RESPONSE OF PLANKTON COMMUNITY STRUCTURE TO TEMPORAL HETEROGENEITY AND PRODUCTIVITY

by

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ABSTRACT

This thesis investigates the effects of different frequencies of temporal heterogeneity in vertical mixing on diversity and composition of phytoplankton communities. I examine the issue theoretically and experimentally in lake mesocosms for systems of different average productivity and for communities embedded within food webs of increasing complexity.

The stochastic resource competition model shows that temporal heterogeneity in nutrient supply can be a feasible mechanism by which phytoplankton community diversity can be enhanced, mainly because demographic stochasticity can lead to a storage effect that precludes competitive exclusion. Responses by phytoplankton communities in oligotrophic Placid Lake to different experimental frequencies of vertical mixing depend on the nutrient status of the system and on the structure of higher trophic levels. Major effects of mixing on phytoplankton communities occur with enrichment, with shifts in community structure to larger, more filamentous types and to more diverse communities with increased water column stability (or decreased frequency of perturbations). Under low nutrient conditions, but when *Daphnia* was present, phytoplankton community structure also responded to different frequencies of mixing with lower community richness with more frequent mixing. This was attributed to an increase in predator-prey encounter rates with more frequent mixing. The inclusion of the entire natural plankton community led to a diminished response to frequency of mixing in phytoplankton, but to size-structure shifts in the top trophic level of invertebrate predators (i.e. *Chaoborus* sp.). The results of this study suggest that

temporal heterogeneity arising in lakes as a result of storm events may have little influence in oligotrophic systems, contrary to the general conclusions drawn mainly from eutrophic laboratory systems that predict large responses in phytoplankton community structure.

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TO MY PARENTS,

For always believing.

All things by immortal power
Near or far, hiddenly
To each other linked are
That thou canst not stir a flower
Without troubling of a star.

- Francis Thompson, 1859-1907
"The Mistress of Vision"

CHAPTER 1

General Introduction

Heterogeneity in Plankton Environments

It has long been recognized that environments fluctuate in time and space in ways that may be important to community structure and assembly (e.g. Hutchinson 1961, Wiens 1977, Connell 1978, Chesson and Case 1986, Menge and Sutherland 1987). Despite this recognition, much work remains to be done to understand the role of heterogeneous features and the interaction of different scales of fluctuation in abiotic and biotic components of ecosystems. In investigations of the effects of temporal variability on community or food web structure, the most commonly examined process is competition (e.g. Richerson *et al.* 1970, Connell 1978, Armstrong and McGehee 1980, Robinson and Sandgren 1983, Abrams 1984, Sommer 1985, Gaedecke and Sommer 1986, Ebenhöh 1988, Grover 1990, Chesson 1994, Chesson and Huntly 1997) because of an historical precedent in the "paradox of the plankton" proposed by Hutchinson (1961). Hutchinson questioned the lack of correspondence between empirical observations of high plankton diversity in spatially unstructured environments with scarce resources and theoretical predictions for low diversity based on the competitive exclusion principle (Hardin 1960). Alterations to standard competition theory, including the addition of temporal and spatial variability, can augment the predicted diversity levels to various degrees, depending on the relative abiotic and biotic scales (Richerson *et al.* 1970, Grenney *et al.* 1973, Connell 1978, Abrams 1984, Chesson 1994, Chesson and Huntly 1997, Durrett and Levin 1998).

Large temporal fluctuations in abiotic forces should alter vital rates of affected populations. Thus, they can involve changes to birth or death rates, or to the spatial distribution of organisms such that changes in population dynamics and interactions are possible. Community-wide consequences of abiotic variability are commonly observed, but the degree of response depends on the relative scales of fluctuation in populations and the environment (Connell 1978). Abiotic fluctuations are generally regarded as an interruption to the natural successional sequence including processes of competition and predation. Fluctuations that occur too frequently may reduce diversity by creating environments that are too harsh while those that are too infrequent resemble non-fluctuating conditions where succession goes to completion. At some intermediate frequency (based on generation time of organisms involved), maximal diversity is maintained (Connell 1978).

Again, largely because of the historical precedent set by examining plankton diversity (Hutchinson 1961), much community ecology has been done with these systems. Plankton communities, which occupy the pelagic zone of lakes and oceans, are exposed to various sources of temporal variability in their environment. At semi-annual time scales in temperate freshwater lakes, most undergo a large environmental shift with spring and fall lake turnover (Goldman and Horne 1983). But even within a growing season in such environments, fluctuations in the abiotic conditions can occur (Harris and Griffiths 1987, Reynolds 1993). Most of these are associated with weather events like storms or high winds that lead to deep water vertical mixing, even in highly stratified systems. Movements of water associated with these events leads to an influx of nutrient-rich, plankton-poor water from the deeper zones to the warm, well-lighted but nutrient-

depauperate euphotic zone (Klein and Coste 1984). Such abiotic events can occur at various temporal and spatial scales, with the predominant scale expected to change as weather patterns are altered with the large-scale climatic effects of global warming (Walker 1991, Carpenter *et al.* 1992, Lathrop *et al.* 1999).

Although the effect of mixing has most commonly been associated with fluxes of nutrients, several other associated effects are possible and relevant for plankton community structure. Vertical fluxes can change the light regime to which phytoplankton are exposed and can affect the composition of their communities (Brzezinski and Nelson 1988, Huisman *et al.* 1999). Changes in turbulence and spatial positions of organisms relative to each other are effects of incomplete mixing that may have consequences at the community level because of changes in population interaction rates (Luckinbill 1974, Murdoch and Oaten 1975, Yamazaki 1996). Vertical mixing also resuspends particulate matter, resulting in a change in recycling rates or allowing detritus to be directly used by heterotrophic phytoplankton or zooplankton.

In examining the role of such abiotic fluctuations for plankton community structure, most work has involved laboratory chemostat studies on the effect of nutrient pulses on competition between two species (Quarmby *et al.* 1982, Brzezinski and Nelson 1988, Grover 1988, 1991a,b, Spijkerman and Coesel 1997), among several species within an assembled community (Robinson and Sandgren 1983, Gaedecke and Sommer 1986), or in natural communities removed from the field to the laboratory (Turpin and Harrison 1979, 1980, Sommer 1984, 1985, Suttle *et al.* 1987, 1988, Sommer 1995). In these experiments, nutrient inputs are pulsed at various frequencies and the outcome for community diversity and composition have been examined. Generally, short time scales

are used with either physiological or population dynamic responses measured. Time scales that may affect the production dynamics and behaviour of higher trophic levels, like herbivorous zooplankton and invertebrate predators, have not been considered. From this body of work in chemostats, a general conclusion is that elevated diversity of phytoplankton communities is promoted under only certain frequencies of nutrient pulsing. However, the frequency at which this occurs varies; two studies from eutrophic systems (Gaedecke and Sommer 1986, Flöder and Sommer 1999) conclude that maximum diversity occurs with 7-day pulsing regimes, while Robinson and Sandgren (1983) find maximum diversity at 28 days. It has been concluded from laboratory studies that phytoplankton diversity maxima occur for intervals that are on the time scale of 2-4 generations (Sommer 1989). Chemostat experiments show very strong effects of intermittent nutrient pulsing on the structure (composition, size, life history strategy) of phytoplankton communities.

Because of chemostat design constraints, it has been difficult to incorporate higher trophic levels. Phytoplankton in natural systems are always exposed to predation by zooplankton and whether the strong effects observed continue to hold when herbivores are present is unknown. Fluctuations in deep water mixing and effects on plankton communities must be examined in a more natural field context to determine whether such forces are relevant for plankton and for higher trophic levels. If natural scales of temporal heterogeneity are likely to change through anthropogenic effects, an understanding of food web effects is necessary. However, a trade-off in realism versus mechanistic understanding arises in a move to field studies. As discussed, vertical mixing events incorporate several important features for plankton that must be examined

phenomenologically in a field context rather than mechanistically through experimental separation into nutrient, light and turbulence effects. By studying natural communities in a lake context, the problems associated with absence of higher trophic levels, spatial homogeneity and assembled (i.e. non-evolved) groupings can be reduced.

The Role of Predation

The influence of predation on prey competition and diversity will depend on the food preferences of predators and their functional responses (Begon *et al.* 1986). Predators can alter competitive outcomes by applying differential mortality, thereby affecting prey composition and diversity (Paine 1966, Cramer and May 1972, Caswell 1978, Lubchenco 1978, Armstrong 1979, Abrams 1987). Predation differs from abiotic fluctuations because there is feedback between predator effects and their prey populations. However, because the density of predators fluctuates in time and space, their effect on plant communities may also vary (Huntly 1991) and this variability in mortality can increase prey diversity (Caswell 1978). This occurs because fluctuating predator populations open up regions in space and time that allow inferior competitors to persist. Because the intensity of predation is temporary at any one location or time, the system does not reach a new steady state equilibrium with possibly fewer species than in the absence of predation (Caswell 1978). When interaction rates between predators and prey are very high, as they are in fully mixed systems, neutral stability and a lowering of prey diversity is expected and observed (Caswell 1978, Hixon and Menge 1991, Hixon and Beets 1993, Caley and St. John 1996).

When considering the role of predation in temporally varying environments, it may be important to understand the effect not just of the type of predator but also the

spatial context within which dynamics are played out. Incomplete mixing, through its effect on spatial structure of plankton communities, may alter the relative importance of predators in determining the diversity levels of phytoplankton.

The Role of Productivity

A large effort in community ecology has gone towards understanding the relationship between diversity and environmental productivity (e.g. Ricklefs and Schluter 1993, Schulze and Mooney 1993, Huston 1994). Simultaneous examination of productivity-diversity relationships has been noticeably absent from empirical studies that consider temporal heterogeneity. Is temporal heterogeneity in nutrient supply (among other effects) equally important in nutrient-poor and nutrient-rich environments? The limited theory that exists suggests that succession (or competitive exclusion) should occur more quickly in enriched environments, and as a result environmental fluctuations must be more frequent under these conditions if competitive exclusion and low diversity are to be avoided (Huston 1979). Many ecosystems are becoming increasingly enriched, and it is important to understand how such systems are affected by temporal variability.

Approach

In this thesis, I examine the consequences for natural plankton communities of the scale at which temporal variability in mixing occurs. The main focus is on the response of phytoplankton, although some higher trophic level (zooplankton and invertebrate predator) responses are also considered. The work consists of theory and experimental treatment and observation of plankton communities in lake mesocosms exposed to various temporal regimes of deep water mixing.

The theoretical component discussed in Chapter 2 is an attempt to extend existing heuristic models of community structure in fluctuating environments to incorporate realistic aspects of the variability to which plankton are exposed. The outcome of resource competition in a stochastic two-species model is considered under different conditions of variability and productivity. The model differs from previous work mainly by the incorporation of both environmental and demographic stochasticity and examines the relative effects of inclusion of either or both of these types of stochasticity for competitive coexistence.

The next three chapters present the results of three factorial experiments on natural communities of plankton in mesocosms. Chapter 3 is closest to the model and to previous chemostat experiments in that it involves a single trophic level of phytoplankton and their responses to vertical mixing events applied at different frequencies in environments that are naturally oligotrophic or artificially enriched. Many empirical studies on phytoplankton community structure in the laboratory and a few field studies have examined systems close to the eutrophic end of the spectrum (Robinson and Sandgren 1983, Sommer 1985, Grover 1991a,b, Flöder and Sommer 1999). Whether oligotrophic systems (without any nutrient addition) respond as strongly to different scales of vertical mixing remains to be determined. The experiment in Chapter 3 examines this question and compares the responses with enriched systems.

Adding another level of complexity to the system in Chapter 4, I examine the role of predation (grazing) and how it might interact with various scales of temporal heterogeneity in oligotrophic environments. Populations of important herbivores like *Daphnia* sp. also fluctuate on time scales that may influence phytoplankton succession.

In addition, the *Daphnia* populations themselves may be affected by the scale of temporal variability, and this could feed back to the phytoplankton community structure and biomass. On the other hand, generalist herbivores may overwhelm any community-wide bottom-up effect of intermittent mixing through strong predation. In this study, I examine the interaction of intermittent vertical mixing at three different frequencies and herbivory by *Daphnia rosea* to determine whether the pattern of responses observed in the absence of the predator continue to hold in its presence.

Finally in Chapter 5, I examine the response of phytoplankton to various temporal scales of mixing in the presence of a full zooplankton community. The full zooplankton community in this system entails two additional trophic levels – macrozooplankton herbivores like cladocerans and copepods, and invertebrate carnivores (*Chaoborus flavicans* Diptera, Chaoboridae). Additionally, in this chapter I examine the responses of higher trophic levels to intermittent mixing to see whether they are also affected by different temporal scales.

CHAPTER 2

Fluctuating Environments and Phytoplankton Community Structure: A Stochastic Model

This chapter is based on a paper to be published in The American Naturalist in April, 2000. It is co-authored by J.M. Anderies who performed the computer simulations. We worked together on development of the model, interpretation of results, and in the writing of the paper. By his signature on this page, Dr. Anderies approves of the inclusion of the work here:

The full reference for the paper is:

Anderies, J.M., and B.E. Beisner. 2000. Fluctuating environments and phytoplankton community structure: a stochastic model. 155:556-569.

Introduction

There has been a tradition of questioning why ecological communities are diverse (e.g. Hutchinson 1961, Richerson *et al.* 1970, Sale 1977, Connell, 1978, Birch 1979) in contrast to the simple communities predicted by the competitive exclusion principle (Hardin 1960). Hypotheses proposed fall into three major categories: predator-mediated coexistence (Paine 1966, Lubchenco 1978, Armstrong 1979), spatial interactions (Richerson *et al.* 1970, Grenney *et al.* 1973, Malchow 1994, Durrett and Levin 1998, Pacala and Levin 1997), and temporal variability (Connell 1978, Levins 1979, Armstrong and McGehee 1980, Abrams 1984, Chesson and Huntly 1988, Ebenhöh 1987, Grover 1990, Chesson and Huntly 1997). Temporal variability in the environment can provide temporal niche opportunities for some types of organisms. The aim of this chapter is to extend work on temporal variability as a possible diversity-promoting mechanism by examining a stochastic version of resource competition models.

In lake environments, nutrients and planktonic organisms are subject to various episodic sources of forcing. Such events operate at several characteristic spatial and temporal scales. Large-scale events include climatic forcing such as large storm events within seasons on time scales of 1 to 2 weeks (Harris and Griffiths 1987). In the north temperate zone, these events act to stir the pelagic zones of lakes, resulting in mixing and a vertical redistribution of nutrients and organisms. On daily to weekly time scales, the movement of fish on and offshore can act as a source of nutrient redistribution (i.e. from littoral to pelagic zones) (Schindler *et al.* 1993) and as a temporally variable source of predation pressure for zooplankton. At smaller scales, heterogeneity in distribution of plankton results from the limited mobility of organisms. Variation in excretion of

nutrients by zooplankton is a small-scale source of variability in resources for phytoplankton (Lehman 1984, Shapiro and Wright 1984). Forcing processes such as these, especially at larger scales, lead to a redistribution of organisms and resources, such that competing phytoplankton observe a temporally fluctuating resource base, not a constant one.

Organisms have a variety of physiological strategies to deal with variability in resource availability. In response to temporal variability in resources, there are three major life history strategies. The first is "storage", the ability of an organism to store resources as a buffer against resource variability. Resource storage plays a very important role in phytoplankton population dynamics in variable environments as several studies have shown (Grover 1991a,c, Sommer 1991, Docubo *et al.* 1998). However, including resource storage in a competition model adds a level of complexity that we would rather avoid at this stage. Because our focus is stochasticity which is challenging in itself, we have chosen to work with the two other simpler life history strategies that represent the ends of a spectrum of responses resulting from a trade-off between acquiring resources and reproduction. At one end of this spectrum are "gleaners"; organisms which specialize in resource acquisition at the expense of a high birth rate. Gleaners always win competition experiments (Tilman 1977, Hansen and Hubbel 1980) under constant resource supply because they can survive at low resource levels (Tilman 1982). In a fluctuating environment, "opportunism" becomes a feasible strategy. Opportunists have lower resource acquisition abilities when resources are scarce but have high birth rates when resources are plentiful. Opportunists are always competitively excluded from constant environments (Tilman 1982), but can survive and coexist with

gleaners under a limited set of fluctuating conditions (Robinson and Sandgren 1983, Sommer 1985, Grover 1988, 1991c, MacIsaac and Gilbert 1991). Considering just these two simple life history types (gleaners and opportunists), it becomes evident that community diversity, both in terms of numbers and types of species, is potentially greater in fluctuating than in constant environments. The question that arises, however, is what types of conditions favour increased diversity and which life history strategies are optimal under various scenarios of resource fluctuation?

The role of temporal fluctuations in resource supply as a diversity-promoting mechanism has been studied in considerable detail via mathematical models, (e.g. Levins 1979, Smith 1980, Grover 1990, Chesson and Huntly 1997). Smith (1980) has shown that for a deterministic model in which two species compete for a sinusoidally fluctuating resource, coexistence is possible. Thus fluctuations alone can facilitate coexistence, but the mathematical proofs give little insight as to what are the mechanisms. Chesson (1994) has provided a framework (Variable Environment Theory) to understand coexistence-promoting mechanisms at work in variable environments for a particular class of models. In this framework, two general mechanisms of species coexistence are identified: relative nonlinearity and the storage effect (not to be confused with the physical ability to store resources). Several authors (Levins 1979, Armstrong and McGehee 1980, Grover 1990) had suggested that it is the former mechanism, in which species competing for a common resource have different shapes of curves describing the dependence of their growth rates on the resource, that allows coexistence in the type of model studied by Smith (1980). However, Chesson (1994) showed that relative nonlinearity was the less important of the two mechanisms for coexistence because, at

most, two species could coexist via this mechanism and only over a very narrow region of parameter space. On the other hand, Hale and Somolinos (1983) have shown that arbitrarily many species can coexist in the model studied by Smith (1980). The discrepancy arises because Hale and Somolinos (1983) and Chesson (1994) studied slightly different models and because of several assumptions about the way competition occurs that underlie Chesson's (1994) results. The differences provide a clue as to where we might look for mechanisms other than relative nonlinearity at work.

The discussion above and the extremely small regions in parameter space that allow for coexistence in the model studied by Smith (1980) might lead one to discount the role of temporal fluctuations alone as a realistic diversifying mechanism. This conclusion comes, however, with many simplifying assumptions that, when relaxed, may result in improved possibilities for coexistence. For example, the coexistence-promoting mechanism in these models is the creation of temporal niche opportunities that allow competitors to utilize the limiting resource at different times. For the types of models studied by Chesson (1994) under the assumptions he makes, relative nonlinearity may be the only mechanism that accomplishes this. Under more general or realistic conditions, other mechanisms may play important roles.

With this in mind, we extend the family of deterministic models proposed by Smith (1980) and Grover (1990) and study their stochastic analogue. First, Chesson's (1994) results depend on the fact that his model is in discrete time, that the "competitive effect" experienced by each population can be written in terms of the competitive effects felt by other populations, and a quadratic approximation of the actual nonlinear dynamics in the system. Discrete time models may not capture the time structure of birth-death

processes that may be important because these processes do not occur simultaneously for the entire population at any point in time. Also, as Chesson (1994) notes, the truncation of higher order terms in the equations of motion of the system necessarily neglects more subtle interactions that may facilitate coexistence. We can explore these points by building a stochastic model and comparing it to the deterministic analogue. We can tease out the effects of random versus deterministic fluctuations, of demographic stochasticity, and of the time structure of the species-resource interaction.

In addition to these interesting issues, we seek to show how changing time scales of nutrient input regimes and changing overall nutrient levels influence relative abundance of the two competitors in the hope that these results can be compared to field experiments with lake plankton communities. Thus, community structure could be related to environmental fluctuation regimes, and more importantly, the sensitivity of community structure to changes in these regimes could be assessed.

The Model and Background

The Deterministic Model

To begin, we briefly review the classic model of phytoplankton competition in chemostats which we then adapt to represent a lake system. This adapted model is used as a baseline, which, when compared to the stochastic lake model developed later, will enable us isolate the role environmental and demographic stochasticity play in the model.

The model for 2-species competition in a chemostat with constant resource delivery rate and Monod phytoplankton growth is (Tilman 1977, Hsu 1980):

$$\frac{dS_t}{dt} = D(S_o - S_t) - \frac{m_1 S_t x_1}{y_1(a_1 + S)} - \frac{m_2 S_t x_2}{y_2(a_2 + S)} \tag{1}$$

$$\frac{dx_1}{dt} = \left(\frac{m_1 S}{a_1 + S} - d_1 \right) x_1 \tag{2}$$

$$\frac{dx_2}{dt} = \left(\frac{m_2 S}{a_2 + S} - d_2 \right) x_2 \tag{3}$$

where x_i is the population density of each competing species, S_o is the (constant) nutrient concentration in the fluid stream entering the chemostat, S_t is the nutrient concentration at time t within the chemostat, and D is the dilution rate in the chemostat. Population i grows at the per capita rate m_i, with a half-saturation constant for growth a_i, and dies or is removed from the chemostat at the rate d_i. Finally, y_i is a conversion factor of resources into organisms (with units of organisms/resource). Solving for the equilibrium population densities (i.e. setting $\frac{dx_i}{dt} = 0$) we get

$$\frac{dx_i}{dt} = \left(\frac{m_i S_t}{a_i + S} - d_i \right) x_i = 0 \tag{4}$$

which implies that at a non-trivial equilibrium

$$S = \frac{a_i d_i}{m_i - d_i}. \tag{5}$$

This equilibrium resource level is often referred to as R^* (Armstrong and McGehee 1980, Tilman 1982). To be consistent with other periodically forced models, let us define this resource level as λ_i after Hsu (1980). λ_i is the resource concentration at which the net growth rate of the i^{th} phytoplankton species is zero. The deterministic model with

constant resource supply predicts that at equilibrium, the population with the lowest λ_i (i.e. the gleaner) will always exclude all others.

The above model has been modified to allow for temporal fluctuations in resource supply (e.g. Smith 1980, Grover 1990). For the gleaner to exclude the opportunist, the system must tend towards the equilibrium resource level of the gleaner λ_i. Fluctuations in resource levels can prevent this. A species with a high growth rate (higher $\frac{m_i}{d_i}$), which can quickly take advantage of high resource levels (opportunists), might coexist with a slower-growing but more efficient (lower λ_i) population of gleaners.

From now on, gleaners and opportunists will be labeled as species one and two, respectively. For species two to be more opportunistic yet less efficient at acquiring resources, we must have:

$$\lambda_1 < \lambda_2 \text{ and } \frac{m_1}{d_1} < \frac{m_2}{d_2}. \tag{6}$$

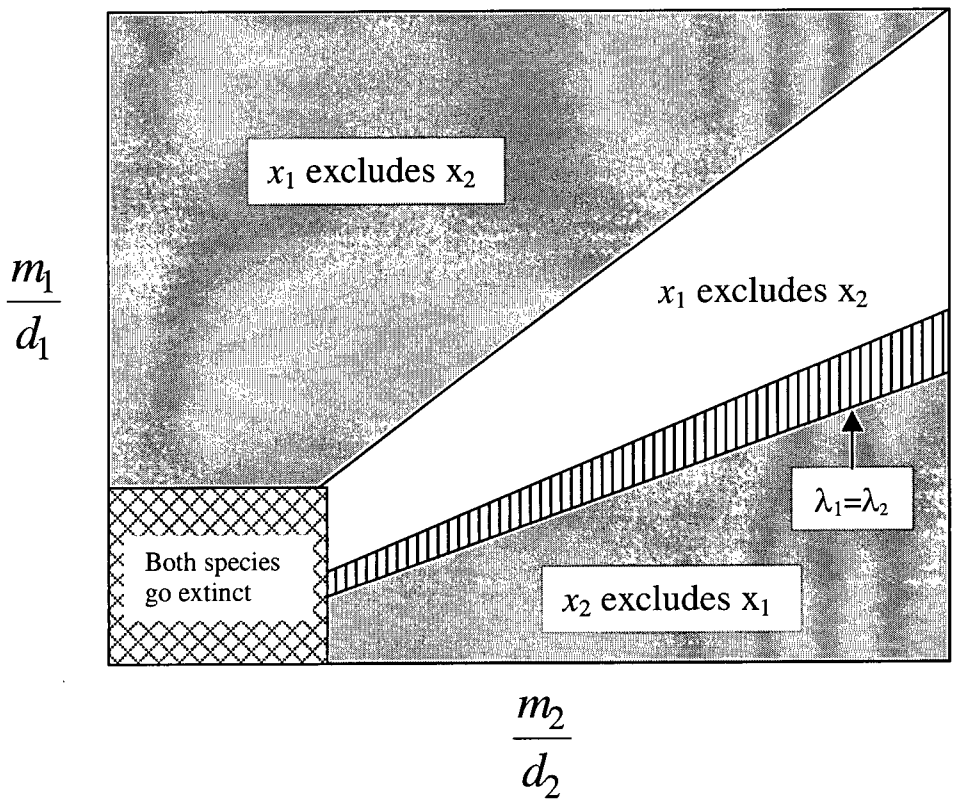
Studying the behavior of the different models in the region of the parameter space defined by these inequalities is the focus of the rest of this chapter.

The model studied by Smith (1980) is generated by replacing equation 1 with

$$\frac{dS}{dt} = D(S_o + be(t) - S) - \frac{m_1 S x_1}{y_1(a_1 + S)} - \frac{m_2 S x_2}{y_2(a_2 + S)}, \tag{7}$$

which includes the periodic environmental forcing term *e(t)* with amplitude *b*. The forcing function is often taken as sinusoidal for mathematical convenience (Hsu 1980, Smith 1980, Hale and Somolinos 1983, Grover 1990). Characteristics of such systems can be summarized by regions of coexistence and exclusion, as shown in Figure 2.1. With constant flow through the chemostat, the gleaner (x_1) will always exclude the

Figure 2.1: Generalized competition results for the original deterministic model with sinusoidal fluctuations in resources. The region between the two gray areas (including the solid white and vertical bar regions) represents the opportunist-gleaner trade-off. For parameter combinations in the white region, although the conditions for the trade-off are satisfied, for a given $\frac{m_1}{D_1}, \frac{m_2}{D_2}$ is not large enough for the opportunist to invade. Only those parameter combinations that fall in the region with the vertical bars within the trade-off region allow for coexistence. Adapted from Smith (1980).



opportunist (x_2) (Hsu *et al.* 1977). With oscillatory forcing, Hsu (1980) showed that, if m_i/d_i is large enough to prevent the organisms from being washed out of the chemostat faster than they reproduce and if $\lambda_i < \lambda_j$ and $m_i/d_i > m_j/d_j$, then species i would exclude species j. Biologically, this means that organisms that are both better competitors and faster growers will exclude all others. These results completely characterize the outcomes of competition outside of the gleaner-opportunist trade-off region defined by equation 6 (the region between the solid gray regions and outside the cross-hatched box in Figure 2.1). Smith (1980) then established the existence of a sub-region of the trade-off region close to the line $\lambda_1 < \lambda_2$ where, depending on b, two species could coexist (the region with the vertical bars in Figure 2.1). Over most of the parameter space, competitive exclusion is observed (Hsu *et al.* 1981). It is also possible to have more realistic pulsed oscillations in resource availability rather than sinusoidal forcing (e.g. Grover 1990). These types of fluctuations increase the likelihood of coexistence but only to a small degree, and competitive exclusion is still the rule over most of the parameter space (Grover 1990).

We have modified the pulsed chemostat model to be more representative of phytoplankton competition in a lake or ocean subject to episodic mixing events. There is no direct outflow of nutrients from the system as in a chemostat. Nutrients are consumed by phytoplankton, which are in turn consumed in a density-independent manner, in this case by herbivores at a constant, species-independent rate d (a simplifying assumption). Thus d_1 and d_2 in equations 2 and 3, respectively, are replaced with d, and the term $D(S_o + be(t) - S)$ for nutrient inflow in equation 7 is replaced by $e(t)$ where

$$e(t) = A\exp(\beta(\cos\omega t - 1)). \tag{8}$$

Equation 8 produces pulses of nutrients of amplitude A with frequency $\frac{\omega}{2\pi}$, where β is a parameter that controls the duration of the pulse. The amplitude and duration of the pulse are chosen so that the average nutrient input is one unit per unit time.

The choice of this function is motivated by two factors. First it generates a more realistic representation of the short-duration, sharp pulses one would expect with nutrient pulses due to large storm events. Second, this function is the "regular" analogue of a Poisson process where discrete packets of nutrients arrive at specific intervals. In the deterministic case, the time between the arrivals of nutrient packets is always $\frac{2\pi}{\omega}$. In the stochastic case, the inter-arrival times are exponentially distributed with a mean of $\frac{2\pi}{\omega}$. The relationship between the deterministic and stochastic models will be discussed in more detail below.

The stochastic model

There are two classes of stochastic effects in population models: environmental and demographic. The stochastic model developed in this section will enable us to study each of these effects separately, as well as their combined effect on competition. Thus, we introduce and study the stochastic analogue of the deterministic model in which births and deaths of competitors along with resource pulses follow jump processes. In the deterministic system, birth, death, and resource pulse rates represent averages of those occurring in nature. A stochastic analogue of this deterministic model is achieved by assuming that discrete births occur at random times that, on average, produce the same

rate as in the deterministic model. A common way to do this is to assume that times between events are exponentially distributed with an average given by the rates in the deterministic case. In our case, this leads to

$$x_i(t) \to x_i(t)+1 \text{ at rate } \frac{m_i S x_i}{a_i + S} \tag{9}$$

$$x_i(t) \to x_i(t)-1 \text{ at rate } Dx_i \tag{10}$$

for $i = 1, 2$. The resource level at time t is given by the expression

$$S(t) = p(t) - E(t) \tag{11}$$

where $p(t)$ is a jump process describing the pulsing of resources into the system. $p(t)$ is given by

$$p(t) \to p(t)+s \text{ at rate } f \tag{12}$$

where s is the size of packets of nutrients, and f is the pulse frequency. In order to maintain an average inflow of nutrients of 1 unit per unit time, s must be taken as $\frac{1}{f}$.

The term $E(t)$ is the total amount of resources utilized by the phytoplankton up to time t. Phytoplankton utilize resources continuously when in contact with them, which makes the stochastic model more difficult to analyze. We thus make the simplifying assumption that phytoplankton utilize resources in discrete packets. Between birth events, $\frac{1}{y_i}$ resources must have been consumed. We assume that this consumption occurs at the

instant the birth occurs so that

$$E(t) = \frac{b_1(t)}{y_1} + \frac{b_2(t)}{y_2} \tag{13}$$

where $b_i(t)$ represents the total number of birth events for species i up to time t. This assumption is reasonable if inter-birth intervals are short and the resource level in the system, $S(t)$, is large compared to the resources consumed per birth, $\frac{1}{y_i}$. The randomness of phytoplankton-resource contact rate is contained in the demographic stochasticity inherent in jump process models of populations. Before analyzing the model, some subtle points must be addressed about what we mean when we say that the stochastic and deterministic models are analogous.

The assumption underlying a differential equation model for a population is that the number of individuals present is changing continuously, i.e. in infinitesimally small jumps. In the stochastic model (as in real life) populations change in discrete units. By replacing the jump size in equations 9 and 10 by $\frac{1}{\theta}$ and the rates by $\frac{\theta m_i S x_i}{a_i + S}$ and $\theta D x_i$ respectively, the scale by which time and births are measured can be controlled. Increasing θ increases the rate at which jumps occur and decreases the size of jumps inversely proportionately. Taking the limit as $\theta \to \infty$ removes the discreteness and exponentially distributed time structure in the population dynamics. Similarly, if we take the limit as $s \to 0$ in the resource dynamics given by equation 12, the behavior of the stochastic model will approach that of the deterministic model with a constant resource influx of one unit per unit time.

By modifying these scale parameters, the effects of each type of stochasticity (environmental and demographic) can be studied. For example, if we take the pulse frequency to be once every 6 days, with a pulse size of 6 units and with θ very large (e.g. θ =100), the model would represent a system with only environmental stochasticity and almost no demographic stochasticity. The only difference between this model and the deterministic pulsed model is that the times between pulses are exponentially distributed with mean of 6 days rather than constant with period of 6 days. By comparing the behavior of these two models, the effect of environmental stochasticity alone on competition can be explored. By another choice of scaling, the effects of demographic stochasticity alone can be assessed. Finally, the combined effects of both sources of randomness on competition can be explored by yet another choice of scaling.

Model Analysis

Several cases were studied with the objective of assessing the effect of stochasticity, relative harshness (defined as potential environmental productivity), and relative time scales of environmental fluctuations on competitive outcomes. The results of the comparisons are presented in terms of the relative sizes of the coexistence region for the deterministic and stochastic models. The results are then related to the general mechanisms of coexistence in variable environments proposed by Chesson (1994) in an effort to present a systematic way of understanding the problem.

Table 2.1 summarizes the parameter values that characterize the different cases. The parameters are held constant for population 1 which is defined as the gleaner population with a lower half-saturation constant for growth (a_i). Parameter values were

Table 2.1: Parameter values used in the deterministic versus stochastic model comparisons. The last two columns represent simulations done to estimate the effects of different frequencies of nutrient pulsing on the composition

Parameter (units)	**Definition**	**Nutrient-Poor**	**Nutrient-Rich**	**Case 4 – Frequent pulsing**	**Case 4 – Frequency on coexistence**
D (day^{-1})	Death rate	0.05	0.05	0.05	0.05
s (mass)	Resource pulse size	2π	2π	$\frac{1}{2}$	$\frac{1}{f}$
m_i (day^{-1})	Max. per capita growth rate	$m_1 = 0.5$ $0 < m_2 < 1$	$m_1 = 0.5$ $0 < m_2 < 1$	$m_1 = 0.5$ $0 < m_2 < 1$	$m_1 = 0.5$ $m_2 = 0.9$
a_i (mass)	Half-saturation constant	$a_1 = 9$ $a_2 = 19$	$a_1 = 0.9$ $a_2 = 1.9$	$a_1 = 9$ $a_2 = 19$	$a_1 = 9$ $a_2 = 19$
$\left(\frac{y_i}{\frac{\#}{\text{resource mass}}}\right)$	Conversion factor for individuals per unit of resource	$y_1 = 0.1$ $y_2 = 0.1$	$y_1 = 0.1$ $y_2 = 0.1$	$y_1 = 0.1$ $y_2 = 0.1$	$y_1 = 0.1$ $y_2 = 0.1$
f (day^{-1})	Pulse frequency	$\frac{1}{2\pi}$	$\frac{1}{2\pi}$	2	$\frac{1}{30}$ to 2
θ	Scale parameter	1 or 10	1	1	1

chosen to generate a scenario where a trade-off in life history strategy occurs and to represent a realistic time scale of reproductive rates for phytoplankton. It is reasonable to assume that phytoplankton reproduce every one to two days when sufficient resources are present suggesting that m_i should be order one. In order to generate a trade-off, m_2 must be larger than m_1 (i.e. the opportunist must be a faster grower), and a_2 must be larger than a_1 (i.e., the gleaner may have a positive growth rate at lower resource levels). Choosing $m_1 = 0.5$, $a_1 = 9$, and $a_2 = 19$ will produce a trade-off region when $0.5 < m_2 < 1$. Choosing the a_i to be order 10 corresponds to nutrient-poor conditions when average resource input is 1 unit per day because nutrient levels are below the half-saturation levels most of the time. For the nutrient-rich case, the a_i are scaled down by a factor of 10. This maintains the same trade-off region, but now, the organisms will experience nutrient pulses well above their half-saturation constants much more frequently. Although changing parameters that measure the characteristics of organisms in order to compare different environments may seem strange, it is most natural in our case. This is discussed in more detail in a later section.

Examination of the model behavior is performed using an invasion analysis. If a small population of one competitor can increase in density while the other is near its equilibrium value (i.e. initially dominates the system), then invasion is said to have occurred. If both species can invade the system under these conditions, coexistence is possible. Applying the invasion criterion was carried out as follows. In the deterministic case, the coexistence region can be determined very accurately using bifurcation techniques. With no opportunists present, the gleaner population will follow a periodic orbit driven by the environment. If stable, when perturbed by the introduction of a few

opportunists, the gleaner population will return to this periodic orbit. The opportunist cannot invade in this case. If, on the other hand, the periodic orbit is unstable, perturbation by the introduction of a few opportunists will cause the system to move away from this orbit, i.e. the opportunists can invade. Thus, by detecting a change in stability of this orbit as m_2 is increased, the minimum value of m_2 that will allow the opportunists to invade a system dominated by gleaners can be located. In the same way, monitoring the stability of the orbit of the opportunist population with no gleaners present as m_2 is reduced allows us to determine the maximum value of m_2 for which the gleaners can invade a system dominated by opportunists. By using the numerical technique of pseudo-arclength continuation implemented in the computer package Auto (Doedel 1982), these points of transition from stability to instability can be accurately determined.

For the deterministic model, the invasion coexistence criterion is equivalent to requiring that the two populations be present in the system for arbitrarily long times. For the stochastic model, these criteria are not equivalent, because both species will go extinct eventually. Chesson and Ellner (1989) developed a definition for coexistence in such models which they call "stochastically bounded persistence". With this definition, they show that if both species can invade (have positive growth rates when the other species is resident in a stationary state), then both species will be present in the sense that they are each bounded below by a positive random value. Although Chesson and Ellner (1989) give a proof for a discrete time model, we assume that the result can be carried over to the continuous time case. To test for invasion, we computed the mean of the invading population after 100 days for 200 to 500 realizations. Invasion was said to have occurred if the mean increased. This criterion would give the same results as the

bifurcation technique if applied to the deterministic model (but is much less efficient), and thus we believe a fair comparison of the models can be made using this criterion.

