SYNOPSIS

Project Number: _____ **Start date:** 01 March 2003

End date: 28 February 2004

Title:

Algal-nutrient dynamics in fresh waters: direct and indirect effects of zooplankton grazing and nutrient remineralization

Investigator:

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Congressional District:

4th Congressional District of Oklahoma

Descriptors:

nutrient-algal ecology, zooplankton, grazing, nutrient cycling, nutrient supply, water quality, freshwater reservoirs, Lake Texoma

Problem and Research Objectives:

Lake Texoma is a large (360 km^2) impoundment of the Red and Washita Rivers draining more than 100,000 km^2 in Oklahoma, Texas and New Mexico. Although Lake Texoma was designed primarily for flood control and hydropower generation, like many reservoirs throughout Oklahoma, water supply and tourism (including fishing) have become primary year-round uses for the reservoir. As such, water quality is a major focus of lake management. With respect to quality of water supply, chloride concentrations receive much emphasis, however, nutrient (especially phosphorus and nitrogen) loading and concentrations are also critical factors affecting water quality for both water supply and tourism. High nutrient loading from the water shed yields relatively high productivity, especially in mid-summer, with potentially nuisance cyanobacteria (bluegreen algae) *Aphanizomenon*, *Anabaena* and *Microcystis* dominating the phytoplankton (algal) assemblage. With most nutrient sources today being non-point sources (e.g., agricultural runoff) that are difficult to regulate, management of internal lake nutrient cycling via food web management may be a viable alternative for regulating water quality. In many lakes worldwide, managers attempt to manipulate lake food webs toward systems in which phytoplankton are suppressed by intensified grazing and reduced rates of nutrient cycling by zooplankton (Drenner and Hambright 1999).

The objective of this research was to examine the role and magnitude of planktonic consumer-driven nutrient regeneration in mesotrophic Lake Texoma. Using laboratory mesocosm experiments based on consumer-food encounter rate models, I attempted to quantify

Indeed, looking to the future, a few successes can be noted for this project. First, all equipment necessary for successful completion of grazing experiments are now available in my laboratory and will therefore contribute to ongoing and future research in zooplankton ecology in Lake Texoma. Second, two students received direct, hands-on training in project methodologies and learned valuable lessons with respect to experimental design and the potentially low success rates in experimentation with living animal and plant assemblages. The first student, a 3rd-year undergraduate at OU, has decided to pursue graduate-level education in limnology, with emphasis relating to water-quality research. The other student is currently pursuing his PhD (2nd year) at OU in my laboratory.

Thus, while I have so far been unable to obtain many of the specified project objectives, I am confident that the ground work laid during this project will serve great impetus in ongoing and toward future experimental analyses of zooplankton grazing and nutrient remineralization, both in my research and in that of my students.

Awards

None

Grants obtained from this Grant

Ms. Nicole Luke (OU undergraduate 3rd year) received a $500 OU Honors College UROP (Undergraduate Research Opportunities Program) grant entitled "Grazing and nutrient remineralization in *Daphnia lumholtzi*" to support her independent research related to this project. She will be presenting her research findings at the OU Undergraduate Research Day in April 2005.

PUBLICATIONS

There are no current publications relating to this project.

STUDENTS SUPPORTED BY THIS PROJECT

	Number	Discipline
Undergraduates:	1	Zoology
Masters		
Ph.D.	1	Ecology & Evolutionary Biology
Post Doc		
Total	2	

grazing rates and nutrient remineralization rates by both macro- and micro-zooplankton assemblages.

Methodology:

The basic design employed for determining zooplankton grazing and nutrient recycling rates consists of measuring changes in abundances of bacteria and algae and in concentrations of dissolved nutrients over a 24-h period in experimental mesocosms containing a gradient of plankton densities, and hence a gradient of consumer-food encounter rates (Lehman 1980a, b, Landry and Hassett 1982).

A benefit of this approach is that the confounding effects of simultaneous uptake of nutrients by bacteria and algae are absent. By adding excess nutrients at the beginning of an experiment, rates of nutrient uptake by bacteria and algae are constant and independent of nutrient recycling by the zooplankton during the short duration of the experiment (24 h). Hence growth rates of bacteria and algae are not positively affected by density-dependent zooplankton nutrient excretion. Zooplankton grazing rates, zooplankton nutrient excretion rates, and maximum potential nutrient uptake rates by bacteria and algae (in the absence of zooplankton) can then be determined from the changes in abundances of bacteria, algae, and nutrient concentrations as functions of zooplankton biomass. Experiments can be performed at different times of the year to provide insight into seasonal variability in grazing and nutrient remineralization.

