The ecological consequences of unpalatable prey: phytoplankton response to nutrient and predator additions

Thomas Bell

Recent theoretical and laboratory studies have suggested that unpalatable prey modify the degree to which trophic levels are regulated by bottom-up or top-down forces such that primarily edible communities are regulated by predation and primarily unpalatable communities are regulated by resource supply. Despite the hypothesised importance of prey edibility, experiments have only rarely examined the response of primary producers to nutrient enrichment or the addition of a top trophic level following the reduction or elimination of inedible species. I performed two experiments in aquatic enclosures in which the prey (phytoplankton) had been manipulated to create treatments composed either nearly exclusively of only edible phytoplankton or with both edible and inedible phytoplankton. In the first experiment, the two types of phytoplankton community were subjected to either high or low nutrient concentrations. In the second experiment, the two types of phytoplankton community were present in enclosures with either 2 or 3 trophic levels. I found that the impact of both the nutrients and the number of trophic levels on total phytoplankton biomass was modified by the edibility of the phytoplankton community. Although enclosures with only edible phytoplankton were able to increase with enrichment, there was a greater overall phytoplankton biomass in enclosures with both edible and inedible phytoplankton. The addition of a third trophic level had a positive effect on phytoplankton biomass when only edible phytoplankton were present, but had no effect on phytoplankton biomass when both edible and inedible phytoplankton were present. These results therefore provide support for the hypothesis that the proportion of inedible phytoplankton determines the degree to which communities are regulated by top-down or bottom-up forces.

T. Bell, Dept of Zoology, Univ. of British Columbia, 6270 Univ. Blvd., Vancouver, BC, Canada, V6T 1Z4 (tbell@zoology.ubc.ca).

A number of studies have recently suggested that the impact of nutrients or predators on trophic level biomass is determined, at least in part, by the architecture of the food web (Hunter and Price 1992, Leibold and Wilbur 1992, Strong 1992, Persson et al. 2001). Food web architecture generally refers to the pattern and strength of feeding relations between organisms in a community. It includes such features as the edibility of prey species, the average connectance, and the prevalence of omnivory, shared predators, and intraguild predation (Holt and Lawton 1994, Morin and Lawler 1995, Holt and Polis 1997, Diehl and Feißel 2000). For example, recent work has shown that the food web architecture can affect trophic level biomass (Kaunzinger and Morin 1998), and the response of the whole community (Hulot et al. 2000) and the primary producers (Cottingham and Schindler 2000) to enrichment.

There has been particular recent interest in the role of prey edibility in determining the biomass of trophic levels. The "edibility hypothesis" (Leibold 1989) focuses on how the presence of less-edible prey alters the predictions of food web theory. The model of Leibold (1989) and subsequent extensions and alternatives explore the consequences of inedible species on the distribution of biomass (Grover 1995, Bohannan and Lenski 1999) and the stability (Abrams and Walters 1996,

Leibold 1996, Genkai-Kato and Yamamura 1999, Huxel 1999) of trophic levels and of predator-prey systems. If a tradeoff between competitive ability and edibility is assumed, as appears to be the case for freshwater algae (Agrawal 1998) and terrestrial plants (Agren and Schemske 1993, Mutikainen and Walls 1995), then the models suggest that both the standing crop biomass and the stability or variability of trophic levels are altered by the presence of inedible species. In general, the models make the following predictions. First, that the inedible species (but not the edible prey) will increase with increasing resource levels in systems with 2 trophic levels. Second, that edible species (but not inedible species) will increase with the addition of a third trophic level. Edible and inedible functional groups therefore play a critical role in communities because the balance between these two functional groups determines the degree to which the prey are regulated by bottom-up or top-down forces.

Comparative studies provide some support for these predictions. Such studies suggest that communities composed of only edible prey (phytoplankton) are regulated by resource levels, and that prey communities heterogeneous in their edibility to predators are regulated by predation (McCauley et al. 1988). As predicted, lakes with greater nutrient concentrations also tend to have a greater proportion of inedible phytoplankton (Watson et al. 1988, 1992, Masson et al. 2000). Despite the obvious appeal of the theory in resolving the debate between proponents of the top-down and the bottom-up views, experimental tests of these hypotheses are rare. However, some examples exist from terrestrial (Schmitz 1994), aquatic (Leibold 1989, Hansson et al. 1998, Persson et al. 2001), and microbial systems (Bohannan and Lenski 1999). These studies manipulated resource levels and the presence of the top trophic level and measured the response of the edible and inedible species. In general, these studies have provided only mixed support for the qualitative predictions of the models. Although enrichment and the addition of a third trophic level often results in increases in inedible and edible prey respectively (Leibold 1989, Hansson et al. 1998, Bohannan and Lenski 1999, Schmitz et al. 2000), a number of experiments report contrary patterns (Leibold 1989).

These correlational studies effectively document the consequences of nutrient and food web manipulations on the proportion of edible to inedible biomass. However, notably absent are studies that investigate how the response to these manipulations is altered by also controlling prey edibility. Apart from studies using highly simplified microbial food webs (Bohannan and Lenski 1999, 2000), the experiments that have been performed to date did not explicitly examine the role of prey edibility because it was not manipulated in the experiments. The present study is among the first that investigates the consequences of inedibility by manipulating the edibility of phytoplankton in natural food webs of phytoplankton, zooplankton, and zooplanktivorous fish in aquatic enclosures. In particular, I examine the response of total phytoplankton biomass to nutrient and secondary consumer (zooplanktivorous fish) additions to food webs in which the proportion of inedible phytoplankton has been manipulated. This study tests the following hypotheses: (1) There is a positive response to enrichment in Mixed (unpalatable + edible) but not Edible prey communities in systems with 2 trophic levels. (2) There is a positive response to the addition of a third trophic level in Edible but not Mixed communities.