Results and Discussion

The results of the model comparison are presented for four different cases: (1) Nutrient-poor conditions with only one type of stochasticity (either environmental or demographic), (2) Both environmental and demographic stochasticity in nutrient-poor conditions, (3) Both environmental and demographic stochasticity in nutrient-rich conditions, and (4) Varying resource pulse frequency (both environmental and demographic stochasticity in nutrient-poor conditions). All models are compared to the baseline deterministically pulsed model to assess the role of stochasticity. By comparing cases (1) and (2), the importance of the interaction between environmental and demographic stochasticity is highlighted. By comparing (2) and (3), the influence of harshness on the relative importance of fluctuations on competition can be assessed. Cases (1) to (3) will be presented first, along with a discussion of the mechanisms by which stochasticity promotes coexistence. Table 2.1 lists the parameter values used for all simulations and Table 2.2 summarizes the results in terms of the parameter ranges over which coexistence occurs. Finally, case (4) will be presented, which allows for an exploration of the importance of the relative time scales of environmental and demographic fluctuations on stabilizing competition.

Table 2.2: Summary of results of competition for cases 1-3. Parameter values for each case studied are given in Table 2.1.

Case	Description	Coexistence Region	% of Trade-off Region
Baseline	Nutrient-poor, deterministic	$0.9296 < m_2 < 0.9325$	0.58
	Nutrient-rich, deterministic	$0.7161 < m_2 < 0.7242$	1.58
1	Nutrient-poor, environmental stochasticity only	$0.868 < m_2 < 0.872$	0.8
	Nutrient-poor, demographic stochasticity only	$0.94 < m_2 < 1$	12
2	Nutrient-poor, environmental and demographic stochasticity	$0.82 < m_2 < 0.91$	18
3	Nutrient-rich, environmental and demographic stochasticity	$0.6 < m_2 < 0.67$	14

The Role of Stochasticity

The role that both environmental and demographic stochasticity play in promoting coexistence was studied under oligotrophic conditions, which are modelled by setting the half-saturation constants well above the mean resource inflow rate (Table 2.1). We specify the coexistence regions for each case, adding complexity one layer at a time. In the deterministic version of the lake model (given by equations 2, 3, 7 and 8 with $\omega = 1$, pulse frequency $=1/2\pi$, $A = 13.7$, and $\beta = 30$ to give a mean resource inflow of one unit per unit time) where there is neither environmental nor demographic stochasticity, coexistence occurs if $0.9296 < m_2 < 0.9325$, which is equivalent to 0.58% of the trade-off region of the parameter space.

Next we add environmental stochasticity alone by setting $\theta = 10$ and simulating the stochastic model as described above. The region of coexistence was determined to be $0.868 < m_2 < 0.872$ or 0.8% of the trade-off region of the parameter space. Based on this, irregularity in inter-pulse times alone does not significantly improve the possibility of coexistence, but it does shift the region of coexistence downward and makes it easier for the opportunist to invade the system. We hypothesize that this slight improvement and shift is the result of the clustering of pulse arrivals which generates more variance in the equilibrium resource distribution faced by the invader. This is consistent with Chesson's (1994) conclusion that for relative nonlinearity to promote coexistence there must be differences in the variance of the limiting resource generated by the resident species. If this is the mechanism, larger coexistence regions should be associated with larger differences in the variances in the limiting resource distribution produced by each competitor when present in monoculture (Chesson 1994).

To relate our results to Chesson's (1994) framework, we have to assess the amount of variation in resources faced by a potential invader. To do this for the deterministic model, imagine sampling the resources at random times and then computing the variance of this sampling distribution. Table 2.3 displays the mean and variance of the resource distributions generated by each competitor when present in monoculture. The first two lines of Table 2.3 compare the deterministic and environmentally stochastic cases. First note that fluctuations, in general, weaken the ability of the gleaner to drive the resource level down. In a constant environment, the resource level with a gleaner present would be 1, whereas with pulsed resources the resource level is 1.125 (deterministic case) and 1.281 (environmental stochastic case). Deterministic fluctuations produce a small difference in the variance of the resource supplies when each competitor is present alone, which, according to Chesson's (1994) framework, will generate modest possibilities for coexistence (i.e. the small coexistence region predicted by our model) via relative nonlinearity. Adding stochasticity to the resource pulses significantly increases the overall variation in resource supplies. Because relative nonlinearity is a weak mechanism for promoting coexistence (Chesson 1994), one would expect this increase to have a small effect on coexistence possibilities. Again our model results are consistent: increased resource variance generated by environmental stochasticity increases the size of the coexistence region but only very slightly (Table 2.2). Finally, we hypothesize that the downward shift of the coexistence region is due to the higher mean resource level when the gleaner is present alone under stochastic conditions (Table 2.3). This enhances the conditions for the opportunist, allowing it to survive with lower maximum population growth rates.

Table 2.3: Statistics for resource distribution when the resident species is present in monoculture for the nutrient-poor conditions.

	Gleaner Resident		*Opportunist Resident*	
Case	**Mean**	**Variance**	**Mean**	**Variance**
Deterministic, nonfluctuating	1	1	0	0
Deterministic, fluctuating	1.125	1.402	1.170	1.369
Environmental Stochasticity	1.281	3.956	1.378	4.683
Demographic Stochasticity	1.044	0.624	1.086	0.674
Environmental + Demographic Stochasticity	1.220	3.956	1.179	3.445

In the case where the system is dominated by demographic stochasticity ($\theta \leq 1$) and the environment approaches a more constant resource influx (pulse period =1 day or less), the trade-off region for coexistence is significantly larger. Now the coexistence region is $0.94 < m_2 < 1$ which corresponds to 12% of the trade-off region (Table 2.2). This expanded region suggests that demographic stochasticity plays a much more important role than environmental stochasticity. If the mechanism were relative nonlinearity, the same argument as above would apply. Compare row three in Table 2.3 to the previous two cases. First note that since the environment is more constant than in the case with environmental stochasticity (one versus five day pulse intervals), the gleaner can drive the mean resource supply lower. This may account for the upward shift of the coexistence region (Table 2.2), i.e. the opportunist must be able to tolerate harsher conditions. The increased size of the coexistence region, however, does not seem to be consistent with relative nonlinearity as a mechanism. The addition of environmental stochasticity alone produces far more overall variance and a greater difference between variances in resource supply than does the addition of demographic stochasticity alone (Table 2.3), yet has a far less significant effect on the size of the coexistence region (Table 2.2). It appears therefore that either demographic stochasticity alone promotes coexistence via another mechanism, or the limitations of Chesson's (1994) analysis by considering only small fluctuations make it difficult to see the role that demographic stochasticity plays in promoting coexistence via relative nonlinearity. We believe it is probably the former for the following reason. Recall that Chesson (1994) assumes that the competitive factor of an invader can be written in terms of the competitive factor of

the other competitor(s). In a system where the only interaction between species occurs through a single resource, which is modelled explicitly, this assumption implies that the resource density at any time t can be written in terms of the population density of the species in the system. In physical terms, this means that resource density responds instantaneously to changes in species density. When resources are modelled with a differential equation or stochastic process involving species density, this is simply not the case. There are significant lags between changes in species density and resource density in our model. Thus, we must be careful not to apply results from Chesson's (1994) framework to our model in too much detail. Also, Chesson (1994) assumes that the size of the environmental fluctuations are "small" in some sense, and he comments that this is the most troublesome limitation of his framework. In our model, the fluctuations are very large. Having said this, we believe the general mechanisms Chesson (1994) describes are still at work here.

Finally, the stochastic model with both demographic and environmental stochasticity (with $\theta = 1$) is the most likely to result in coexistence (Table 2.2). Now coexistence occurs for m_2 satisfying $0.82 < m_2 < 0.91$. This represents 18% of the parameter region, or a 31-fold increase over the deterministic model. The large increase in the coexistence regions for this model suggests that there is an interaction between demographic and environmental stochasticity in the model that helps stabilize coexistence.

The second coexistence-promoting mechanism in Chesson's (1994) framework is referred to as the storage effect. As Chesson (1994) (page 233) notes, "storage is a metaphor for the potential for periods of strong positive growth that cannot be cancelled

by negative growth at other times". We believe that this mechanism is at work in our model. To investigate, we examined correlations between competitor population densities in the deterministic and stochastic versions of the model. If an opportunist efficiently exploits fluctuations with periods of strong positive growth, it should be doing so at different times than the gleaner; the two populations should be out of phase, or negatively correlated. Figure 2.2 shows the phase plane and time trajectories for the deterministically fluctuating model for m_2 = 0.93. Clearly, the populations are only slightly out of phase, with the correlation between densities of opportunists and gleaners being 0.999. The competitors are using the resource in periods that almost completely overlap, which may explain the narrowness of the region of coexistence.

Compare these results to those obtained in the stochastic versions of the model. Figure 2.3A shows the mean population sizes for the two species under nutrient-poor conditions with both demographic and environmental stochasticity and figure 2.3B shows the correlation of the gleaner and opportunist population sizes. In the region of coexistence, the correlation becomes negative, i.e. as one population declines, the other increases. At the point where the correlation is most negative, the mix of species (i.e. the means of the stationary distribution of populations) shown in Figure 2.3A is most even. For example, when the birth rate of the opportunist is 0.88, the equilibrium population means are roughly 100 each, and the correlation is approximately -0.5. Towards the edges of the coexistence region, the populations become less negatively correlated (more in phase), and one species begins to dominate. To illustrate the importance of the

Figure 2.2: (A) Phase plane diagram and (B) time trajectories for the deterministically fluctuating system. In Figure (B) the solid line represents gleaners and the dotted line represents opportunists and the x-axis represents model time steps.

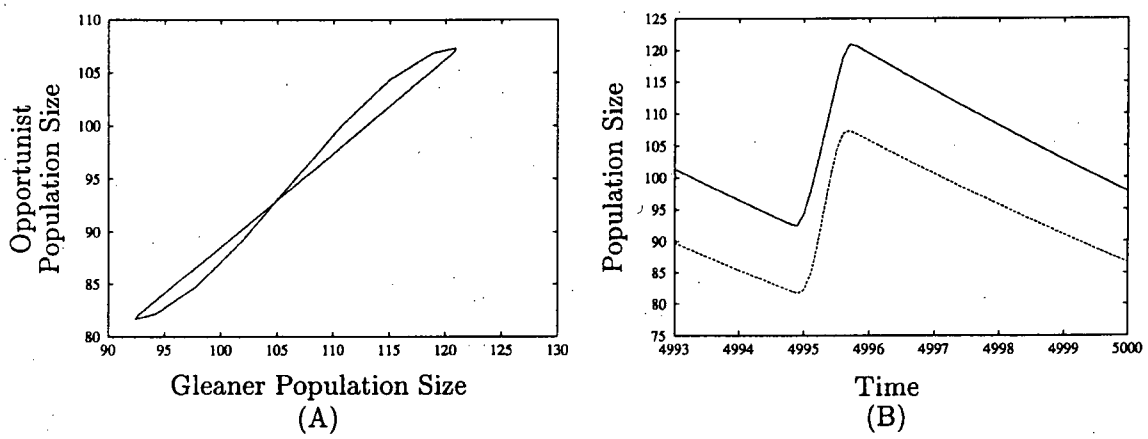
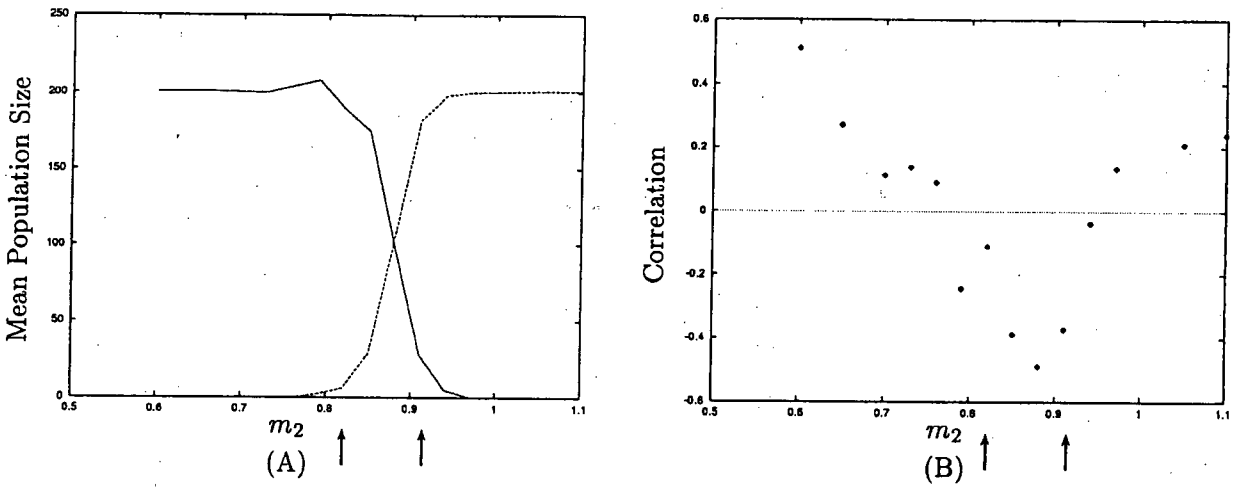


Figure 2.3 (A) Equilibrium population sizes (solid = gleaner, dotted = opportunists) and (B) correlation of gleaner and opportunist population sizes for the fully (demographic and environmental) stochastic model with 300 realizations run for 3000 time steps. The arrows denote the boundaries of the coexistence region which are for values of $0.82 < m_2 < 0.91$.



interaction between demographic and environmental stochasticity, consider that the minimum correlation between populations for the case with only environmental stochasticity is around 0.5 versus around -0.9 for the case with only demographic stochasticity. Based on these correlation results we suggest that demographic stochasticity is responsible for putting the populations out of phase, allowing each to experience periods of strong positive growth, while environmental stochasticity increases the variance in the resource base which can increase the magnitude of the growth that takes place in each of these periods.

The Role of Population Size

It is well known that demographic stochasticity is most relevant in the context of low population size. In our model, we are working with population sizes of around 200 individuals. In contrast, samples from natural waters often contain on the order of 10^3 to 10^6 phytoplankton cells per milliliter of water. For example, if we run our model with population sizes on the order of 2000 individuals in nutrient-poor conditions, the coexistence region shrinks to $0.87 < m_2 < 0.88$ in comparison with populations on the order of 200 where the coexistence region is $0.82 < m_2 < 0.91$. This is not surprising, as running the model with population sizes of order 10^3 with jump sizes of 1 is similar to running the model with population sizes of order 10^2 with jump sizes of 0.1. Recall that increasing θ decreases jump sizes, and we have already seen that doing so reduces the size of the coexistence region. In short, the effects of demographic stochasticity can be removed by increasing either population sizes or θ.

This does not, however, reduce the interest in the results of our model. It merely forces one to think about the spatial and temporal scales at which one believes the

mechanism in question operates. We believe the choice of population sizes of 200 to be appropriate because phytoplankton competition likely occurs at microscale patches under quiescent conditions (Siegel 1998). Given natural densities for phytoplankton in lakes (especially more oligotrophic ones), 200 individual cells corresponds to densities at the μl scale. According to Siegel's (1998) calculations, phytoplankton are distributed discretely relative to their nutrient resources because characteristic length scales for nutrients are on the order of 5-10 μm, while for phytoplankton the scale is greater than 500 μm. Because of these differences in characteristic length scales, the environment appears patchy to phytoplankton, and their population dynamics should be modelled by keeping track of individuals (Siegel 1998) or using a birth/death approach as we have done here.

The Role of Nutrient Levels

The relative richness of an environment as perceived by an organism is related to the magnitude of that organism's average growth rate. Thus, in the context of a model where this is a matter of scaling, enriching an environment can be accomplished by increasing the nutrient levels in the system or increasing the growth rate response of an organism to a given environment. In the models we discuss, there is no benchmark for nutrient rich or nutrient poor. The distinction is relative, characterized only by the growth rate of all individuals in one system as compared to another.

To generate a basis for comparison, we could either leave the organisms alone and change the environment (the intuitively more obvious approach) or leave the environment alone and change the organisms. Either approach generates two models that can be compared to one another: one where all organisms grow relatively fast (nutrient rich), and one were they all grow relatively slow (nutrient poor). We chose to change the growth

characteristics of species so that we could keep the effects of demographic stochasticity and nutrient levels separate. Note that the stationary average population level is given by $S_o y/d$ where S_o is the average resource influx rate. Thus, increasing the "richness" of the environment by the former method also increases the equilibrium population level, which reduces the effect of demographic stochasticity. Comparing rich and poor environments this way is really comparing models with different levels of demographic stochasticity, which is not what we want to do. Notice that these equilibrium population levels do not depend on half-rate constants (i.e. a_i). Thus we can scale the half-saturation constants for both competitors to smaller values while maintaining the same relative values (Table 2.1). For example, in the presence of one unit of nutrient, the gleaner with a half-rate constant of 19 would have a per capita growth rate of 0.025 while a gleaner with a half-rate constant of 1.9 would have a rate of 0.1742. This is roughly equivalent to increasing the nutrient level by a factor of approximately 7 for the gleaner with a half-rate constant of 19. Finally, our approach allows us to maintain the influx of nutrients at one unit per unit time so that all the cases can be compared to the same baseline model.

For the nutrient-rich deterministic model, coexistence occurs if $0.7161 < m_2 < 0.7242$ which represents 1.58% of the parameter trade-off region (Table 2.2). Increasing nutrients has a greater effect than does adding environmental stochasticity alone. The fully stochastic (environmental and demographic) model again does better, with values of m_2 satisfying $0.6 < m_2 < 0.67$, leading to coexistence. This represents 14% of the trade-off region which is a nearly 9-fold increase over the deterministic case. The regions of coexistence have shifted downward in comparison with the nutrient-poor case and the percentage of trade-off region is smaller in the nutrient-rich case. This is probably due to

the fact that in the nutrient-rich case, resource fluctuations are larger and therefore enhance the competitive ability of the opportunist as discussed above.

The role of harshness (i.e. average environmental productivity) in promoting coexistence is also linked to rare events. Chesson and Huntly (1997) note that harshness and fluctuations cannot promote coexistence unless they allow species to use ecological conditions that occur in ways that are non-additive or nonlinear. For the deterministic model, under harsh or oligotrophic conditions, the coexistence region is smaller than the nutrient-rich case, 0.58% versus 1.58% of the trade-off parameter region. Environmental and demographic stochasticity reverses this relationship and the trade-off region under nutrient-poor conditions is greater than under nutrient-rich conditions, 18% versus 14% of the trade-off parameter region. With stochasticity, harshness increases the importance of rare events, improving the competitive ability of an organism which can exploit them. Harshness enhances the storage effect in the same way as environmental stochasticity, by increasing the resource variability.

The Role of the Temporal Scale of Fluctuations (Case 4)

Next, we investigate the relationship between pulse frequency, harshness, and life history strategies. First we will examine the effect of pulse frequency on composition and life history strategies in the stochastic model. Then we will fix life histories and look at the effect of stochasticity on the environmental conditions (frequency of pulsing) necessary for coexistence.

The influence of the average frequency of pulses on the composition of these simple communities can be examined by modifying the parameter f. Consider the nutrient-poor case with pulses that are more frequent ($f = 2$) than the previous cases

(Table 2.1, "frequency on coexistence" column). Under these conditions, coexistence in the fully stochastic (environmental and stochastic) model occurs when $0.94 < m_2 < 1$. This region of coexistence represents 12% of the trade-off parameter range, and the correlation between population densities is approximately -0.85. Recall that previously, when $f = 1/2\pi$ (less frequent pulsing), coexistence occurred when $0.82 < m_2 < 0.91$ (18% of the trade-off region). With more frequent pulsing, m_2 approaches 1 and as this occurs, λ_2 approaches λ_1, which means that the minimum resource requirements for positive growth are the same for both species, and there is no longer a trade-off in terms of λ, the resource level. Recall that the wait-times between pulses are exponentially distributed with a mean given by the inverse of the frequency and variance given by the square of the inverse of the frequency. As the frequency of pulses goes up, the variance of the pulse arrival times goes down, reducing the irregularity that is required for the relative nonlinearity mechanism to work. Thus, the opportunist must maximize its growth rate to compete. Increasing the frequency of small nutrient pulses therefore should favour maximum differences in life histories with very fast growing opportunists coexisting with slower growing gleaners.

Secondly, how does adding stochasticity influence the predictions for the frequencies of environmental variation that favour coexistence? To explore this question we computed the region of coexistence as a function of pulse frequency for two fixed life history strategies (Table 2.1, last column). Two factors are important in assessing the possibility of coexistence: the ability to invade and the ability to persist after invasion. These are the same for the deterministic model but are slightly different in the stochastic case.

We perform a bifurcation analysis on the deterministic version of the model with the period (*1/f*) as the bifurcation parameter, with the parameters for life history set for the nutrient-poor case and with m_2 fixed at 0.9. The analysis identified the region of coexistence for a pulse frequency between 8.678 and 9.079 days. There is a very narrow band of frequency ranges for the fluctuations that allow individuals with the life history strategies described by this set of parameters to coexist.

The results for the stochastic model are markedly different (Figures 2.4 and 2.5). Figure 2.4A shows the mean gleaner population size (based on 300 realizations run for 100 time steps) starting from an initial condition with 10 gleaners and 200 opportunists, while Figure 2.4B shows the probability that there will be more than 10 gleaners after 100 time steps - a measure of the probability of invasion. By considering pulse periods where the means increase for both populations (Figure 2.4A and 2.5B), coexistence is possible for pulse periods in the range of 2 to 10 days, because both competitors can invade under these conditions. Using the pulse periods where the probability of invasion for the opportunist begins to increase (Figure 2.5B), one might choose the coexistence region to be in the range of 2 to 25 days. In either case, the region is much larger than for the deterministic model. Figure 2.5 illustrates the mechanism by which environmental fluctuations enhances the storage effect discussed earlier. Based on the simulations, the probability of invasion seems to approach a maximum of approximately 0.65 (Figure 2.5B), but the population mean continues to increase (Figure 2.5A). In the region where the relationship between pulse period and probability of invasion is flat (see Figure 2.5B),

Figure 2.4: Statistical data for frequency-dependent coexistence for the case of gleaners invading a population of opportunists. Graph (A) shows the mean gleaner population after 100 time steps (days) from an initial condition of x_1=10, x_2=200. Graph (B) shows the probability that the gleaner population will be greater than 10 at t=100, which is a measure of the probability of invasion.

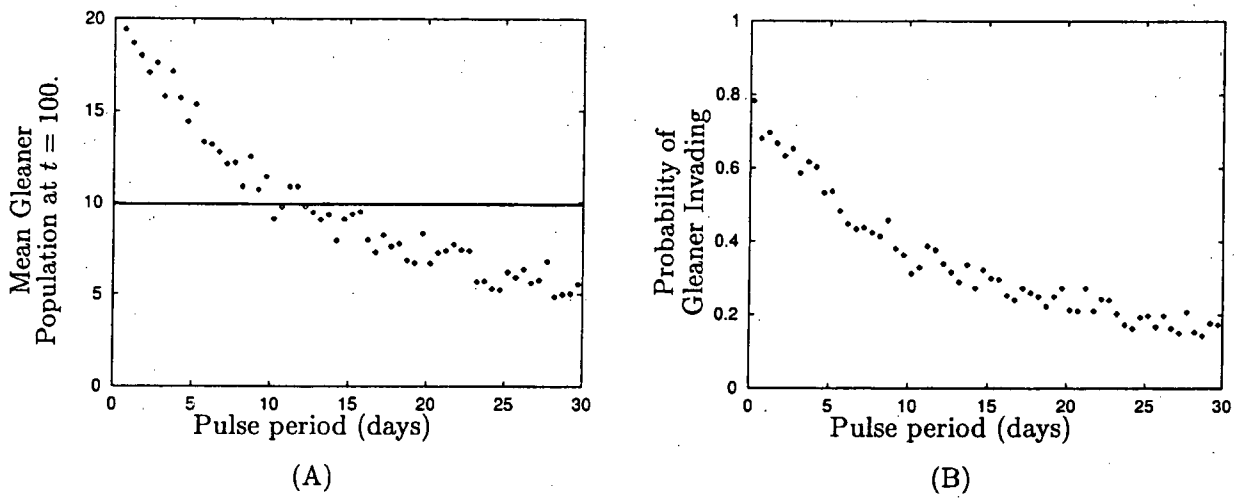
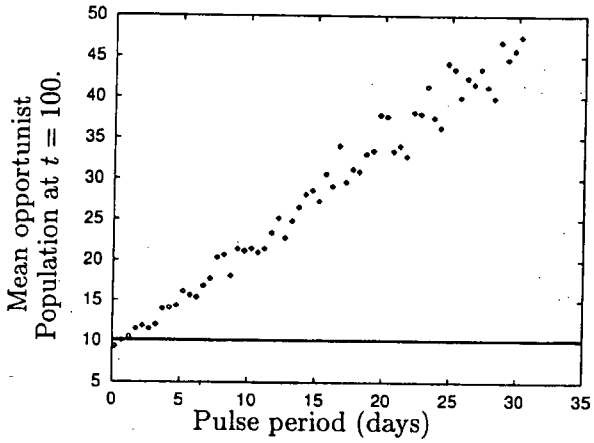
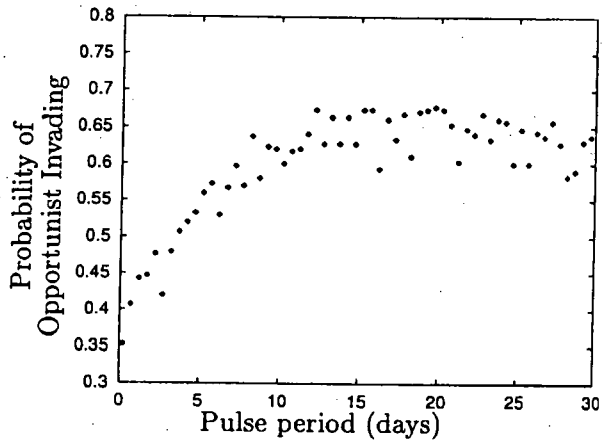


Figure 2.5: Statistical data for frequency-dependent coexistence for the case of opportunists invading a population of gleaners. Graph (A) shows the mean opportunist population after 100 time steps (days) from an initial condition of x_1=10, x_2=200. Graph (B) shows the probability that the opportunist population will be greater than 10 at t=100, which is a measure of the probability of invasion.



(A)



(B)

the same proportion of realizations are remaining above 10 individuals. The increasing mean must then be attributed to the fact that as the pulse period increases, those populations that do survive, reach higher population levels. As the pulse period increases, resource variability increases allowing for rare events of large resource influxes that enable the opportunists to grow very quickly, capitalizing on the storage effect.

Conclusions

In this chapter we have studied the role of stochasticity in enabling similar species to coexist while competing for a common fluctuating resource. Environmental stochasticity led to a greater variance in resources, which would invoke the mechanism of relative nonlinearity (Chesson 1994). As Chesson (1994) noted, and our results support, relative nonlinearity is a very weak coexistence-promoting mechanism. Demographic stochasticity appears to promote the storage effect (Chesson 1994) in our model by introducing a time lag between changes in population sizes and the resource pool. This reduces the correlation between competing population sizes, thereby allowing each to experience periods of strong positive growth that counteract periods of negative growth. Thus, the temporal dynamics generated by demographic stochasticity are another type of storage effect, not previously considered as part of Chesson's framework (1994). The storage effect (demographic stochasticity) has a much larger potential to promote coexistence than relative nonlinearity (environmental stochasticity) as noted by Chesson (1994). When both types of stochasticity are present, an interaction occurs. Environmental stochasticity enhances the storage effect by increasing the variance in the resource base that can increase the magnitude of growth that can occur during pulse

periods. In addition, the marked difference between the effect that deterministic and stochastic fluctuations have on the frequencies that favour coexistence (8-9 day versus 2-25 day periods respectively) suggests that taking advantage of a rare event which allows the opportunist to grow very quickly is relatively more important than regularly periodic fluctuations alone.

From this work, it is evident that both abiotic and biotic factors interact to determine community structure. Diversity levels and composition are both functions of the structure of the environment (i.e. when and how fluctuations arrive) and of the types of organisms present (i.e. their life history strategies). Fluctuations in the environment in no way obviate the role of competition in affecting ecological communities but rather interact with this biotic process.

CHAPTER 3

Phytoplankton Community Structure: Responses to Temporal Heterogeneity in Environments of Contrasting Productivity

This chapter presents an empirical study on the roles of temporal heterogeneity in vertical mixing and environmental productivity and their interaction on phytoplankton community structure. Higher trophic levels were initially excluded from the systems.

Introduction

Planktonic organisms in the epilimnion of north temperate lakes are exposed to environmental variability at various frequencies, even within a single growing season. Deep-water mixing in the summer can lead to an influx of nutrient-rich water into the generally nutrient-poor photic zone. This mixing can produce a nutrient pulse to phytoplankton in the epilimnion (Klein and Coste 1984, Reynolds 1993). The average phytoplankton cell is likely to experience a nutrient pulse with vertical mixing, as organisms and resources are redistributed relative to each other, with the magnitude of the effect depending on the degree of mixing. Phytoplankton exposure to nutrient variation in their natural environment has been the impetus for a large number of chemostat and batch culture studies in the laboratory (e.g. Turpin and Harrison 1979, Sommer 1984, 1985, Gaedecke and Sommer 1986, Suttle and Harrison 1986, Brzezinski and Nelson 1988, Reynolds 1988, Grover 1988, Lindenschmidt and Chorus 1998). These small-scale experiments have focussed on the effects of nutrient pulses at various time scales on phytoplankton community structure (diversity and composition). The general conclusion from these is that the temporal scale of fluctuation has important

consequences for community structure (diversity and composition) that might apply at the macroscale (lake) level (Reynolds 1993). Theory tells us that diversity changes occur because a larger number of life history strategies can be accommodated under fluctuating conditions (Chesson and Case 1986). The actual diversity achieved depends on the frequency of fluctuation relative to the cell division rates (or generation times) of the organisms involved. Changes in diversity levels can also lead to modifications in species composition through exclusion or addition, usually of different life history strategists.

Theory (Levins 1979, Chesson and Huntly 1988, Grover 1990, 1991c) and empirical studies (Sommer 1984, 1985, Gaedecke and Sommer 1986, Grover 1991a,b) have identified generally successful life history strategies resulting from competitive interactions under fluctuating resource conditions. There are three major strategies that represent trade-offs in the numerical and functional responses: gleaner, opportunist or storage strategies. Organisms with steep functional responses (specifically, high affinity for resources or low half-saturation constant for growth) but with moderate maximum growth rates, are called gleaners. Gleaners always dominate in the long-term when environmental conditions are steady (Tilman 1982). They also do well under conditions that approach steady state with small, frequent nutrient pulses (Grover 1990). Organisms that have high population growth rates, but weaker resource acquisition abilities at low resource densities are called opportunists. Persistence of opportunists require moderately large pulses of resources that are not too far apart in time relative to their generation times (Grover 1990). Finally, storage specialists are organisms that have steep functional responses (low half-saturation) and an ability to sequester large quantities of resources within their large bodies, but maintain relatively low maximum population growth rates.

They are much like opportunists in that they rely on large pulses of resources, but instead of putting those resource into population growth (i.e. more individuals), they put resources into bigger individuals and into sustaining cell divisions (over a long period of time) between nutrient pulses. These organisms should dominate under conditions where very large resource pulses occur very infrequently.

Based on allometric relationships that relate physiology or life history to cell size (Grover 1989), predictions can be made for changes in phytoplankton community size-structure under disturbed conditions. Conditions that approach steady state promote growth of gleaners, which tend to be small to intermediate-sized cells with high surface area to volume ratios (Reynolds 1989). Frequent fluctuations should favour the development of a community consisting of small opportunists while infrequent, large fluctuations should favour large storage specialists (Turpin and Harrison 1980, Gaedecke and Sommer 1986, Reynolds 1988).

A general assumption of laboratory pulsing experiments is that the main effect of episodic upwelling in natural systems from vertical mixing events is to introduce a pulse of nutrient resources. However, in addition, vertical mixing events can have other important effects, like modifying the distribution of phytoplankton cells with respect to sunlight. The physical process of mixing can also destroy any natural spatial structure (like vertical "thin-layers" or horizontal patchiness) in the distribution of phytoplankton species within a lake (Talling 1957, Richerson *et al.* 1970, Jones and Ilmavirta 1988, Reynolds 1992) or small nutrient patches that arise from zooplankton excretion (Lehman and Scavia 1982). The consequences of these larger scale spatial processes cannot be examined in laboratory chemostats, which are fully mixed.

The average productivity of the environment may influence the predicted effects of fluctuating nutrient levels on community structure (Huston 1979). If increasing average productivity acts to boost population growth rates among competitors but not to eliminate competition for resources, it should more quickly lead to decreased diversity because, for a given frequency of fluctuation, resource depletion and competitive exclusion are more likely to occur before the next abiotic event (Huston 1979). In addition, in stratified aquatic environments where resource fluctuations should accompany vertical mixing, nutrient pulses should be larger in enriched systems than in oligotrophic ones for the same degree of mixing. Some have argued that tighter recycling by the microbial loop occurs in the photic zone occurs under oligotrophic conditions, resulting in fewer losses to the hypolimnion (Goldman 1984, Stone and Weisburd 1992). In enriched systems, the proliferation of larger cells, which take longer to decompose, can result in higher losses of biomass and nutrients to the hypolimnion (Wehr *et al.* 1994). As a result, enrichment may have two effects: increase the magnitude of the pulse that accompanies vertical mixing, and increase the characteristic frequency of fluctuations relative to generation times.

The temporal scale of mixing and its interaction with average nutrient loading have been studied in the laboratory, but there are very few experimental field data to date. The goal of this chapter is to examine the consequences of different temporal scales of mixing in determining natural phytoplankton community structure and the influence of background environmental productivity on the responses in lake mesocosms.

Methods

Mesocosm experiments consisted of replicate plankton communities isolated and suspended in partly darkened polyethylene bags (~ 4000 l = 1 m diameter x 5 m deep, open at surface) in Placid Lake at the Malcolm Knapp Research Forest, southwestern British Columbia, Canada. This montane lake (550 m elevation) is oligotrophic, slightly stained and approximately 2.5 ha in area (Northcote and Clarotto 1975). The maximum depth of the lake is 7 m and it is well stratified in the summer with transparency as measured by Secchi depth of 3.5 m. Because the photic zone is likely to extend to the bottom of the lake, and in order to maximize the difference between the hypolimnion and the epilimnion, the lower 2.5 m of the bags was covered in black plastic.

The experimental treatments consisted of two factors with three levels for vertical mixing and two levels for productivity. There were two replicate bags per treatment for a total of 12 bags. Mixing treatments were: (i) frequent mixing (every 3 days), (ii) infrequent mixing (21 days) and (iii) unmixed (∞ days). Mixing was accomplished by bubbling air into the bottom of the bag using a bilge pump. Frequency and intensity of the mixing treatments were inversely related to hold constant the overall amount of mixing, and to manipulate only the frequencies. As such, the 21 day treatments were bubbled for 3.5 min, a period 7 times longer than the 3 day treatments (30 s). Thirty seconds was chosen as the time for the most frequently mixed systems because it represented an amount of mixing that disrupted the temperature gradient without having too large of an effect (see Fig. 3.1 in Results). The nutrient treatments were a high and low productivity treatment. Enriched (high) treatments had 100 times the nutrients (phosphate and nitrate) added initially, compared to the unenriched (low) ones. The

natural 25:1 N:P ratio (Butler 1986) was maintained in all treatments. Nutrients were added every 2 days over a 2 week period before the start of the experiment. 1μg l^{-1} of PO_4^{-3} d^{-1} was added to the high treatments and 0.01μg $l^{-1}$of PO_4^{-3} d^{-1} added to low treatments. Nutrients were added as solutions of KH_2PO_4 and $NaNO_3$. The quantity of nutrient added to the "low" nutrient treatments is minimal compared to average measured levels of total P in Placid Lake of 10 μg l^{-1} (Werring 1986) and is unlikely to push the system far from its natural oligotrophic state. Placid Lake is considered an oligotrophic system in large part because nutrients are relatively inaccessible owing to the high humic content of the lake (Krause 1984). The addition of a total of 14 μg l^{-1} PO_4^{-3} to the enriched bags would place these systems in the meso-eutrophic category of Vollenweider (1968).

The bags were filled initially (June 7 1999) with lake water that was filtered to 54 μm to exclude initially crustacean zooplankton. At the midpoint of the experiment, there were no crustacean zooplankton present. However, by the end of the experiment, low average numbers (<1 l^{-1}) of juvenile *Daphnia* were present in two bags (bags 1 and 10). These bags represented one of two replicates and I compared the diversity measures and community composition for these to other treatments (Appendix 1). The variability between treatments was higher than within, even for cases where a small *Daphnia* invasion had occurred. It appears to be very difficult to completely exclude zooplankton from field mesocosms of this size for extended periods of time (see also Chapter 4). I suspect that these zooplankton entered the systems during the initial filling of the bags (despite filtration) but remained as eggs or at undetectable and insignificant numbers for

most of the experiment. For the majority of the experimental period, these systems did not have crustacean herbivores and these mesocosms represent the most herbivore-free conditions of this entire project. For this reason, and because of the small effect on critical community measures (Appendix 1), they will be compared to other experiments within the thesis as being a single trophic level.

The experiment began on June 19, 1999 with mixing events for the 3 and 21 day treatments. The experiment ran for two full cycles of the 21 day treatments (or 14 cycles for the 3 day treatments). Phytoplankton inocula from a eutrophic lake (Trout Lake, Vancouver) were added weekly (25 ml sample filtered to 54 μm) as a potential seed of competitors for the community originally drawn from the more oligotrophic Placid Lake. The experiment ended on July 31 1999.