Grazing: Theoretical Framework of Approach

During an experiment, biomass of bacteria or algae A can potentially change as

$$dA / dt = r_A A \qquad (1)$$

where rA is the net rate of change in biomass due to grazing mortality g and reproduction or growth k, (i.e., $r_A = k - g$) and is calculated as

$$r_A = (lnA_t - lnA_0) / \Delta t \qquad (2)$$

where A_t and A_0 are biomasses of bacteria or algae present at the end and beginning of the experiment ; Δt is 1 day. No net change is indicated by $r_A = 0$; a net increase by $r_A > 0$, and a net decrease by $r_A < 0$. According to the Lehman model, grazing rates can be quantified by linear regression of r_A on zooplankton biomass, Z (Figure 1).

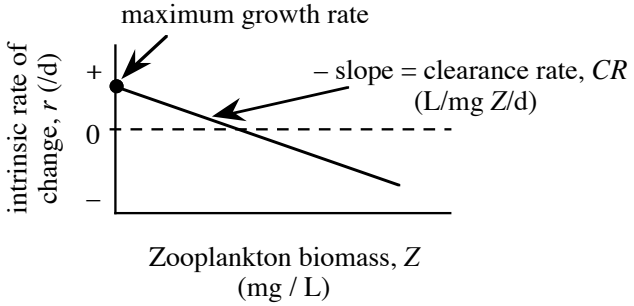

Figure 1. Demonstration of the relationship between bacterial or algal rate of change, r, and zooplankton biomass, Z, showing estimation of clearance rate, CR, (the amount of water cleared of bacteria or algae per biomass of zooplankton per day) and maximum growth rate of the bacteria or phytoplankton at saturating nutrient concentrations in the absence of grazing.

Because nutrients are added at the beginning of an experiment at algal and bacterial growth-saturating concentrations, enhanced growth is expected. The y-intercept of this regression, where zooplankton biomass = 0, indicates the maximum rate of growth for the bacteria or algae *A* in the absence of grazing and has units of per day. The slope of this regression indicates zooplankton-dependent effects; its negative equals the clearance rate (*CR*) in units of L Z^{-1} d^{-1}. A slope of 0 indicates that the bacteria or algae under consideration was not grazed.
Grazing rates, *GR*, can be calculated as the product of the mean biomass of the bacteria or algae and the clearance rate ($GR = CR * A$), where *A*, the mean biomass of bacteria or algae is calculated as

$$A = (A_0 - A_t)\ [(r_A)\ (\Delta t)]^{-1}. \qquad (3)$$

The basic assumptions of the Landry-Hassett model are similar in nature to those of the Lehman model. The principle difference between the two models is the manner in which the encounter-rate dependency of the grazer-food (i.e., zooplankton-algae and bacteria) relationship is manipulated although both manipulations yield the same result. In the Lehman model, the grazer densities are manipulated directly by adding increasing amounts of macro-zooplankton to a series of experimental bottles; in the Landry-Hassett model, both grazer and food densities are manipulated by diluting whole lake water (containing ambient densities of grazers and food) with lake water in which all grazers and food have been removed by filtration. According to the Landry-Hassett model, the probability of a food item being grazed is a direct function of the rate of encounter of grazers with food items and that encounter rates are directly proportional to grazer and food densities, thus $r_A = k - g$ becomes

$$r_A = k - Xg, \qquad (4)$$

where *X* is the dilution factor (i.e., the fraction of non-filtered water in the experimental bottles). Therefore, any observed rates of change in food taxa during an experiment (Eq. 1) at different dilutions are linearly related to the dilution factor (Figure 2).

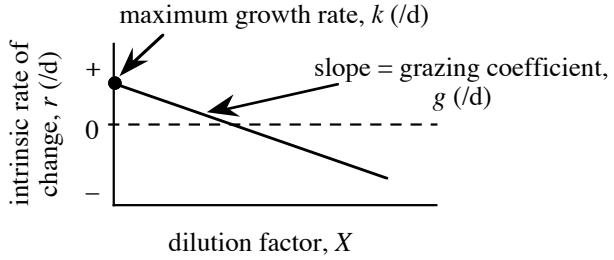

Figure 2. Demonstration of the relationship between phytoplankton rate of change, *r*, and the dilution factor (ratio of unfiltered to filtered lake water) allowing calculation of the grazing coefficient, *g*, where $r = k - Xg$. The maximum growth rate of the phytoplankton at saturating nutrient concentrations in the absence of grazing, *k*, is estimated as the y-intercept.

Nutrient Remineralization: Theoretical Framework of Approach.