Methods

Study site and enclosures

The study was conducted in enclosures in an experimental pond at the University of British Columbia, Canada. The pond is 23×23 m^2 and slopes to a maximum depth of 3.5 m. This pond has never contained fish since the ponds were built in 1991. Phytoplankton biomass in previous years was dominated by highly edible small flagellates, especially *Chlamydomonas* and *Cryptomonas*, although blooms of *Tetraedron* and small (< 50 μm) dinoflagellates occasionally occurred. The larger phytoplankton, thought to be inedible to most zooplankton, are dominated by *Ceratium* and by filamentous blue-green (*Anabaena*) and green algae. My unpublished data indicate that the pond is eutrophic. Zooplankton biomass was dominated by *Daphnia pulex* and calanoid and cyclopoid copepods. Smaller cladocerans, such as *Chydorus*, *Diaphanosoma*, *Bosmina longirostrus*, and rotifers were also common, but most often contributed little to the total biomass.

The study was conducted in enclosures in the limnetic zone of an experimental pond from 13 July to 5 October 2000. The enclosures were constructed from UV-protected plastic bags suspended from a floating wooden frame. The bags were 1 m^2 and 2.5 m deep, and contained approximately 1000 l of water. They were closed to the sediment because the conditions were intended to mimic those of the pelagic zone of a small lake.

Experimental design

I created two types of phytoplankton community. Only edible phytoplankton were present in the first (Edible), and both edible and inedible phytoplankton were present in the second (Mixed). To estimate the response of the phytoplankton community to nutrient enrichment, I added nutrients to half of the replicates (High

nutrients) or left nutrients at ambient levels (Low nutrients). In a second experiment, zooplanktivorous fish were added to half of the High nutrient replicates (3 trophic levels) while the rest remained without fish (2 trophic levels). Although the study is treated as two separate experiments, the results of the High nutrient + 2 trophic level treatment is used in both experiments (Table 1). Fish were also added to half of the Low nutrient replicates, but these were not used in the analysis of the results because of unexpected fish mortality. There were 2 replicates for each treatment combination.

Manipulation of the phytoplankton community

Previous studies have shown that phytoplankton larger than approximately 30 µm are for the most part inedi-

Table 1. Summary of the experimental design for the 2 experiments in this study. Each cell is a replicate (2 replicates for each treatment combination).

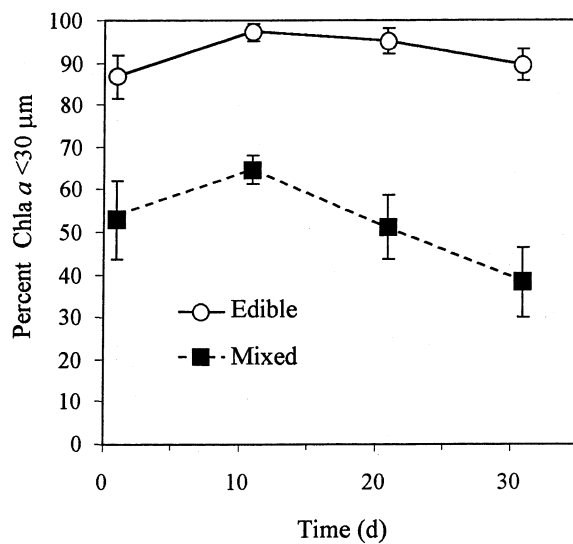| | Low nutrients | | High nutrients | | | |
	2 trophic levels		2 trophic levels		3 trophic levels	
Edible	1	2	3	4	5	6
Mixed	7	8	9	10	11	12

Experiment 1 ▲▲ Experiment 2 ▲

Fig. 1. Mean percent of the total phytoplankton biomass (Chl a) in the edible (< 30 µm) fraction for the Edible and Mixed enclosures. Error bars are the standard error of 4 enclosures for each sampling date.

ble, or at the least highly unpalatable, to common zooplankton grazers (Burns 1968, Vanderploeg 1981, Lehman and Sandgren 1985, McCauley and Downing 1985). As a consequence, phytoplankton size is often used as a rough estimate of their edibility. I therefore attempted to eliminate these unpalatable phytoplankton from half of the enclosures by filtering the water through fine netting as it was pumped into the enclosures. It is impossible in practice to eliminate all large phytoplankton from the enclosures. The purpose of the manipulation was rather to create treatments with ambient and very low concentrations of unpalatable phytoplankton.

Water was added from the pond to the enclosures from 3 July to 12 July 2000. All water was first filtered through 202 µm Nitex netting to remove the macrozooplankton. In half of the enclosures (Edible treatment), the water was also filtered through 20 µm Nitex netting as it was added to the enclosures to remove the large inedible phytoplankton. The water that had been added to each enclosure was re-filtered for the amount of time that it would take to pump approximately 2000 l of water. I therefore estimate that the water in each enclosure was filtered approximately 3 times before initiating the experiments. The macrozooplankton trapped on the 202 µm netting were subsequently introduced into the enclosures. Although the filtering procedure eliminates the smaller zooplankton (20–202 µm) from the Edible enclosures, previous unpublished work in the same ponds suggests that small zooplankton have little effect on phytoplankton dynamics when large cladocerans are present in the system, as was the case in this study.

Size-fractionated phytoplankton samples were taken once every 10 days for the first 31 days of the experiment to check the efficacy of the phytoplankton manipulation. Water was withdrawn from the center of each enclosure and deposited in a jar using a hollow glass tube (1.5 m long, 18 mm diameter) fitted with a removable stopper. Two samples of 120 ml each were withdrawn from the jar. To estimate total phytoplankton biomass, one of the 120-ml samples was passed through a 25 mm diameter Whatman GF/F glass-fiber filter in situ. To estimate the contribution of the inedible phytoplankton (> 30 µm), the same procedure was followed but the 120-ml sample was first passed through 30 µm Nitex netting. Both samples were incubated in 95% acetone overnight (> 18 h) at 4C. Phytoplankton Chl a was estimated using the fluorometric technique (Parsons et al. 1984). The contribution of the edible phytoplankton (< 30 µm) was obtained by subtracting the > 30 µm Chl a from the total phytoplankton Chl a.

Analysis of these samples indicated that the filtering procedure described above was successful in creating a higher proportion of edible phytoplankton in the Edible treatment, and that this manipulation was sustained at least throughout the first half of the experiment (Fig.