At the end of the experiment, samples were taken from two depths (1.5 and 4 m from the surface) for phytoplankton and chlorophyll measurements using an opaque 2 l Van Dorn bottle. A 250 ml sample from each depth was preserved immediately using several drops of Lugol's iodine. In the lab, identification, enumeration, measurements of cell size (maximum linear dimension; MLD), and descriptions of shape were done using an inverted microscope and 50 ml of preserved samples that had been settled for 20 h. Cells were counted along transects at 200X magnification until at least 100 cells of the most common group had been enumerated. In addition, a 60 ml sample was filtered in the field onto a GF/F filter for chlorophyll *a* analysis. Filters were kept in the dark and frozen until analysis one week later. Fluorometric (Turner Designs, Model 10 Analog Fluorometer) determination of chlorophyll *a* was done with 90% ethanol extraction (Nusch 1980). Nutrient data for PO_4^{-3} (SRP) taken on August 30, 1998 in a similar

experimental setup are presented for high and low nutrient level treatments from bags subject to mixing every 15 days (since none were collected in 1999 owing to sample contamination problems). Although these data are taken from systems where zooplankton were present (while they were absent from the current study), they are shown to help explain differences in nutrient pulses associated with mixing events in low and high nutrient systems. The water samples were filtered using a combusted GF/F filter and frozen until later analysis with an autoanalyser.

Part way through the experimental period (July 19 1999), estimates of the effect of mixing were done using a Self-Contained Autonomous MicroProfiler (SCAMP), which measures temperature at 1 mm intervals through a column of water. It also provides a measure of turbulence dissipation rates (ε) using the Batchelor method (Batchelor 1967). Estimates were done in extra enclosures set up at the same time as the experimental ones. Using the SCAMP, I was able to get estimates of the short and medium-term effect of various mixing times on the thermal profile in the bags.

Data and Statistical Analyses

The structure of the major statistical analyses for this and subsequent chapters (4 and 5) are outlined in Appendix 2. The analyses were hierarchical in nature with primary tests consisting of either 2-way factorial ANOVAs or MANOVAs depending on the types of data.

Several aggregate measures of community structure were calculated. The Shannon diversity index was calculated as: $H' = -\sum_{i=1}^{s} (p_i)(\log_e p_i)$ where S is the number of species (richness) and p_i is the proportion of total sample abundance belonging to

species *i*. Evenness was calculated as: $J' = \frac{H'}{\log_e S}$. Aggregate community measures (H', S and J'), chlorophyll data, and compositional data were compared in 2-way ANOVAs with enrichment (2 levels) and mixing frequency (3 levels) as factors. Significant main effects were further analyzed with Tukey's test, and significant interactions were explored using orthogonal contrasts.

Biovolume in each taxon was calculated based on cell size measurements and formulae for geometric shapes that approximated cell shapes. Biovolume ($\log_{10}$ transformed for statistical analysis to reduce heteroscedasticity), in addition to chlorophyll *a*, was used as a measure of phytoplankton biomass to look for quantitative differences in the biomasses of various groups. To investigate differences in community composition, I also used the relative abundances (arcsine square root transformed) of selected taxa based on cell densities (not biomass). Cell densities represent the numbers upon which mathematical models are generally formulated, and changes in relative abundance indicate how community changes are manifested, disregarding the confounding change in overall community cell density.

For the statistical analyses, phytoplankton taxa (based on biovolumes and relative abundances) were combined into descriptor classes based on aspects of (1) size (four classes: <5, 6-20, 21-35 and >35μm), (2) taxonomy (by phylum), (3) morphological features (six classes: round, long, filamentous, spiny, hairy, or big flagella present, and amorphous), and (4) mobility (four classes: solitary nonflagellates, solitary flagellates, colonial nonflagellates, colonial flagellates). Taxa encountered and classification into these various groups are given in Table 3.1. These community composition variables

Table 3.1: Phytoplankton species encountered in the Placid Lake experiments.

Genus	Size (MLD) in μm	Phylum	External Features	Mobile?	Habit
Ankistrodesmus fractus	>35	Chlorophyta	spiny	no	solitary
Arthrodesmus sp.	21-35	Chlorophyta	spiny	no	solitary
Arthrodesmus octocornus	21-35	Chlorophyta	spiny	no	solitary
Asterionella formosa	>35	Bacillariophyta	long	no	colonial
Cerasterias sp.	21-35	Chlorophyta	spiny	no	solitary
Chaetosphaeridium globosum	6-20	Chlorophyta	round	yes	solitary
Chlamydomonas sp.	6-20	Chlorophyta	round	yes	solitary
Chlorogonium elongatum	6-20	Chlorophyta	round	yes	solitary
Chrysidiastrum sp.	21-35	Chrysophyta	spiny	no	solitary
Chrysosphaerella sp.	>35	Chrysophyta	spiny	yes	colonial
Closteriopsis longissima	>35	Chlorophyta	spiny	no	colonial
Cosmarium sp.	6-20	Chlorophyta	round	no	solitary
Crucigenia tetrapedia	6-20	Chlorophyta	round	no	solitary
Cryptomonas erosa	21-35	Cryptophyta	hairy	yes	solitary
Cylindrocystis sp.	>35	Chlorophyta	long	no	solitary
Denticula sp.	6-20	Bacillariophyta	spiny	no	solitary
Dinobryon sp.	>35	Chrysophyta	spiny	yes	colonial
Euastrum sp.	6-20	Chlorophyta	round	no	solitary
Eudorina elegans	21-35	Chlorophyta	hairy	yes	colonial
Euglena sp.	21-35	Euglenophyta	hairy	yes	solitary
Fragilaria sp.	21-35	Bacillariophyta	round	no	colonial
Frustulia sp.	>35	Bacillariophyta	spiny	no	solitary
Genicularia sp.	>35	Chlorophyta	long	no	solitary
Gloeocystis major	6-20	Chlorophyta	round	no	colonial
Golenkinia sp.	6-20	Chlorophyta	spiny	no	solitary
Gymnodinium sp.	6-20	Pyrrhophyta	hairy	yes	solitary
Gyrosigma sp.	>35	Bacillariophyta	long	no	solitary
Mallomonas caudata	6-20	Chrysophyta	hairy	yes	solitary
Melosira sp.	21-35	Bacillariophyta	long	no	solitary
Meridion circulare	21-35	Bacillariophyta	long	no	colonial
Merismopedium sp.	21-35	Cyanophyta	amorphous	no	colonial
Mesotaenium sp.	6-20	Chlorophyta	long	no	solitary
Microcystis aeruginosa	21-35	Cyanophyta	amorphous	no	colonial
Nanoflagellates	<5	Chrysophyta	round	no	solitary
Non-motile Chlorococcales	<5	Chlorophyta	round	no	solitary
Ochromonas sp.	6-20	Chrysophyta	round	yes	solitary
Oocystis sp.	6-20	Chlorophyta	round	no	colonial
Opephora martyi	21-35	Bacillariophyta	long	no	solitary

Table 3.1 (continued): Phytoplankton species encountered in the Placid Lake experiments.

Genus	Size (MLD) in μm	Phylum	External Features	Mobile?	Habit
Oscillatoria sp.	>35	Cyanophyta	filamentous	no	solitary
Pediastrum sp.	21-35	Chlorophyta	round	no	colonial
Penium sp.	>35	Chlorophyta	long	no	Solitary
Pleurotaenium trabecula	>35	Chlorophyta	long	no	Solitary
Pseudostaurastrum hastatum	21-35	Chrysophyta	spiny	no	Solitary
Roya obtusa	>35	Chlorophyta	long	no	Solitary
Scenedesmus incrassatulus	6-20	Chlorophyta	spiny	no	solitary
Schroederia setigera	>35	Chlorophyta	spiny	no	solitary
Spinocosmarium quadridens	6-20	Chlorophyta	spiny	no	solitary
Spirogyra sp. 1	>35	Chlorophyta	filamentous	no	solitary
Spirogyra sp. 2	>35	Chlorophyta	filamentous	no	solitary
Spirotaenia condensata	>35	Chlorophyta	filamentous	no	solitary
Spirulina laxissima	>35	Cyanophyta	filamentous	no	solitary
Spirulina subsalsa	>35	Cyanophyta	filamentous	no	solitary
Spondylosium planum	>35	Chlorophyta	filamentous	no	colonial
Staurastrum cornutum	21-35	Chlorophyta	spiny	no	solitary
Staurastrum rotula	21-35	Chlorophyta	spiny	no	solitary
Synedra sp.	>35	Bacillariophyta	long	no	solitary
Synura uvella	6-20	Chrysophyta	round	yes	colonial
Tabellaria sp.	>35	Bacillariophyta	long	no	colonial
Terpsinoe americana	6-20	Bacillariophyta	round	no	solitary
Tetradesmus smithii	6-20	Chlorophyta	round	no	colonial
Tetrastrum sp.	6-20	Chlorophyta	spiny	no	solitary
Trachelmonas ampulla	21-35	Euglenophyta	round	yes	solitary
Treubaria crassispina	21-35	Chlorophyta	spiny	no	solitary
Uroglena volvox	6-20	Chrysophyta	round	yes	colonial

were used as multivariate descriptors of each community type in each treatment. Communities were defined by one vector of descriptor class at a time and a factorial MANOVA was run on each to determine whether there were treatment effects. Thus a total of four MANOVAs were run. Significance in the MANOVA was determined by a significant value for the Hotelling-Lawley Trace, Wilks' Lambda, Pillai's Trace and/or Roy's Greatest Root. Roy's Root was not used on its own as an indicator of significance because it is the most prone to Type I errors (Bernstein 1988, p. 328). A stepwise discriminant analysis indicated which groups in each descriptor class contributed most to main effect treatment differences. It turned out, however, that most significant effects in the MANOVA were interaction effects, and for these, contrasts were used to determine where differences occurred (i.e. simple effects between treatments). Finally, factorial ANOVAs were done on each significant variate describing communities to determine where the significant interactions lay.

In addition, in order to determine which species were producing the observed patterns at higher levels of resolution (i.e. diversity measures or aggregate community descriptors just described), I ran ANOVAs on the relative abundances of common species (>1% of total density) to determine main and interaction effects of the applied treatments. These results are presented in the section entitled "Species Composition".

To address concerns about multiple comparisons with the number of statistical tests described here, I have outlined the structure of the analyses for all data chapters in Appendix 1. Where there are causes for concern of type I error because of a large number of tests, I will address them as the results are presented.

Results

Physical Effects

Mixing of the water column by bubbling had large effects on stratification in the bags. The upper panels of Figure 3.1 show the effects of mixing on July 19 in the 3 day treatments (30 s). The immediate effect (i.e. 3 min after mixing) was to accentuate the temperature gradient and induce a deeper thermocline. Mixing also reduced the surface temperature. These effects lasted for at least 30 min after mixing. The second part of Figure 3.1 shows that the effect of mixing for 3.5 min (the 21 day treatment) was enough to make the water column in the bag completely isothermal.

Table 3.2 shows the maximum turbulence dissipation rates measured by SCAMP under various conditions (i.e. in the bag both before and after mixing). Maximum turbulence dissipation rates were one to two orders of magnitude higher after mixing of either 30 s or 3.5 min had been imposed.

The effect of mixing on nutrient levels (SRP) in the epilimnion after mixing differed with treatment in measurements done in 1998. At low nutrient levels, there was a net increase in the epilimnion (1.5 m depth) by 4 $\mu g\ l^{-1}$ of PO_4^{-3} (from 7 to 11$\mu g\ l^{-1}$) while the epilimnetic region of the enriched system showed a much larger net increase of 14 $\mu g\ l^{-1}$ (from 1 to 15 $\mu g\ l^{-1}$) with mixing.

Phytoplankton Community Diversity

There was a significant interaction of frequency of mixing and nutrient levels in the response of the phytoplankton community diversity measures: the Shannon diversity index ($P_{interaction}$=0.0071)(Figure 3.2a) and community evenness ($P_{interaction}$=0.0187) (Figure 3.2b). Species richness was not significantly affected by any treatment factor.

Figure 3.1: Temperature-depth profiles before and after two imposed mixing events. In the upper plots, the results for 3 min and 30 min after a 30 s bubbling event (equivalent to the 3 day mixing regime) are displayed. The bottom graph shows the effect 3 m after bubbling for 3.5 min (21 day regime).

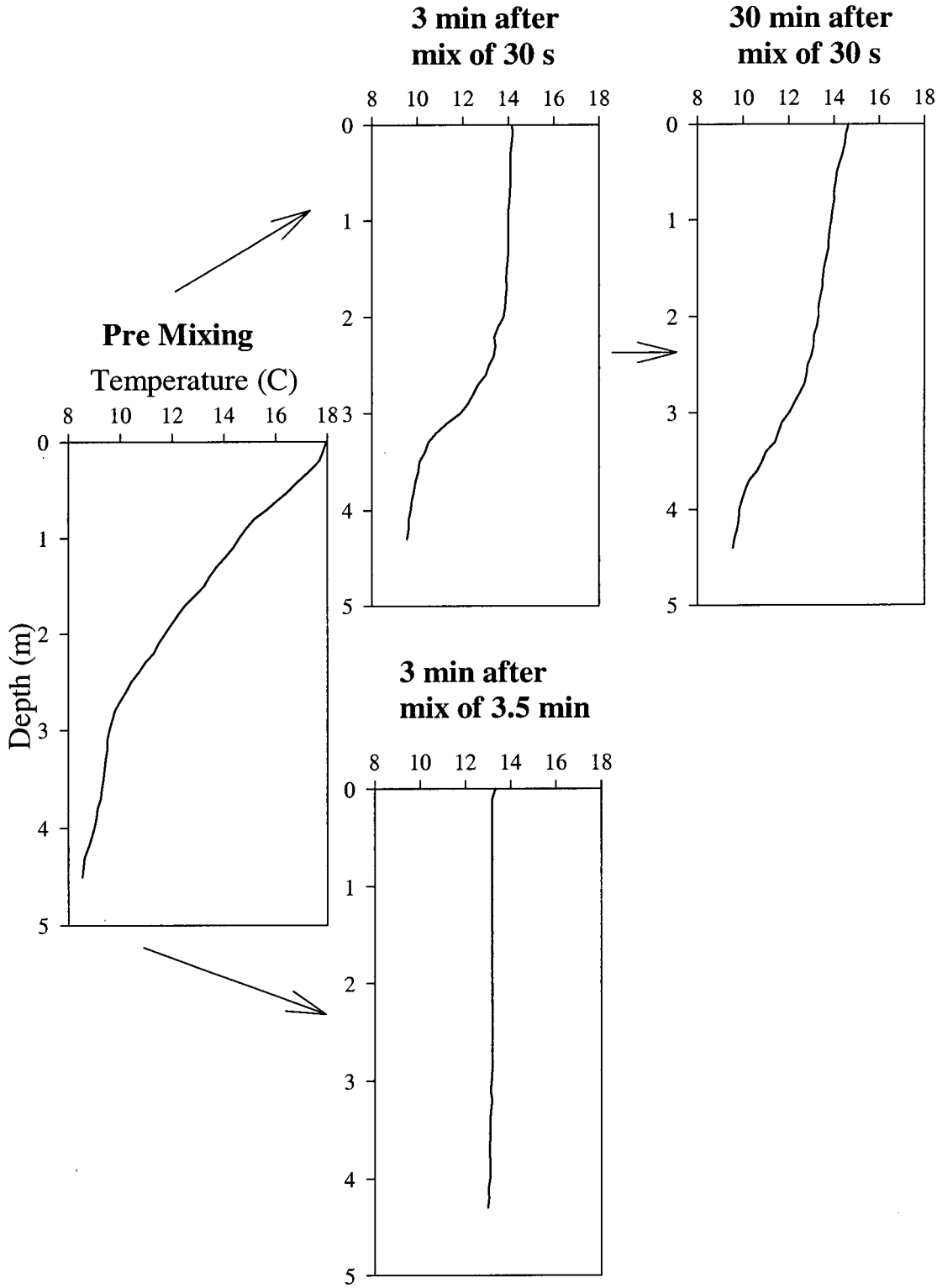
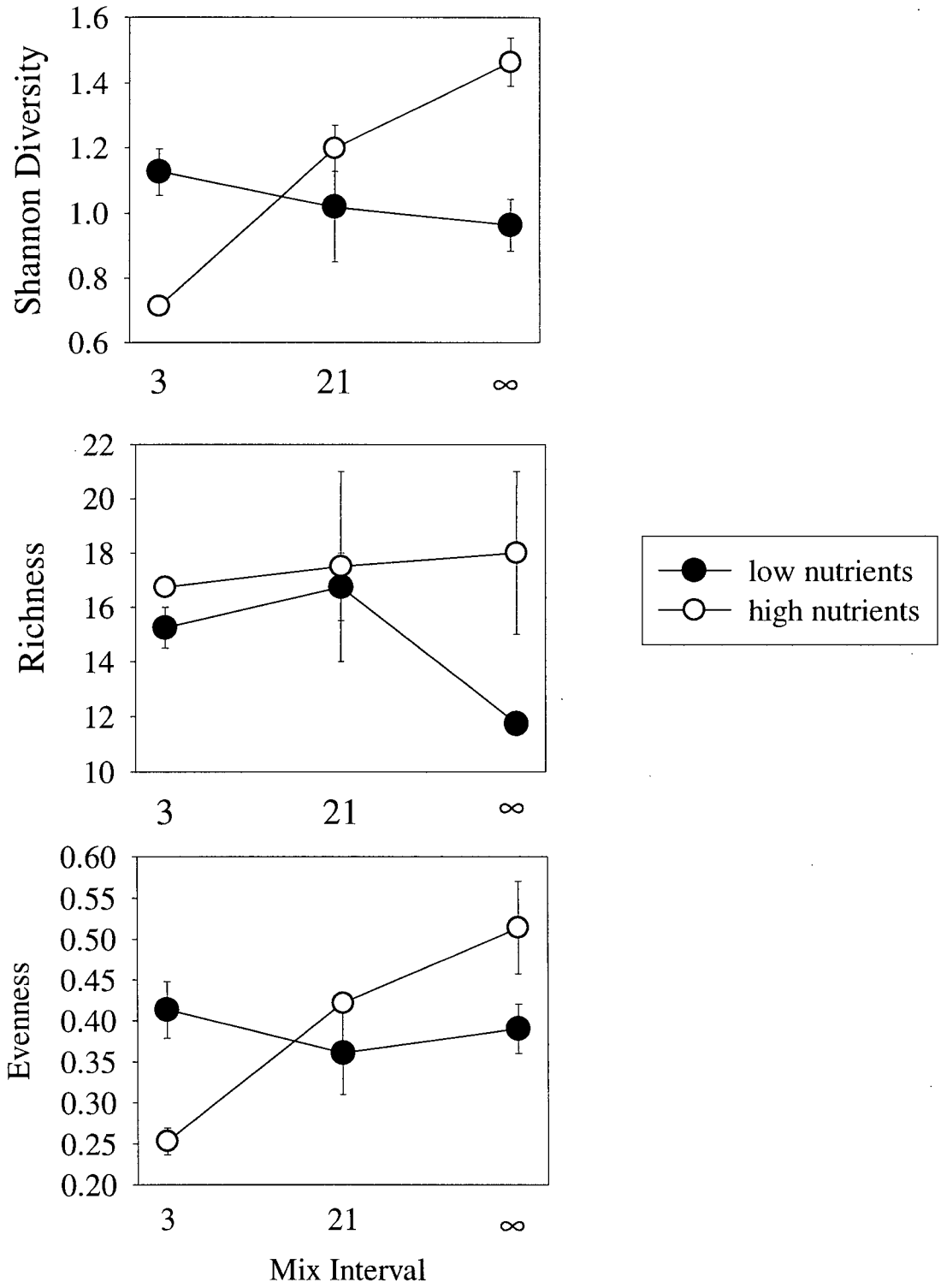


Table 3.2: Maximum turbulence dissipation rates (ε) before and after various imposed mixing events as measured using the SCAMP.

	Length of Mixing (minutes)	**Time since mixing (minutes)**	**Maximum ε ($m^2 s^{-3}$)**
Pre-mixing	-	-	1.59×10^{-7}
Post-mixing	0.5	3	3.89×10^{-6}
Post-mixing	0.5	30	3.88×10^{-6}
Post-mixing	3.5	3	9.68×10^{-6}

Figure 3.2: Interaction diagrams for the aggregate measures of mean community diversity (± standard error).



Enriched systems responded to mixing with significantly lower diversity and evenness levels in the systems mixed frequently (every 3 days) than in those mixed infrequently or not at all (unmixed vs. 3 day $P_{diversity}$=0.0012; 21 day vs. 3 day $P_{diversity}$=0.01; unmixed vs. 3 day $P_{evenness}$=0.0024; 21 day vs. 3 day $P_{evenness}$=0.02). Oligotrophic systems did not show any differences in aggregate community measures with different frequencies of mixing (Figure 3.2). For systems mixed frequently, diversity and evenness were lower with enrichment ($P_{diversity}$=0.0196; $P_{evenness}$=0.0212) while unmixed systems had higher aggregate community measures with enrichment ($P_{diversity}$=0.0085; $P_{evenness}$=0.05) (Figure 3.2).

Biomass Levels

Chlorophyll *a*, a measure of total phytoplankton biomass, was slightly higher in the enriched treatments by the end of the experiment (+nutrients $\log_{10}$ mean ± standard error = 4.12 ± 0.40 μg l^{-1}; -nutrients $\log_{10}$ values mean ± standard error = 2.34 ± 0.68μg l^{-1}) (P=0.0878), regardless of mixing frequency.

Community Composition

(i) Size Classes

The results of the MANOVA showed a weakly significant interaction effect of mix frequency and nutrient levels on the relative abundance size class vectors (Hotelling-Lawley Trace=15.91, P=0.0991; Roy's Greatest Root=15.412, P=0.011). The significant simple effects included the influence of nutrient level for frequently mixed systems (P=0.0597) and at high nutrients, a difference between frequently mixed systems (3 days) and infrequently (3 day vs. 21 day; P=0.0778) and unmixed (3 day vs. unmixed P=0.0349) systems. Examination of the univariate ANOVAs for treatment effects on

each size class revealed that the classes showing interaction responses were the <5 μm ($P_{interaction}$ =0.0528) and the 6-20 μm ($P_{interaction}$ =0.0833) groups (Figure 3.3). The main responses by these 2 groups occurred for the frequently mixed systems. The smallest phytoplankton group showed very large increases in relative abundance with enrichment where mixing was frequent (P=0.0207), at the expense of the phytoplankton in the next larger size class (6-20 μm) (P=0.012) (Figure 3.3).

For biovolumes, the multivariate analysis again indicated a weak interaction effect of nutrient and mix frequency levels (Pillai's Trace=3.8, P=0.0385). Univariate ANOVAs on the various size class responses indicated that the smallest size class (<5 μm, P=0.0099) and the largest size class (>35 μm, P=0.0569) were responsible for the interaction effect (Figure 3.4). For the smallest size class, biovolumes were highest under enriched conditions for both the unmixed (P=0.0336) and the 3 day (P=0.0004) mixing regimes. Under enriched conditions however, there was also a significantly higher biovolume of the smallest class when systems were mixed frequently (3 days) than less frequently (21 days) (P=0.0015) or not at all (P=0.0019). For the largest size class, higher biovolumes in the enriched treatments were observed when mixing was absent (P=0.0096) (Figure 3.4). When systems were enriched, the unmixed treatment always had higher biovolumes than either of the mixed ones (unmixed vs. 3 days, P=0.0301; unmixed vs. 21 days, P=0.0313).

Figure 3.3: Interaction diagrams for phytoplankton community composition by size class. Values represent mean relative abundance (± standard error) of each class as a proportion of total density. Results are for significant interaction effects in the MANOVA. Solid lines represent low nutrient conditions and dashed ones are for enriched conditions.

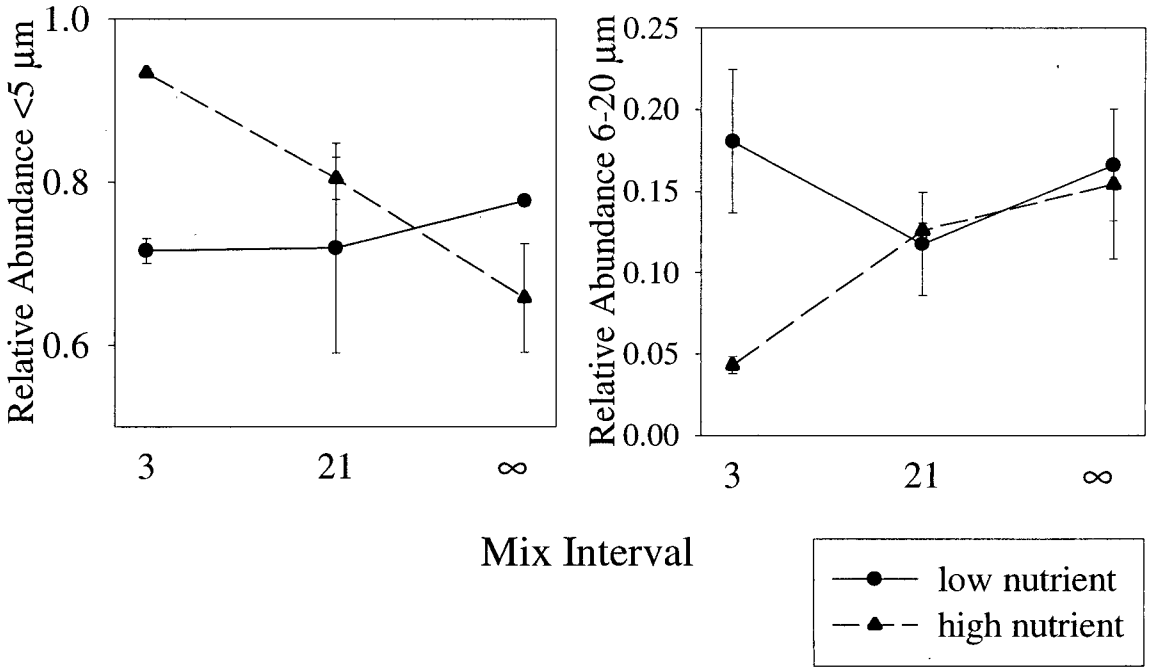
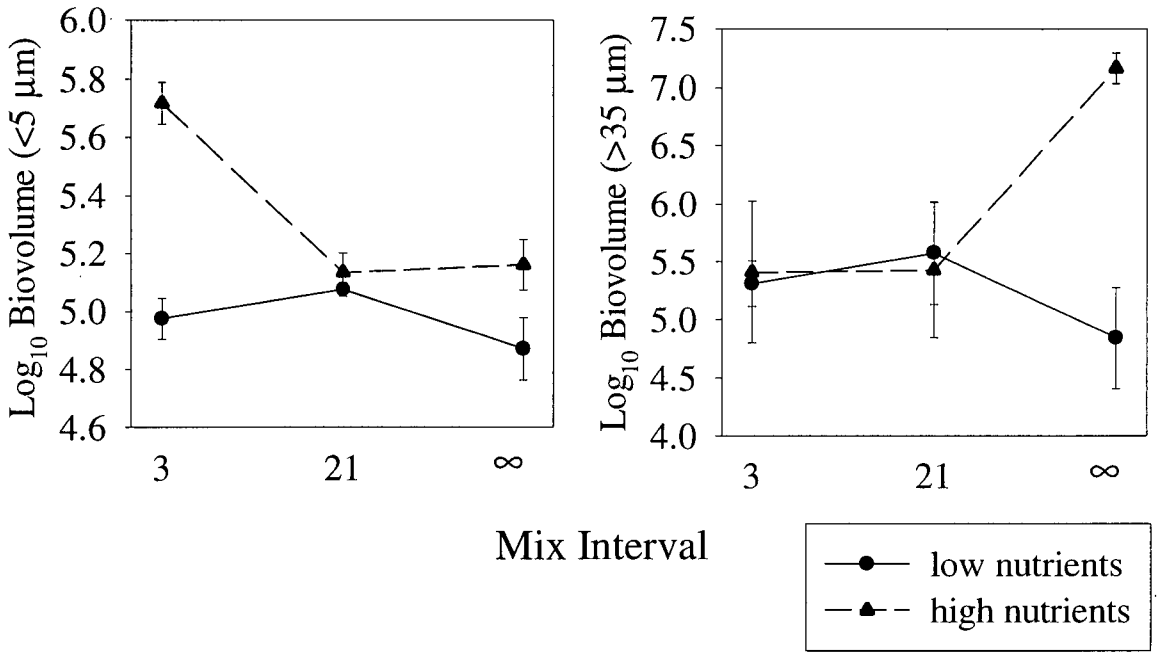


Figure 3.4: Interaction diagrams for mean $\log_{10}$ phytoplankton biovolume (± standard error) (μm^3 ml^{-1}) by size class. Results are for significant interaction effects in the MANOVA. Solid lines represent low nutrient conditions and dashed ones are for enriched conditions.



(ii) Taxonomic Classes

There were no significant responses in the relative abundance or the biovolume vectors representing the taxonomic divisions at the phylum level to the main effects of mix frequency, nutrient levels, or to the interaction of these factors in the MANOVA.

(iii) Morphological Features

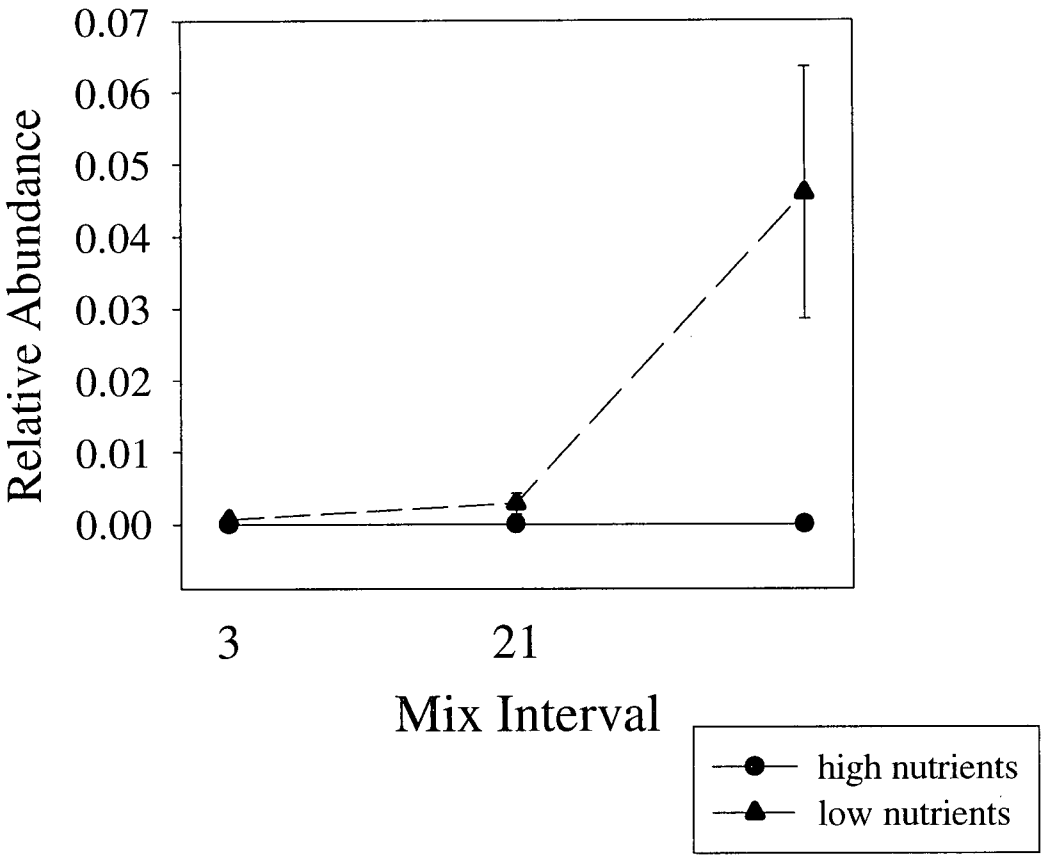
The communities, as described by relative abundance vectors of morphological features, showed a significant interaction response to mixing frequency and nutrient levels (MANOVA: Wilks' Lambda=0.0001, P=0.0028). Analysis of the simple effects of the interaction in the MANOVA revealed that all differences (at all levels of all factors) were significant at the α=0.05 level. From the univariate ANOVA on each class, it became apparent that the interaction effect was due mainly to changes in the filamentous class ($P_{interaction}$=0.0047) (Figure 3.5). When systems were unmixed, the filamentous group did significantly better in enriched than in unenriched systems (P=0.0002). In fact this group was completely absent under oligotrophic conditions. For enriched conditions, lack of mixing promoted higher relative abundances of this group than either infrequent mixing (P=0.0009) or frequent mixing (P=0.0004).

In terms of absolute and relative biovolume changes, there were no significant responses of the various morphological classes to the mixing and nutrient treatments.

(iv) Mobility Classes

The MANOVA on mobility class relative abundance vectors revealed no significant main or interaction effects of mix frequency and nutrient levels.

Figure 3.5: Interaction diagram for abundance of the filamentous morphological features class. Values represent mean relative abundance (± standard error) as a proportion of total density. This group represents the significant interaction effect in the MANOVA. Solid lines represent low nutrient conditions and dashed ones are for enriched conditions.

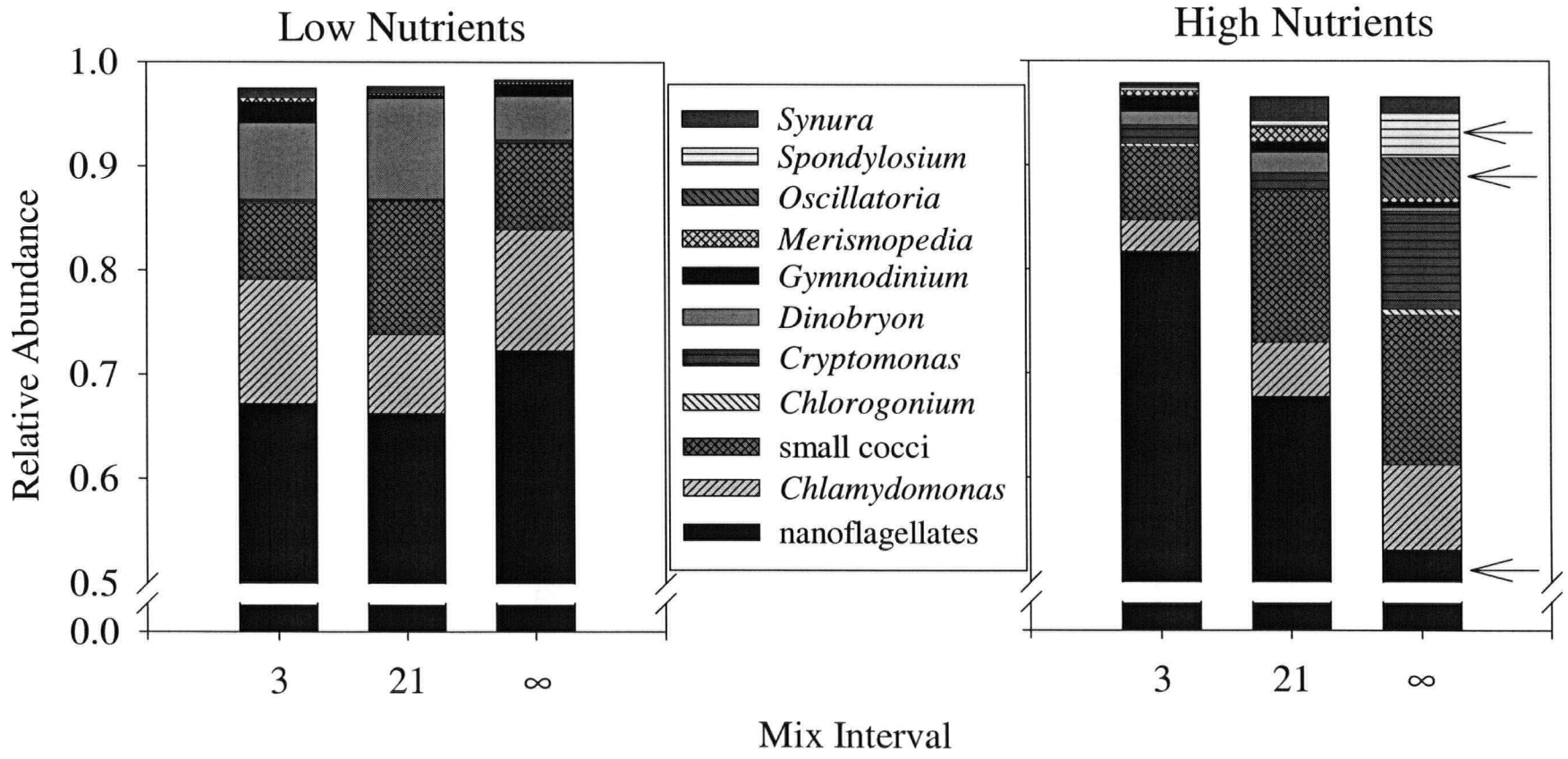


For biovolumes, there was a significant main effect of nutrient levels (Wilks' Lambda=0.038, P=0.0182). Discriminant analysis revealed that the groups leading to the effect were the solitary nonflagellates (Wilks' Lambda=0.417, P=0.0038) and solitary flagellates (Wilks' Lambda=0.29, P=0.0038). Univariate analyses indicated that both of these groups had higher biovolumes under enriched conditions. Solitary nonflagellates had total log biovolumes of 5.98 ± 0.43 μm^3 ml^{-1} under enriched conditions as compared to 4.18 ± 0.22 μm^3 ml^{-1} without enrichment (P=0.0034). For solitary flagellates the means were 5.72 ± 0.1 μm^3 ml^{-1} with enrichment and 5.31 ± 0.05 μm^3 ml^{-1} without (P=0.0016).