During an experiment, changes in nutrient concentrations can be described as

$$dS/dt = -uA + cZ \qquad (5)$$

where *S* is the nutrient concentration, *A* is the biomass of food taxa (i.e., algae and bacteria), *Z* is the biomass of zooplankton estimated by direct counts, u is the rate of nutrient uptake by the phytoplankton and bacteria, and c is the rate of nutrient recycling by the zooplankton (Lehman 1980a, b, Landry-Hassett 1982). Because nutrients are added at the beginning of the experiment at saturating conditions such that $du / dS = 0$, equation (5) can be integrated over a time period of 1 day and rewritten as

$$-\Delta S / A = - cZ / A + u. \qquad (6)$$

Both *c* and *u* can then be computed by linear regression of $-\Delta S / A$ on Z / A (Figure 3).

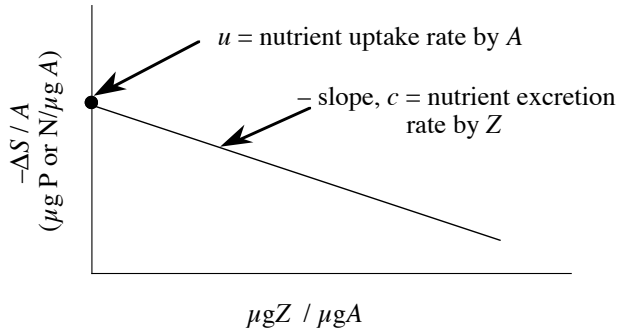

Figure 3. Demonstration of the relationship between changes in nutrient concentrations over 24 hrs as a function of zooplankton biomass. The maximum nutrient uptake rate, u, for the phytoplankton (or other food taxon), A, and the excretion rate of that nutrient by the grazing zooplankton, c, are calculated when both the change in nutrient concentrations and zooplankton biomass are standardized to the biomass of phytoplankton (or other food taxon).

The negative of the slope of this regression equals the rate at which a nutrient is excreted by zooplankton. The intercept of this regression equals the maximum potential rate of nutrient uptake by the phytoplankton and bacteria. Because of the initial nutrient-saturated conditions, it could be considered that the obtained values of excretion may be maximum estimates. However, results of radio-isotope tracer experiments (Hambright et al. in prep.) confirmed, at least for P, that such estimates of excretion by this method are realistic.

Experimental Protocol: Macro-zooplankton:

Macro-zooplankton grazing and nutrient recycling rates were measured using an experimental protocol according to Lehman (1980a, b) and as determined in my previous research with Lake Kinneret plankton assemblages (Hambright et al. 2001a, b). The experiments were conducted in 10-L, clear polystyrene bottles filled with lake water (from 5m depth at a central lake station) filtered through 150 μm-mesh netting (to remove macro-zooplankton but retain the natural assemblage of algae and bacteria). Bottles were placed on a laboratory bottle roller (apparatus for maintaining optimal light and preventing settling of suspended particles, including algae) inside a large walk-in growth chamber that allowed for maintenance of natural light and thermal regimes for a given season. Four bottles were stocked with zooplankton at naturally-occurring densities; four with 2X naturally-occurring densities; four with 4X naturally-occurring densities; and four remained without macro-zooplankton. All 16 bottles were enriched with inorganic nitrogen (500 μg N as NH_4Cl) and phosphorus (50μg P as Na_2HPO_4),

concentrations sufficient for saturating algal and bacterial uptake rates (Hambright et al. 2001a). All bottles were sampled at 0 and 24h to determine initial and final concentrations of size-fractionated chlorophyll *a* , NH_4^+ , SRP-PO_4^{-3}, total nitrogen (TN) and total phosphorus (TP), zooplankton, phytoplankton, bacteria and protists as described below. Because other N and P sources can be important seasonally, I also monitored initial and final concentrations of NO_3^- and total dissolved P (TDP).

These data were analyzed as functions of zooplankton biomass according to Lehman (1980a, b) to assess grazing and nutrient remineralization rates of macro-zooplankton. Because micro-zooplankton were also present in the bottles, the final calculated rates of grazing and nutrient remineralization were corrected to account for effects of micro-zooplankton using results from paired micro-zooplankton experiments described in the following section.