1). Small edible phytoplankton contributed on average 93.1% of the total phytoplankton biomass in Edible enclosures, and only 51.7% in Mixed enclosures. Repeated measures analysis of variance (ANOVA) confirmed that this difference was statistically significant ($F_{1,10} = 84.2$, $P < 0.0005$). There was furthermore no difference in the total phytoplankton biomass between Edible and Mixed enclosures immediately following the manipulation of the phytoplankton community ($F_{3,4} = 0.51$, $P = 0.69$). Although the removal of the unpalatable phytoplankton should result in a loss of nutrients in the Mixed enclosures, the absence of a difference in the total phytoplankton biomass at the outset of the experiment suggests that this loss was minimal.

Experiment 1: the effect of enrichment

The experiment was initiated on 24 July 2000 when 0.175 µg 1^{-1} KH_2PO_4 and 3.883 µg 1^{-1} $NaNO_3$ were added to the High nutrient enclosures. The nutrients were first dissolved in 5 l of water from the enclosure into which the nutrients were being added. The water in the enclosures was mixed briefly immediately following the nutrient addition to ensure an initial even distribution of the nutrients within the enclosures. No nutrients were added to the Low nutrient enclosures, but these enclosures were also briefly mixed. Phytoplankton were usually sampled twice weekly beginning 20 July 2000. Total phytoplankton biomass was measured as described in the previous section.

Zooplankton were sampled near the beginning (7 August 2000) and conclusion (20 September 2000) of the experiment. I used the same glass tube to collect the zooplankton as was used to collect the phytoplankton. Fifteen liters of water were obtained from each enclosure and was sieved through 100 µm Nitex netting. The zooplankton trapped on the netting were then placed in scintillation vials in 95% ethanol. Zooplankton were enumerated and measured under a dissecting microscope after the conclusion of the experiment. Zooplankton biovolume was estimated from the maximum length and width of each individual. Because the 100 µm netting is unreliable for sampling the smallest zooplankton (e.g. rotifers and copepod nauplii), total zooplankton biomass reflects biomass of the macrozooplankton only (post-naupliar cladocerans and copepods). Smaller (1-l) zooplankton samples were also taken weekly throughout the experiment. Such small samples were taken to minimize the impact of the sampling procedure on the zooplankton community.

Experiment 2: the effect of zooplanktivorous fish additions

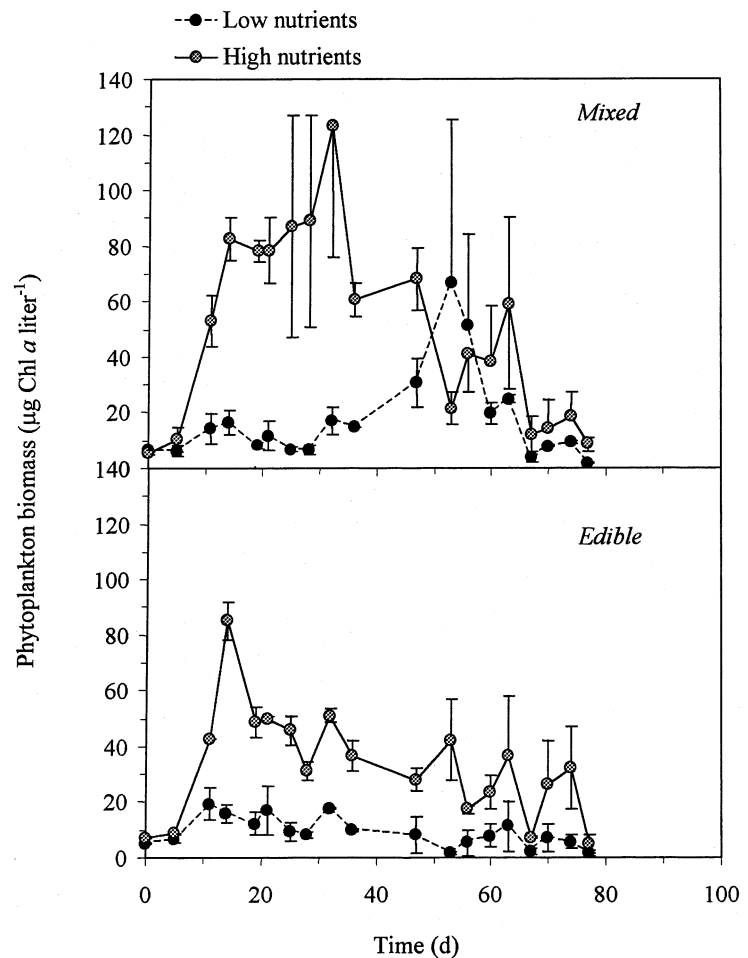I used the limnetic species of the threespine stickleback (*Gasterosteus* sp.), a zooplanktivorous fish (Schluter 1993), as the top predator trophic level. Parental fish were captured from Paxton Lake, British Columbia, Canada (49°43′N, 124°31′W) during May 2000. Eggs from a single female were fertilized with the sperm from a single male in the laboratory. The progeny were raised in the laboratory for 6 weeks before being added to the appropriate enclosures at the initiation of the experiment (4 September 2000). Five individuals (mean weight = 88.9 mg, mean standard length = 21.1 mm) were added to each of the appropriate enclosures. The fish added to each enclosure were drawn haphazardly from the available stock. There was no difference in the mean weight of the fish added to different enclosures (ANOVA: $F_{3,16} = 0.83$, $P = 0.50$). Although the addition of predators was 46 days after the initial phytoplankton manipulation, there was little change in the proportion of edible phytoplankton in the Edible enclosures (Fig. 1), indicating that the manipulation of the phytoplankton community persisted throughout the experiment.

The fish were transported to the enclosures and allowed to acclimatize to pond temperatures overnight in plastic bags half filled with aquarium water before being added to the enclosures. They were removed from the enclosures at the conclusion of the experiment first using minnow traps overnight for 3 consecutive nights, and then by adding rotenone ($C_{23}H_{22}O_6$). Phytoplankton and zooplankton sampling methods were the same as for Experiment 1. Phytoplankton were sampled every 10 days beginning 9 days prior to the addition of the fish (26 August 2000).