(v) Species Composition

Further univariate analyses on the communities of abundant species (>1% relative abundance) demonstrated that the species causing interaction effects in relative abundances were: nanoflagellates ($P_{interaction}$ =0.0471), *Oscillatoria* sp. ($P_{interaction}$ =0.0062), and *Spondylosium* sp. ($P_{interaction}$ =0.0574) (Figure 3.6). Nanoflagellates were more dominant when systems were frequently mixed and nutrient levels were high (3 day vs. unmixed P=0.0004; 3 day vs. 21 day P=0.0004; 3 day, mix -nutrients vs. +nutrients P=0.0002). *Oscillatoria* had higher relative abundances when systems were unmixed and nutrient levels were high (unmixed vs. 21 day P=0.0005; unmixed vs. 3 day P=0.0005; unmixed, -nutrients vs. +nutrients P=0.0005). *Spondylosium* responded exactly as *Oscillatoria* with associated P values of: unmixed vs. 21 day P=0.0145; unmixed vs. 3 day P=0.0129; unmixed, -nutrients vs. +nutrients P=0.0087.

Figure 3.6: Composition plots based on relative abundances of common genera (>1% relative abundance) as a fraction of the total cell density in the various treatments. Arrows indicate groups that showed a significant interaction effect between mix frequency and nutrient levels.



In terms of absolute biovolumes in the abundant taxa, nanoflagellates were the only group that showed an interaction effect ($P_{interaction}$=0.0067) for the treatment applications. For this group, biovolumes were highest under enriched conditions if systems were mixed frequently (P=0.0003). Under enriched conditions, frequent mixing always led to higher biovolumes (all Ps<0.001). Several genera had higher biovolumes under enriched conditions, regardless of mixing frequency, including: *Cryptomonas* sp. (P=0.0032), *Oscillatoria* sp. (P=0.0198) and *Spondylosium* sp. (P=0.0064). Only *Dinobryon* sp. had higher biovolumes under oligotrophic conditions (P=0.0219).

Discussion

The mixing imposed in this experiment was greater than what would commonly be observed in small sheltered lakes like Placid Lake and even in larger lakes where turbulent mixing should be most prevalent. Imboden (1990) summarized turbulence dissipation rates for larger lakes throughout the world and found that typical summer ε values are 10^{-7} m^2 s^{-3} in the epilimnion, 10^{-9} m^2 s^{-3} at the thermocline and 10^{-10} m^2 s^{-3} in the hypolimnion. In a study of Lake Neuchâtel in Switzerland, Kocsis *et al.* (1999) found that high wind conditions (14.6 ms^{-1}) generated turbulence levels in the upper epilimnion of 10^{-6} m^2 s^{-3} and these were associated with waves of up to 0.6 m in height. Some of the highest turbulence dissipation rates recorded for lakes and oceans are on the order of 10^{-5} m^2 s^{-3} and are associated with breaking waves (MacIntyre *et al.* 1999). In my experiment, the values for turbulence that I observed (maximum ε = 10^{-6} m^2 s^{-3}) following mixing were an order of magnitude higher than typical values. Values were in the range associated with high wind conditions, which suggests that plankton in this

experiment were experiencing levels of mixing that should be associated with large mixing events and that are probably rarely observed in such a small lake. Thus the applied treatments were imposing abiotic events with potential to influence vital rates.

The frequency with which competitive systems are disturbed has the potential to affect phytoplankton community structure in terms of diversity or composition. This has been the major tenet of nonequilibrium competition theory such as the intermediate disturbance hypothesis (Richerson *et al.* 1970, Connell 1978, Levins 1979, Armstrong and McGehee 1980, Chesson and Case 1986, Chesson 1994). It is commonly observed in small-scale laboratory experiments with phytoplankton (Robinson and Sandgren 1983, Sommer 1984, 1985, 1995, Gaedecke and Sommer 1986, Brzezinski and Nelson 1988, Grover 1988, 1991a,b). The results of my large-scale experiment suggest that the influence of mixing, which can alter the conditions for phytoplankton in a number of ways (i.e. nutrients, light, proximity of competitors, resuspension of sedimented cells), depends on background environmental conditions. Specifically, the strength of the response by the community to various frequencies of mixing depended on the average environmental productivity.

My results suggest that oligotrophic systems are much more constrained in their responses than eutrophic ones. There are several possible reasons for a dependency on environmental productivity. There may be less "scope" for response by nutrient-deprived communities, with life history strategies constrained by the harshness of the oligotrophic environment. Only the true gleaners with high ratios of surface area to volume, which allow them to maintain high uptake rates and relatively high growth rates, appear to be favoured under such conditions. Thus, nutrient-poor communities have a reduced range

of possible life history types with which to respond to different frequencies of environmental perturbations. Additionally, tighter nutrient recycling through an efficient microbial loop in oligotrophic systems (Goldman 1984, Stone and Weisburd 1992, Wehr *et al.* 1994), may result in smaller nutrient "pulses" when vertical mixing occurs. Measurements taken in 1997 (Chapter 4) and 1998 (presented in this chapter) on the nutrient (orthophosphate) pulse accompanying a mixing event indicate that the pulse was smaller in the oligotrophic treatments. Finally, based on local stability arguments, it is possible that a large scale perturbation like enrichment that moves populations away from their natural equilibria could sensitize phytoplankton communities to further perturbations like vertical mixing occurring at different scales.

It appears that the life history strategy that best allows phytoplankton to survive under very low nutrient conditions constrains the community composition such that the same species are seen regardless of mixing frequencies. Solitary nanoflagellates, non-motile chlorococcales, and species like *Chlamydomonas* sp. and *Dinobryon* sp. dominated to similar extents in all the oligotrophic communities. These groups (small chlorophytes and small chrysophytes) are known to do better in oligotrophic environments where they compete well for scarce nutrients (Reynolds 1984a). Some, like the chrysophytes, can even adopt heterotrophic strategies when required (Sandgren 1988). Thus, we should consider these communities as consisting mainly of gleaners. *Chlamydomonas* sp. is generally thought of as a good competitor in mixed environments (Reynolds 1984a), and their small size with high surface area may enable them to do well under low mixing conditions as well (i.e. gleaner types) (Happey-Wood 1988). *Dinobryon* spp. are considered to be restricted to oligotrophic environments (Reynolds

1984a) and are mixotrophic so their success under my low nutrient conditions was expected. Very small opportunists like chlorococcales and nanoflagellates also benefit from their ability to use microzones of nutrients efficiently with high ratios of surface area to volume and high growth rates (Reynolds 1984a, Happey-Wood 1988, Sandgren 1988). Given that nutrient pulses are so small in my oligotrophic systems, it is unlikely that a "storage" strategy would be viable. This is supported by the absence of very large cells from the nutrient-poor mesocosms even though seed densities of larger phytoplankton from a eutrophic lake were added weekly. This observation supports cross-lake empirical relationships for phytoplankton size structure as a function of lake productivity, which have shown that large cells are generally excluded from oligotrophic lakes (Paloheimo *et al.* 1982, McCauley *et al.* 1989, Watson *et al.* 1992).

Enriched conditions appear to permit a greater range of responses by the phytoplankton communities under different mixing regimes. The nutrient data collected in 1998 shows that pulses in orthophosphate were larger in the enriched bags. Biovolumes or biomass levels were higher for several phytoplankton groups under enriched conditions. Increases were observed mainly in nanoflagellates (especially with frequent mixing) such as *Cryptomonas* sp. and in *Oscillatoria* sp. and *Spondylosium* sp. Small opportunists like nanoflagellates appear to respond well to very frequent but larger nutrient pulses, while water column stability favours larger filamentous types. I hypothesize that different processes are in operation in each case, that are not simply related to the frequency of nutrient pulsing. A complete lack of mixing allowed the larger filamentous types to thrive, an observation that has been noted by others (e.g. Reynolds 1984a, Paerl 1988). Filamentous algae (cyanobacteria especially) can

compensate for poor nutrient uptake ability by modifying their buoyancy and thus moderating their position in the water column and their access to light (Paerl 1988). Under conditions of frequent mixing as in this experiment, they lose this competitive advantage and are forced to compete more intensely because of a disrupted spatial distribution (on centimetre to metre scales). On the other hand, small opportunists like nanoflagellates are dominant when mixing is frequent. This is likely due to their ability to respond to pulses in nutrients that mixing provides because of high growth rates and high surface area to volume ratios that allow for high nutrient uptake (Reynolds 1988). In fact, these types did so well under nutrient-enriched conditions that they reduced the evenness and therefore diversity index when mixing was frequent.

At high nutrient levels, the growth of many different types of phytoplankton (high diversity and evenness) was favoured when mixing was less frequent or completely absent. This result suggests that spatial structuring naturally present in phytoplankton communities (i.e. vertical thin layers, small-scale horizontal patchiness) plays an important role in mediating coexistence of competitors. Lack of frequent mixing resulted in a water column with very little to no forced interruption to the spatial structuring. Frequent mixing led to fewer local interactions (at mm to cm scales) and more global ones among species, which implies an increased intensity of population interactions from the point of view of mass-action modelling. Indeed, theory predicts that more intense population interactions should lead to less stable dynamics and a greater likelihood of extinctions (Durrett and Levin 1994, Holmes *et al.* 1994). Note, however, that this argument appears to hold only under enriched conditions. The strategy that best allows species to exist under oligotrophic conditions may be one that allows for tolerance of low

nutrients with a higher tolerance for patchy resource distributions. Thus, the spatial distribution of competitors relative to each other may be of less consequence in nutrient-poor environments, especially where most community members are mobile, as they are here. Adaptations to low nutrient levels and mobility may reduce the importance of heterogeneity in nutrient supply for oligotrophic communities.

It appears that the role of vertical mixing for phytoplankton community structure goes beyond simple nutrient pulsing. In fact, nutrient pulsing in lakes may be a minor consequence of mixing in natural systems, despite the strong responses observed in laboratory studies that consider pulses of nutrients (Quarmby *et al.* 1982, Robinson and Sandgren 1983, Sommer 1984, 1985, 1995, Gaedecke and Sommer 1986, Brzezinski and Nelson 1988, Grover 1988, 1991a,b). In my study under semi-natural conditions, the pulse sizes of PO_4^{-3} even under enriched conditions, were small (14 $\mu g\ l^{-1}$) compared to laboratory studies which include additions of 35 $\mu g\ l^{-1}$ (Sommer 1985) or 0.3 d^{-1} medium dilution rates (Gaedecke and Sommer 1986). Rather, it appears that at the community level, the spatial disruption induced by mixing is more important. Whether phytoplankton systems respond with changes in diversity and composition under mixing regimes with different time scales depends on the nutrient status of the system. Communities in nutrient-poor systems have fewer species and a limited ability to respond to different frequencies of mixing even with the addition of "seed" exotics. High nutrient levels allow for a larger range of species of phytoplankton to persist, leading to a greater observed diversity of forms and a greater diversity of responses to different scales of mixing.

CHAPTER 4

Phytoplankton Community Structure: The Role of Herbivory in Variable Environments

This chapter presents an experiment I conducted under oligotrophic (natural) conditions to investigate how the addition of a common generalist herbivore might affect or induce a response of phytoplankton communities to different frequencies of environmental heterogeneity.

Introduction

Equilibrium resource competition theory predicts low diversity in ecological systems in the absence of elaborate niche partitioning. In contrast, natural plankton systems have high diversity, and this is the source of Hutchinson's (1961) oft-cited "paradox of the plankton". A major focus of ecological research has been the search for mechanisms that allow for high diversity in competitive systems. Identified mechanisms fall into three major categories: temporal environmental variability, spatial heterogeneity in resource and/or population distributions and predator-mediated coexistence. Each of these factors may play roles in determining the diversity of real phytoplankton communities.

Role of Environmental Heterogeneities

Phytoplankton in epilimnetic waters of north temperate lakes are exposed to several sources of temporal and spatial variability in important growth factors like nutrients and light. These heterogeneities are related largely by the turbulent movement of water that occurs with different frequencies and magnitudes during the growing season. Mixing events that occur as a result of episodic storms throughout the growing

season can lead to an influx of nutrient rich, plankton-poor water from the deeper zones into the epilimnion of lakes (Harris and Griffiths 1987, Reynolds 1993), as well as transport plankton themselves from one region to another. Water movements can result in a redistribution of phytoplankton cells, thereby altering the nutrient and light regimes to which they are exposed. Because they occur intermittently, these water movements expose plankton to temporal heterogeneity in ecological conditions that could have consequences for competition. Anthropogenic events such as the global climate warming predicted to occur over the next 50 years may change the pattern of storm events and alter the frequency and intensity of episodic mixing events (Walker 1991, Carpenter *et al.* 1992, Lathrop *et al.* 1999).

It is difficult to separate temporal and spatial environmental variability for phytoplankton systems where the temporal fluctuations in resource and light levels arise from the physical movement of water, organisms and nutrients. As phytoplankton are moved, the most likely change an individual cell will experience is in nutrient and light availability. In general, this change should be a net positive one, because of either the bulk upward movement of nutrient-rich hypolimnetic water, or eradication of nutrient-depleted patches, especially in meso- to eutrophic lakes (Reynolds *et al.* 1983, 1984b). This should occur despite the potential redistribution of high nutrient patches that occur at small scales from zooplankton excretion (e.g. Lehman and Scavia 1982). Plankton which have fallen below the thermocline may be re-exposed to high light levels with the upwelling of water, while those originally in the photic zone may be displaced to lower light-level regions in the hypolimnion with mixing events. But mixing may also reduce mean light levels if detritus is resuspended (Reynolds 1989). Finally, for the

heterotrophic nanoflagellates which are commonly dominant in more oligotrophic systems (Malone 1980, Porter *et al.* 1988), bacteria from deeper waters (with rich sources of dissolved organic carbon) may provide a pulse of resources with mixing. Therefore, temporal heterogeneity in resource and light supply will be the most obvious consequences of vertical mixing in lakes.

Given phytoplankton reproductive rates, ecologically meaningful high frequency within-season fluctuations should occur every few days (time scales that match cell generation times). Intermediate scales should be on the order of several days to a couple of weeks, and very low frequency fluctuations should occur less than once every few weeks (Reynolds 1993). Phytoplankton community composition changes in response to nutrient fluctuations that may accompany vertical mixing should be determined in large part by competitive interactions, which are related to the physiological capabilities of the organisms involved (Quarmby et al. 1982, Robinson and Sandgren 1983, Sommer 1984, 1985, Gaedecke and Sommer 1986, Brzezinski and Nelson 1988, Reynolds 1988, Lindenschmidt and Chorus 1998). In Chapter 3, I noted that temporal heterogeneity might in fact be less important than vertical spatial heterogeneity in permitting and promoting diversity. Enrichment also helps promote higher diversity, when coupled with stability in vertical structure (see Chapter 3). To my knowledge, no other studies have experimentally examined the role of intermittent vertical mixing in oligotrophic systems.

Role of Herbivory

Another factor that can influence the diversity of competitive systems, and which should be relevant for phytoplankton in most freshwater systems, is predation (Paine 1966, Lubchenco 1978, Armstrong 1979). In lakes, the cladoceran zooplankton *Daphnia*

sp. is generally considered the herbivore of greatest importance because of its high grazing rates, generalist foraging, and quantitative effects on the success of higher trophic levels (reviewed in Sterner 1989). *Daphnia* can have major effects on the size structure and composition of phytoplankton communities (e.g. McCauley and Briand 1979, Lynch and Shapiro 1981, Lehman and Sandgren 1985, Kerfoot *et al.* 1988, Leibold 1989).

The influence of predation on prey competition, and ultimately, on diversity will depend on the predator population dynamics as compared to rates of competitive exclusion. Although predation may fluctuate in time or space, it can have different effects than abiotic forces because of the possibility of strong feedback between predator and prey populations. Diversity of prey can be enhanced by predation that reduces densities of competitive dominants (e.g. Paine 1966, Murdoch and Oaten 1975, Lubchenco 1978, Armstrong 1979, Abrams 1987, Holt and Lawton 1994). On the other hand, indiscriminate predation does not necessarily increase diversity and may actually reduce it because the most productive prey species support high predator numbers (Cramer and May 1972).

Herbivorous zooplankton can discriminate among prey, often based on prey cell sizes (Bergquist *et al.* 1985, Lehman and Sandgren 1985, Gilbert 1988, Sterner 1989, Edgar and Green 1994). In addition, differences in functional and numerical responses (i.e. attack and reproductive rates) among herbivore species lead to differences in both the size-structure and taxonomic diversity of the phytoplankton. Communities dominated by crustacean zooplankton like *Daphnia* have higher grazing rates than those dominated by smaller zooplankton like rotifers (see Sterner 1989). *Daphnia* grazing can increase phytoplankton community richness (McCauley and Briand 1979) when it does not exceed

the capacity of many phytoplankton taxa to reproduce. Herbivory by larger zooplankton like *Daphnia* tends to result in high losses for smaller, more spherical phytoplankton (competitive dominants), which allows for the proliferation of larger, more inedible types (e.g. Leibold 1989, Watson *et al.* 1992, Cottingham 1999).

Interaction of Environmental Heterogeneity and Predation

The impact on community diversity of interactions between resource fluctuation and predation are not well understood. Menge and Sutherland (1987) suggest that the role of predators in regulating prey densities, and therefore the outcome of competition among prey species, can be dependent on temporal changes in the environment. How intermittent mixing on various time scales influences phytoplankton community structure in the presence of predators is unknown.

In the absence of mixing in lakes, there is potential for natural spatial structure to form in the distribution of phytoplankton owing to local abiotic (i.e. temperature, light) preferences of mobile taxa, differential sinking rates (Talling 1957, Jones and Ilmavirta 1988, Reynolds 1992), and patchy distributions of zooplankton (Colebrook 1960, Steele 1974, Steele 1978, Okubo 1978, Tessier 1983, Threlkeld 1983, Urabe 1989). Zooplankton patchiness can arise at small scales (<1 m) because of limited mobility and a tendency of zooplankton like *Daphnia* to aggregate in areas of high food density (Young and Getty 1987, Dibble 1993, Cuddington and McCauley 1994, Neary *et al.* 1994). Frequent mixing should disrupt the natural spatial structure of both phytoplankton and zooplankton, thereby increasing the encounter rates between different species (Haury *et al.* 1990, Browman 1996, Yamazaki 1996), especially if patches are generally monospecific. More intense population interactions can lead to greater instability and

higher likelihood of extinctions in both competitive and predator-prey systems (Luckinbill 1974, Murdoch and Oaten 1975, de Roos *et al.* 1991, McLaughlin and Roughgarden 1991, Timm and Okubo 1992, McCauley *et al.* 1993, Wilson *et al.* 1993, Durrett and Levin 1994, Holmes *et al.* 1994). Instability arises essentially because of a lack of spatial and temporal refuges in fully mixed systems (Murdoch and Oaten 1975, McLaughlin and Roughgarden 1991).

This chapter presents the results of an experiment to determine the influence of intermittent mixing (temporal and spatial heterogeneity), different types of predation, and the interaction of these factors on phytoplankton community structure in an oligotrophic lake. Community structure encompasses both diversity (species numbers and evenness) and composition (i.e. size, morphology and taxonomy).

Methods

Mesocosm experiments consisted of replicate plankton communities isolated and suspended in partly darkened polyethylene bags as described in Chapter 3. The experimental treatments consisted of two factors with three levels for vertical mixing and two levels for herbivory. There were three replicate bags per treatment for a total of 18 bags. Mixing treatments were (i) frequent mixing (every 5 days), (ii) intermediate frequency mixing (15 days), and (iii) infrequent mixing (25 days). These time scales were chosen to correspond to both phytoplankton and zooplankton generation times and expected rates of response. Frequency and intensity of the mixing treatments were inversely related such that the 25 day treatments were mixed for a period of time 1.6 times longer than the 15 day treatments and 5 times longer than the 5 day treatments to ensure that the total mixing associated with each treatment was similar over the longest

time scale (i.e. each 25 day period). The mixing events involved turnover of 1/2 of the volume (~2000 l) in the bags for the 25-day treatment (approximately 1 h of mixing time), 1/4 of the volume (~1000 l) for the 15-day treatments and 1/10 of the volume (~ 400 l) in the 5 day treatments so that the volume of water turned over in each bag was the same for each 25 day period. Mixing was accomplished by the vertical movement of water using a bilge pump to bring water from lower depth (4 m) to the surface of the bag. Herbivore (predator) treatments consisted of systems with mature *Daphnia rosea* excluded or *Daphnia rosea* added.

The bags were filled initially with lake water from several depths, filtered to 54μm to exclude crustacean zooplankton. The bags were filled on May 13 and 14, 1997. Over a period of two weeks (May 22 to June 3 1997), a total of 1.2 individuals l^{-1} of *Daphnia rosea* were inoculated into half the bags to provide the plus-*Daphnia* treatment. *Daphnia* were collected by gentle vertical tows in the lake with a plankton net and were separated by hand-picking from other zooplankton before inoculation into the appropriate bags.

Unfortunately, as previous experience has shown (W.E. Neill, *personal communication*), *Daphnia* have an uncanny ability to invade experimental enclosures from which they have been apparently excluded, perhaps through bag to bag transfer through contamination of sampling gear. All *Daphnia*-free mesocosms in this study were subject to a small invasion of *Daphnia* by the mid-point of the experiment (i.e. starting on approximately Julian day 208 for all bags). Nevertheless, there were still large enough differences between the treatments to allow for meaningful comparisons. First, there were 40 days during which no *Daphnia* were detected and treatment comparisons can

therefore be made on the basis of whether *Daphnia* were initially present. Second, even though *Daphnia* invaded, the population size structure in the two cases differed radically. Systems that had *Daphnia* stocked initially were composed of very large, adult individuals, while those undergoing invasion consisted of small populations of juvenile *Daphnia* during the time period for which phytoplankton counts were done. Communities of smaller zooplankton in general consume lower quantities of phytoplankton over a smaller size range (Leibold 1989). For these reasons, the treatments will hereafter be referred to as initially planned, as either + or - *Daphnia*.

The first mixing application occurred on June 9 1997 (Julian day 160), and this was also the start of the sampling period.

(i) Sampling

Phytoplankton were sampled immediately before mixing, whenever mixing events occurred for each bag. Bags were sampled three days following mixing and every five days thereafter until the next mixing event occurred, at two depths (1.5 m and 4 m from the surface), using a 2 l Van Dorn bottle. A 250 ml sample was preserved immediately using several drops of Lugol's iodine. In the laboratory, phytoplankton were counted over a full 25 day cycle during the warmest period of the summer, from July 24-August 23 (Julian days 205-235), when the lake was maximally stratified. Enumeration and measurements of cell size and shape were done using an inverted microscope and 50 ml of preserved sample that had settled for 20 hours. Transects were counted at 200X magnification until at least 100 cells of the most common group had been enumerated. Phytoplankton were identified to genus except for small unidentified flagellates (<3 μm MLD or maximum linear dimension), which were counted as one

group. A list of taxa encountered and classification into various functional groups is given in Table 3.1.

Samples for chlorophyll *a* extraction were taken at each depth on every sampling occasion. A 60 ml water sample taken with the Van Dorn sampler at each sampling depth was filtered onto a GF/F filter. Filters were kept in the dark and frozen for a period no more than 3 weeks until analysis could be done. Fluorometric (Turner Designs, Model 10 Analog Fluorometer) determination of chlorophyll *a* was done with 90% ethanol extraction (Nusch 1980). Nutrient samples for PO_4^{-3} (Soluble reactive phosphate or SRP) were taken from one replicate per treatment prior to and just after mixing events on July 4 and August 23, 1997. The water samples were filtered using a combusted GF/F filter and frozen until later analysis with an autoanalyser.

In 1999, *in situ* video measurements of *Daphnia* distributions were done inside a bag set up with phytoplankton and *Daphnia* earlier that summer. A video camera with a field of view of 7cm x 7cm x 1cm was lowered to 1.5 m from the surface of the water. Video footage of *Daphnia* individuals was shot *in situ* for 30 min before mixing, during a mixing event, and for another 30 min post-mixing. For analysis, *Daphnia* positions from still photographs from 5 min intervals pre- and post-mixing were digitized (total of 7 photos pre-mixing and 5 photos post-mixing). Inter-neighbour distances were calculated based on Euclidean distances, and from these an index of dispersion (variance/mean) was calculated for each photograph. The values before and after mixing were compared with a t-test, with each photo representing a pseudo-replicate (because of temporal auto-correlation) measure.

(ii) Data and Statistical Analyses

Rainfall and temperature data were obtained from the weather station at the Malcolm Knapp Research Forest and from these, three periods for some data analyses were determined (see Results).

Because few differences and trends in the time series for individual phytoplankton densities were observed, average values of relative abundance over the 30 day period for which samples were counted (July 24-Aug. 23; Julian days 205-235) were used for statistical analyses. In addition, unless otherwise indicated (i.e. for analyses of spatial distributions), samples were averaged over the two depths measured. Calculations for diversity indices and analyses for these and for community composition (i.e. relative abundances and biovolumes of major groups and taxa) are outlined in the methods section of Chapter 3 and a major overview of the tests are given in Appendix 2.

To assess vertical spatial structure in the distributions of phytoplankton and zooplankton in the bags, the coefficient of variation (CV) between the shallow and deep samples in each bag was used. Higher coefficients correspond to greater differences between sites in the variables measured, i.e. more spatial heterogeneity. CVs were calculated for chlorophyll, phytoplankton diversity measures and total *Daphnia* densities. These coefficients were averaged over time and analyzed in a 2-way ANOVA to detect treatment differences in the amount of variation between spatial locations in measured variables (average values over each climatic period for chlorophyll and over the entire period counted for phytoplankton diversity).

Results

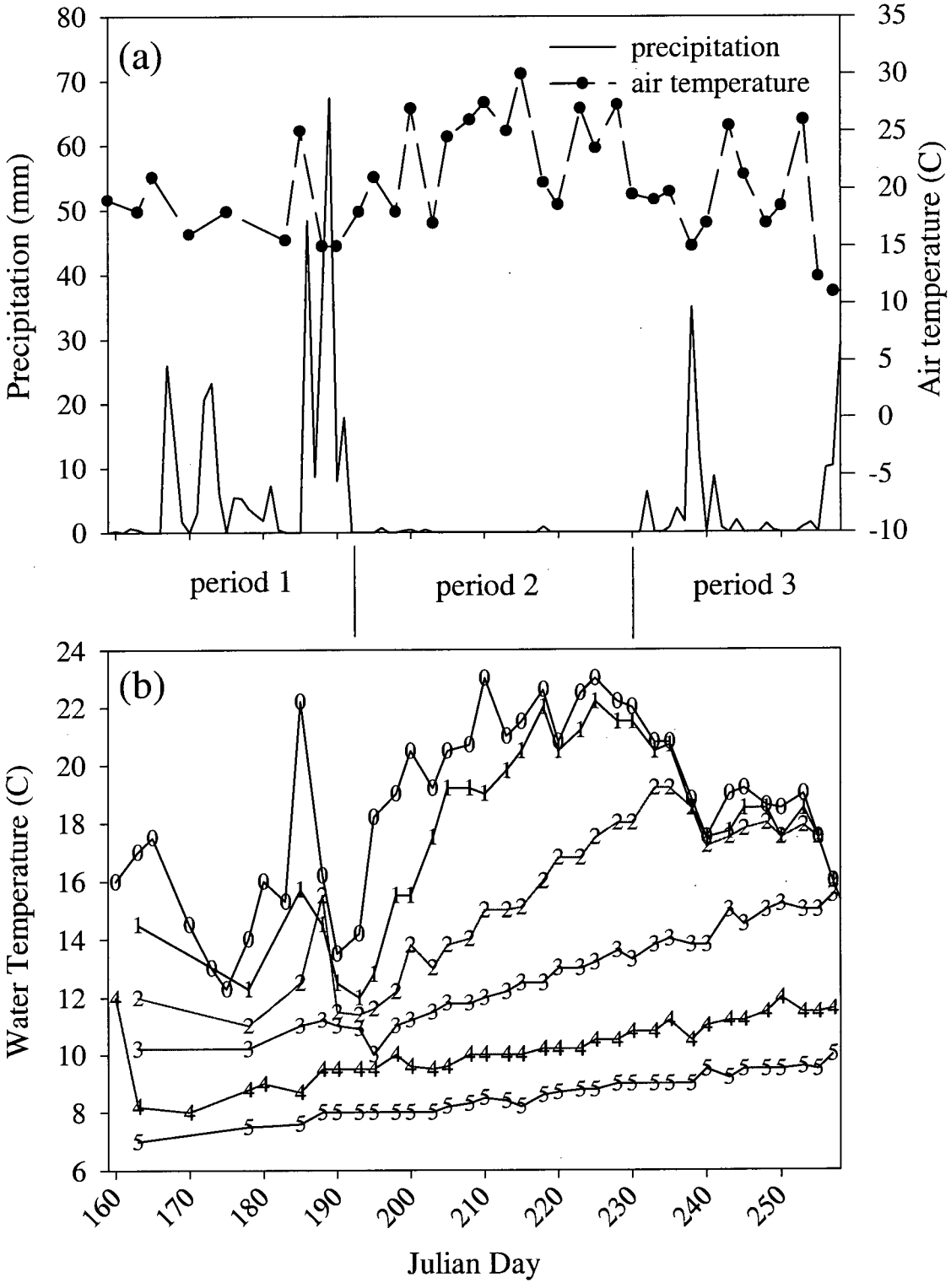
Physical Effects

Precipitation and temperature data are shown in Figure 4.1. From these data it became apparent that there were 3 key climatic periods during the summer of 1997 in the region around Placid Lake. Period 1 consisted of cool and especially wet days, from the beginning of the experiment, June 9 (Julian day 160) to July 11 (Julian day 192). Period 2 (July 11-Aug. 21; Julian days 193-232) was dry with warm summer conditions. Finally, cold, rainy weather dominated again during period 3 (Aug. 21-Sept. 16; Julian days 233 to 258). The thermocline of the lake did not become firmly established until period 2 and then broke down again during period 3 (Figure 4.1). For this reason, most of the focus of the results and analyses was on data collected during period 2. Phytoplankton data were collected and counted for a period that overlapped mostly with period 2.

Water temperatures indicated that strong lake density stratification occurred only after July 19 (Julian day 200), when the thermocline was at a depth of approximately 1.5 m (Figure 4.1b). The depth of the thermocline reached 2.5 to 3 m after Aug. 8 (Julian day 220) (Figure 4.1b).

Mixing events in this experiment did not cause phosphate concentrations to increase above detection limits (soluble reactive phosphate or SRP $< 1\ \mu g\ l^{-1}$). Nutrient levels were unmanipulated in this experiment (i.e. represented the oligotrophic lake conditions).

Figure 4.1: (a) Air temperatures and precipitation levels and (b) water temperatures at depths (in meters indicated by the symbols) in Placid Lake during the summer of 1997.



Phytoplankton Biomass

Mean chlorophyll levels (estimates of phytoplankton biomass) were calculated over the three climatic periods identified from the physical data (Table 4.1). The mean chlorophyll levels were not affected by either main or interaction effects of *Daphnia* presence/absence and mix frequency. Over time, however, chlorophyll levels increased in all treatments (Table 4.1).

Phytoplankton Community Diversity

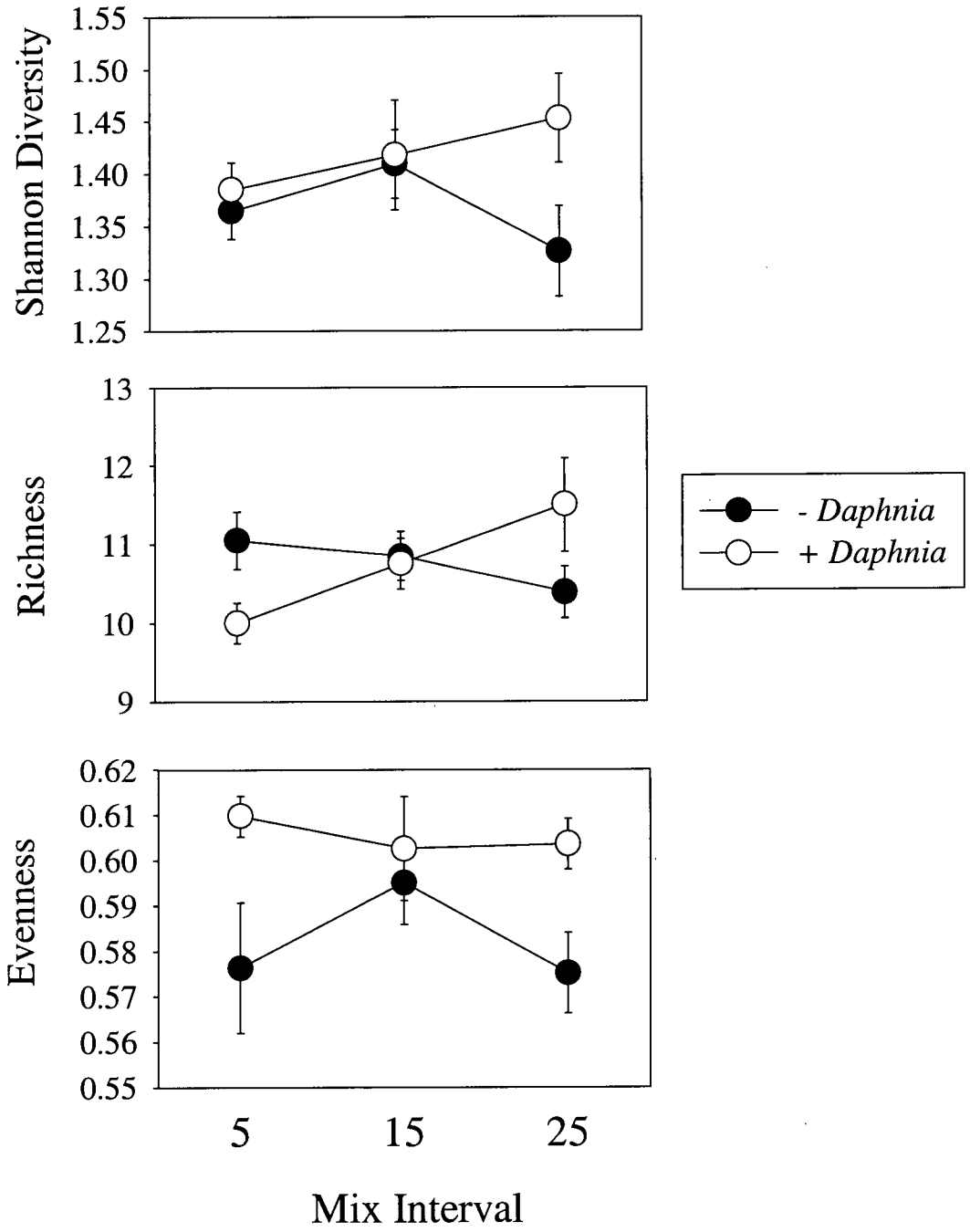
For phytoplankton community richness, there was a significant interaction between frequency of mixing and the presence of *Daphnia* ($P_{interaction}$=0.0285) (Figure 4.2). When mixing occurred least frequently (25 days), richness was highest in the presence of *Daphnia* (P=0.0474). An opposite trend was found when mixing occurred frequently (5 days). Here, richness was slightly less in the presence of *Daphnia* (P=0.0614). At intermediate mixing frequencies (15 days) there was no effect of *Daphnia* on richness levels. In the presence of *Daphnia* only, richness was significantly higher for the 25 day mixing regime than for the 5 day mixing (P=0.0095). In the absence of the herbivore, there was no significant effect of mixing schedule on richness (P>0.05) (Figure 4.2). Phytoplankton species diversity (the Shannon index) was unaffected by the treatments (no significant main effects nor a significant interaction of *Daphnia* and mix frequency (Figure 4.2)).

Daphnia significantly increased the evenness of phytoplankton communities (P=0.03; +*Daphnia* mean ± 1 s.e. = 0.61± 0.006 (n=3), -*Daphnia* = 0.58± 0.009). Phytoplankton community evenness was not significantly affected by mixing frequency nor an interaction of mix frequency and *Daphnia* (Figure 4.2).

Table 4.1: Mean (± standard error) chlorophyll *a* values ($\mu g\ l^{-1}$) in each treatment averaged over time in each climatic period.

Mix Period (days)	*Daphnia* Level	Climatic Period 1	Climatic Period 2	Climatic Period 3
5	-	1.80 ± 0.17	6.00 ± 3.24	35.39 ± 8.06
5	+	1.83 ± 0.08	1.49 ± 0.18	27.31 ± 11.89
15	-	1.75 ± 0.17	2.82 ± 0.88	25.37 ± 13.23
15	+	1.92 ± 0.09	2.88 ± 1.45	26.37 ± 16.86
25	-	2.00 ± 0.48	1.79 ± 0.24	32.07 ± 5.75
25	+	1.95 ± 0.09	3.71 ± 0.95	31.31 ± 4.85

Figure 4.2: Interaction effects of levels of *Daphnia* and mixing frequency on measures of phytoplankton community diversity. Error bars represent ± one standard error of the mean. Data are from averages calculated over the entire period counted.



Phytoplankton Community Composition

(i) Size Classes

For relative abundances, a MANOVA based on size class vectors revealed significant main effects of both mixing frequency (Table 4.2) and *Daphnia* presence (Table 4.3) on the phytoplankton communities, but no interaction (Table 4.4). A discriminant analysis on these main effects showed that the mixing effect was mainly due to changes in the 6 to 20 μm class, which had highest relative abundances in the very frequent and infrequently mixed systems and lower abundances in the intermediate (15 day) treatments. For the *Daphnia* effect, the main groups leading to differences based on the discriminant analysis were: the 21-35 μm and the >35 μm class. Both of these larger size classes were significantly more abundant in the presence of *Daphnia*. For biovolume measurements, there was a significant main effect of only *Daphnia* (Table 4.3). The 21-35 μm and the >35 μm classes did significantly better in the presence of *Daphnia* while the <5 μm and the 6-20 μm groups had higher total biovolumes when *Daphnia* were initially excluded.