Experimental Protocol: Micro-zooplankton:

Micro-zooplankton grazing and nutrient recycling rates were measured using an experimental protocol according to Landry and Hassett (1982). Experiments were conducted in 2-L polystyrene bottles maintained in the walk-in growth chamber as detailed above for macro-zooplankton. Forty liters of lake water were collected from a central lake station, filtered through 150 μm mesh to remove macro-zooplankton and returned to the laboratory. Half of this water was filtered through 0.2μm mesh to remove all plankton, including algae and bacteria (checked microscopically). This filtered lake water was combined with the remaining non-filtered lake water in ratios of unfiltered to filtered water of 1:0 (100% unfiltered), 3:1 (75%), 1:1 (50%), and 1:3 (25%). Four 2-L bottles were filled with each dilution mixture and excess N and P added to each bottle similar to the paired experiment for macro-zooplankton describe above. All 16 bottles were sampled as in the macro-zooplankton experiments, with data analyzed as functions of the dilution mixture according to Landry and Hassett (1982) to assess grazing and nutrient remineralization rates for micro-zooplankton. Additionally, these resultant micro-zooplankton grazing and nutrient remineralization rates were used to "correct" the rates calculated for the paired macro-zooplankton experiment (i.e., micro-zooplankton effects were subtracted from the combined effects of macro- and micro-zooplankton in the 10-L mesocosms).

Sample analyses

Chlorophyll: Chlorophyll concentrations were determined fluorometrically on whole and filtered (2, 25 μm) water following methanol-chloroform extraction (Wood 1985). Net-chlorophyll was calculated by subtraction of the <25 μm fraction from whole-water chlorophyll; nano-chlorophyll by subtraction of the <2 μm from the <25μm fraction; pico-chlorophyll as the <2 μm fraction. Chlorophyll values were converted to carbon assuming C:chl = 50 (Hambright et al. 2001a).

Phytoplankton: Phytoplankton were preserved in Lugol's iodine solution and examined microscopically to determine dominant taxa in each algal size class.

Bacteria: Bacteria were preserved in 10% filtered (0.45μm) Formalin, stained using DAPI and enumerated and measured using epifluorescent microscopy and Image-Pro software. Densities and biovolumes were converted to carbon according to Simon and Azam (1989).

Zooplankton: Zooplankton biomass was determined by direct microscopical counts of ethanol-preserved samples taken from the beginning and end of each experiment followed by conversion to carbon according to Culver et al. (1985) and Hambright et al. (2001a).

Protozoans: Ciliated-protozoans were enumerated on 5X concentrated (by sedimentation), Lugol's-preserved samples using an inverted microscope. Flagellated protozoans were enumerated with epifluorescent microscopy following preservation in 10% filtered Formalin and staining using DAPI. Densities and biovolumes were converted to carbon according to Putt and Stockner (1989).

Nutrients: Nutrients were analyzed following persulfate digestion (at 100°C for 1 hour) of whole water (total phosphorus and total nitrogen) or filtered (0.2 μm) water (total dissolved phosphorus and nitrate). Soluble reactive phosphate and ammonia was analyzed on filtered (0.2 μm) water. All procedures were according to standard methods (APHA 1995).

Statistical analyses: Zooplankton grazing and nutrient remineralization rates were examined using linear regression (by least squares) of the relationships detailed in Figs. 1, 2, and 3. Significance for the null hypothesis that the slope of the regression is equal to or greater than zero (H_0: slope ≥ 0) was set at $\alpha = 0.05$.

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Principal Findings and Significance:

In this project, I set out to experimentally quantify zooplankton grazing and nutrient remineralization rates in Lake Texoma. Initially, I planned to conduct two experimental series during the year, each series including macro- and micro-zooplankton grazing experiments run in tandem. An additional experimental series was made possible through an OU-UROP grant to an undergraduate student working in my lab. Each experiment, lasting only 24 hours, required extensive set-up and preparation, including collection and sorting of zooplankton, calibration of methodology in accordance with ambient plankton and nutrient concentrations and actual preparation of experimental treatments. Following 3 months of preliminary testing during summer 2003, six experimental series were attempted, two of which were aborted before completion, leaving four series run through completion. In none of the four complete series of experiments did we detect significant grazing by either macro- or micro-zooplankton. Most of these findings can be attributed to either insufficient densities of zooplankton in the mesocosms or failure of the mesocosm roller unit (Fig. 4). After extensive delay in obtaining appropriate

Figure 4. UOBS bottle roller, showing 16 macrozooplankton (12-L bottles; bottom) and 12 microzooplankton (2.5-L bottles; top) mesocosms. Bottles rotate at 1-4 revolutions per minute and change directions every 5 minutes to prevent sedimentation and aggregation of planktonic particles. Ambient temperature and light are maintained to mimic lake conditions of interest.

parts (mostly relating to electronic controls regulating rotation speed, direction and timing), the mesocosm roller is now in working condition, and should therefore not be an issue with future experiments. Likewise, after more than a year of working with Texoma plankton in the mesocosm facility, I am better able to judge appropriate densities conducive to successful experiments. Thus, although this project is officially finished, I will continue to pursue this issue of zooplankton grazing and nutrient remineralization in Lake Texoma.