Analyses

For Experiment 1, I tested for differences in the phytoplankton biomass among the treatments using repeated measures analysis of variance (ANOVAR), where the phytoplankton community (Edible and Mixed) and nutrient concentration (High and Low) were the dependent variables. To investigate more general patterns in the data, I calculated the grand mean across the time series for each treatment, and compared treatments using a factorial ANOVA. For Experiment 2, I wanted to test the hypothesis that zooplanktivorous fish had a greater positive effect on phytoplankton biomass in the Edible treatment than in the Mixed treatment. Phytoplankton biomass was therefore converted to effect sizes by dividing the phytoplankton biomass in each replicate enclosure with 3 trophic levels by the phytoplankton biomass in the appropriate controls (2 trophic levels). The time series were compared using repeated measures ANOVA. All statistical analyses were performed on log-transformed data to homogenize the variances. The statistics were computed using SYSTAT 5.05.

Fig. 2. Mean phytoplankton biomass
($\pm$ range) in the High and Low nutrient
enclosures for Edible and Mixed
phytoplankton communities.

Results

Experiment 1: the effect of enrichment

In this experiment, I tested the prediction that phytoplankton biomass would increase after nutrient additions in the Mixed enclosures but not in the Edible enclosures. There was a marginally non-significant increase in the phytoplankton biomass with enrichment in the Edible treatment (ANOVAR: $F_{1,2} = 13.6$, $P = 0.065$), and a significant increase in the Mixed treatment (ANOVAR: $F_{1,2} = 47.6$, $P = 0.020$) (Fig. 2). For the latter, there was also a significant time $\times$ nutrient interaction (ANOVAR: $F_{18,36} = 2.3$, $P = 0.018$). However, when the status of both the phytoplankton community (Edible and Mixed) and the nutrient concentration (High and Low) were included in a repeated measures analysis of variance, the nutrient concentration but not the edibility of the phytoplankton community had a significant effect on the total phytoplankton biomass (Table 2). The significant time $\times$ nutrient $\times$ edibility interaction is perhaps because the phytoplankton biomass increased in the Low Nutri-

ent + Mixed treatment near the conclusion of the experiment but not in the corresponding Edible treatment (Fig. 2).

I averaged the phytoplankton biomass across the time series of each enclosure to look at coarser trends in the data. The data show (Fig. 3) that the biomass of the Mixed phytoplankton communities was able to increase substantially with enrichment to 3.0-times the biomass in enclosures that did not receive any nutrients. However, the Edible phytoplankton communities were also able to increase with enrichment to 3.6-times the biomass without nutrient inputs. When both nutrients and phytoplankton edibility are included in a fully factorial ANOVA, both nutrients ($F_{1,5} = 28.71$, $P = 0.006$) and phytoplankton edibility ($F_{1,5} = 8.35$, $P = 0.045$) had a significant effect on total phytoplankton biomass, but there was no interaction between the two ($F_{1,5} = 1.07$, $P = 0.40$). However, there was no difference in the phytoplankton biomass between High nutrients + Edible and Low nutrients + Mixed.

If nutrient enrichment results in a higher density of edible phytoplankton, then zooplankton density should

Table 2. Results of a repeated measures ANOVA to determine the effect of nutrient enrichment (High and Low nutrients) and of the phytoplankton community (Edible and Mixed) on total phytoplankton biomass.

	Source of variation	F	Num. df	Den. df	P
Between subjects	nutrients	14.55	1	4	0.019
	edibility	1.01	1	4	0.372
	nutrient $\times$ edibility	0.49	1	4	0.523
Within subjects	time	5.16	18	72	<0.0005
	time $\times$ nutrients	2.22	18	72	0.009
	time $\times$ edibility	0.76	18	72	0.740
	time $\times$ nutrient $\times$ edibility	1.94	18	72	0.026

also increase with enrichment. Analysis of the larger (15 l) samples indicated that there was no difference in the total zooplankton biomass among the treatments near the beginning (7 August 2000) of the experiment (ANOVA: $F_{3,4} = 1.22$, $P = 0.41$). Manipulation of the phytoplankton community and nutrient concentrations resulted in a difference in the zooplankton biomass among the treatments near the conclusion of the study (ANOVA: $F_{3,4} = 13.73$, $P = 0.014$) because of a significantly higher zooplankton biomass in the enriched Edible enclosures (Fig. 4). There is therefore a significant interaction between nutrient levels and phytoplankton community structure when the data are included in a factorial ANOVA ($F_{1,4} = 12.0$, $P = 0.026$). Weekly zooplankton samples collected from only 1 l of water indicated that there was a substantial increase in the zooplankton abundance during the first half of the experiment for one of the replicates in both the Edible treatments, but not in the Mixed treatments (Fig. 5). When both the nutrient levels and the phytoplankton community type were included in a repeated measures ANOVA, there was no nutrient or nutrient $\times$ edibility interaction ($F_{1,4} = 0.29$, 0.01 respectively, both $P > 0.5$). There was some indication of an effect of Edibility, but this was not significant ($F_{1,4} = 4.61$, $P = 0.09$).

Experiment 2: the effect of zooplanktivorous fish additions

There was a lower zooplankton biomass in enclosures with 3 trophic levels ($t_6 = 2.87$, $P = 0.028$). I tested the prediction that the addition of zooplanktivorous fish would have a positive effect on phytoplankton biomass in both Edible and Mixed treatments, but that this effect would be more pronounced in the Edible enclosures. There was no significant effect of fish on phytoplankton biomass in the Edible enclosures (ANOVAR: $F_{1,2} = 3.57$, $P = 0.20$) but there was a significant time effect ($F_{3,6} = 28.3$, $P = 0.001$) and time $\times$ treatment interaction ($F_{3,6} = 8.9$, $P = 0.013$) (Fig. 6). The biomass of phytoplankton was higher (marginally non-significant) in enclosures with fish for the Mixed treatment (ANOVAR: $F_{1,2} = 15.02$, $P = 0.061$).