(ii) Taxonomic Classes

There was no significant main effect of mixing frequency (Table 4.2) nor an interaction effect (Table 4.4) in the MANOVA for relative abundances. There was a significant effect of *Daphnia* presence (Table 4.3). The cyanophytes and the cryptophytes were relatively more abundant in the presence of *Daphnia*, while the chlorophytes, and the pyrrhophytes (dinoflagellates) were more abundant in the absence of *Daphnia*.

Table 4.2: The *P*-values and Wilks' Lambda values for the main effect of mixing frequency in the 2-way MANOVAs on relative abundances and biovolumes. Values for the groups within each class are based on the stepwise discriminant analysis. The final group (5) represents the *P*-values from the 2-way ANOVA on relative abundances and biovolumes for each species separately.

Group	*Relative Abundance*		*Biovolume*	
	***P*-value**	**Wilks' λ**	***P*-value**	**Wilks' λ**
1. Size Classes (μm)				
MANOVA	**0.0205**	**0.17**	**ns**	**ns**
<5	ns	ns	ns	ns
6-20	0.016	0.58	ns	ns
21-35	ns	ns	ns	ns
>35	ns	ns	ns	ns
2. Taxonomic Classes				
MANOVA	**ns**	**ns**	**ns**	**ns**
3. Morphological Features				
MANOVA	**ns**	**ns**	**ns**	**ns**
4. Mobility Classes				
MANOVA	**ns**	**ns**	**ns**	**ns**
5. Species Composition				
Chlamydomonas	0.0338	n/a	ns	n/a
Nanoflagellates	0.0238	n/a	ns	n/a

Table 4.3: The *P*-values and Wilks' Lambda values for the main effect of *Daphnia* in the 2-way MANOVAs on relative abundances and biovolumes. Values for the groups within each class are based on the stepwise discriminant analysis. The final group (5) represents the *P*-values from the 2-way ANOVA on relative abundances and biovolumes for each species separately.

Group	*Relative Abundance*		*Biovolume*	
	***P*-value**	**Wilks' λ**	***P*-value**	**Wilks' λ**
1. Size Classes (μm)				
MANOVA	**0.0002**	**0.11**	**0.0001**	**0.04**
<5	ns	ns	0.0001	0.08
6-20	ns	ns	0.0001	0.12
21-35	0.0001	0.38	0.0001	0.33
>35	0.0001	0.27	0.0001	0.07
2. Taxonomic Classes				
MANOVA	**0.0014**	**0.05**	**0.0045**	**0.07**
Chlorophyta	0.0001	0.11	ns	ns
Chrysophyta	ns	ns	ns	ns
Bacillariophyta	ns	ns	ns	ns
Cyanophyta	0.0001	0.14	ns	ns
Cryptophyta	0.0001	0.17	0.0001	0.13
Euglenophyta	ns	ns	ns	ns
Pyrrhophyta	0.0001	0.31	0.0001	0.17
3. Morphological Features				
MANOVA	**0.0252**	**0.19**	**ns**	**ns**
Round	0.0005	0.46	ns	ns
Long	ns	ns	ns	ns
Filamentous	ns	ns	ns	ns
Spiny	ns	ns	ns	ns
Hairy/large flagella	ns	ns	ns	ns
Amorphous	ns	ns	ns	ns
4. Mobility Classes				
MANOVA	**0.0003**	**0.11**	**0.0059**	**0.22**
Solitary, nonflagellates	0.0001	0.22	0.0007	0.38
Solitary, flagellates	ns	ns	ns	ns
Colonial nonflagellates	0.0001	0.34	0.0005	0.46
colonial flagellates	ns	ns	ns	ns
5. Species Composition				
Ankistrodesmus	0.0001	n/a	0.0001	n/a
Chlamydomonas	0.0003	n/a	0.009	n/a
Cryptomonas	0.0003	n/a	0.0006	n/a
Gymnodinium	0.0001	n/a	0.0001	n/a
Mallomonas	0.0036	n/a	0.0051	n/a
Nanoflagellates	0.003	n/a	ns	n/a
Non-motile chlorococcales	0.0001	n/a	0.0001	n/a
Oocystis	0.0001	n/a	0.0001	n/a

Table 4.4: The *P*-values and Wilks' Lambda values for the interaction effect of *Daphnia* and mixing frequency in the 2-way MANOVAs on relative abundances and biovolumes. Values for the groups within each class are based on the stepwise discriminant analysis. The final group (5) represents the *P*-values from the 2-way ANOVA on relative abundances and biovolumes for each species separately.

Group	*Relative Abundance*		*Biovolume*	
	***P*-value**	**Wilks' λ**	***P*-value**	**Wilks' λ**
1. Size Classes (μm)				
MANOVA	**ns**	**ns**	**ns**	**ns**
2. Taxonomic Classes				
MANOVA	**ns**	**ns**	**ns**	**ns**
3. Morphological Features				
MANOVA	**ns**	**ns**	**ns**	**ns**
4. Mobility Classes				
MANOVA	**ns**	**ns**	**ns**	**ns**
5. Species Composition				
Non-motile chlorococcales	ns	n/a	0.01	n/a
Merismopedia	ns	n/a	0.05	n/a
Tetradesmus	ns	n/a	0.058	n/a
Staurastrum cornutum	ns	n/a	0.03	n/a

Examination of the biovolumes revealed similar trends for the cryptophytes and for the pyrrhophytes (dinoflagellates) with the former having higher biovolumes in the presence of *Daphnia* and the latter having higher biovolumes in the absence of *Daphnia* (Table 4.3).

(iii) Morphological Features

The MANOVA based on vectors of morphological features for relative abundances showed only a significant effect of *Daphnia* presence (Table 4.3). The discriminant analysis revealed that this was due to a significant decline in the "round" group in the presence of *Daphnia*.

There were no significant effects in the biovolume MANOVA for morphological feature classifications.

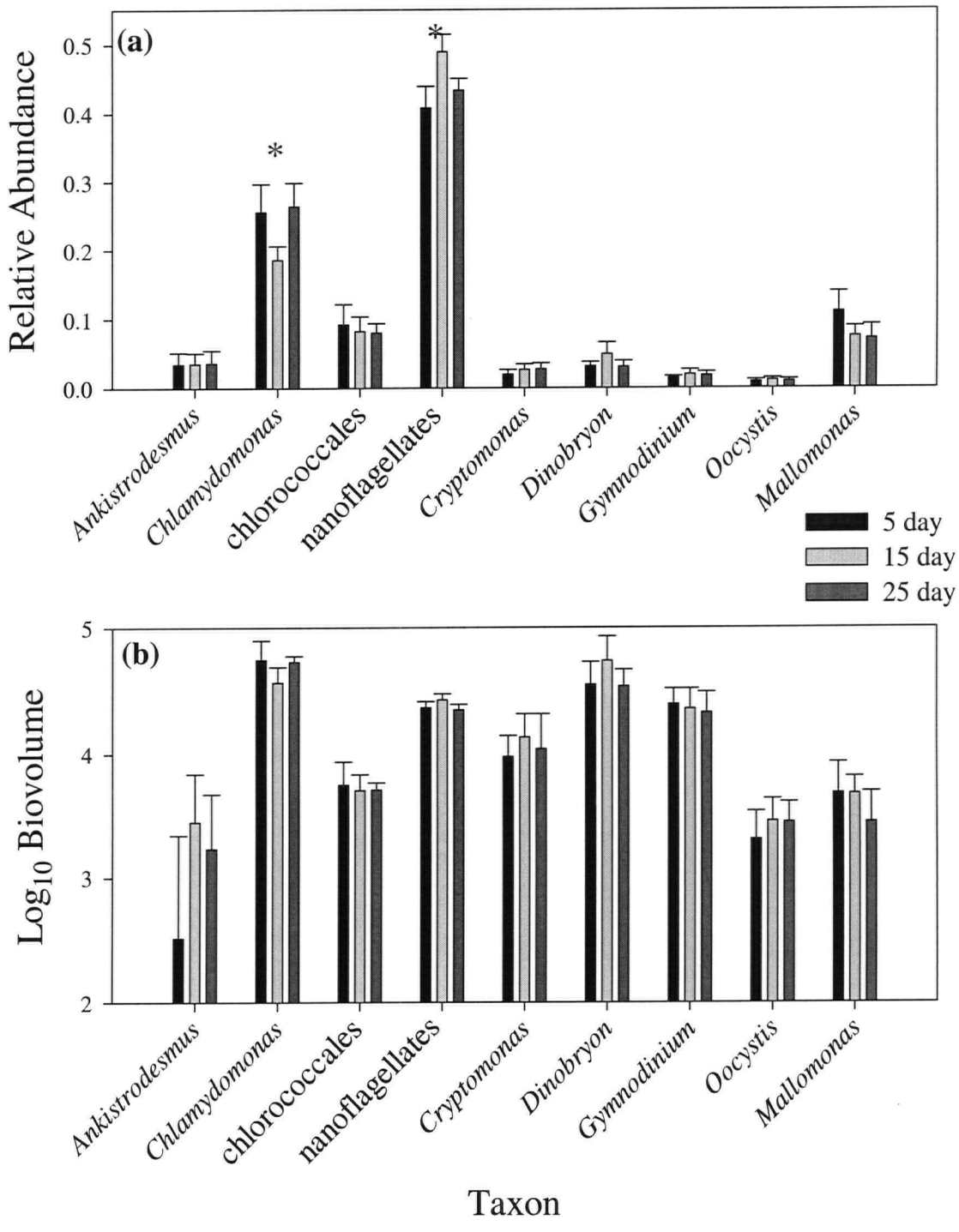
(iv) Mobility Classes

For relative abundances, the only significant difference in the case of the vector defined by mobility class, was for the effect of *Daphnia* (Table 4.3). The important groups leading to this main effect were the solitary nonflagellates and the colonial nonflagellates. The solitary types were more abundant in the presence of *Daphnia*, while the colonial types had lower abundances when the herbivore was present. The relationships were the same for the biovolume comparisons.

(v) Species Composition

The relative abundance of *Chlamydomonas* and nanoflagellates showed a main effect of mixing frequency in univariate ANOVAs (Figure 4.3a and Table 4.2). *Chlamydomonas* were relatively more abundant in infrequently mixed systems (25 days); the nanoflagellates also did better with less frequent (15 and 25 day) mixing. There were

Figure 4.3: Effect of mixing interval on (a) the relative abundance (mean ± standard error) and (b) the biovolume ($\mu g\ l^{-1}$) of common taxa (>1% relative abundance). An asterisk indicates a significant main effect as discussed in the text.



no significant biovolume effects in response to the frequency of mixing (Figure 4.3a and Table 4.2).

The *Daphnia* treatments had effects on the relative abundances of more individual species than did mixing (Figure 4.4a and Table 4.3). *Daphnia* significantly reduced the abundances of *Chlamydomonas*, non-motile chlorococcales, *Gymnodinium* and *Oocystis*. Species that did proportionately better in the presence of *Daphnia* were *Ankistrodesmus* sp., *Cryptomonas erosa*, *Mallomonas* sp. and nanoflagellates.

For biovolumes, all the same groups responded in the same way as for relative abundances (Figure 4.4b and Table 4.3). The one exception was the nanoflagellates, which showed no significant change in biovolume as a result of changes in the *Daphnia* treatment. There were no significant interaction effects on relative abundances of common (>1% relative abundance) species (Table 4.4). For biovolumes, the non-motile chlorococcales was the only common group (>1% relative abundance) to show an interaction effect (Figure 4.5, Table 4.4). This group had highest biovolumes in the absence of *Daphnia* when systems were frequently mixed (5 day P=0.0001 and 15 day P=0.0007). In the presence of *Daphnia* only, their biovolumes were significantly lower for frequently mixed systems than for infrequently mixed ones (5 vs. 25 day P=0.0456). The rare species *Merismopedia* sp., and *Tetradesmus* sp. also displayed significant interaction effects, and both displayed similar responses (Figure 4.5). These species had lowest biovolumes, and in the case of *Merismopedia*, were excluded from systems mixed frequently when *Daphnia* were present (+ vs. – *Daphnia* for 5 day mix: P=0.0636 for *Merismopedia* and P=0.0171 for *Tetradesmus*). In the presence of *Daphnia*, both of

Figure 4.4: Effect of *Daphnia* on (a) the relative abundance (mean ± standard error) and (b) the biovolume ($\mu g\ l^{-1}$) of common genera (>1% relative abundance). An asterisk indicates a significant main effect as discussed in the text.

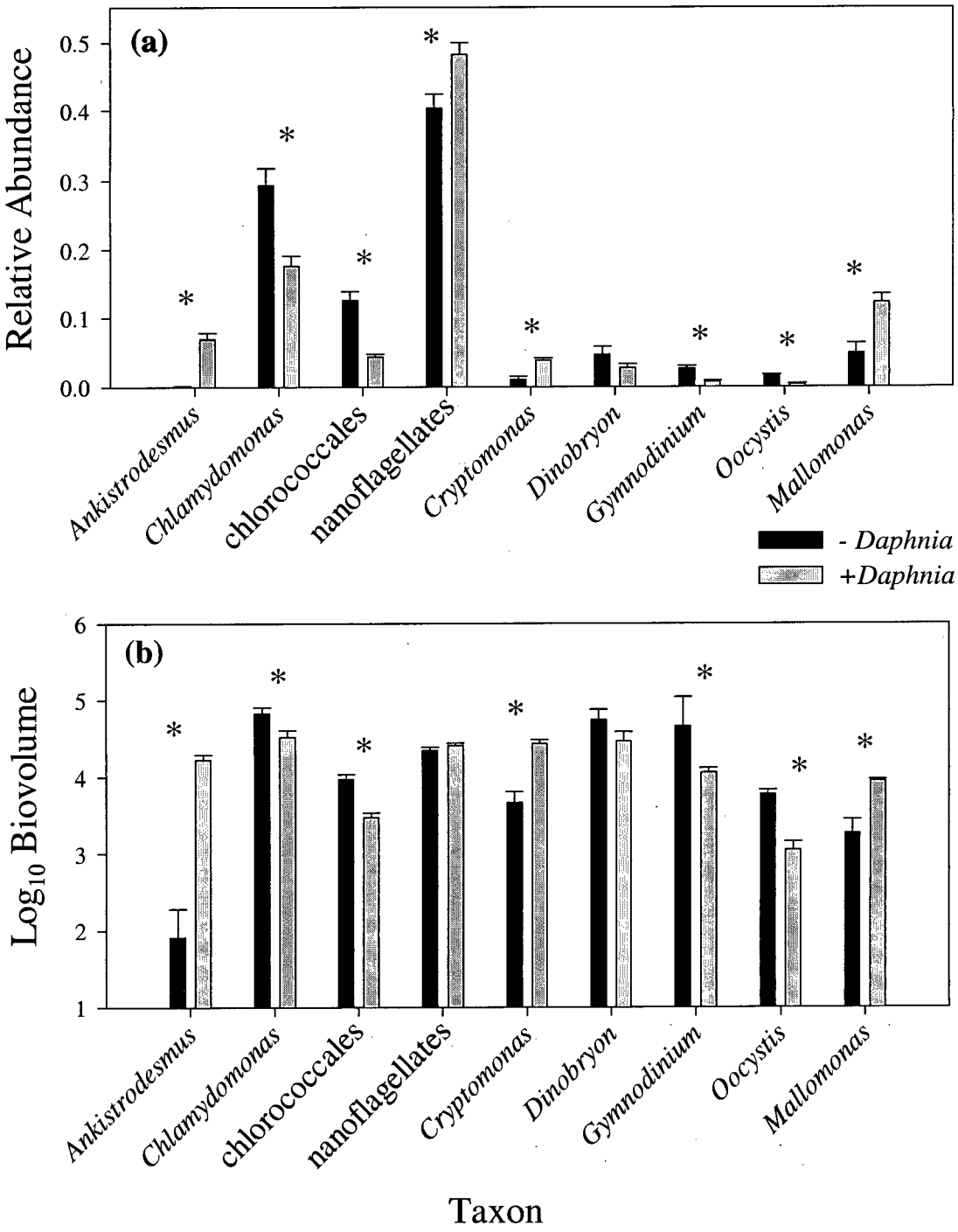
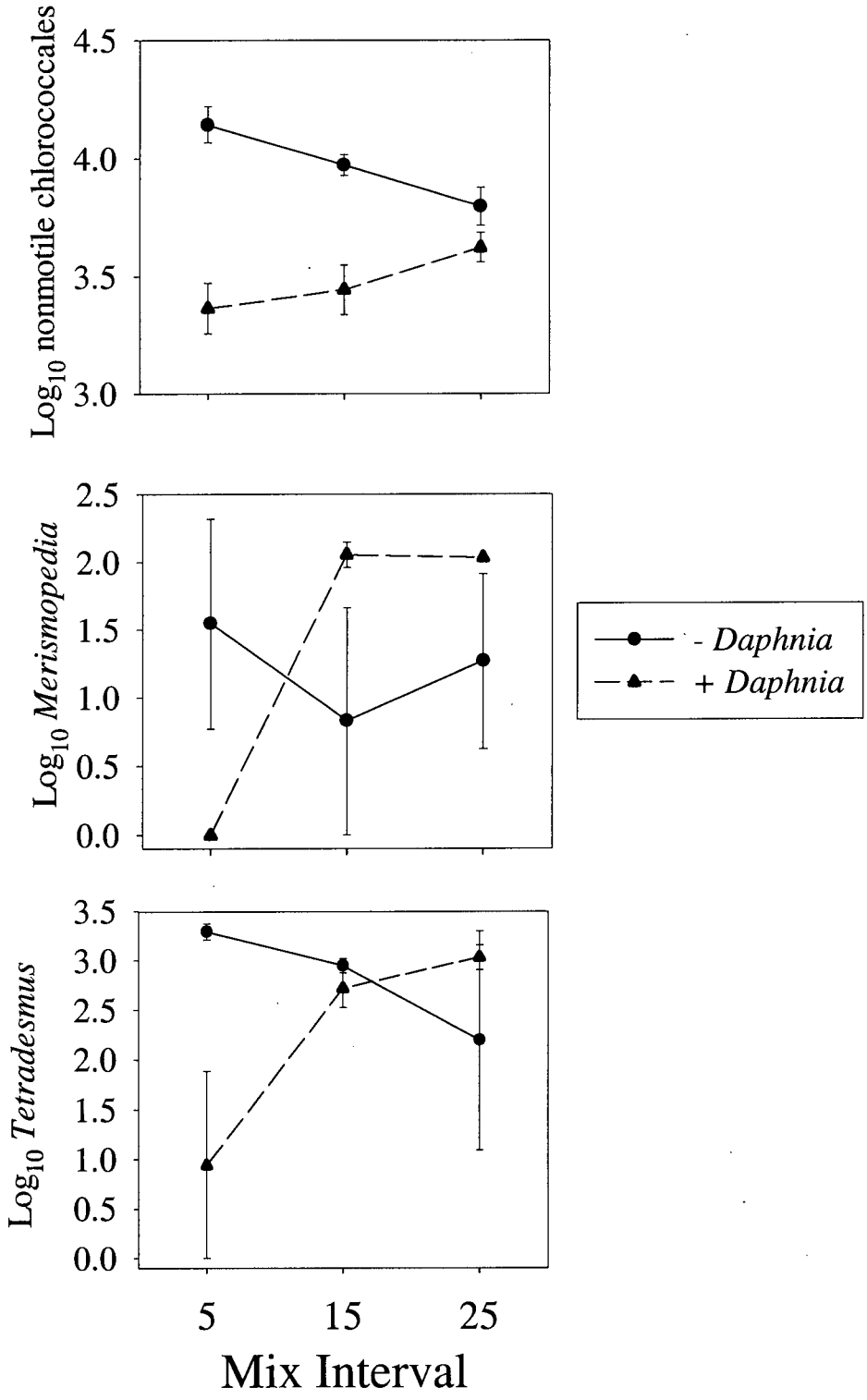


Figure 4.5: Interaction diagrams for species whose biovolumes ($\mu g\ l^{-1}$) responded to the effects of *Daphnia* and mixing frequency. Points are means ± standard errors.



these groups had significantly lower biovolumes in frequently mixed than in infrequently mixed systems (5 day vs. 25 day for +*Daphnia*: P=0.0199 for *Merismopedia* and P=0.03 for *Tetradesmus*). However, the interaction effect for *Merismopedia* here may represent a type I error given the large number (i.e. 62) of tests done for this section (see Table A2.2 in Appendix 2).

Assessing Spatial Heterogeneity

For chlorophyll levels, the average coefficient of variation between shallow and deep samples in each bag over the entire summer was used to assess vertical patchiness in phytoplankton. During the mid-summer period only (when phytoplankton counts were done), there was a significant interaction effect of *Daphnia* and mixing frequency ($P_{interaction}$=0.0204) (Figure 4.6). When bags were frequently mixed (5 or 15 days), there was greater variability between shallow and deep samples when *Daphnia* were excluded (5 day P=0.0464, 15 day P=0.0929). The opposite effect of *Daphnia* was observed when mixing was very infrequent (P=0.0667). For infrequent mixing, the coefficient of variation was higher when *Daphnia* were present. Overall, the infrequently mixed systems had higher coefficients than the more frequently mixed ones (5 and 15 day), but only when *Daphnia* was present (P-values<0.02).

To examine the short-term effect of physical mixing on large scale *Daphnia* distributions, I measured the variation between shallow and deep samples in one replicate immediately before and after mixing in the 5 and 25 day treatments on a single date. Results showed that mixing reduced the variation between the two spatial locations (Figure 4.7), which suggests that *Daphnia* were redistributed more evenly by the mixing

Figure 4.6: Interaction diagram for the mean (± standard error) coefficient of variation between deep and shallow chlorophyll *a* samples during period 2.

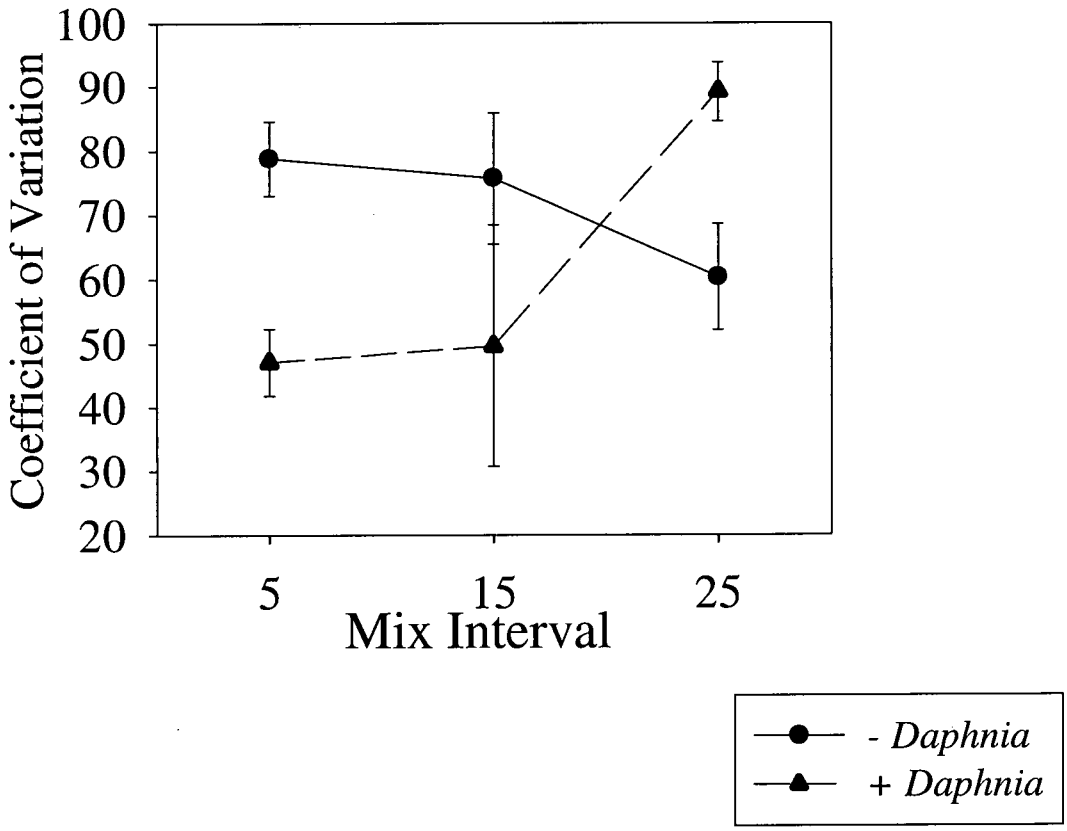
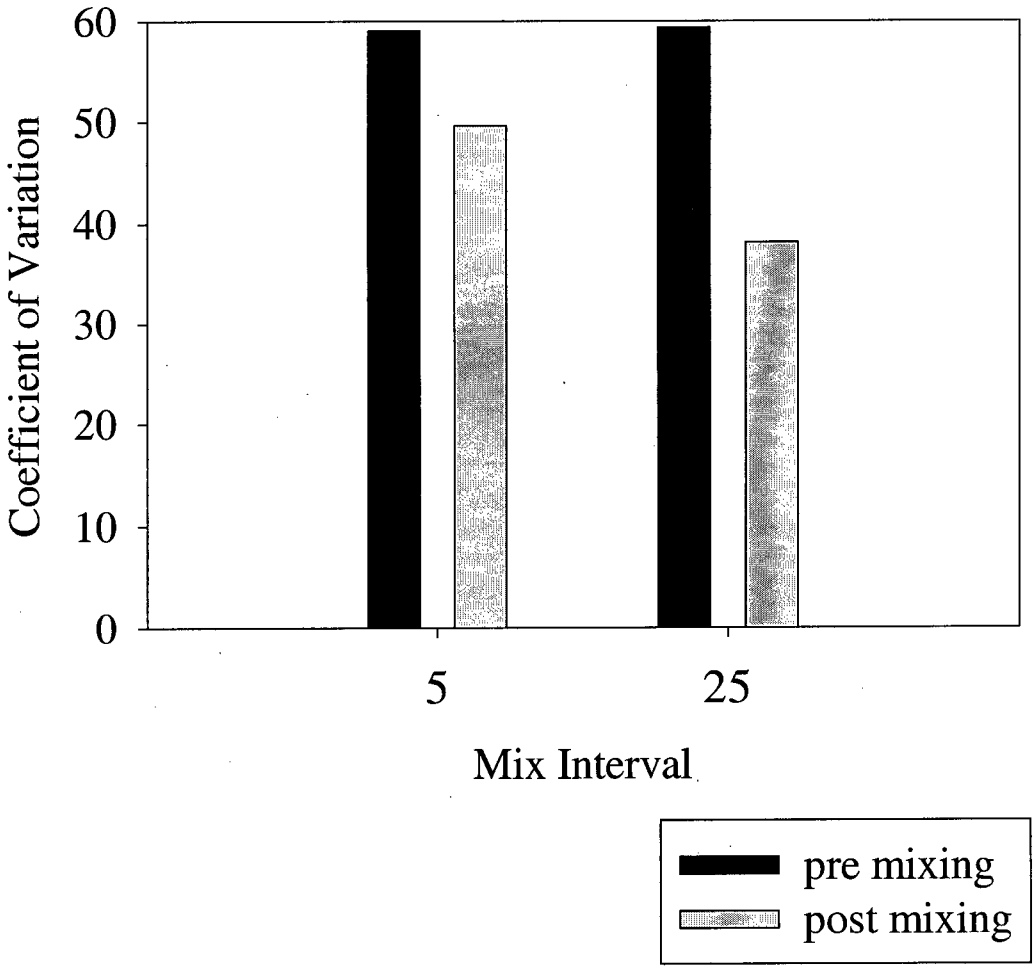


Figure 4.7: Immediate effects of the frequent (5 day) and infrequent (25 day) mixing application on the disparity (coefficient of variation) in *Daphnia* densities between two spatial locations (4 m and 1.5 m depths).



process. Also, the effect of the larger 25 day mixing was greater than the effect for the low intensity mixing done in the 5 day treatments (Figure 4.7).

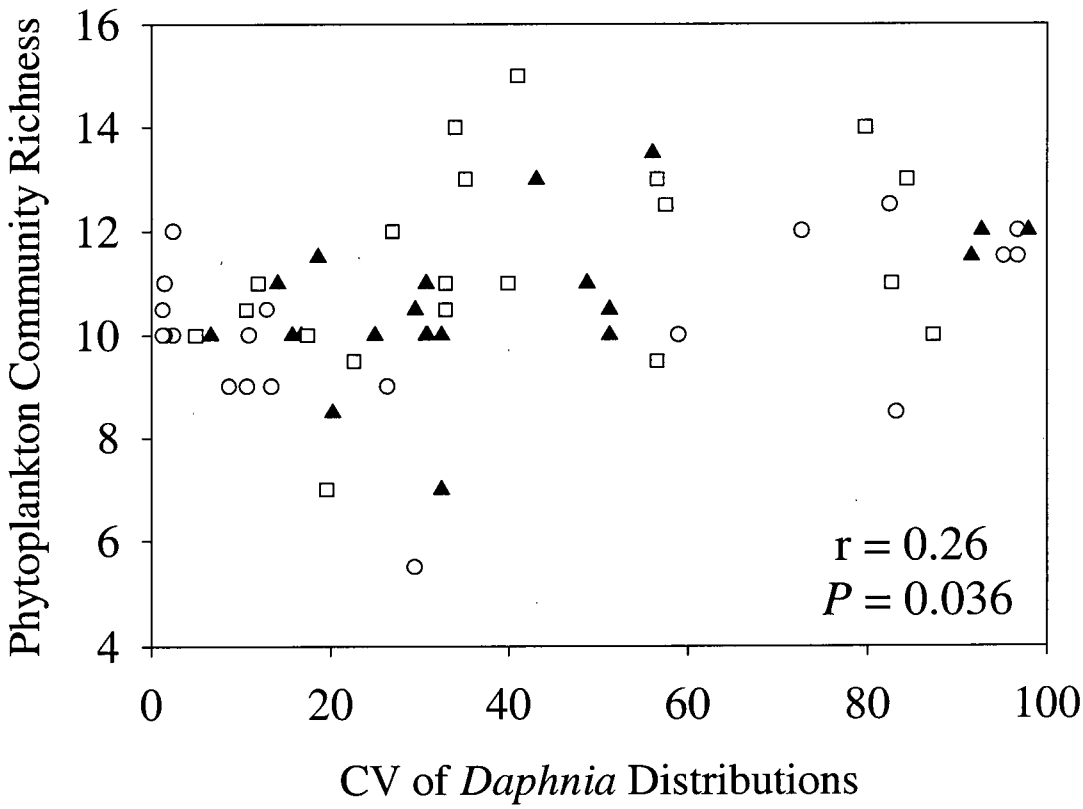
The results of the video analysis for small scale (temporal and spatial) effects of mixing indicate that the distribution of *Daphnia* was more clumped prior to mixing. The index of dispersion (CV of nearest neighbour distances) was 12.2 ± 1.2 before mixing as opposed to 6.8 ± 0.7 after mixing (P=0.005).

Finally, phytoplankton community richness was assessed as a function of the spatial variation (CVs) in *Daphnia* distributions (large temporal and spatial scales). Over the time period during which phytoplankton counts were done, the CVs for the two depths were calculated for the *+Daphnia* treatments. Richness was correlated with this variable (Figure 4.8) and a significant response was observed (P=0.036; r=0.26). Phytoplankton community richness increased with greater spatial variability (CV) in *Daphnia* vertical distributions.

Discussion

The role of mixing, and specifically vertical destratification, in conjunction with food web structure, has been the focus of this experiment. Community-level attributes of diversity, richness and evenness were not affected by the frequency of mixing alone (although there were interaction effects with predation to be discussed later). Previous empirical work had demonstrated that phytoplankton diversity can be influenced by mixing, mainly ascribed to associated changes in rates of nutrient pulsing (e.g. Turpin and Harrison 1980, Robinson and Sandgren 1983, Sommer 1984, 1985, Gaedecke and Sommer 1986, Brzezinski and Nelson 1988, Reynolds 1988, Lindenschmidt and Chorus

Figure 4.8: Correlation of phytoplankton species richness with the coefficient of variation in the vertical distribution of *Daphnia*. Circles indicate the values for the 5 day mixing treatments, triangles are for the 15 day treatments and squares represent the 25 day treatments.



1998). The lack of effect here is surprising. The only significant effect was a minor one on the size structure of the communities (lower relative abundances of the 6-20 μm size class with intermediate frequencies of mixing). The largely negative results here support the observation of Chapter 3 i.e., there is little effect of mixing in oligotrophic systems without herbivores.

Mixing Effects

Mixing effects in this experiment were minimal under oligotrophic conditions. The general lack of mixing effect can be attributed to two factors: low between-species contact rates on large spatial scales and low nutrient levels. Small-scale chemostats incorporate high contact rates between species because systems are fully mixed. Stronger results from these competition experiments would be expected as compared to this current field study, because the laboratory systems are always fully mixed and population interactions are therefore more intense. When spatial structure or limited interaction is included in a model of phytoplankton competition (albeit, implicitly, through rendering growth rates stochastic), as in the model of Anderies and Beisner (2000; Chapter 2), little effect of pulsing interval on diversity levels is expected for frequencies extending over the range of 2 to 25 days. In addition, as discussed in Chapter 3, high nutrient levels may be required to see major changes in phytoplankton community composition with different vertical mixing regimes, because they likely provide larger nutrient pulses with mixing episodes and permit a greater range of life history types to persist.

Herbivore Effects

The herbivore *Daphnia rosea* appears to play a stronger structuring role for phytoplankton communities than does mixing frequency. *Daphnia*, a generalist herbivore

that is a relatively unselective feeder over intermediate size classes of phytoplankton (e.g. Sterner 1989), increased the evenness of the phytoplankton communities in this study. Given the lack of response of average chlorophyll levels to the presence of *Daphnia*, it is likely that *Daphnia* enhanced community evenness by reducing the densities of competitive dominants, rather than by simply reducing the population densities of all species.

A closer examination of the composition of phytoplankton reveals that larger size classes (greater than 20 μm) of phytoplankton were more dominant in the presence of *Daphnia*, a result that is consistent with the observation that *Daphnia* feeding is strongest for phytoplankton between 3 and 20 μm (see Sterner 1989). In terms of biovolume changes, the smaller size classes (<20 μm) appeared to be preferentially consumed by *Daphnia* while the larger ones (>20 μm) responded with higher total volumes. Nanoflagellates, which are cells less than 3 μm long, also had higher relative abundances in the presence of *Daphnia*, perhaps because they are relatively inaccessible to the herbivore because of a small body size refuge (see Sterner 1989). Alternatively, because total biovolume of this group was unaffected by the herbivore, these small organisms may also be counteracting any effects of predation with very high population growth rates. In fact, it has been shown that these very small phytoplankton can grow at bacterial rates with several generations per day (Fenchel 1986), and they should be able to outgrow zooplankton grazing effects as a result.

Relative abundances of taxonomic groups like chlorophytes and dinoflagellates (similarly, biovolume for dinoflagellates) were reduced in the presence of the herbivore,

which suggests that they were preferentially consumed by *Daphnia*. Dinoflagellates consisted only of the species *Gymnodinium* sp. The chlorophyte species that displayed this pattern (again, both by changes in relative abundance and biovolumes) were *Chlamydomonas*, *Oocystis* and the non-motile chlorococcales. Cryptophytes and cyanophytes were more dominant numerically (and in the case of cryptophytes, in terms of biovolume as well) in the presence of the herbivore. Cyanobacterial trichomes, of which the "filamentous" group mostly consisted, are relatively inedible by *Daphnia* and this taxon displays greater dominance when high levels of herbivory are present (Lampert 1987). Cryptophytes were represented by only one species: *Cryptomonas erosa*, which appears to be "resistant" to *Daphnia* grazing. Others have found that *Cryptomonas* sp. can dominate where intense grazing occurs (Fott *et al.* 1979, Shapiro and Wright 1984), which suggests that this genus can avoid or compensate for predation.

As filter feeders, *Daphnia* are generally rather unselective feeders over a broad intermediate range of cell sizes (see Sterner 1989). When phytoplankton were grouped based on morphological features, there was little evidence to indicate differential susceptibilities. The only response observed was for the "round" class, which had higher relative abundances when *Daphnia* were initially excluded. This result is likely due to the responses of non-motile chlorococcales, which had significantly lower relative abundances in the presence of *Daphnia*. However, it is still possible that the "resistance" to *Daphnia* consumption of several species is due to their morphology (i.e. sharply pointed, hairy or large flagella). This result may have disappeared when aggregate measures of morphological features were used as a basis for comparison, but was observed in the univariate comparisons for *Ankistrodesmus*, *Mallomonas* and

Cryptomonas. *Ankistrodesmus* may be less edible to *D. rosea* because of its sharply pointed crescent shape, and it responded with both higher relative abundance and biovolumes in the presence of the herbivore. *Ankistrodesmus* is edible to the larger *Daphnia pulex* (Lynch and Shapiro 1981), but it may be more difficult for the smaller *D. rosea* to handle (A. Mazumder *personal communication*). Similarly, *Mallomonas* (which had higher relative abundances and biovolumes when *Daphnia* were present) may be less edible to the herbivore because it has a large number of cilia on the surface of the cell. In short-term experiments, Lehman and Sandgren (1985) demonstrated that *Mallomonas* spp. are not grazed by *Daphnia*. *Cryptomonas* may use its large flagella and strong swimming ability to escape predation.

