphnia*) did not survive the severe algal blooms (Shapiro and Wright 1984; Carpenter et al. 1985; Brett and Goldman 1996; MacKay and Elser 1998). Eutrophication constitutes a serious environmental problem which usually results in the occurrence of spectacular algal blooms not only in lakes and reservoirs but in marine coastal systems as well (Sellner 1997). Eutrophication is not a new water quality problem;

however, there is evidence that aquatic systems are experiencing more severe and more frequent algal blooms (Moss 1996). As zooplankton communities constitute a crucial link for energy transfer through the foodweb, it is important to better understand how large cladocerans are more affected then other zooplankters by increased cyanobacterial biomass in lakes.

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Chapitre 6

Conclusion générale et perspectives futures

Une des questions fondamentales qui occupe les écologistes est la relation entre la structure de l'écosystème et son fonctionnement. Chaque écosystème est unique par la nature des interactions entre ses différentes composantes d'un côté, et par la nature des interactions entre ces mêmes composantes et leur milieu abiotique d'un autre côté (Levin 1999). Très tôt les écologistes ont adopté une approche holistique ou intégrative afin de pouvoir comprendre le fonctionnement des écosystèmes à travers l'étude des interactions trophiques (Hairston et al. 1960, Tilman, 1999). Ceci a conduit au développement de toute une sous discipline en écologie à savoir l'étude des réseaux trophiques et de leurs interactions (Schoener 1989). Cette sous-discipline a trouvé une application très large dans les systèmes aquatiques et a beaucoup contribué à notre compréhension du fonctionnement de ces systèmes.

Dans le cadre de cette thèse, je me suis attaqué à un de maillons les plus importants des réseaux trophiques lacustres à savoir l'interface zooplancton-phytoplancton dans les lacs eutrophes. L'approche expérimentale à plusieurs échelles d'observation m'a permis de comprendre certains aspects de ces interactions.

Les expériences en enclos limniques (Chapitre 2), ont révélé que les cladocères, et principalement les daphnies, répondaient d'une manière très rapide aux changements au niveau de leurs ressources. Ainsi, l'augmentation de la biomasses des algues non-ingérables principalement dominées par des espèces de cyanobactéries coloniales, a produit une réponse au niveau de la population qui s'est manifestée par une baisse de la biomasse des cladocères marquée par la réduction importante du nombre d'individus de grande taille. D'un autre côté, l'examen des appendices de filtration des daphnies a révélé l'existence d'une réponse au niveau phénotypique chez ces populations de daphnies (Chapitre 3). Suite à la diminution de la biomasse des algues ingérables, les daphnies ont augmenté la taille de leurs filtres afin de maximiser la collecte des particules algales. À une plus petite échelle spatio-temporelle, l'étude du comportement alimentaire nous a permis de constater que les daphnies réagissaient à la présence des cellules et colonies de cyanobactéries ou des cyanotoxines par une diminution drastique de l'assimilation des particules

alimentaires (Chapitre 4). Ces résultats nous ont permis de poser une hypothèse sur un mécanisme expliquant la diminution des cladocères de grande taille en présence de forte biomasse de cyanobactéries tel qu'observé en enclos limniques. Il apparaît que l'augmentation des particules algales non-ingérables, particulièrement des colonies et filaments de cyanobactéries produit une inhibition de l'alimentation des daphnies qui conduirait en premier lieu à la diminution ou la perte des gros individus. Les gros individus semblent en effet être plus affectés par la présence des cyanobactéries coloniales que les plus petits (Webster and Peters 1978).

La question sur la possibilité de l'extrapolation de ces résultats au milieu naturel a été évaluée dans le cadre d'une étude expérimentale de déforestation faisant partie du projet TROLS (Chapitre 5). Cette étude à grande échelle ayant résulté par un enrichissement trophique et une augmentation des cyanobactéries coloniales dans certains lacs dont le bassin versant avait été déboisé (Prepas et al. 2001) a constitué une opportunité unique pour tester notre hypothèse d'interaction négative entre les cladocères et les cyanobactéries dans le milieu naturel et se s'assurer de sa validité. Ainsi, nous avons pu observer la même tendance le long d'un gradient de cyanobactéries caractérisée par des corrélations négatives entre les cladocères et l'augmentation de la biomasse des algues, dominée par les cyanobactéries. Nous avons constaté que

l'augmentation expérimentale du niveau trophique dans certains lacs, caractérisée par une forte abondance de cyanobactéries et de cyanotoxines a causé une diminution de la structure en taille et la biomasse des cladocères (surtout les daphnies). Comme dans les expériences en enclos, nous avons assisté à une réduction de la dominance des cladocères en faveur des copépodes.

Ces résultats ont des implications aussi bien sur le plan théorique que fondamental, mais aussi sur le plan appliqué. Ainsi, les tendances observées dans le cadre des expériences *in situ* (enclos et lacs) seraient facilement applicables en modélisation des interactions trophiques. On pourrait ainsi considérer les relations phytoplancton-zooplancton comme étant des relations non-linéaires. Jusqu'à présent, la plupart des modèles se basent sur la prémisse que le facteur limitant de la production zooplanctonique est la quantité de ressources algales. Nos résultats démontrent clairement que la nature et la qualité des algues peuvent aussi limiter le développement du zooplancton de grande taille surtout dans les systèmes eutrophes. D'un autre côté, l'observation d'une réponse au niveau de la plasticité phénotypique des tailles des filtres chez les daphnies montre que ces organismes sont capables d'adopter des stratégies pour augmenter leur pouvoir de filtration en conditions défavorables. Il reste que nous ne pouvons pas connaître les répercussions de ce type de

stratégie à long terme, au niveau de la population ou de la communauté, sans la réalisation de nouvelles expériences.

Sur le plan appliqué, la connaissance des conditions favorables au développement des floraisons de cyanobactéries augmente notre capacité de prédire leurs avènements et les répercussions sur les autres composantes de l'écosystème. Cependant, ces phénomènes sont encore très peu connus ce qui constitue une perspective de recherche intéressante. Notre besoin de créer un gradient de cyanobactéries pour la réalisation des expériences en enclos, nous a poussé à explorer partiellement la question relative au développement des floraisons algales dans les systèmes aquatiques. En effet, les conditions de déclenchement des phénomènes spectaculaires de floraisons de cyanobactéries sont encore mal connues. Nos résultats nous suggèrent que la lumière peut être un facteur déterminant dans le développement de ces floraisons, car avec les mêmes conditions en éléments nutritifs nous n'avons obtenu des floraisons que dans les enclos peu profonds et pas dans les enclos profonds. L'étude plus en détail de ces phénomènes fait partie de mes perspectives de recherche future dans le cadre de mon stage post-doctoral.

Chapitre 7

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