After calculating phytoplankton effect sizes (Fig. 7), there was no significant difference between phytoplankton effect sizes in the Edible and Mixed treatments (ANOVAR: $F_{1,2} = 0.52$, $P = 0.54$). However, the significant time $\times$ treatment interaction ($F_{3,6} = 50.0$, $P < 0.0005$) suggests that the qualitative divergence between the Edible and Mixed effect sizes as the experiment progressed is real. Pairwise comparisons at each sampling date indicated that there was a significantly higher effect of zooplanktivorous fish on phytoplankton biomass in the Edible enclosures on the final sampling date (Tukey test: $q_{10,5} = 2.25$, $P = 0.032$), but there was no significant difference on any of the other sampling dates. I therefore conclude that the fish only had a positive effect on phytoplankton biomass in the Edible enclosures.
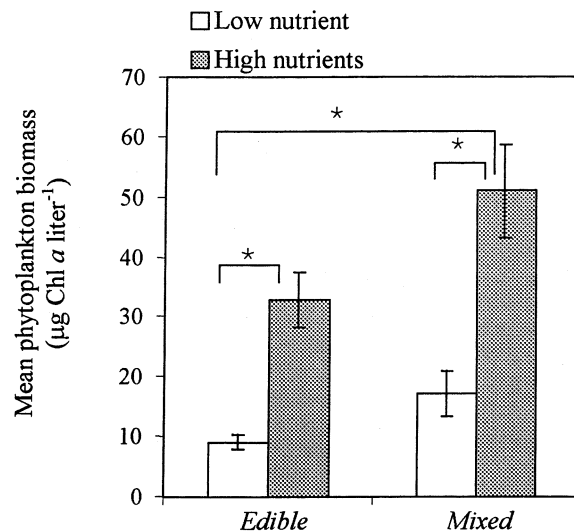

Fig. 3. Grand mean of the phytoplankton biomass ($\pm$ SE) averaged over the course of the experiment in the two types of phytoplankton community (Edible and Mixed) and under the two nutrient regimes (High and Low nutrients). The lines connecting the columns represent pairwise comparisons between treatments using the Tukey test on log-transformed data (* $P < 0.05$). Non-significant comparisons are not indicated.
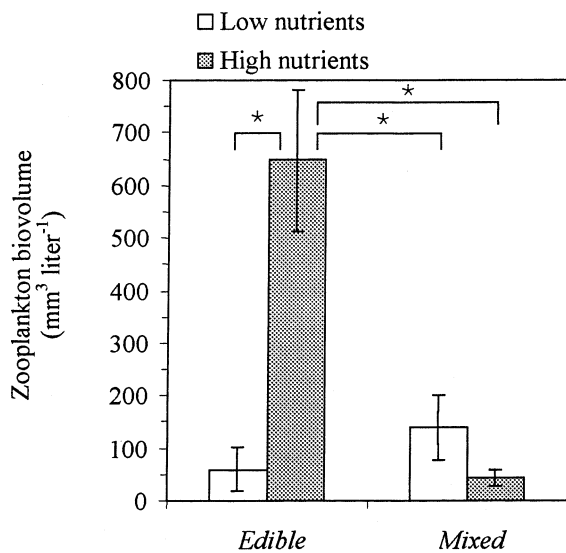
64

Fig. 4. Mean (± range) zooplankton biomass on a single sampling date near the conclusion of the experiment (20 September 2000) in the two types of phytoplankton community (Edible and Mixed) and under the two nutrient regimes (High and Low nutrients). The lines connecting the columns represent pairwise comparisons between treatments using the Tukey test on log-transformed data (* $P < 0.05$). Non-significant comparisons are not indicated.

Discussion

The experiments in this study confirm the suggestions of previous authors (Leibold 1989, Agrawal 1998) that prey (phytoplankton) edibility, here represented as phytoplankton size, can have considerable effects on the dynamics of the phytoplankton trophic level. The results demonstrate that the phytoplankton respond differently to nutrient enrichment in the two types of phytoplankton community (Edible and Mixed). Theory predicts that prey biomass should increase with enrichment in 2-trophic level systems only if some of the prey are inedible (Leibold 1989, Kretzschmar et al. 1993, Bohannan and Lenski 2000) or unpalatable (Grover 1995). If there are only edible prey, any increases in primary production would simply result in increases in primary consumer biomass as they offset increases in their prey. If inedible phytoplankton are present, not only are edible algae susceptible to grazing losses, but the inedible algae are at a competitive advantage because they act as nutrient "sponges" by sequestering the available nutrients (Watson et al. 1988, Bohannan and Lenski 1999), which might therefore lead to further reductions in the biomass of the edible phytoplankton.

As predicted, there was an increase in the phytoplankton biomass with enrichment in the Mixed enclosures. Contrary to the predictions, there was also an increase in the phytoplankton with enrichment in the Edible enclosures, and as a result there was no interaction between nutrients and prey community categories.

Despite the absence of an interaction, there was a differential effect of enrichment on the two types of phytoplankton community that was manifest in an overall higher total phytoplankton biomass in enclosures with both edible and unpalatable phytoplankton. The time series indicate that this higher phytoplankton biomass in the Mixed enclosures was the result of a larger and more sustained increase following enrichment in the High nutrient enclosures, and an increase near the conclusion of the experiment in the Low nutrient enclosures.

There has been considerable recent interest in the effect of biodiversity on ecosystem functioning, such as primary producer biomass. This work has shown that plant biomass often increases with increasing biodiversity as a result of differences in resource use among species (Tilman et al. 2002). Analogously, edible and unpalatable phytoplankton differ in their resource requirements. In particular, phytoplankton with anti-predator traits, such as thicker cell walls, require different ratios of the common micronutrients, as is the case for many inedible blue-green algae (MacKay and Elser 1998). The relatively higher phytoplankton biomass in the Mixed phytoplankton communities might therefore reflect the higher functional group di-
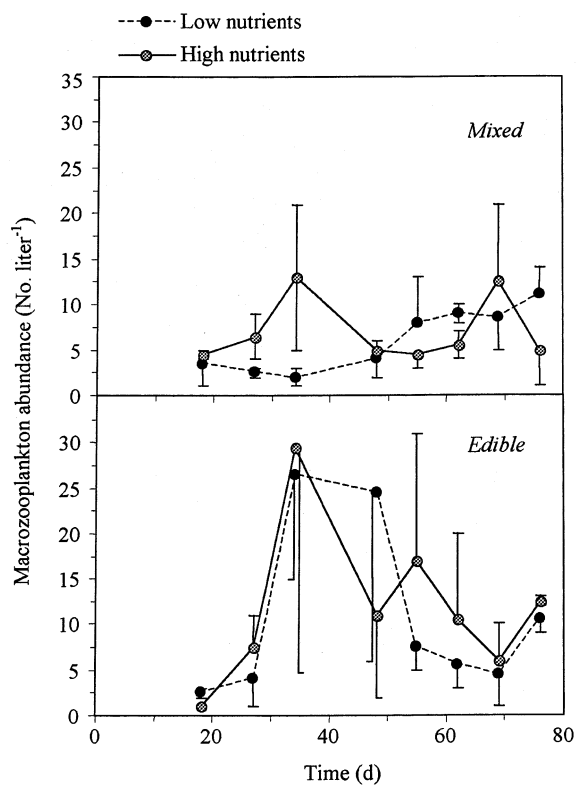