In terms of mobility classes, solitary nonflagellates appear to be most resistant to *Daphnia* grazing, both in terms of relative abundances and biovolumes. We might naively expect this group to be most susceptible to grazing by *Daphnia* because of the lack of mobility and the simpler structure of each edible unit (Lehman and Sandgren 1985). *Ankistrodesmus* and *Mallomonas* are likely the responsible for this unexpected response. The colonial nonflagellates also responded in a surprising way because we might expect this group to be susceptible to herbivory by *Daphnia*. This group did proportionately and absolutely better in the absence of *Daphnia* in this experiment. The only species in this group that also responded significantly in the same way was *Oocystis*, and it primarily drove the response of the entire class. *Oocystis* is a small, two to four cell colonial type within the size class that *Daphnia* preferentially consumes, and has few morphological features like trichomes to protect it from herbivory.

Finally, a genus which did not drive any of the class responses but which did show susceptibility to *Daphnia* predation in this study is *Chlamydomonas*. This genus is a preferred food source of *Daphnia galeata mendota* (Porter 1973), and *Chlamydomonas* sp. population growth rates often decline in the presence of zooplankton (Bergquist and Carpenter 1986).

Interaction of Mixing and Herbivory

A major question of interest here is whether zooplankton community structure and episodic mixing events can interact to influence phytoplankton communities. Both processes alone have been shown in the past to affect phytoplankton composition and diversity, but how they interact is unknown. The process of vertical mixing is likely to alter the distributions of predator and prey individuals relative to each other and as a result could affect the strength of both competitive and predator-prey interactions. In fully mixed systems, there may be a lack of temporal and spatial refuges, and more intense and unstable population interactions may lead to species extinctions (Murdoch and Oaten 1975, McLaughlin and Roughgarden 1991).

The results of this field experiment suggest that for a strictly competitive process, the extent to which a system approaches fully mixed has little effect on phytoplankton community structure (*Daphnia*-absent cases). As in Chapter 3, where zooplankton were excluded and conditions were oligotrophic, no response to frequency of mixing was observed at the community level. Frequent disruption of any natural patchiness in distributions of phytoplankton cells probably has minor effects because of the extremely low nutrient levels everywhere and the high mobilities of most phytoplankton (i.e. ~90% flagellates) present in these enclosures. Vertical layering and patchiness at the scale of

these enclosures alone do not seem to be major mechanisms for maintaining diversity, at least in my oligotrophic systems when crustacean zooplankton were initially excluded. Vertical thin layers of phytoplankton (on scales of centimetres to a few metres) have been observed in oligotrophic systems (e.g. Cowles *et al.* 1993, Sullivan *et al.* 1999, Wingard and Cowles 1999), and they are generally dependent on the stability of the water column in lakes and degree of wind shelter (Lindholm 1992) rather than on nutrient levels. However, the ecological role that this structure serves in oligotrophic systems has not been examined in detail.

For the predator-prey interactions (i.e. when *Daphnia* were included), frequency of mixing affected phytoplankton richness. More frequent mixing led to lower species richness levels among the phytoplankton prey. Turbulent mixing has been shown in other studies (e.g. Haury *et al.* 1990, Browman 1996, Yamazaki 1996) to alter zooplankton distributions and increase encounter rates with phytoplankton prey. In strongly linked predator-prey systems such as the *Daphnia*-phytoplankton one, increasing encounter rates between species or decreasing the availability of refuges in time or space can decrease population stability (Luckinbill 1974, Murdoch and Oaten 1975). Most work on these stability relationships has focussed only on single predator, single prey combinations (e.g. Luckinbill 1974, Murdoch and Oaten 1975, de Roos *et al.* 1991, McLaughlin and Roughgarden 1991, Timm and Okubo 1992, McCauley *et al.* 1993, Wilson *et al.* 1993). Where a food web approach has been taken to examine the influence of predator encounter rates on prey community structure (Caswell 1978, Hixon and Menge 1991, Hixon and Beets 1993, Caley and St. John 1996), a decline in species richness with increased encounter rates is observed, just as seen in this experiment.

Several lines of evidence from this experiment support the idea that frequent mixing alters encounter rates between predators and prey and reduces the availability of prey refuges for supporting high community richness. The correlation of phytoplankton richness with the spatial disparity (CV) of *Daphnia* densities indicates that the number of prey genera present is positively related to the degree of spatial heterogeneity or patchiness in *Daphnia* distributions at relatively large spatial and temporal scales (i.e. on mesocosm spatial and monthly time scales). The reduction in the discrepancy (CV) between the *Daphnia* density samples taken at two depths immediately following a mixing event suggests that *Daphnia* were distributed patchily prior to mixing on large spatial (mesocosm-level) but short time scales (before vs. after mixing events). Mixing reduces the degree of spatial heterogeneity in predator distribution on this vertical scale. In addition, the video measurements taken in 1999 indicate that even on very small (centimetre) scales, *Daphnia* distributions become less patchy under imposed mixing. In the presence of *Daphnia*, chlorophyll levels were less variable between depths with frequent mixing, but more variable in the infrequently mixed systems. This suggests that mixing leads to a breakdown of potential natural vertical structure in the phytoplankton, especially if *Daphnia* is present. When vertical heterogeneity is seldom disturbed, *Daphnia* presence leads to a greater patchiness - at least at the gross level of phytoplankton biomass across two depths. Taken together, these results provide evidence for the idea that mixing destroys the natural patch structure in less disturbed columns of water that may form by *Daphnia* feeding.

The species that are most likely responsible for the changes in community richness are the abundant non-motile chlorococcales group and rare genera like

Tetradesmus sp. and possibly *Merismopedia* sp. Based on the arguments just presented, it appears that these groups rely on a spatial or temporal refuge from predation by *Daphnia* in the absence of mixing. When mixing is frequent, these refuges are removed, and these groups are the main ones to suffer great losses to predation.

Another possible explanation for the observed richness pattern is an indirect effect of *Daphnia* on phytoplankton growth through the production of small-scale nutrient patches by excretion (Lehman and Scavia 1982). A lack of forced mixing leading to a patchy distribution of *Daphnia* can therefore result in a heterogeneous distribution of recycled resources available for phytoplankton. This mechanism could produce the same outcome at the aggregate community level (i.e. species richness) as observed here and as attributed to predator-prey encounter rates. However, where the two mechanisms make different predictions is in terms of community composition. For the excretion hypothesis, we would expect that when interactions between species and resources are most heterogeneous, as they are when mixing is infrequent, systems should be dominated by small opportunists, which can take advantage of pulses of excreted nutrients. On the other hand, if patchy distributions are mainly caused by predation, herbivore-tolerant groups should dominate when mixing is frequent and interactions between species are most intense. From the species composition data, the dominant group that shows an interaction response is the small round chlorococcales. These phytoplankton are known to be relatively resistant to predation and, in fact, may have higher productivities because of ingestion by *Daphnia* (Porter 1973). This group, probably dominated by an opportunist life history strategy because of their small size, did less well with infrequent

mixing in the presence of *Daphnia*, an observation that does not support the idea that the main effect of *Daphnia* is to promote a patchy distribution of nutrient resources.

To summarize, strong herbivory by *Daphnia* does enhance phytoplankton community richness, as generally predicted (McCauley and Briand 1979), but only if systems are relatively undisturbed. Otherwise, systems often disturbed by mixing experience reduced phytoplankton richness when *Daphnia* is present. The mixing treatments, which occur with differing frequencies, can be regarded as temporally altering the degree of enemy-free space for phytoplankton. Caswell (1978) predicted that for open, nonequilibrium predator-prey systems, prey diversity should be lowest in systems that act as a single patch (i.e. fully mixed) and highest in those where predation acts intermittently with occasional releases of predation pressure (i.e. less frequently mixed systems).

Overall, the results of this experiment indicate that the frequency with which vertical mixing of the water column occurs in lakes can have consequences for phytoplankton community structure even in oligotrophic systems. However, this is only the case in the presence of strong herbivory. The direct responses of aggregate community measures, individual taxa and size structure of the phytoplankton to mixing frequency alone are not large. *Daphnia* have strong effects, and the role of mixing probably operates in terms of changing the encounter rates between predators and prey in these systems. In this way, environmental forcing can have an influence on plankton community structure. This view is different from that arising from pulsed nutrient studies in the laboratory, probably because the study system here is oligotrophic. Relevant life history characteristics for responses to mixing are not just those associated with nutrient

uptake abilities, because the range of abilities in such a community, adapted to low nutrient conditions, is necessarily small. Rather, the characteristics that become relevant are those associated with edibility (i.e. avoiding herbivory) and mobility (i.e. acquiring nutrients). The interaction of predation by *Daphnia* (top-down force) with vertical mixing appears to be far more important than the interaction with nutrient acquisition (bottom-up force) in this type of oligotrophic system.

CHAPTER 5

Plankton Community and Food Chain Structure in Fluctuating Environments of Varying Productivity

In this chapter I further extend the "realism" in the experimental setup by considering the role of environmental productivity and temporal heterogeneity for phytoplankton communities in the presence of the entire planktonic food web.

Introduction

A major goal in community ecology is to determine the causes of variation in the structure of communities in space and time in response to many physical and biotic forces (Menge and Sutherland 1987). Of the forces influencing communities, many of these are intermittent in time or "nonequilibrial" in nature (Wiens 1977, Price 1984). Examining the role of resource fluctuations and other intermittent forces has increasingly been a focus in community ecology.

Plankton systems are good model ecosystems to further our understanding of ecological processes, including those related to temporal heterogeneity in environmental conditions and the effects on community structure. Plankton are naturally exposed to variation in ecological conditions because of turbulent mixing events that occur in their pelagic environments. Vertical mixing in the water column at many different temporal and spatial scales is a common event in lakes and in the ocean (Harris and Griffiths 1987). For the most part, plankton ecologists have focussed attention on understanding the effect of intermittent abiotic processes on only a single trophic level, usually the phytoplankton. They have also generally adopted a reductionist approach by examining

separately each of the abiotic processes that mixing affects, including various pulsed regimes of nutrients (Tilman 1977, Turpin and Harrison 1979, Robinson and Sandgren 1983, Scavia *et al.* 1984, Sommer 1984, 1985, Suttle *et al.* 1988, Grover 1991a,b, Rothhaupt 1996, Docubo *et al.* 1998, Huisman 1999), light (Denman and Marra 1986, Huisman *et al.* 1999), temperature (Eddison and Ollason 1978) and turbulence (Estrada *et al.* 1988). The approach has also involved either removing natural phytoplankton communities from their pelagic settings to well-mixed chemostats (e.g. Turpin and Harrison 1979, Scavia *et al.* 1984, Sommer 1985, Suttle *et al.* 1988) or using assembled communities from stock cultures (e.g. Tilman 1977, Robinson and Sandgren 1983). The few studies that have examined intermittent vertical mixing within a lake setting (in mesocosms), have similarly only considered a single trophic level, the phytoplankton (e.g. Reynolds *et al.* 1983, 1984, Estrada *et al.* 1988). Lake studies that examine the responses of entire plankton communities at more than one trophic level to various intermittent mixing scenarios are lacking (but see Flöder and Sommer 1999).

From the numerous chemostat experiments, it is fairly well understood that the structure of phytoplankton communities is sensitive to the frequency of abiotic fluctuations. Resource fluctuations may introduce temporal niche opportunities that can have consequences for the diversity and composition of ecological communities (Chesson and Huntly 1997, Anderies and Beisner 2000, see Chapter 2). It is not known whether phytoplankton continue to respond to such bottom-up forces as fluctuations in resource supply when higher trophic levels are present. Similarly, the inputs of intermittent bottom-up forces (i.e. fluctuating abiotic conditions) for biomass and population dynamics higher in the food chain, where dynamics are generally slower, are also not

well known. However, some laboratory studies with zooplankton exposed to pulsed phytoplankton food supplies have shown that population growth and biomass can differ between constant and fluctuating prey conditions and that there are differential species responses (e.g. Lampert and Muck 1985, Kremer and Kremer 1988, MacIsaac and Gilbert 1991). For example, intermittency in food supply more negatively affects survivorship and growth of the cladoceran *Daphnia*, compared to a constant food condition. *Diaptomus* copepods are much less affected by similar intermittency (Lampert and Muck 1985). On the other hand, *Daphnia* are more successful than rotifer species under pulsed phytoplankton conditions than constant ones (MacIsaac and Gilbert 1991). There exists, therefore, a potential for higher trophic levels in the plankton food chain to be affected by fluctuating abiotic conditions.

Characteristic time scales for vertical mixing as a result of wind events range from several days to a few weeks, with an average frequency of approximately 11 days (Harris and Griffiths 1987). Moving up through the pelagic food chain, characteristic time scales of the populations gradually increase. Generation times for phytoplankton range from hours to a few days, for freshwater zooplankton from days to a year, and for invertebrate carnivores like *Chaoborus*, over a year. The question arises as to whether the influences of vertical mixing, which occur at characteristically short time-scales, can also be observed at higher trophic levels where generation times are long. Although the biomass may respond more slowly at higher trophic levels, behavioural responses, on much faster time scales are possible and can affect trophic dynamics.

The immediate effect of vertical mixing may be different for each trophic level. Turbulent vertical mixing results in the movement of organisms and nutrients.

Resuspension of non-motile phytoplankton, nutrients, detritus and deep-water dwelling organisms is a common effect. Turbulence at microscales, which can accompany mixing, can also alter biotic interactions. Moderate mixing can increase encounter rate and feeding success of planktonic organisms as well (Kiørboe and Saiz 1995, Yamazaki 1996, Sanford 1997, Petersen *et al.* 1998), although severe turbulent mixing can have destructive effects especially on gelatinous forms of zooplankton (Petersen *et al.* 1998). In studying the role of intermittent vertical mixing in natural systems at various trophic levels, an inclusive view has to be taken of all these possible effects. A mixing event probably has some mixture of positive and negative consequences for each trophic level.

On longer time scales (i.e. not just during a mixing event), vertical mixing can introduce both bottom-up and top-down forces that may affect planktonic food chains. Resource pulses (nutrient, light or detritus) can act as bottom-up forces for various trophic levels in the food chain. Increased encounter rates between predators and prey may lead to higher zooplankton biomass, as has been observed following upwelling stimulated by wind events in marine systems (Cowles *et al.* 1987), and thus turbulent mixing can alter the role of top-down forces (also see Chapter 4). Because of the dual nature of the influences of vertical mixing, it is unclear how higher trophic levels will affect the response of the phytoplankton and how they themselves will be affected by any bottom-up forces that may be introduced. However, given the lack of studies incorporating higher trophic levels, it is essential to gain at least an empirical understanding of the response of entire food chains in a natural plankton community to intermittent forces like vertical mixing.

The diversity and composition of communities is also affected by productivity (reviewed in Rosenzweig and Abramsky 1993). In Chapter 3, I demonstrated that the response of communities to vertical mixing frequency may be related to the degree of environmental stress (nutrient status in the system), with enriched systems being more responsive. Many laboratory studies on the input of intermittent abiotic factors on plankton community structure have involved enriching the systems (e.g. Tilman 1977, Turpin and Harrison 1979, Robinson and Sandgren 1983, Scavia *et al.* 1984, Sommer 1985, Suttle *et al.* 1988, Grover 1991a,b, Rothhaupt 1996, Docubo *et al.* 1998, Huisman 1999). Oligotrophic systems may be less responsive to intermittent vertical mixing for a variety of reasons including: a more restricted range of life history strategies is possible at low nutrient levels, and less internally-generated detrital accumulation occurs (with increased recycling in the epilimnetic zone) so that pulses may be smaller in terms of actual resource content. A common feature of vertical mixing that is independent of environmental productivity, however, is the turbulence and movement of organisms that are introduced episodically.

The role of enrichment has been well studied within the context of food chain theory (e.g. Oksanen *et al.* 1981, Leibold 1989, Abrams 1993). A prevailing view for three trophic level food chains, such as the ones studied here, is that the first and third trophic levels are most likely to be responsive to changes in resource abundance because their biomasses are limited by competition rather than predation (Hairston *et al.* 1960, Oksanen *et al.* 1981). If competition is the major biotic interaction affected by mixing events, we might similarly expect these trophic levels to be the most responsive to

changes in the temporal scale of the vertical mixing regime, while the second trophic level should be least affected.

This chapter presents an experiment aimed at several questions. First, in the presence of higher trophic levels, is the structure of phytoplankton communities affected by various frequencies of vertical mixing? Does the background productivity or harshness of the environment alter the pattern of response? Given a three-trophic level food web, how and to what extent are higher trophic levels (population dynamics and biomass) affected by intermittent mixing processes?

Methods

Mesocosms were set up as described in the previous two chapters with the modification that mixing treatments were (i) frequent mixing (every three days), (ii) intermediate frequency mixing (15 days) and (iii) infrequent mixing (30 days). Frequently mixed systems were bubbled with air from the bottom of the bag for 30s while the most infrequently mixed ones were bubbled for a period 10 times longer (i.e. 5 minutes). The other difference compared to previous experiments is that the mesocosms all contained natural unfiltered zooplankton communities (including microzooplankton, crustacean macrozooplankton and the invertebrate carnivore *Chaoborus flavicans*) in addition to the phytoplankton complement from the lake. As in chapter 3, the experiment was a two-way factorial design with three levels of mixing frequency and two levels of nutrients (ambient low levels and enriched levels).

The bags were filled initially with lake water from several depths, filtered to 54 μm to exclude crustacean zooplankton. The bags were filled on May 11 and 12, 1998. Over the next two days, zooplankton from the lake were collected with gentle tows using

a plankton net (mesh size of 64 μm) and inoculated into the bags at natural densities. Zooplankton density calculations were based on the volume of water filtered during the tows and the necessary dilution factor to compensate for the volume of the bags. Over the two weeks following the bag setup and prior to the initial mixing, bags were fertilized every second day at the same high (1 $\mu g\ l^{-1}$ of $PO_4^{-3}\ d^{-1}$) and low levels (0.01 $\mu g\ l^{-1}$ of $PO_4^{-3}\ d^{-1}$) as in Chapter 3 (N:P ratio of 25:1). The first mixing application occurred on June 1, 1998 (Julian day 152).

(i) Sampling

Phytoplankton were sampled only at the end of the 60 day experimental period, on July 31 (day 212) at two depths (1.5 and 4 m from the surface), using a 2 l Van Dorn bottle. A 250 ml sample was preserved immediately using several drops of Lugol's iodine. Additional sampling was not considered worthwhile in view of the largely negative time series results found in the more extensively sampled experiment of Chapter 4. Enumeration was done using an inverted microscope and 50 ml of sample that had been settled for 20 hours. Transects were counted at 200X magnification until at least 100 cells of the most common group had been enumerated.

Zooplankton were sampled mid-morning, every six days throughout the experimental period, just before a mixing event when mixing coincided with sampling. Because the zooplankton community might take longer to respond than phytoplankton, mixing treatments actually continued for another 60 day period, and zooplankton were again sampled throughout August and on September 29 1998. These results are shown in Appendix 3. Samples for zooplankton were taken at the same two depths (1.5 and 4 m from the surface) as for phytoplankton. For zooplankton samples, 25 l of water was

pumped from each location using a bilge pump and filtered through a 64 μm mesh plankton net. Zooplankton were anaesthetized with soda water (to prevent loss of eggs) and preserved in 4% sugared formalin. Samples were counted using a dissecting microscope.

(ii) Data and Statistical Analyses

For the phytoplankton data, diversity indices, relative abundances, and biovolumes of major groups and taxa were calculated and analyzed as outlined in the methods section of Chapter 3 and Appendix 2.

For zooplankton and *Chaoborus*, average densities (# l^{-1}) over the entire summer and over both depths were calculated and compared. MANOVAs based on taxonomic groupings were done, followed by discriminant analyses and univariate two-way factorial ANOVAs as outlined for phytoplankton in Chapter 3. Biomasses for various zooplankton groups were also calculated based on average measured dimensions of the groups and published length-weight regressions (McCauley 1984).

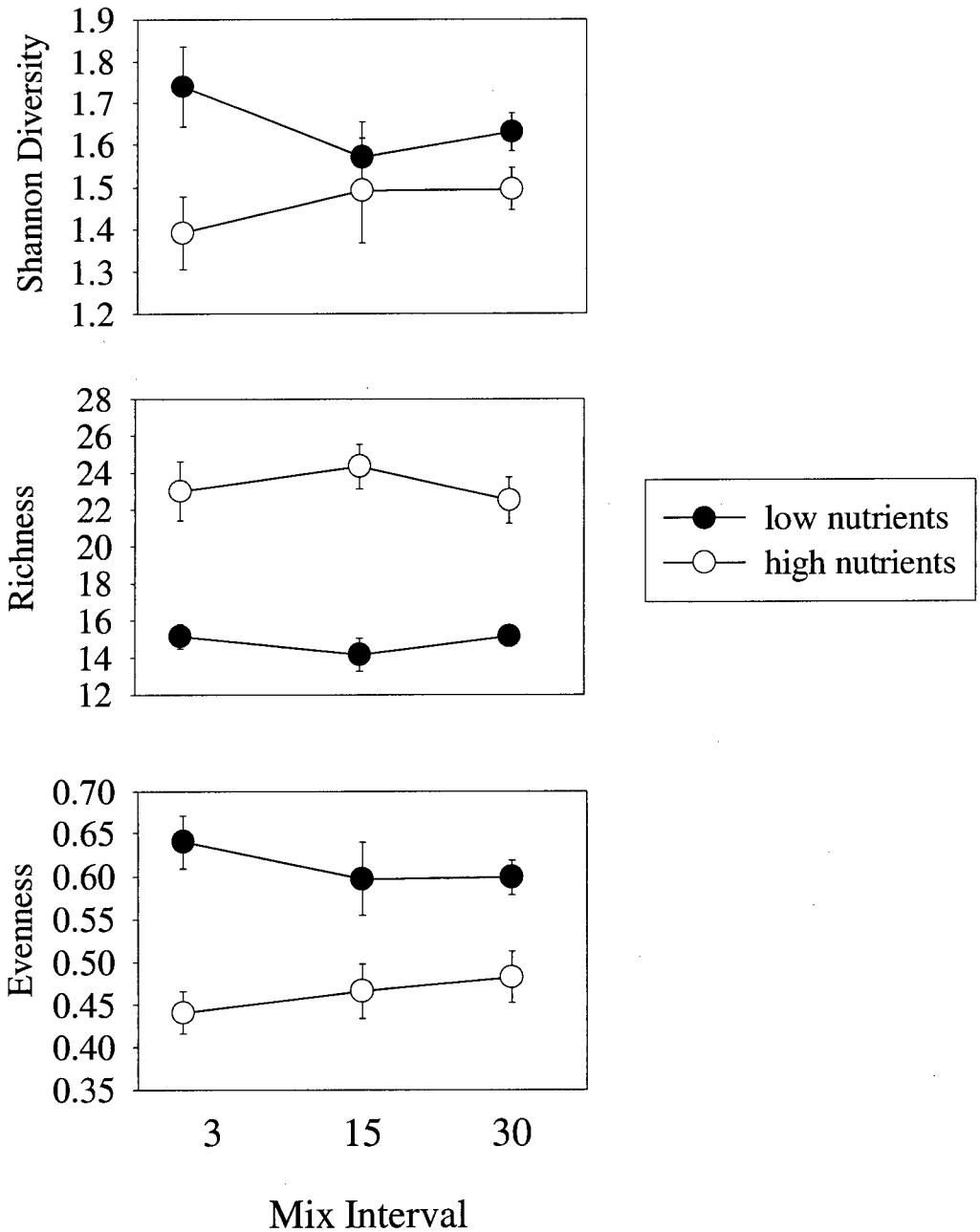
Results

PHYTOPLANKTON COMMUNITY STRUCTURE

Community Diversity

Phytoplankton community diversity measures only responded to the main effect of nutrient level (Figure 5.1). Diversity was significantly higher in the unenriched treatments (P=0.0196), mainly because evenness was higher (P=0.0001). On the other hand, enriched communities had higher richness levels (P=0.0001). There was no effect of the frequency of mixing on this aggregate community measure.

Figure 5.1: Phytoplankton diversity at the end of the experimental period. Values are the mean of three replicates ± one standard error.



Biomass

Total biomass at the primary producer trophic level was unaffected by both mixing frequency and nutrient levels (all $P>0.1$).

Community Composition

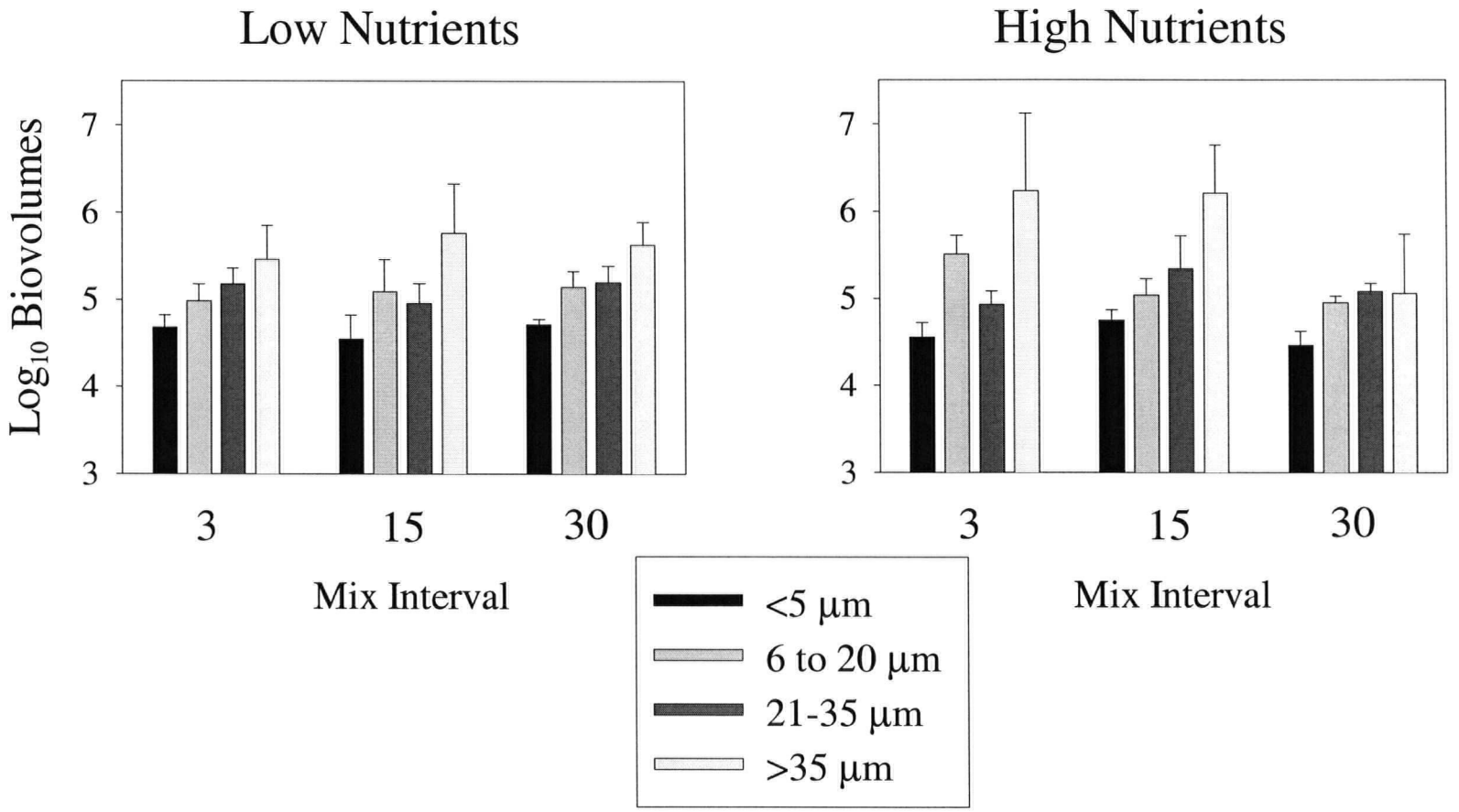
(i) Size Classes

There were no significant effects on phytoplankton size structure when relative abundances were considered. Using biovolumes in each size class as vectors, there was a significant mixing by nutrient interaction (Wilks' $\lambda=0.08$, $P_{interaction}= 0.008$) from the MANOVA (Figure 5.2). The frequently mixed systems (3 days) differed from the less frequently mixed ones (15 day mix Wilks' $\lambda=0.12$, $P=0.0023$; 30 day mix Wilks' $\lambda=0.15$, $P=0.0056$) when systems were enriched. In the absence of nutrients as well, the frequently mixed systems differed in size structure from the ones mixed every 15 days (Wilks' $\lambda=0.3$, P=0.0497). Also, for the frequently mixed systems, the size structure of low and high nutrient communities differed significantly (Wilks' $\lambda=0.99$, $P=0.0013$). The discriminant analysis and further univariate contrasts did not reveal that any one particular group led to the significant differences, however.

(ii) Taxonomic, Morphological and Mobility Classes

There were no significant effects of mixing frequency or nutrient levels for vectors composed of either relative abundances or biovolumes for taxonomic, morphological, or mobility classes.

Figure 5.2: Phytoplankton community composition at the end of the experimental period based on $\log_{10}$ biovolumes ($\mu g\ l^{-1}$) in various size classes. Bars are means ± one standard error.



(iii) Species Composition

The most abundant phytoplankton species showed very little change in relative abundance or dominance with differences in treatments. Figure 5.3 shows composition for abundant (>3% relative abundance) species of phytoplankton. Two-way univariate ANOVAs on the relative abundances of these species showed that there was a significant effect only for *Cryptomonas* sp., which showed an interaction response to the effects of mix frequency and nutrient levels ($P_{interaction}$=0.0285). This species was relatively more abundant under nutrient poor conditions when mixing was frequent (P=0.046) but more abundant under enriched conditions when mixing was very infrequent (P=0.046). However, it is likely that the significance here represents a Type I error given that there was only one significant test of the 18 ANOVAs done for species composition (Table A2.3 in Appendix 2).

ZOOPLANKTON COMMUNITY STRUCTURE

Community Diversity

Zooplankton community diversity and evenness showed no significant response to either mixing frequency or nutrient levels (Figure 5.4). The richness of the zooplankton communities did respond to the input of nutrients however. Richness was higher in the enriched treatments (P=0.0001).

Biomass

Total biomass at the herbivore trophic level was higher under nutrient-poor conditions (two-way ANOVA, P=0.0226). Cladocerans composed the major part of the

Figure 5.3: Relative abundances of common phytoplankton genera at the end of the experiment. These are represented by groups with greater than 3% of total abundance in at least one treatment. Bars do not add up to one, with the remainder representing genera of low abundance (<3%).

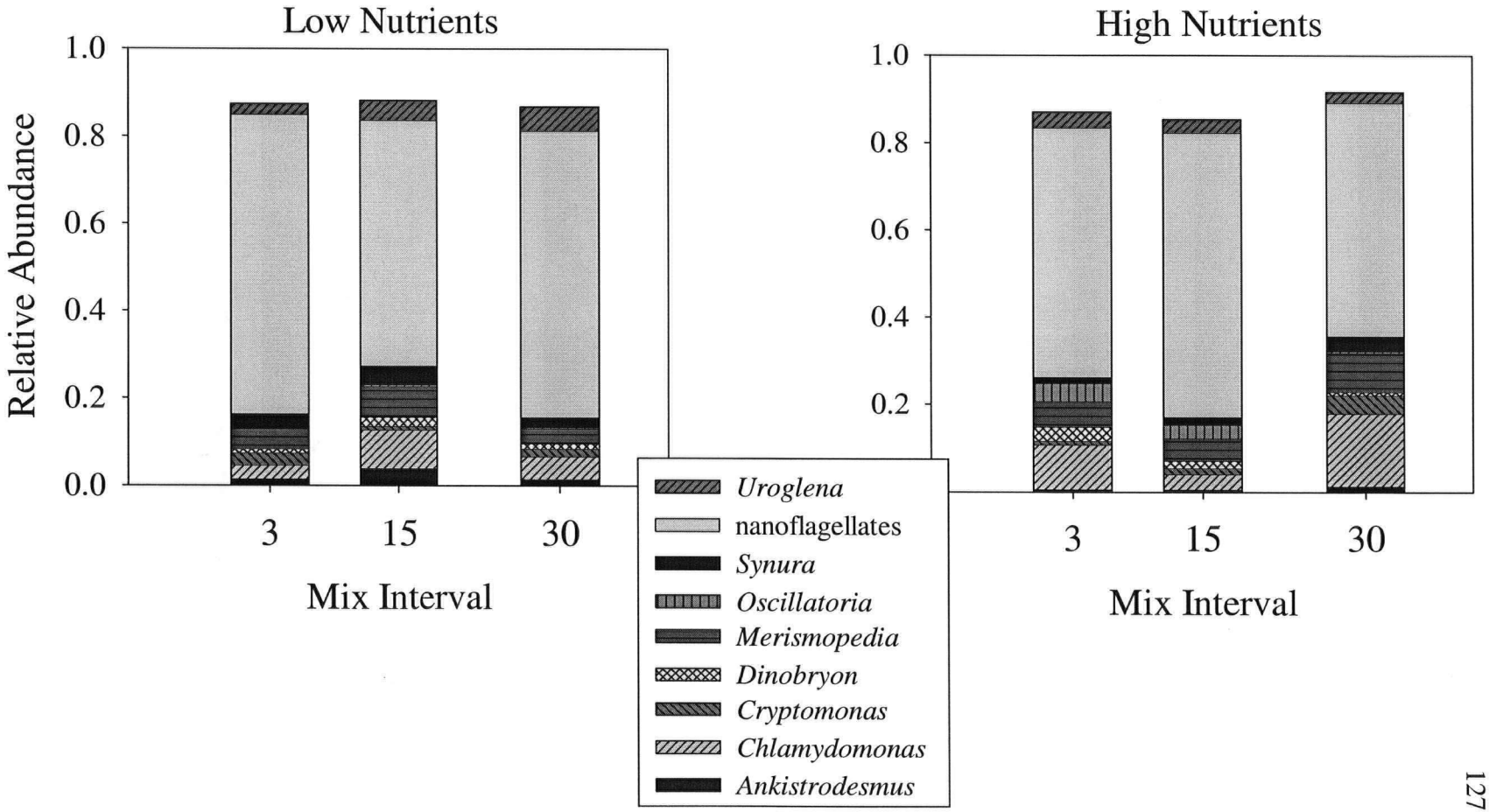
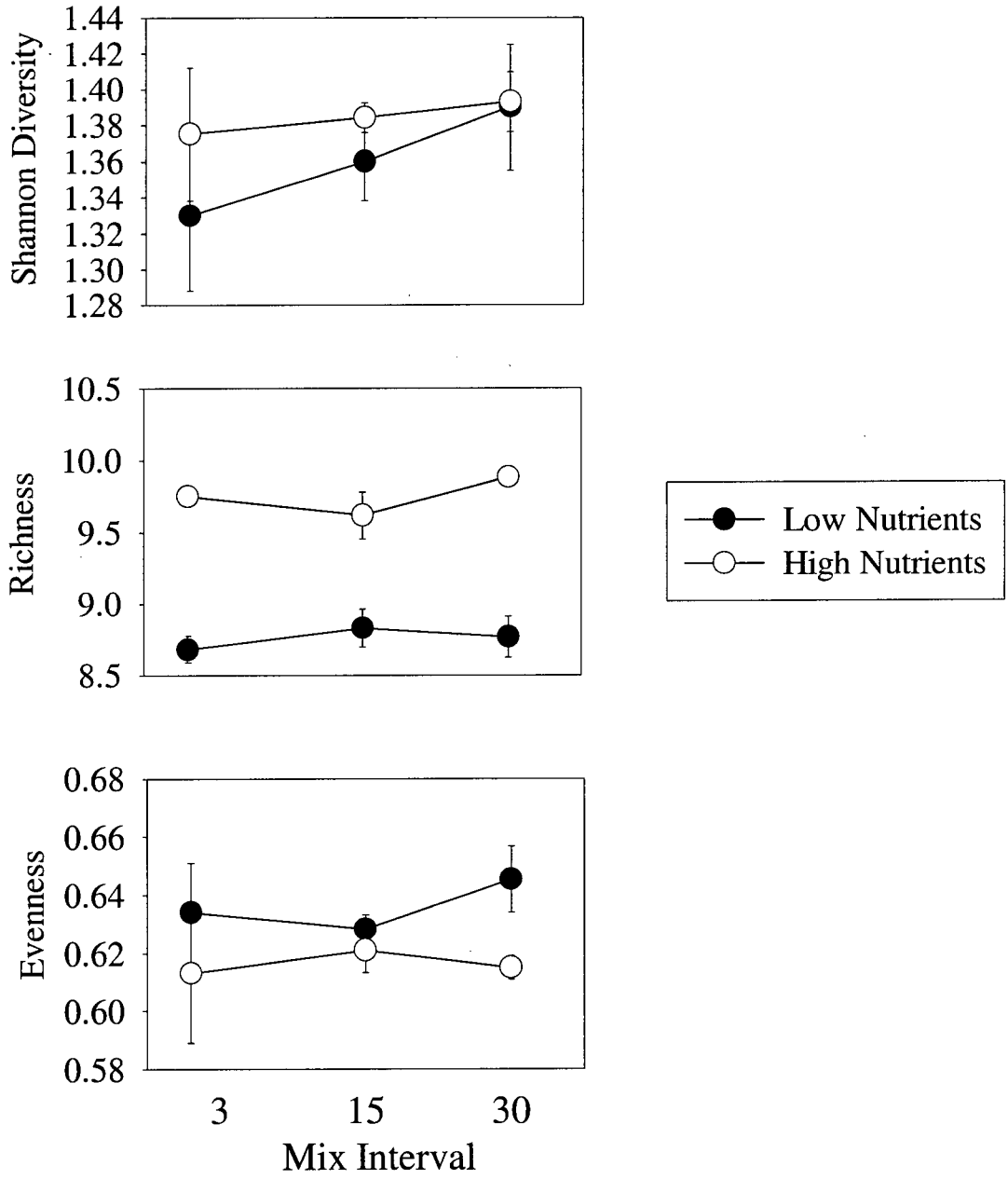


Figure 5.4: Average zooplankton diversity measures in the various experimental treatments. Values are the means of three replicates ± standard errors.



zooplankton biomass followed by copepods and rotifers (Figure 5.5). A MANOVA and discriminant analysis indicated that all three groups (cladocerans, copepods and rotifers) were responsible for biomass responses to nutrient levels (all $P<0.0001$). Cladocerans had higher biomass levels under normal low-level lake nutrient conditions ($P=0.0202$) while both copepods ($P=0.0029$) and rotifers ($P=0.0001$) responded with increases in biomass when nutrient levels were increased (Figure 5.5).