Fig. 5. Mean (± range) zooplankton abundance over the course of the experiment in the two types of phytoplankton community (Edible and Mixed) and for High and Low nutrient concentrations.
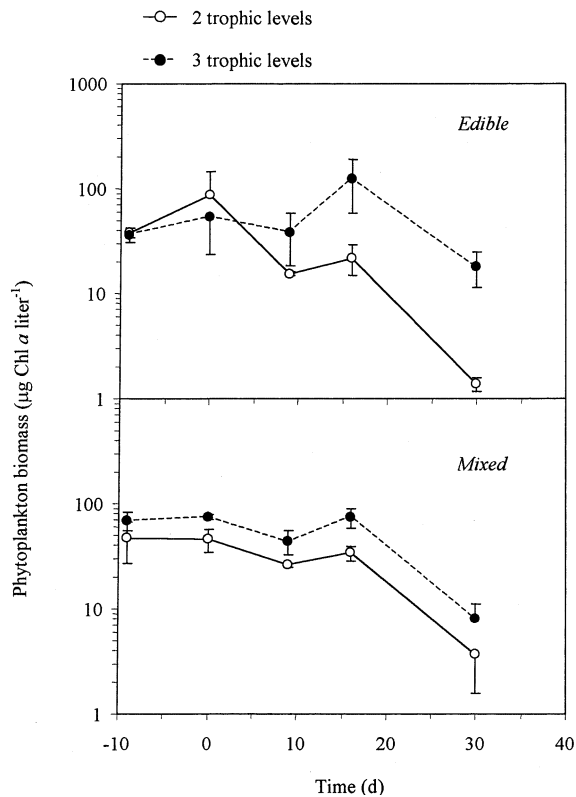
Fig. 6. Mean (± range) phytoplankton biomass in enclosures to which zooplanktivorous fish have (3 trophic levels) or have not (2 trophic levels) been added for the two types of phytoplankton community (Edible and Mixed). Negative values on the x-axis are data from before the start of the experiment.

versity in these treatments (Hulot et al. 2000). Further replication would be necessary to see whether this result is repeatable.

The mechanism by which phytoplankton were able to increase in the Edible enclosures despite concomitant increases in the zooplankton also remains unclear. It is possible that the zooplankton were not given sufficient time to respond to increases in edible phytoplankton biomass (Hansson 1992), but experiments show that zooplankton are able to approach equilibrium densities over comparable time scales (Walters et al. 1987, Attayde and Hansson 2001). Alternatively, increased interference among zooplankton with increased zooplankton densities (McCann et al. 1998), including interference competition and intraguild predation, could also lead to the observed increase in edible phytoplankton with enrichment. It is also possible that small but inedible algae became abundant in the Edible enclosures. Finally, there are no size-fractionated phytoplankton after the midpoint of the experiment. An increase in large unpalatable phytoplankton would therefore not have been detected. Unfortunately, the methods used during the experiment were insufficient to account for this possibility. Further work would be required to distinguish among these hypotheses.

The zooplankton dynamics suggest that zooplankton were able to increase in the Edible enclosures, but not in the Mixed enclosures. However, because only a small volume of water was sampled from each of the enclosures, abundance is often estimated from only a few individuals per sample. It is therefore unclear whether the data are truly representative of actual zooplankton abundance. The larger and presumably more accurate samples indicate that the zooplankton are affected both by the phytoplankton community type and the nutrient concentrations. These data therefore suggest that these bottom-up signals are able to also cascade up through the food web.

There is now considerable evidence that top-down forces are important in regulating trophic level biomass in aquatic systems (Brett and Goldman 1996). There is a great deal of variability, however, in the degree to which phytoplankton biomass is affected by the trophic levels above. Several authors have suggested that the strength of trophic cascades is contingent on the degree of heterogeneity, or functional complexity, in the lower trophic levels (Hunter and Price 1992) such that trophic cascades are common in "simple" aquatic food webs, but rare in more complex terrestrial systems (Polis 1994, Hansson et al. 1998, but see Hairston and Hairston 1993, 1997). It is evident that the addition of a third trophic level should have little effect on lower trophic levels if the primary producer biomass is dominated by organisms that are inedible to the primary consumers. Primary producer communities that are homogeneous in their edibility to primary consumers should therefore be more tightly regulated by top-down forces than heterogeneous communities.
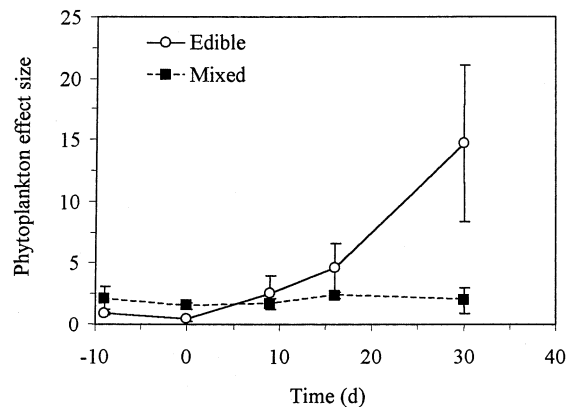

Fig. 7. Mean (± range) effect of fish on phytoplankton biomass in Edible and Mixed enclosures. Effect sizes are calculated by dividing the phytoplankton biomass in the 3 trophic level treatments with the average value of the control (2 trophic levels) for the same date. Negative values on the y-axis are data from before the start of the experiment. Pairwise comparisons were performed at each sampling date using the Tukey test on log-transformed data (* $P < 0.05$).