Community Composition

(i) Major Groups

When densities of zooplankton genera were classified into three major groups for analysis in a MANOVA, there was a significant multivariate effect of nutrient levels (Wilks' $\lambda=0.068$, $P=0.0001$). Discriminant analysis revealed that differences were due mainly to responses by rotifers and cladocerans, both of which had higher densities in the presence of added nutrients (Figure 5.6a). The MANOVA also revealed a slight effect of mixing frequency (Wilks' $\lambda=0.35$, $P=0.0736$) but no single group showed a similarly significant response.

The same analysis on abundances of these major taxa relative to total abundance across all groups showed a strong response to nutrient level (Wilks' $\lambda=0.77$, $P=0.0001$) and also a community-wide response to mixing frequency (Wilks' $\lambda=0.31$, $P=0.0478$) (Figure 5.6b). According to the discriminant analysis, the effect of nutrient levels was mainly a result of changes in the copepod group. From the univariate analyses however, it is clear that all groups responded to the input of nutrients: cladocerans ($P=0.0012$) and

Figure 5.5: Average zooplankton biomass ($\mu g\ l^{-1}$) over time for each major class: cladocera, copopoda and rotifera in the various treatments. Error bars represent ± one standard error of the mean.

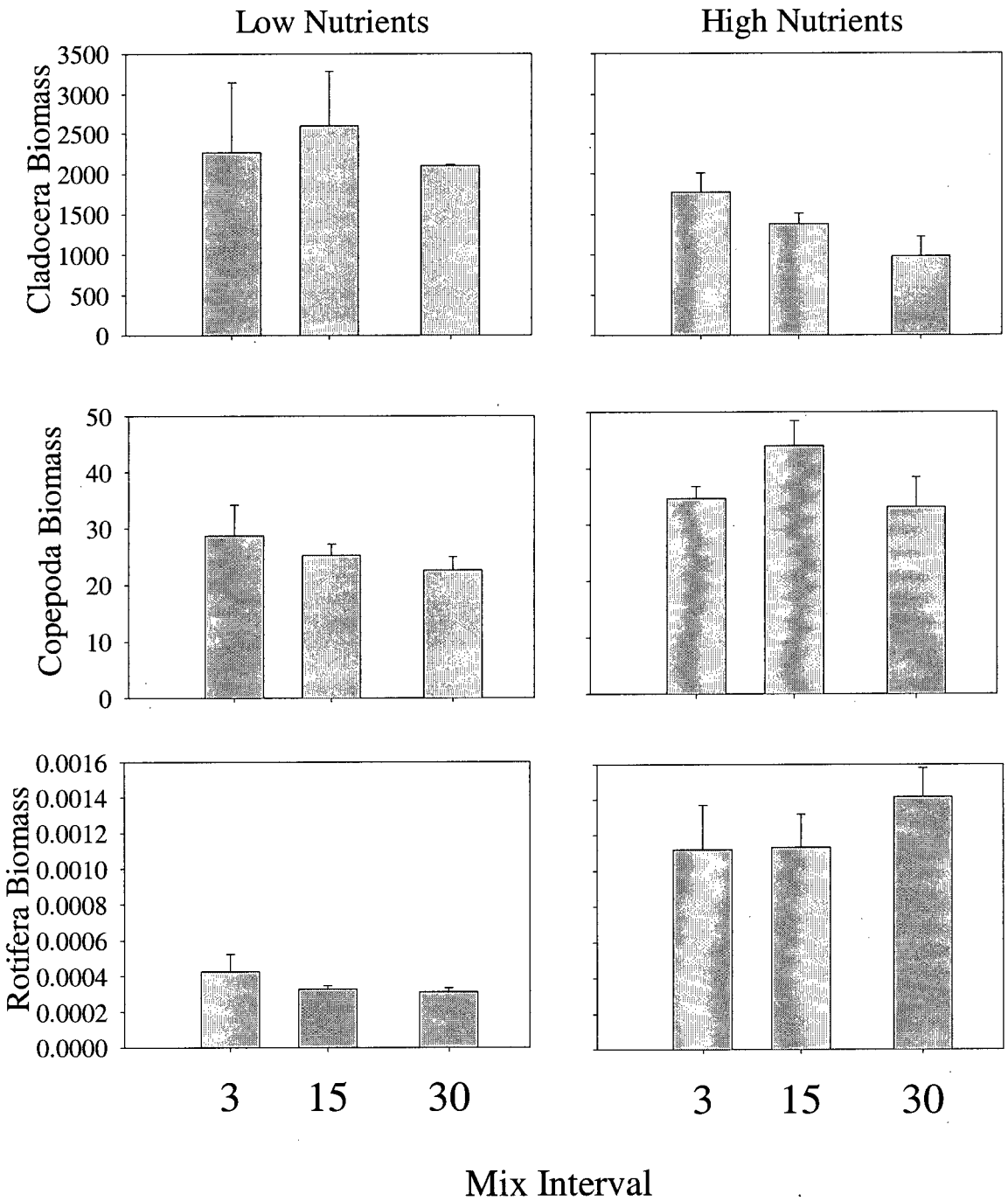
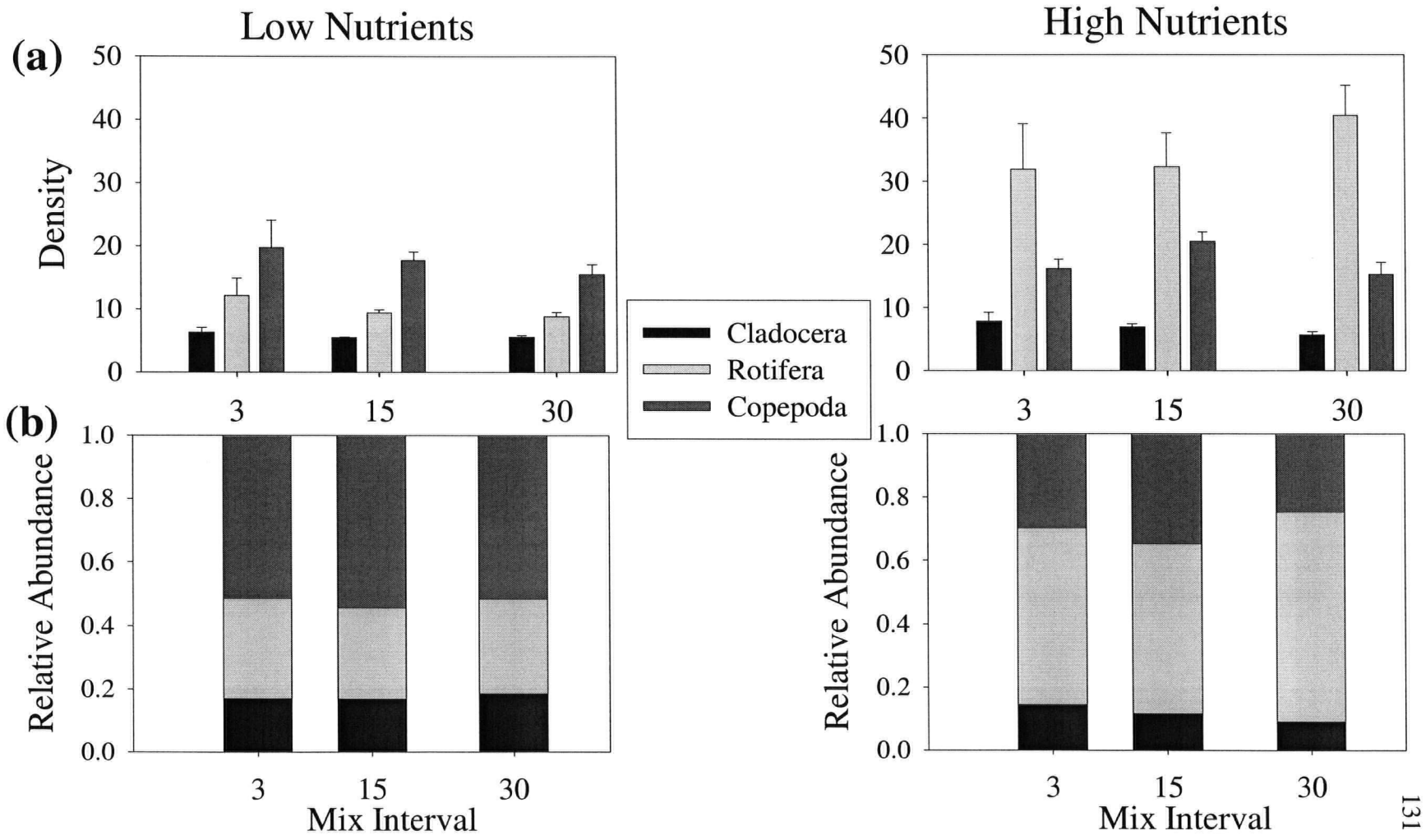


Figure 5.6: Zooplankton community composition averaged over the experimental period based on (a) densities (# l^{-1}) and (b) relative abundances. Error bars in (a) represent ± one standard error.



copepods (P=0.0001) had lower relative abundances because of a large increase in the relative abundance of rotifers (P=0.0001). Poor nutrient conditions favoured communities numerically dominated (52%) by copepods while high nutrient conditions favoured communities dominated by rotifers (58%). Copepods were also the only group to show even a minor response to mixing frequency (P=0.07). They were relatively more abundant in the 15 day treatments than in either the frequently mixed (3 day) or infrequently mixed (30 day) systems.

(ii) Cladocerans

Results of the MANOVA on densities of cladoceran genera indicated a significant nutrient effect (P=0.0002) due mainly to greater abundances of *Chydorus* and *Alona* in the enriched treatments. Univariate analyses revealed that several groups responded to changes in nutrient levels: *Chydorus* and *Alona* had higher densities in the enriched treatments while *Holopedium* did better under unenriched conditions (Table 5.1).

In terms of abundances of genera relative to the density of the entire cladoceran group, the MANOVA similarly indicated a significant effect of nutrient levels (P=0.0001) which was due mainly to the groups: *Chydorus*, *Diaphanosoma*, *Ceriodaphnia*, *Daphnia*, *Polyphemus* and *Alona*. Univariate analysis revealed that *Alona* and *Chydorus* were relatively more abundant with enrichment, while *Daphnia* and *Holopedium* were more dominant under oligotrophic conditions (Table 5.1).

The response of the *Daphnia* populations in particular was of interest since they are the dominant group under natural oligotrophic conditions (Table 5.1). *Daphnia* demography was affected mainly by nutrient levels. Juveniles

Table 5.1: Main effects of nutrient levels on average (± one standard error) densities (# l^{-1}) and relative abundances of cladoceran species. The demographic data (densities) for the various stage classes in *Daphnia* are also presented. Relative abundance is in terms of total cladoceran density. *P*-values for the univariate ANOVAs for each species are given.

		Low Nutrients	High Nutrients	*P*-value
1 -	**Densities**			
	Alona sp.	0.003 ± 0.001	0.036 ± 0.009	0.0005
	Ceriodaphnia sp.	0.013 ±0.006	0.024 ±0.005	ns
	Chydorus sp.	0.052 ±0.014	1.46 ±0.19	0.0001
	Daphnia rosea sp.	3.91 ±0.18	3.52 ±0.27	ns
	Diaphanosoma sp.	1.44 ±0.09	1.58 ±0.38	ns
	Holopedium sp.	0.34 ±0.05	0.184 ±0.025	0.0282
	Polyphemus sp.	0.010 ±0.003	0.013 ±0.003	ns
2 -	**Relative Abundances**			
	Alona sp.	0.001 ±0.000	0.006 ±0.002	0.0001
	Ceriodaphnia sp.	0.002 ±0.001	0.003 ±0.001	ns
	Chydorus sp.	0.009 ±0.002	0.228 ±0.036	0.0001
	Daphnia rosea sp.	0.68 ±0.01	0.521 ±0.018	0.0001
	Diaphanosoma sp.	0.25 ±0.01	0.213 ±0.036	ns
	Holopedium sp.	0.06 ±0.01	0.027 ±0.004	0.0039
	Polyphemus sp.	0.002 ±0.000	0.002 ±0.001	ns
3 -	**Daphnia Demography**			
	Juveniles	1.2 ±0.05	0.70 ±0.07	0.0001
	Adults without eggs	2.45 ±0.16	1.97 ±0.23	ns
	Adults with eggs	0.26 ±0.02	0.85 ±0.01	0.0001

were significantly more abundant under unenriched conditions. On the other hand, adults with eggs were more abundant under enrichment (Table 5.1).

(iii) Copepods

There was a slight multivariate response of copepod densities to the frequency of mixing (Wilks' λ=0.45, P=0.058) owing mainly to the response by *Diaptomus kenai*. *D. kenai* did significantly better under intermediate mixing regimes than with very frequent (3 day) or very infrequent (30 day) mixing conditions (Table 5.2).

No significant multivariate treatment effects were found when abundances of copepod species (*D. kenai* vs. *D. oregonensis*) were considered relative to the total density of the copepod group. The copepod group was heavily dominated by *D. oregonensis* (99.6% of total copepod abundance).

(iv) Rotifers

Based on densities of rotifer genera, the MANOVA revealed a significant nutrient effect (Wilks' λ=0.15, P=0.0133). Discriminant analysis showed that the major group leading to the difference was *Polyarthra* (P=0.0001). Densities were higher for *Polyarthra, Kellicottia,* and *Lecane* in the presence of added nutrients (from univariate analyses)(Table 5.3).

For the MANOVA based on relative abundances, again, a significant main effect of nutrients was the only response (Wilks' λ=0.18, P=0.0233). Discriminant analysis revealed the effect was due mainly to changes in *Polyarthra*. From the univariate analyses, *Polyarthra* was the only rotifer genus to be relatively more abundant in the presence of added nutrients (Table 5.3). *Gastropus* and *Kellicottia* were more prevalent under low nutrient conditions.

Table 5.2: Main effects of mixing frequency on average (± one standard error) densities (# l^{-1}) of copepod species. *P*-values for the univariate ANOVAs for each species are given.

	3 day	**15 day**	**30 day**	***P*-value**
Diaptomus oregonensis	17.9 ±2.2	19.0 ±1.1	15.3 ±1.14	ns
Diaptomus kenai	0.058 ±0.006	0.081 ±0.008	0.044 ±0.008	0.0139

Table 5.3: Main effects of nutrient levels on average (± one standard error) densities (# l^{-1}) and relative abundances of rotifer species. Relative abundance is in terms of total rotifer density. *P*-values for the univariate ANOVAs for each species are given.

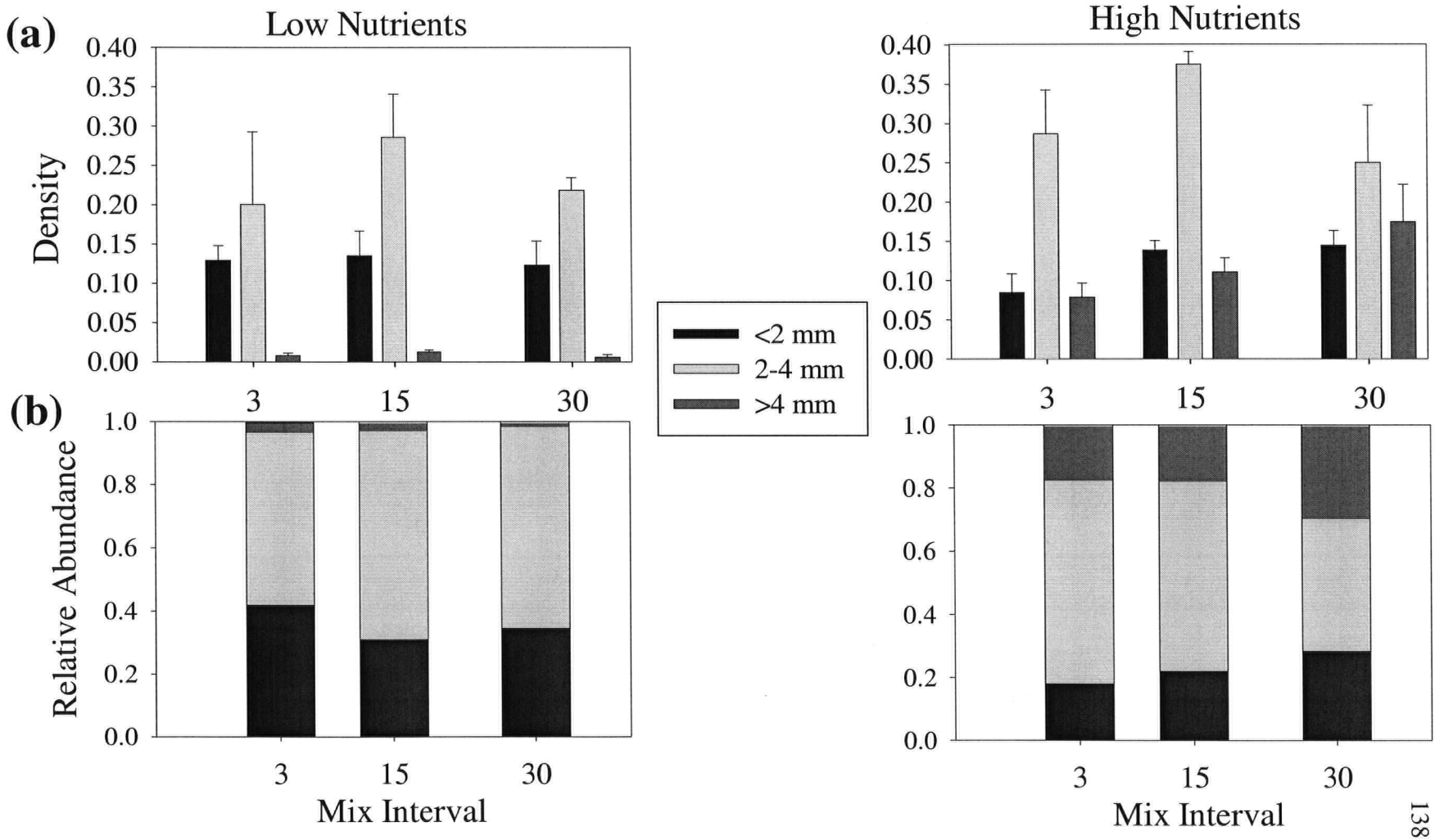
		Low Nutrients	High Nutrients	*P*-value
1 -	**Densities**			
	Filinia sp.	0.03 ±0.01	0.06 ±0.01	ns
	Gastropus sp.	0.48 ±0.09	0.58 ±0.15	ns
	Kellicottia sp.	2.48 ±0.41	5.08 ±0.36	0.0007
	Keratella sp.	0.05 ±0.05	0.03 ±0.01	ns
	Lecane sp.	0.09 ±0.02	0.26 ±0.05	0.0157
	Polyarthra sp.	6.96 ±0.54	28.9 ±3.1	0.0001
2 -	**Relative Abundances**			
	Filinia sp.	0.003 ±0.001	0.002 ±0.001	ns
	Gastropus sp.	0.050 ±0.010	0.018 ±0.005	0.0116
	Kellicottia sp.	0.237 ±0.015	0.155 ±0.018	0.0026
	Keratella sp.	0.003 ±0.003	0.001 ±0.000	ns
	Lecane sp.	0.010 ±0.002	0.007 ±0.001	ns
	Polyarthra sp.	0.697 ±0.014	0.817 ±0.019	0.0001

CHAOBORUS POPULATIONS

The total density of *Chaoborus* was higher under enriched conditions (-nutrients = 0.37 l^{-1} ± 0.04, +nutrients = 0.55 l^{-1} ± 0.05; P=0.0224). There were also demographic shifts. A MANOVA on *Chaoborus* class densities showed a significant effect of nutrient enrichment (Wilks' λ=0.22, P=0.0013) owing mainly to the increase in the largest size class (>4 mm) (P=0.0001) with enrichment.

In terms of relative abundances, the smallest (<2 mm; P=0.0117) and the largest (>4 mm; P=0.0001) groups showed significant responses to nutrient levels (MANOVA Wilks' λ=0.08, P=0.0001)(Figure 5.7b). The smallest group was more abundant under oligotrophic conditions, while the largest group was more abundant with enrichment. There was also a slight mixing effect revealed by the relative abundance MANOVA (Wilks' λ=0.36, P=0.083). Univariate analyses revealed that any effect of mixing was a result of an interaction with nutrient levels. The intermediate size class (2-4 mm) was more dominant with more frequent (3 or 15 day) mixing than for infrequent, but only when systems were enriched ($P_{interaction}$=0.047; +nutrients, 3 day vs. 30 day P=0.015; +nutrients 15 day vs. 30 day P=0.042). For infrequently mixed systems, this size class was more dominant under oligotrophic conditions (P=0.017). The opposite relationship occurred for the largest size class ($P_{interaction}$=0.032; +nutrients, 3 day vs. 30 day P=0.024; +nutrients 15 day vs. 30 day P=0.026), which was least dominant when mixing was infrequent under oligotrophic conditions. Under all mixing regimes, the largest size class was more dominant with enrichment than without (all P<0.0005).

Figure 5.7: Size structure of *Chaoborus flavicans* based on (a) densities (# l^{-1}) and (b) relative abundances of the various size classes in each treatment. Values in (a) represent time and treatment means ± standard error.



ZOOPLANKTON DYNAMICS

In addition to mean biomass and densities for zooplankton, data were collected on the dynamics of both zooplankton and *Chaoborus* populations (Figures 5.8, 5.9 and 5.10; also see Appendix 3 for full dataset). Rotifer populations peaked just before the *Chaoborus* population biomass did under all conditions (Figure 5.8). Overall, however, biomass increases were larger and peaks were maintained for longer under enriched conditions for both rotifers and *Chaoborus* (Figure 5.8) For the cladocerans, *Daphnia* biomass was maintained at constant levels throughout the experimental period, unaffected by nutrient levels and by mixing frequency (Figure 5.9). For all other cladocerans combined, dynamics were similar at both nutrient levels with more temporally variable biomass when systems were mixed frequently than when they were infrequently perturbed. There was an especially large second increase in cladoceran biomass in the enriched systems that were frequently mixed. All changes in total cladoceran biomass were driven by the large changes in *Holopedium* biomass. Finally, for copepods, dynamics were also less variable under oligotrophic conditions and were relatively unaffected by the frequency of mixing (Figure 5.10). Biomasses were much greater and more peaked under enriched conditions.

Discussion

Vertical mixing experiments with pelagic systems have generally ignored the responses of entire plankton communities or food chains with more than one trophic level. This study has attempted to remedy that situation by examining the simultaneous responses of natural lake plankton communities to different frequencies of vertical

Figure 5.8: Time series of the total average (± standard error) rotifer biomass in $\mu g\ l^{-1}$ and for comparison, a standardized relative biomass estimate for *Chaoborus* (based on a biomass of 0.0001 for the smallest size class).

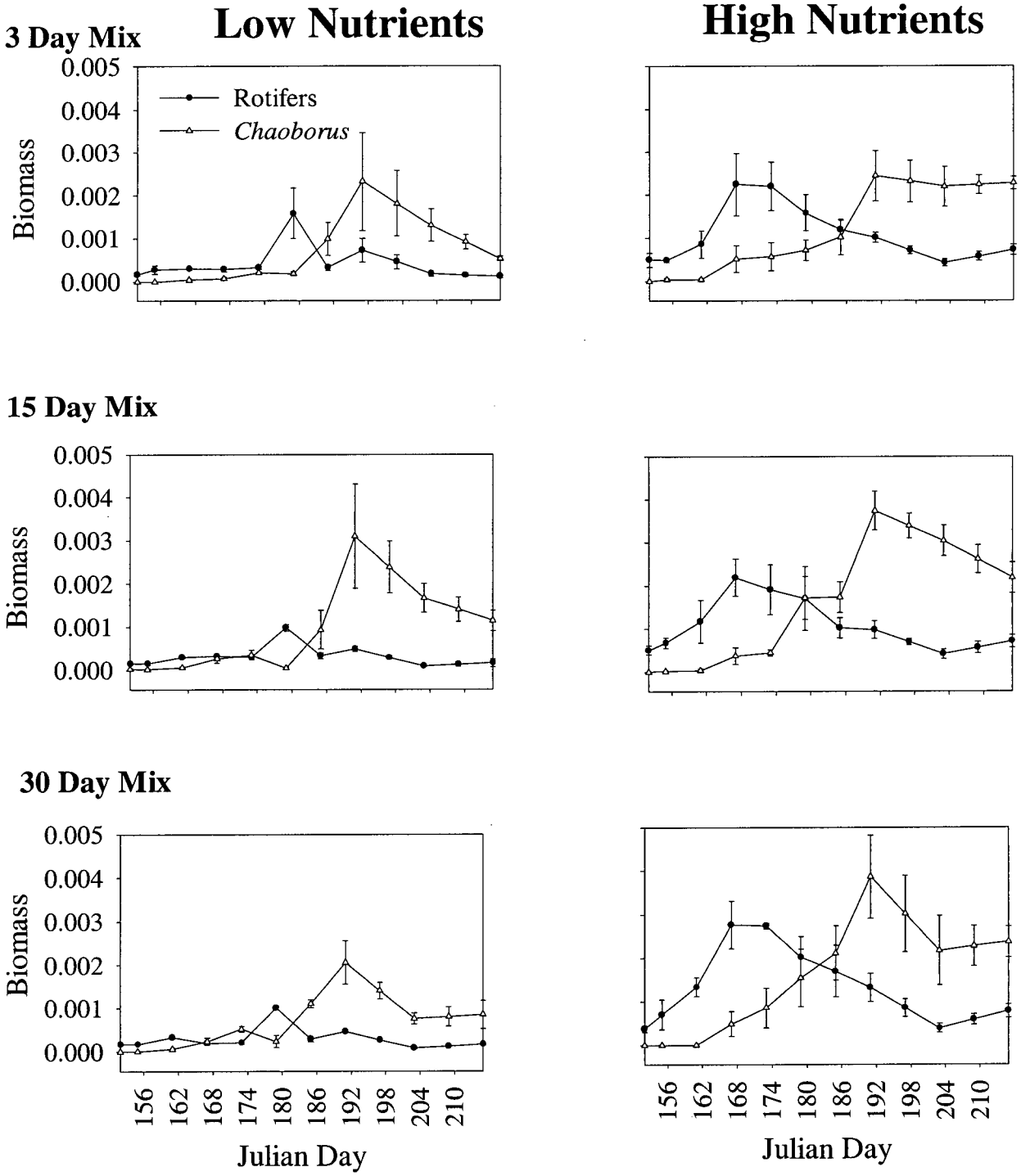


Figure 5.9: Time series of the average (± standard error) biomass of *Daphnia* and the sum of all other cladocera in $\mu g\ l^{-1}$.

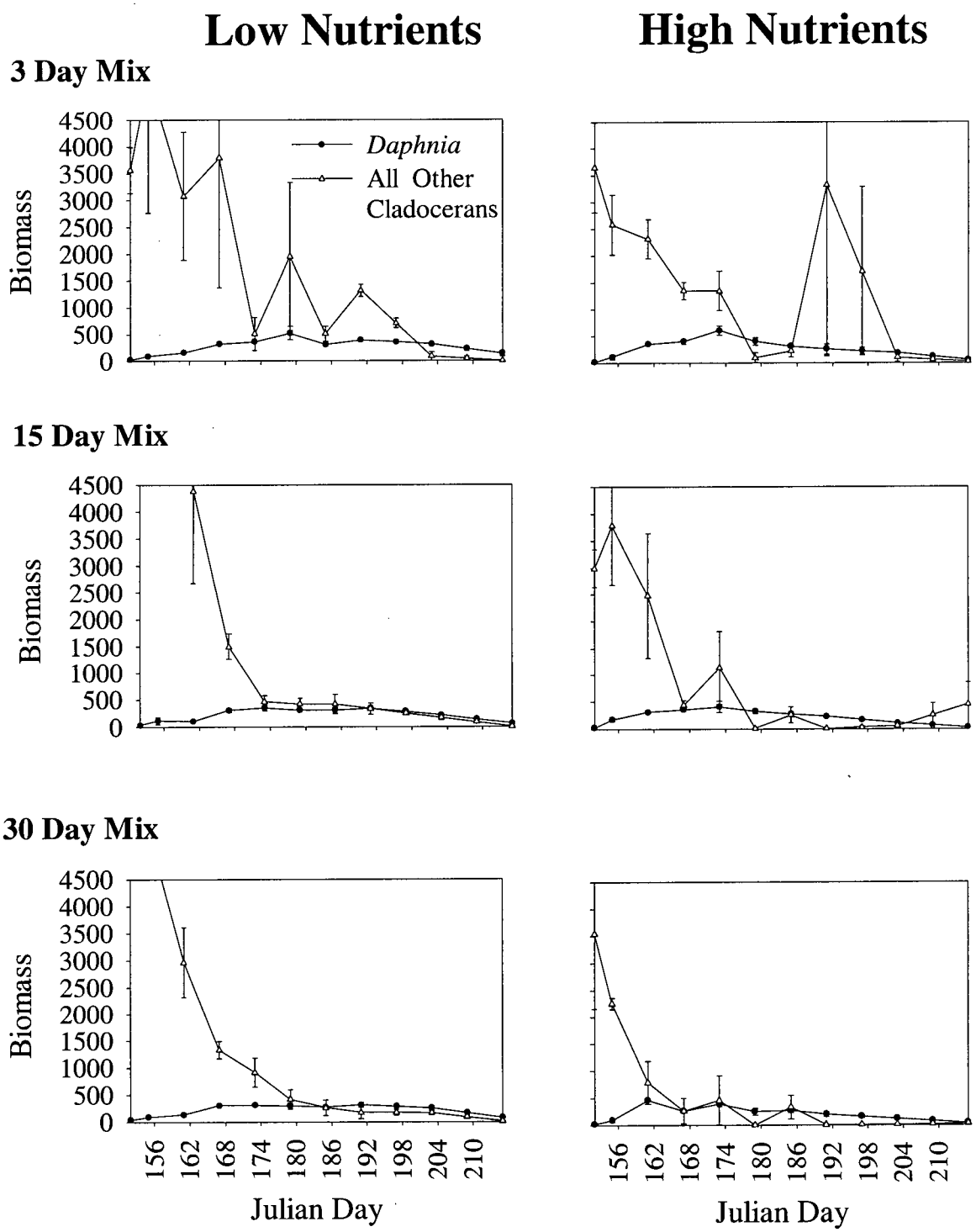
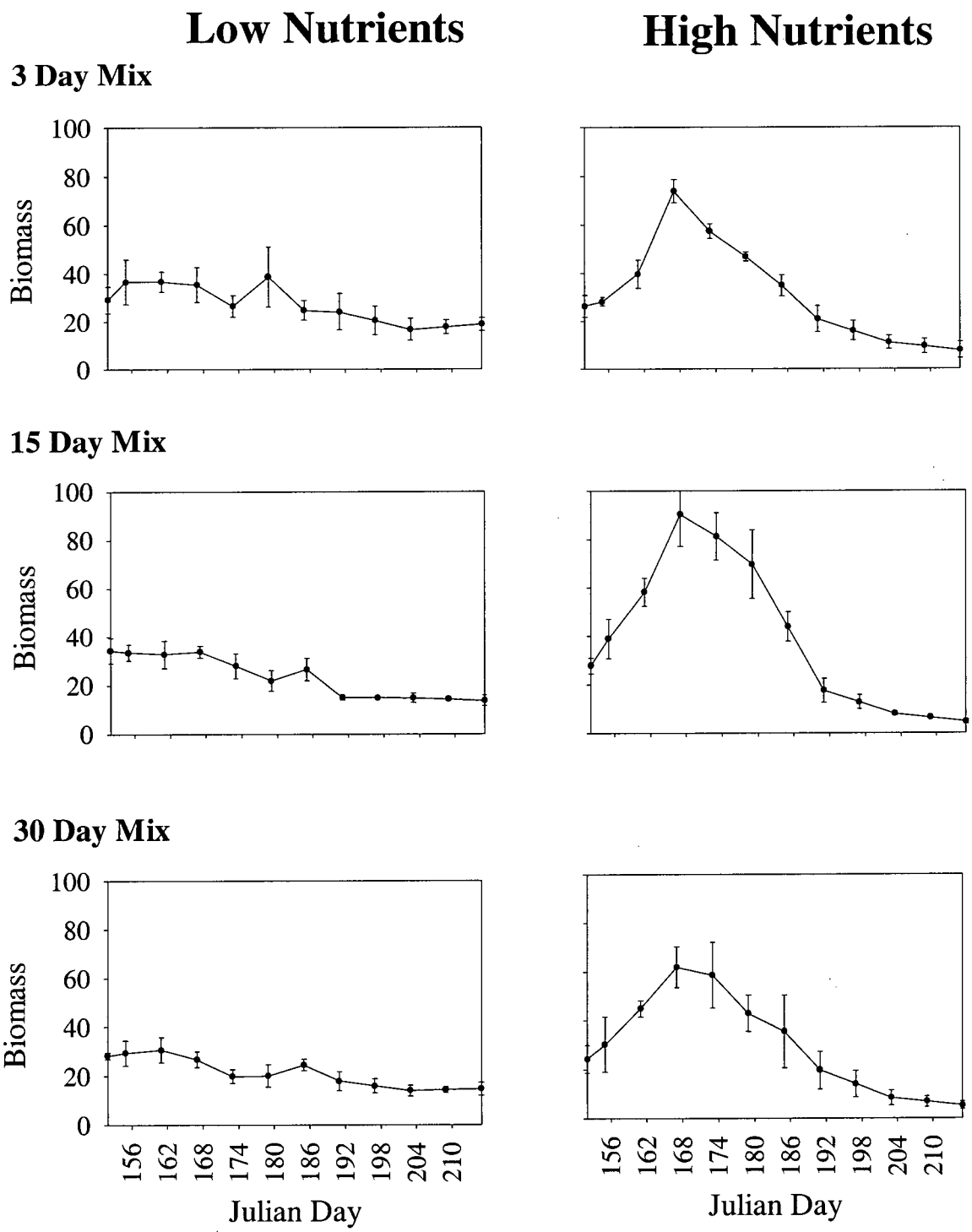


Figure 5.10: Time series of the average (± standard error) biomass of all copepods in $\mu g\ l^{-1}$.



mixing under oligotrophic and nutrient-enriched conditions, in the absence of fish predation.

Mixing Effects

Responses to mixing frequency generally only occurred with enrichment. These plankton communities did not respond to alterations in the frequency of vertical mixing under natural oligotrophic conditions. The main effect on the phytoplankton communities was observed in the response of *Cryptomonas* sp., although this represents a weak result and may be a spurious one when adjustments for multiple comparisons are made. Enrichment led to lower relative abundances for *Cryptomonas* in frequently mixed systems, but higher relative abundances in infrequently mixed systems. *Cryptomonas*, a motile species may gain a competitive advantage when systems are unmixed and nutrients are high. Frequent mixing probably reduces the effective advantage of their motile habit for competition and avoiding predation.

At the next trophic level, there was no effect of mixing frequency on the zooplankton community structure on the final date of the experiment under either oligotrophic or enriched conditions. However, from the dynamical data, the total biomass levels of all cladocerans together fluctuate more with frequent mixing.

The demography of the top trophic level, the invertebrate carnivore *Chaoborus*, was affected by mixing frequency, but only at high nutrient levels. Water column stability appears to favour high survivorship and recruitment of larger *Chaoborus* under enriched environmental conditions where survivorship and recruitment to the largest size class can occur (Neill 1988a). It is possible the mixing process actually imposed significant mortality on larger individuals because of physical damage from turbulent

forces. Although not for *Chaoborus* specifically, such sensitivities to the physical effects of turbulence have been noted for other zooplankton, especially more gelatinous forms (Acuna *et al.* 1994, Petersen *et al.* 1998).

The major effects of frequency of vertical mixing on full plankton communities were two-fold. First, most effects appeared only at the higher trophic levels, and especially in the demography of the population at the top trophic level. It appears that the presence of higher trophic levels (i.e. complete zooplankton community and *Chaoborus* populations) reduces the responsiveness of the phytoplankton community (as compared to the responses seen previously in Chapters 3 and 4). Second, significant effects of mixing only appeared under enriched conditions and not under the natural regime of the lake. This result suggests that these systems are naturally fairly robust to different frequencies of vertical mixing and only respond when major changes to the system through nutrient addition or the removal of food web components occur.

Environmental Productivity Effects

Regardless of the mixing schedule, there were strong responses at all trophic levels to the background environmental productivity of the system. Diversity of the phytoplankton communities was reduced with enrichment, owing to a decline in community evenness and despite a higher species richness. There were no statistically significant shifts in phytoplankton community composition or size structure owing to enrichment alone.