66

When zooplanktivorous fish were added to the two types of phytoplankton community, the data indicate that, by the conclusion of the study, the magnitude of the effect of the secondary carnivore (zooplanktivorous fish) on primary producer biomass depends on the edibility of the primary producer trophic level. Results from previous enclosure experiments have been restricted to the observation that inedible phytoplankton commonly, but not always, increase with increases in zooplankton biomass (Leibold 1989) and therefore dampen the trophic cascade. Similarly, several hypotheses based on comparative data have suggested that the degree to which top-down forces regulate trophic levels depends on nutrient concentrations (Coley et al. 1985, McQueen et al. 1986, Elser and Goldman 1991). Although there is some debate as to whether trophic cascades are important in oligotrophic (nutrient-poor) lakes, there is general agreement that trophic cascades are weak in nutrient-rich aquatic systems because they are dominated by inedible phytoplankton (Watson et al. 1988, 1992). Interestingly, terrestrial plants appear to exhibit the opposite pattern, with decreased plant defense in nutrient-rich environments (Coley et al. 1985). Comparisons between terrestrial and aquatic systems are complicated, however, for example by differences in plant and herbivore life-history strategies between terrestrial plants and phytoplankton (e.g. perenniality, size differences between primary producers and herbivores) as well as by differences in the ratio and absolute concentration of available nutrients, which might influence the relative cost of defense. Unfortunately, comparative studies cannot separate the effects of nutrient concentration from phytoplankton edibility and other covariates of lake nutrient concentrations (such as lake size) on the strength of the trophic cascade. I believe manipulative experiments of the kind described here provide insights that could not be gained by the analysis of comparative data alone.

Although the replication of the study was insufficient to provide definitive answers, the combined results of the two experiments provide useful insights into the importance of prey heterogeneity in determining the balance between top-down and bottom-up regulation. Theoretical (Genkai-Kato and Yamamura 1999, Huxel 1999) and experimental (Bohannan and Lenski 1999) work has further shown that not only the standing crop biomass but also the variability and stability of the biomass is affected by the presence of inedible species. Unfortunately, there are few experiments that explicitly manipulate prey edibility despite the hypothesised importance of inedible species in determining the dynamics of trophic levels. I hope my work will stimulate further efforts. Because phytoplankton size is only a rough estimate of phytoplankton edibility, and in reality there is a gradient in edibility rather than a dichotomy between edible and inedible individuals, the results of these experiments provide only qualitative

support for the theory. I believe the next step is to obtain a thorough understanding of the edibility of a large number of phytoplankton species (i.e. growth rate with and without zooplankton predators for a variety of zooplankton communities). Such work would provide explicit predictions about species replacement along nutrient and predation gradients (Abrams 1993, Holt et al. 1994, Leibold 1996).

The experiments presented in this study also underline the importance of understanding the dynamics of functional groups within a trophic level (see also Hulot et al. 2000). Removal of other types of functional groups, such as omnivores (Diehl 1995), dominant herbivores (Persson et al. 2001) or intraguild predators (Polis and Holt 1992, Rosenheim et al. 1993, Morin 1999) has similarly proved to be a fruitful method of understanding the dynamics of trophic levels and communities. Such studies, including the present one, demonstrate that the debate on whether the abundance of organisms is principally determined by nutrient supply or predation levels will only be resolved in the context of the food web in which the organisms of interest are embedded.

References

Abrams, P. A. 1993. Effects of increased productivity on the abundances of trophic levels. – Am. Nat. 141: 351–371.

Abrams, P. A. and Walters, C. J. 1996. Invulnerable prey and the paradox of enrichment. – Ecology 77: 1125–1133.

Agrawal, A. A. 1998. Algal defense, grazers, and their interactions in aquatic trophic cascades. – Acta Oecologica 19: 331–337.

Agren, J. and Schemske, D. W. 1993. The cost of defense against herbivores: an experimental study of trichome production in *Brassica rapa*. – Am. Nat. 141: 338–350.

Attayde, J. L. and Hansson, L.-A. 2001. Press perturbation experiments and the indeterminacy of ecological interactions: effects of taxonomic resolution and experimental duration. – Oikos 92: 235–244.

Bohannan, B. J. M. and Lenski, R. E. 1999. Effect of prey heterogeneity on the response of a model food chain to resource enrichment. – Am. Nat. 153: 73–82.

Bohannan, B. J. M. and Lenski, R. E. 2000. The relative importance of competition and predation varies with productivity in a model community. – Am. Nat. 156: 329–340.

Brett, M. T. and Goldman, C. R. 1996. A meta-analysis of the freshwater trophic cascade. – Proc. Nat. Acad. Sci. USA 93: 7723–7726.

Burns, C. W. 1968. The relationship between body size of filter-feeding Cladocera and the maximum size of particle ingested. – Limnol. Oceanogr. 13: 675–678.

Coley, P. D., Bryant, J. P. and Chapin, I. F. S. 1985. Resource availability and plant antiherbivore defense. – Science 230: 895–899.

Cottingham, K. L. and Schindler, D. E. 2000. Effects of grazer community structure on phytoplankton response to nutrient pulses. – Ecology 81: 183–200.

Diehl, S. 1995. Direct and indirect effects of omnivory in a littoral lake community. – Ecology 76: 1727–1740.

Diehl, S. and Feißel, M. 2000. Effects of enrichment on three-level food chains with omnivory. – Am. Nat. 155: 200–218.

Elser, J. J. and Goldman, C. R. 1991. Zooplankton effect on phytoplankton in lakes of contrasting trophic status. – Limnol. Oceanogr. 36: 64–90.

Genkai-Kato, M. and Yamamura, N. 1999. Unpalatable prey resolves the paradox of enrichment. – Proc. R. Soc. Lond. B 266: 1215–1219.

Grover, J. P. 1995. Competition, herbivory, and enrichment: nutrient-based models for edible and inedible plants. – Am. Nat. 145: 746–774.

Hairston, N. G. Jr. and Hairston, N. G. Sr. 1993. Cause-effect relationships in energy flow, trophic structure, and interspecific interactions. – Am. Nat. 142: 379–411.

Hairston, N. G. Jr. and Hairston, N. G. Sr. 1997. Does food web complexity eliminate trophic-level dynamics? – Am. Nat. 149: 1001–1007.

Hansson, L.-A. 1992. The role of food chain composition and nutrient availability in shaping algal biomass development. – Ecology 73: 241–247.