More species of zooplankton were supported with added nutrients. For the zooplankton, enrichment led to higher richness but not to significant changes in Shannon diversity or evenness. Changes in the zooplankton species composition accompanied this

shift in richness. Under natural, oligotrophic conditions, the zooplankton communities were dominated numerically by copepods (*Diaptomus oregonensis*) and in terms of biomass, by cladocerans. Enrichment shifted the numerical dominance to rotifers, mainly *Polyarthra* sp., but zooplankton biomass was still dominated by cladocerans. There were important shifts in the species composition of cladocerans however. *Daphnia* and *Holopedium* dominated the natural state, while *Chydorus* and *Alona* came to dominate with enrichment. In general, there was a numerical shift to smaller sizes of macrozooplankton species and rotifers with enrichment. Nutrient enrichment led to an increase in the densities of cladocerans but not to biomass because of this shift in dominant body size within the group.

Neill (1988b) summarizes enrichment experiments in other lakes in the Malcolm Knapp Research Forest. In nearby Gwendoline Lake, enrichment led to dramatic increases in *Daphnia* densities that were not observed here. Rather, these results resemble the responses to enrichment of Eunice Lake where dramatic increases in rotifers, copepod nauplii and small crustacean zooplankton have been observed. Placid Lake grazer communities show the same response with a shift to small-bodied zooplankton.

The third trophic level present in these mesocosms was the invertebrate carnivore *Chaoborus flavicans*. In the past, it has been demonstrated that this species can show strong responses to enrichment of oligotrophic systems because of the enhanced survivorship of young instars that accompanies the proliferation of rotifer prey early in the growing season (Neill 1988a). A similar increase in survivorship and accelerated growth to larger size classes was noted with enrichment in this experiment. *Chaoborus*

populations were denser and dominated more quickly and to a larger degree by the largest size class (>4 mm) when nutrients were added.

Food chain theory is generally concerned with biomass levels at various trophic levels and responses of biomass to increased environmental productivity. In this study, enrichment had no effect on total phytoplankton biomass; it led to a decline in herbivore biomass and an increase in the invertebrate carnivore biomass at the third trophic level. Standard theory (e.g. Oksanen *et al.* 1981, Carpenter *et al.* 1985), that considers trophic levels as a single homogeneous entity, predicts the increase in the top trophic level of carnivores and the decline in herbivores as a cascading effect. However, standard theory also predicts an increase in primary producer biomass. In this study, the biomass levels of primary producers did not follow the predicted pattern in these three-trophic level food chains. As has been proposed by others, the lack of congruence between theory and experiment probably lies in the fact that trophic levels are not homogeneous entities but rather have important structural and biological features that can also change with enrichment (Leibold 1989, Abrams 1993, Grover 1995). The pattern of competition between herbivores can alter the predicted responses of both higher and lower trophic levels when heterogeneous trophic levels are considered (Abrams 1993). As a result, without knowing the exact food web structure, it can be difficult to make predictions for changes in trophic level biomass. In this study, there were important shifts in herbivore species composition that could have compensated for declines in overall biomass, allowing them to maintain control over the phytoplankton biomass even with enrichment. Shifts to numerical dominance by small cladocerans, copepods and rotifers occurred with enrichment. The higher turnover rates of the smaller zooplankton and the efficiency of

copepod feeding may have ensured continued control over phytoplankton biomass despite a decline in absolute biomass of zooplankton. Higher turnover rates can maintain high grazing rates. In any event, the upper two trophic levels in this three-trophic level food chain responded to enrichment in a pattern predicted by food chain theory (Oksanen *et al.* 1981).

As for the effects of temporal heterogeneity, the greatest effects of enrichment on community species composition and population demographies were similarly observed most strongly at higher trophic levels. The *Chaoborus* population was more dense and consisted of larger individuals with added nutrients. Zooplankton community composition also responded to the effect of nutrient levels with greater dominance by smaller species. It is unclear whether their response was due mainly to the presence of added nutrients or to the increased growth and survival of *Chaoborus*. There was most likely a response to both factors, mediated through size-structured responses. From the dynamical data, enrichment promotes more pronounced and earlier rotifer biomass peaks, allowing *Chaoborus* to reach larger sizes in time to take advantage of summer peaks in macrozooplankton numbers. The lack of major shifts in the phytoplankton community structure and biomass with enrichment suggests that zooplankton control to some degree the response of phytoplankton to enrichment. Again, top-down effects seem to be the manifestation of the bottom-up effect of added nutrients. As predicted by food web theory, the responses to increased productivity levels at the base of the food chain are manifested most strongly in the population densities, biomass and size structure of the top trophic level (Abrams 1993).

In trying to disentangle the role of various factors in the ecology of plankton communities, this study has shown that environmental productivity can have more obvious effects than the scale of temporal heterogeneity of vertical mixing. Effects of temporal heterogeneity generally appear only with enrichment as I also found in Chapter 3. Phytoplankton community structure responses to intermittency appear to be muted by the presence of higher trophic levels. This raises the question as to the value of relating studies of intermittent nutrient pulsing in laboratory cultures to oligotrophic lake conditions where higher trophic levels are always present. It appears that a full complement of zooplankton and low nutrient conditions can preclude bottom-up effects of abiotic temporal heterogeneity for phytoplankton.

CHAPTER 6

General Conclusions

In this thesis, I have explored the influence of various scales of temporal heterogeneity in abiotic factors that are commonly thought to influence the structure of plankton communities (e.g. Harris and Griffiths 1987, Reynolds 1993). Among the most significant immediate effects of vertical mixing on pelagic organisms are the alterations to the resource base (nutrients, detritus, and light) and the movement of organisms relative to each other. The mixing treatments in the series of experiments introduce higher levels of turbulence (see Chapter 3) than would commonly occur in small temperate lakes. Levels were similar to those expected for high wind conditions associated with storm events (Kocsis *et al.* 1999, MacIntyre *et al.* 1999). Temporal scales considered ranged from frequent (3-5 days) to intermediate (15 days) to infrequent (21, 25 and 30 days or never mixed). These three time scales span the generation time of many phytoplankton, the approximate frequency of storm events (Harris and Griffiths 1987, Reynolds 1993), the time required for a successional climax in phytoplankton competition experiments (Sommer 1989), and the generation times for important zooplankton like *Daphnia.*

Chapter 2 improves the ability of theoretical models of phytoplankton competition to represent more accurately the results of chemostat studies of nutrient pulsing. This was accomplished by adding demographic and environmental stochasticity. Demographic stochasticity was more important than environmental stochasticity for expanding the ability of the model to display high diversity levels, mainly because it introduced a storage effect along the lines described by Chesson (1994). Through time

lags that arise between resources and populations with demographic stochasticity, the competing populations fluctuate out of phase. Each population experiences periods of strong positive growth that allows persistence of each over longer periods of time. The model demonstrates that temporal heterogeneity in resource supply can structure phytoplankton communities by altering composition or diversity, especially under oligotrophic conditions. The stochastic model also finds that coexistence of gleaners and opportunists is easier when phytoplankton are competing in nutrient-poor environments, while gleaners tend to dominate with enrichment.

The first experiment (Chapter 3) represents the closest test of the model in a natural system. The responses of natural phytoplankton to various frequencies of vertical mixing and to enrichment were examined. Few responses to mixing frequency were found in the natural oligotrophic state of the systems, and communities generally consisted of very competitive gleaners. Experimentally enriched systems would be classified as meso-eutrophic (Vollenweider 1968). With enrichment, a greater diversity of forms was observed with a larger range of size classes represented. Apparently, a wider range of life history types (including opportunists and storage strategists) was able to persist when overall nutrient levels were higher. Responses to different scales of vertical mixing were observed in the enriched communities. The community changes can be attributed not just to responses to pulses in nutrients that generally accompany deep water mixing but also to the frequency with which natural spatial structure is disrupted by turbulent water movements. The results suggest that vertical thin-layers, small-scale patchiness and phytoplankton characteristics like buoyancy may have important

consequences for phytoplankton community structure and its response to intermittent vertical mixing.

In relating this first field study to the model of Chapter 2, I find that the model correctly predicts the pattern of diversity in frequently mixed systems and the response to enrichment but that for composition, the empirical results are opposite to the predictions. The model predicts that diversity should be higher under low nutrient conditions than under high nutrient ones (case 2 *vs.* case 3 in Table 2.2) as is observed in Chapter 3 (see 3 day mixing in Fig. 3.2 diversity plot). For composition however, the model predicts that, with enrichment and nutrient pulses, phytoplankton communities should consist of less disparate life history strategies with surviving species all adopting gleaner strategies. I saw the opposite trend in the experiment with a greater diversity of forms under enriched pulsed conditions and gleaners dominating under oligotrophic conditions. The discrepancy can be attributed to the simplicity of the model that considers only one axis for competition (nutrients) and only the very simplest of life history strategies (gleaners and opportunists). The storage strategists, which are generally the larger phytoplankton (Reynolds 1988), became important under enriched experimental conditions, but they are not incorporated into this first version of the model. Note that the "storage strategy" discussed here involves a qualitative change to the model to incorporate luxury consumption and is not the same as the "storage effect" (*sensu* Chesson 1994) resulting from demographic stochasticity. Grover (1991c) found that considering storage as an alternative strategy in competition models could have significant effects on the outcome of predictions in fluctuating environments. When luxury consumption is included as a strategy, species that have both lower than maximal growth and uptake rates can

dominate. For the sake of tractability, such a strategy was not considered in our model, but the inclusion of it could have led to a greater range of as well as different dominant life history types in the community. This greater range was observed experimentally, with natural communities, where such life history strategies exist.

In Chapter 4, I considered the role of food web structure on the phytoplankton community responses to various scales of vertical mixing by incorporating a generalist herbivore. This experiment was conducted under natural oligotrophic conditions and considered the role of the important herbivore *Daphnia rosea*. The major result of this study was that the role of spatial structuring in ecological interactions and the frequency with which these interactions are disrupted are the biggest consequences of various scales of intermittency in mixing. Systems that were frequently disrupted by mixing had lower phytoplankton richness levels but only in the presence of *Daphnia*. It is hypothesized that frequent mixing increased encounter rates between predator and prey and thereby decreased refuge availability in time and space for prey, leading to less stable interactions and a loss of phytoplankton richness. In the absence of *Daphnia* there were again no effects of different frequencies of mixing on the phytoplankton community structure under oligotrophic conditions, just as observed in Chapter 3.

In the last chapter, I discuss the results of an experiment that examines the response of the entire plankton food chain to various scales of intermittency. Most responses were observed under enriched conditions, but the responses of the phytoplankton communities are minor compared to those observed in the complete absence of higher trophic levels (i.e. Chapter 3). In this study, there were no changes in diversity of the phytoplankton with changes in the frequency of mixing and only one

important compositional change. Zooplankton dynamics may have fluctuated more with changes in the scale of temporal heterogeneity, but responses at this trophic level were also small. There were significant changes to the age-structure of the top trophic level of *Chaoborus* as a result of changes in mixing frequency that appear to be related to the detrimental effect of high turbulence for the larger stages. The results suggest that temporal heterogeneity in abiotic conditions may have little effect on phytoplankton communities under natural conditions when higher trophic levels are present.

Interestingly, in all experiments, the effects of either enrichment or alterations to the food web structure (i.e. addition of *Daphnia*) had larger effects on the community structure of phytoplankton than did the temporal scale at which vertical mixing occurred. However, responses by the phytoplankton communities are much more apparent in the absence of higher trophic levels (Chapter 3) than when full zooplankton communities are present (Chapter 5). The mixing imposed in all of these experiments was at levels higher than would normally occur in north-temperate lakes. As revealed in the first study (Chapter 3), it appears that the important factor is whether systems are mixed at all, and the frequency of this mixing is less important. The second major conclusion from this work is that nutrient pulsing appears to be a minor part of the effects of mixing on these plankton communities. Rather, it appears that the movement of organisms relative to each other and the effect that this has on interspecific interactions of predation and competition are more important.

Finally, phytoplankton communities are most sensitive to different scales of intermittency in their environment when systems are enriched. Why is the response of oligotrophic communities so reduced? I propose three non-exclusive explanations for

reduced responses in nutrient-poor systems. The first is that life histories in harsh oligotrophic environments may be primarily constrained to survive under low nutrient conditions. Under enriched conditions, a wider range of sizes, life histories, forms and mobilities is represented. This may allow for a broader range of potential community states with enrichment that can be observed when the systems are further perturbed at various frequencies. Second, the tight coupling of the microbial loop to phytoplankton production under oligotrophic conditions (Goldman 1984, Stone and Weisburd 1992, Stone and Berman 1993) and the reduced loss of biomass and nutrients to the hypolimnetic zone (Wehr *et al.* 1994) should lead to smaller nutrient pulses when vertical mixing occurs. Finally, the enriched systems may have been less stable because they were already perturbed away from their natural state when nutrients were added. Under natural conditions, mixing itself is only a minor perturbation to which the community is fairly robust. These communities may be well adapted to deep water mixing or turbulence at various scales because of the many potential sources occurring naturally, including fish movements (Schindler *et al.* 1993), zooplankton excretion (Lehman and Scavia 1982) and natural mixing events (Harris and Griffiths 1987, Reynolds 1993). The destabilization introduced by the nutrient perturbation may sensitize the communities to further perturbations such that the scale at which they occur matters.

An important *caveat* to keep in mind however is that these experiments were done in different years without complete replication of baseline treatments in each year. This was unavoidable given the scale of the project and the number of treatments investigated. However, where similar treatment conditions did exist (i.e., low nutrient treatments in 1999, chapter 3 and zooplankton exclusion treatments in 1997, chapter 4), similar results

were obtained. This, and observations by others (e.g., Reynolds 1984b, W.E. Neill personal communication), as well as the predictability of plankton succession within a single lake in different years represented by conceptual frameworks such as the P.E.G. model (Sommer *et al.* 1986), suggest that between-year comparisons may be justified. Nevertheless, a within-year comparison of the effects of enrichment on communities with and without higher trophic levels would be valuable.

It may be useful to compare my results on plankton community structure with the predictions of a general model of plankton community structure, the P.E.G. model (Sommer *et al.* 1986). The P.E.G. model considers the role of species interactions and replacement (autogenic succession) and the role of abiotic perturbations on these interactions (allogenic succession), based on observations from Lake Constance (Bodensee), a nutrient-rich warm monomictic lake. Succession in this system is based heavily on bottom-up effects of nutrient competition for phosphorus, nitrogen and silica. When the physical process of deep water mixing begins in late summer in Lake Constance, it leads to a large change in phytoplankton species composition, despite the presence of grazers. In Placid Lake, under oligotrophic conditions without herbivores, there is little change in phytoplankton composition with mixing. My results only support the P.E.G. model of phytoplankton nutrient competition leading to successional replacement under conditions where the range of life forms is broad enough, or the nutrient pulses are large enough (i.e., with nutrient enrichment). A modified model of plankton community succession may be required for oligotrophic conditions where population interactions based on bottom-up, fluctuating nutrients may be less important. Rather, factors related to top-down effects of zooplankton grazing and encounter rates

may be the more important consequence of physical mixing events in oligotrophic systems.

A related conceptual framework for phytoplankton community structure was developed by Reynolds (1984b, 1989) based on the roles of physical, chemical, and biotic forces. In Reynolds' (1984b) framework, allogenic forces like periodic water column mixing act to reset the successional sequence to an earlier stage where nutrients are more available and competitive exclusion is alleviated. For oligotrophic conditions, this "resetting" should be of smaller consequence because systems are more constrained because of the small size of nutrient pulses and because of the more limited types of phytoplankton that can exist under nutrient-depauperate conditions. Putting the results of my study within Reynolds' (1984b, 1989) framework, it appears that allogenic forces have less influence on phytoplankton community structure in oligotrophic systems.

Some authors have argued that complex systems, like ecological ones, sit "at the edge of chaos" (e.g. Rosenzweig 1971, Abrams and Roth 1994, Huisman and Weissing 1999). This implies that population dynamics and community structure are susceptible to radical changes if perturbed. The results of this empirical study with natural phytoplankton communities suggest that this may not be the case, at least with regard to the influence of mixing frequency. There may very well be compensatory processes in natural communities at the behavioural, population, and evolutionary level that constrain the community responses within certain bounds for commonly encountered abiotic forces like temporal heterogeneity in deep-water mixing. Mathematical models that find large effects on population dynamics or community structure may not consider behavioural or physiological flexibility on the part of the community's members to withstand commonly

encountered perturbations. This study indicates that rarely encountered events like changes in system productivity or, the addition or removal of trophic linkages may have much larger effects than more common events like vertical mixing.

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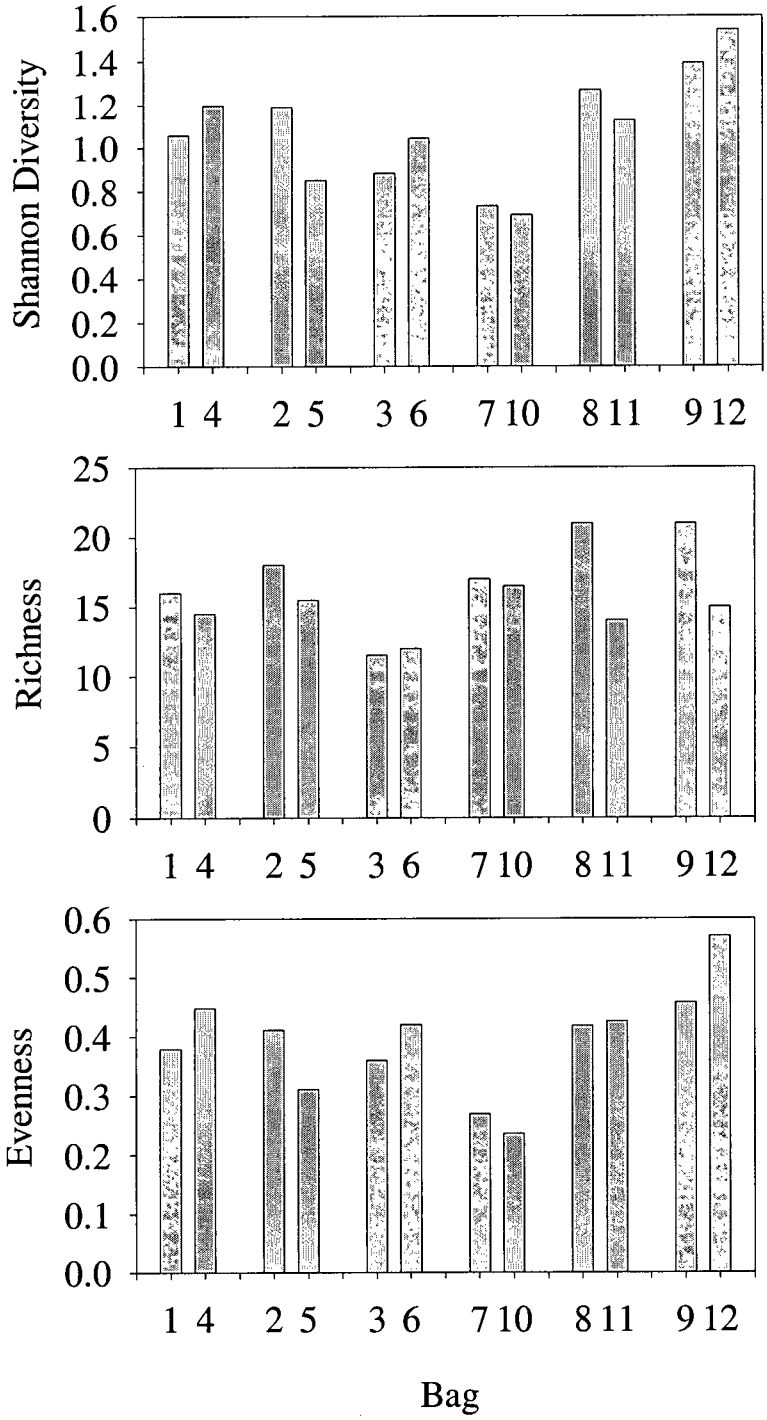
APPENDIX 1

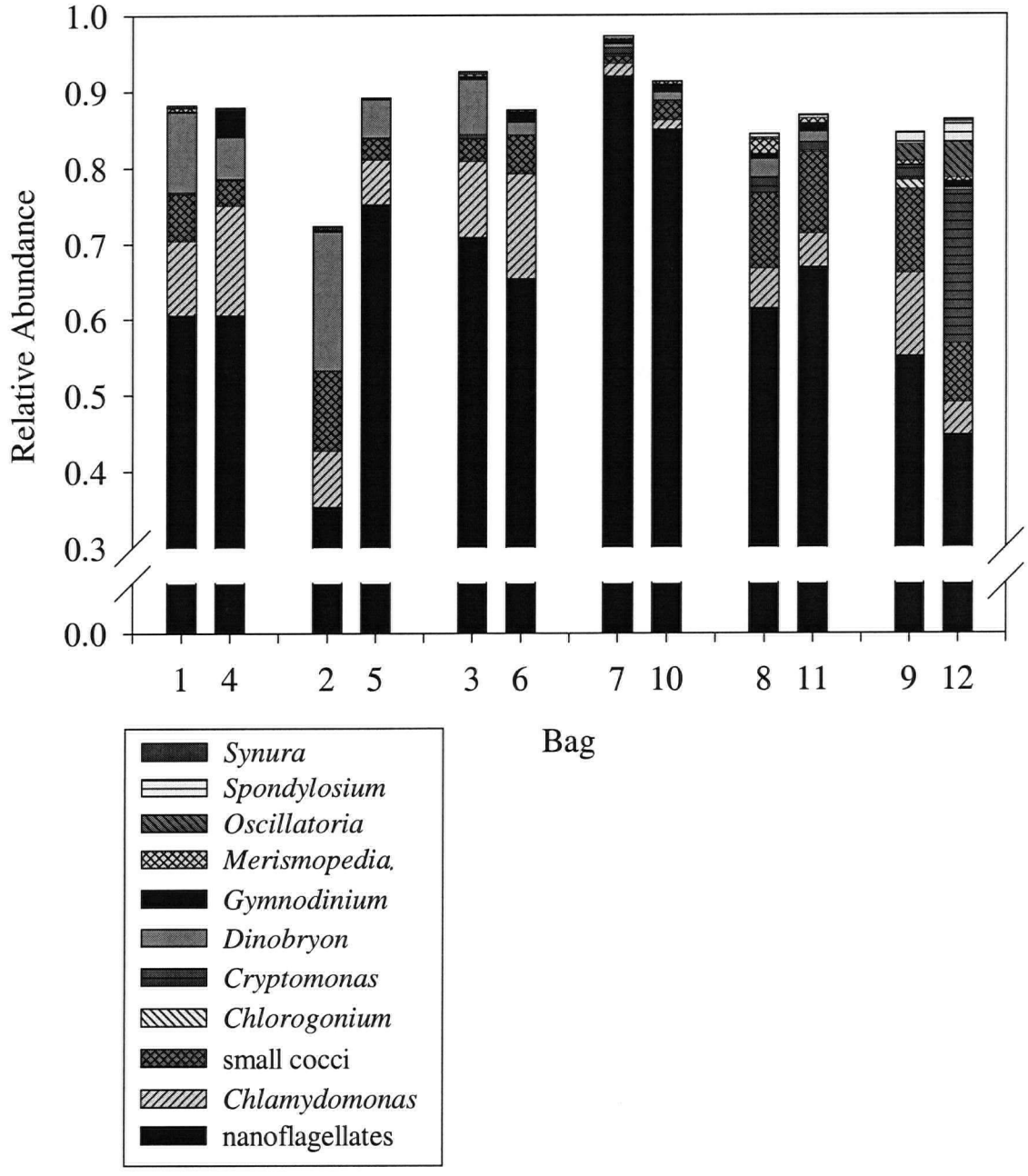
This appendix contains the data for phytoplankton community diversity and composition for the 1999 experiment (Chapter 3). The objective is to show that the within- treatment variability between bags is less than the between-treatment variability in the cases where *Daphnia* invasion occurred (bags 1 and 10).

Fig. A1.1: Phytoplankton community diversity measures for 1999 (chapter 3) to show the minimal effect of a small invasion of juvenile *Daphnia* in bags 1 and 10. Other bags were free of *Daphnia* for the entire experiment.



Treatment	Bags
3d mix/low	1, 4
21d mix/low	2, 5
unmixed/low	3, 6
3d mix/high	7, 10
21d mix/high	8, 11
unmixed/high	9, 12

Fig. A1.2: Phytoplankton community composition for 1999 (chapter 3) for common genera to show the minimal effect of a small invasion of juvenile *Daphnia* in bags 1 and 10. Other bags were free of *Daphnia* for the entire experiment.



APPENDIX 2

This appendix contains tables which outline the hierarchical structure of the statistical analyses performed in the experiments (chapters 3-5). The number of major tests done for each case and the number of significant tests are recorded. Significance was determined by $\alpha < 0.05$ unless indicated with an asterisk (*) in which case $\alpha < 0.01$. The total number of primary tests (other than the Sp. Composition tests which are discussed in the text) are indicated at the bottom of each table.

The total number of major primary tests done amount to 55 comparisons over all experiments discussed in this thesis. As always, there is a chance that some of the 29 significant results are Type I errors. However, this appendix indicates that more than half of the tests were significant, which is unlikely to occur by chance alone, and the majority of results therefore represent real statistical differences.

Table A2.1: Outline of major statistical tests for chapter 3. All ANOVAs and MANOVAs represent full 2-way factorial tests with 3 levels of mix frequency and 2 levels of nutrients. Orthogonal contrasts (simple and main effect *P*-values) for the significant ANOVAs and discriminant analysis *P*-values for the significant MANOVAs are recorded in the body of the text in chapter 3. As indicated here, significant interaction in MANOVAs was followed by univariate ANOVAs to test for the nature of the interaction.

Variable	Primary Test	Secondary Test	Total No. of Tests	No. of tests Significant	Proportion Significant
Community Diversity	ANOVA	--	3	2	0.67
Biomass	ANOVA	--	1	1	1.0
Composition					
1. Size	MANOVA		2	2 *	1.0
	➥	ANOVA	8	4	0.5
2. Taxonomic	MANOVA	--	2	0	0
3. Morphological	MANOVA		2	1	0.5
	➥	ANOVA	6	1	0.17
4. Mobility	MANOVA		2	1	0.5
	➥	ANOVA	4	2	0.5
5. Sp. Composition	ANOVA	--	22	8	0.36
Total			12	7	0.58

Table A2.2: Outline of major statistical tests for chapter 4. All ANOVAs and MANOVAs represent full 2-way factorial tests with 3 levels of mix frequency and 2 levels of *Daphnia*. Orthogonal contrasts (simple and main effect *P*-values) for the significant ANOVAs and discriminant analysis *P*-values for the significant MANOVAs are recorded in the body of the text in chapter 4.

Variable	Primary Test	Secondary Test	Total No. of Tests	No. of tests Significant	Proportion Significant
Community Diversity	ANOVA	--	3	2	0.67
Biomass	ANOVA	--	1	0	0
Composition					
1. Size	MANOVA	--	2	1	0.5
2. Taxonomic	MANOVA	--	2	0	0
3. Morphological	MANOVA	--	2	0	0
4. Mobility	MANOVA	--	2	0	0
5. Sp. Composition	ANOVA	--	62	18	0.29
Total			12	3	0.25

Table A2.3: Outline of major statistical tests for phytoplankton community structure in chapter 5. All ANOVAs and MANOVAs represent full 2-way factorial tests with 3 levels of mix frequency and 2 levels of nutrients. Orthogonal contrasts (simple and main effect *P*-values) for the significant ANOVAs and discriminant analysis *P*-values for the significant MANOVAs are recorded in the body of the text in chapter 5. As indicated here, significant interaction in MANOVAs was followed by univariate ANOVAs to test for the nature of the interaction.

Variable	Primary Test	Secondary Test	Total No. of Tests	No. of tests Significant	Proportion Significant
Community	ANOVA	--	3	3	1.0
Diversity					
Biomass	ANOVA	--	1	0	0
Composition					
1. Size	MANOVA		2	1	0.5
	➥	ANOVA	4	0	0
2. Taxonomic	MANOVA	--	2	0	0
3. Morphological	MANOVA	--	2	0	0
4. Mobility	MANOVA	--	2	0	0
5. Sp. Composition	ANOVA	--	18	1	0.06
Total			12	4	0.33

Table A2.4: Outline of major statistical tests for zooplankton community structure and for *Chaoborus* populations in chapter 5. All ANOVAs and MANOVAs represent full 2-way factorial tests with 3 levels of mix frequency and 2 levels of nutrients. Orthogonal contrasts (simple and main effect *P*-values) for the significant ANOVAs and discriminant analysis *P*-values for the significant MANOVAs are recorded in the body of the text in chapter 5. As indicated here, significant interaction in MANOVAs was followed by univariate ANOVAs to test for the nature of the interaction.

Variable	Primary Test	Secondary Test	Total No. of Tests	No. of tests Significant	Proportion Significant
Community Diversity	ANOVA	--	3	1	0.33
Total Biomass	ANOVA	--	1	1	1.0
Composition Biomass	MANOVA	--	1	1	1.0
Composition					
Major Groups	MANOVA		2	2	1.0
	➥	ANOVA	3	3	1.0
Cladocerans	MANOVA		2	2	1.0
	➥	ANOVA	14	7	0.5
Daphnia	ANOVA		3	2	0.67
Copepods	MANOVA		2	1	1.0
	➥	ANOVA	2	1	0.5
Rotifers	MANOVA		2	2	1.0
	➥	ANOVA	12	6	0.5
Chaoborus Density	ANOVA		1	1	1.0
Chaoborus Demography	MANOVA		2	2	1.0
	➥	ANOVA	3	2	0.67
Total			19	15	0.79

APPENDIX 3

Figures A3.1, A3.2 and A3.3 showing the complete 120 day time series (June 1-September 29 1998) for zooplankton biomass as explained in the Methods section of Chapter 5. Plots show that the general trends in biomass of major groups of zooplankton and *Chaoborus* did not change between the end of the first 60 day period (day 212 or July 31) and the end of the full 120 day period (September 29).

Figure A3.1: Complete time series of the total average (± one standard error) rotifer biomass in $\mu g\ l^{-1}$ and for comparison, a standardized relative biomass estimate for *Chaoborus* (based on a biomass of 0.0001 for the smallest size class).

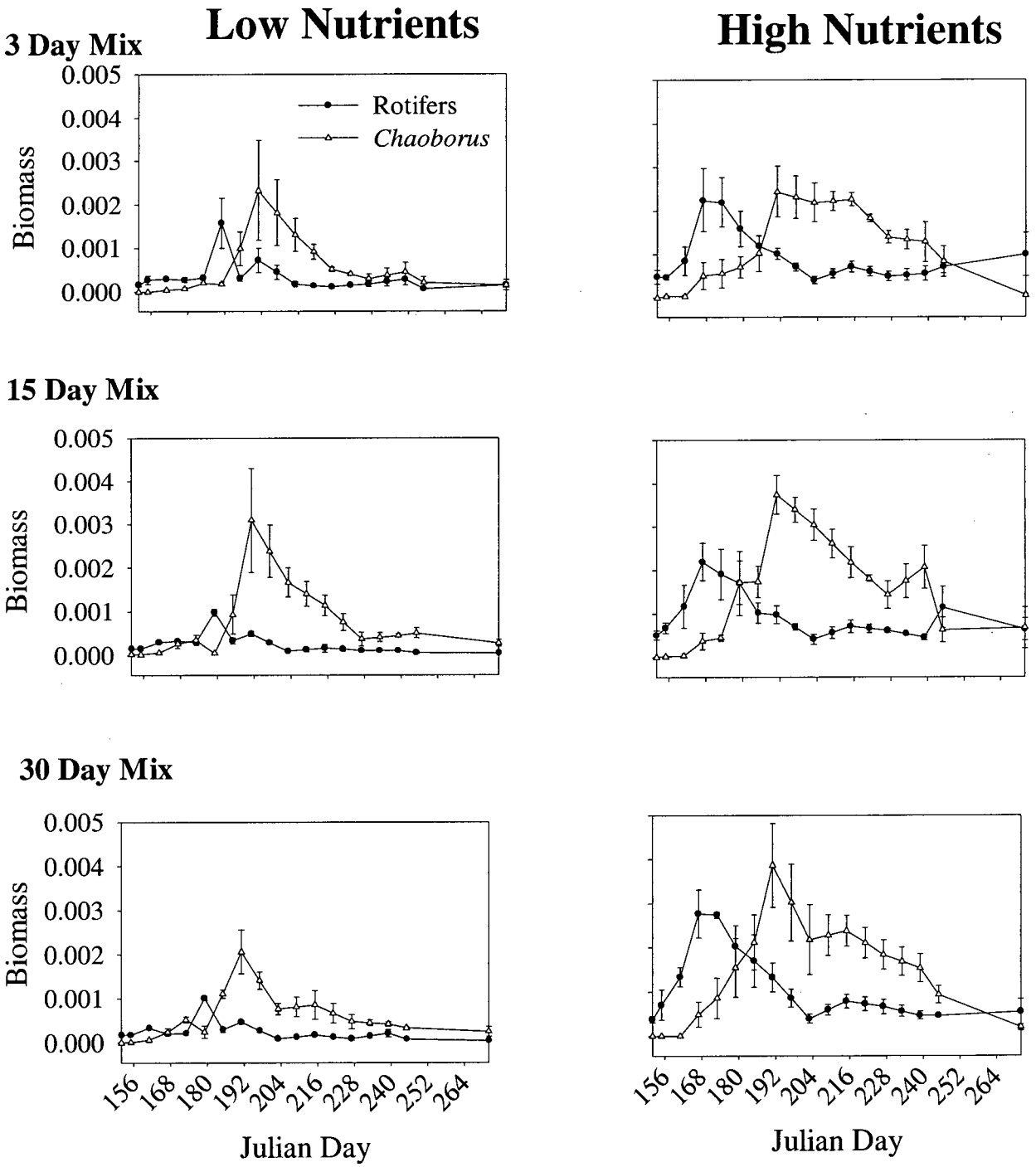


Figure A3.2: Complete time series of the total average (one ± standard error) biomass of *Daphnia* and the sum of all other Cladocera in $\mu g\ l^{-1}$.

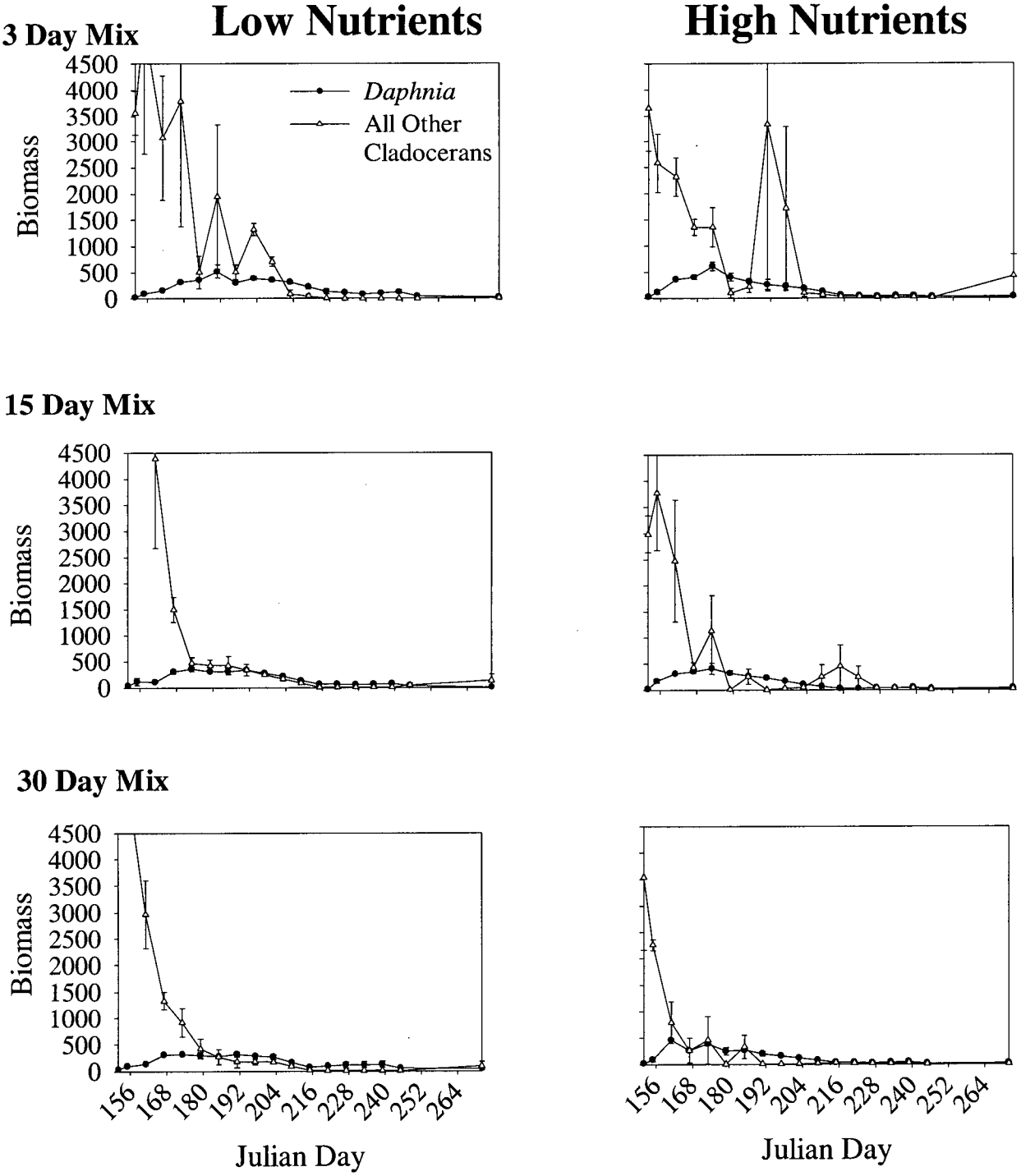


Figure A3.3: Complete time series of the total average (± one standard error) biomass of all copepods in $\mu g\ l^{-1}$.