Hansson, L.-A., Brönmark, C., Nystrom, P. et al. 1998. Consumption patterns, complexity and enrichment in aquatic food chains. – Proc. R. Soc. Lond. B 265: 901–906.

Holt, R. D. and Lawton, J. H. 1994. The ecological consequences of shared natural enemies. – Annu. Rev. Ecol. Syst. 25: 495–520.

Holt, R. D. and Polis, G. A. 1997. A theoretical framework for intraguild predation. – Am. Nat. 149: 745–764.

Holt, R. D., Grover, J. and Tilman, D. 1994. Simple rules for interspecific dominance in systems with exploitative and apparent competition. – Am. Nat. 144: 741–771.

Hulot, F. D., Lacroix, G., Lescher-Moutoué, F. and Loreau, M. 2000. Functional diversity governs ecosystem response to nutrient enrichment. – Nature 405: 340–344.

Hunter, M. D. and Price, P. W. 1992. Playing chutes and ladders: heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. – Ecology 73: 724–732.

Huxel, G. R. 1999. On the influence of food quality in consumer-resource interactions. – Ecol. Letters 2: 256–261.

Kaunzinger, M. K. and Morin, P. J. 1998. Productivity controls food-chain properties in microbial communities. – Nature 395: 495–497.

Kretzschmar, M., Nisbet, R. M. and McCauley, E. 1993. A predator-prey model for zooplankton grazing on competing algal populations. – Theor. Pop. Biol. 44: 32–66.

Lehman, J. T. and Sandgren, C. D. 1985. Species-specific rates of growth and grazing loss among freshwater algae. – Limnol. Oceanogr. 30: 34–46.

Leibold, M. A. 1989. Resource edibility and the effects of predators and productivity on the outcome of trophic interactions. – Am. Nat. 134: 922–949.

Leibold, M. A. 1996. A graphical model of keystone predators in food webs: trophic regulation of abundance, incidence, and diversity patterns in communities. – Am. Nat. 147: 784–812.

Leibold, M. A. and Wilbur, H. W. 1992. Interactions between food-web structure and nutrients on pond organisms. – Nature 360: 341–343.

MacKay, N. A. and Elser, J. J. 1998. Factors potentially preventing trophic cascades: food quality, invertebrate predation and their interaction. – Limnol. Oceanogr. 43: 339–347.

Masson, S., Pinel-Alloul, B. and Smith, V. H. 2000. Total phosphorus-chlorophyll a relationships in southern Quebec lakes. – Limnol. Oceanogr. 45: 732–740.

McCann, K. S., Hastings, A. and Strong, D. R. 1998. Trophic cascades and trophic trickles in pelagic food webs. – Proc. R. Soc. Lond. B 265: 205–209.

McCauley, E. and Downing, J. 1985. The prediction of cladoceran grazing rate spectra. – Limnol. Oceanogr. 30: 202–212.

McCauley, E., Murdoch, W. W. and Watson, S. 1988. Simple models and variation in plankton densities among lakes. – Am. Nat. 132: 383–403.

McQueen, D. J., Post, J. R. and Mills, E. L. 1986. Trophic relationships in freshwater pelagic ecosystems. – Can. J. Fish. Aquat. Sci. 43: 1571–1581.

Morin, P. 1999. Productivity, intraguild predation, and population dynamics in experimental food webs. – Ecology 80: 752–760.

Morin, P. J. and Lawler, S. P. 1995. Food web architecture and population dynamics: theory and empirical evidence. – Annu. Rev. Ecol. Syst. 26: 505–529.

Mutikainen, P. and Walls, M. 1995. Growth, reproduction and defence in nettles: responses to herbivory modified by competition and fertilization. – Oecologia 104: 487–495.

Parsons, T. R., Maita, Y. and Lalli, C. M. 1984. A manual of chemical and biological methods for seawater analysis. – Pergamon Press.

Persson, A., Hansson, L.-A., Brönmark, C. et al. 2001. Effects of enrichment on simple aquatic food webs. – Am. Nat. 157: 654–669.

Polis, G. A. 1994. Food webs, trophic cascades and community structure. – Aust. J. Ecol. 19: 121–136.

Polis, G. A. and Holt, R. D. 1992. Intraguild predation: the dynamics of complex trophic interactions. – Trends Ecol. Evol. 7: 151–154.

Rosenheim, J. A., Wilhoit, L. R. and Armer, C. A. 1993. Influence of intraguild predation among generalist insect predators on the suppression of an herbivore population. – Oecologia 96: 439–449.

Schluter, D. 1993. Adaptive radiation in stickleback size, shape, and habitat use efficiency. – Ecology 74: 699–709.

Schmitz, O. J. 1994. Resource edibility and trophic exploitation in an old-field food web. – Proc. Nat. Acad. Sci. USA 91: 5364–5367.

Schmitz, O. J., Hambäck, P. A. and Beckerman, A. P. 2000. Trophic cascades in terrestrial systems: a review of the effects of carnivore removals on plants. – Am. Nat. 155: 141–153.

Strong, D. R. 1992. Are trophic cascades all wet? Differentiation and donor-control in speciose ecosystems. – Ecology 73: 747–754.

Tilman, D., Knops, J., Wedin, D. and Reich, P. 2002. Experimental and observational studies of diversity, productivity, and stability. – In: Kinzig, A. P., Pacala, S. W. and Tilman, D. (eds), The functional consequences of biodiversity. Princeton Univ. Press, pp. 42–70.

Vanderploeg, H. A. 1981. Seasonal particle-size selection by *Diaptomus sicilis* in offshore Lake Michigan. – Can. J. Fish. Aquat. Sci. 38: 504–517.

Walters, C. J., Krause, E., Neill, W. E. and Northcote, T. G. 1987. Equilibrium models for seasonal dynamics of plankton biomass in four oligotrophic lakes British Columbia, Canada. – Can. J. Fish. Aquat. Sci. 44: 1002–1017.

Watson, S., McCauley, E. and Downing, J. A. 1988. Contrasting patterns of net and nannoplankton production and biomass among lakes. – Can. J. Fish. Aquat. Sci. 45: 915–920.

Watson, S., McCauley, E. and Downing, J. A. 1992. Sigmoid relationship between phosphorus, algal biomass, and algal community structure. – Can. J. Fish. Aquat. Sci. 49: 2605–2610.