FINAL REPORT

AUG 0 4 2006

2003 Project Abstract
For the Period Ending June 30, 2006

TITLE: Maintaining Zooplankton (*Daphnia*) for Water Quality: Square Lake

PROJECT MANAGER: Dr. Leif K. Hembre.
ORGANIZATION: Marine-on-St. Croix Water Management Organization
ADDRESS: c/o Dean I. Tharp, 14089 Oakland Road N.
Stillwater, MN 55082
WEB SITE ADDRESS: NA
FUND:
LEGAL CITATION: ML 2003, Ch., 128, Art. 1, Sec. 9, Subd. 07 (f)
APPROPRIATION AMOUNT: $32,000.

Overall Project Outcome and Results

High frequency sonar and conventional sampling methods were used to assess whether rainbow trout, stocked by the Minnesota DNR, adversely affected the water clarity of Square Lake via an ecological domino effect whereby trout consume *Daphnia*, algae are released from grazing pressure by *Daphnia*, and high algal concentrations result in low water clarity. Square Lake is one of many lakes in Minnesota stocked with rainbow trout. These lakes are valued both for their clear water, and for the recreational opportunity provided by the trout fishery. Understanding the impact of trout stocking on water quality is therefore relevant for the proper management of these popular recreational lakes. The fisheries management of the lake was altered between the first (2004) and second year (2005) of this study to assess whether a respite from trout predation over winter would allow a larger "seed" population of *Daphnia pulicaria* to survive into spring. The "seed" population was expected to grow and create clearer water in the spring and summer of 2005 than in 2004. *Major findings*: 1) Stomach content analyses showed that trout preferentially preyed on *Daphnia pulicaria* over the winter of 2004 and during the ice-free season, but to a lesser extent. When not subject to predation by autumn-stocked trout, *Daphnia* present at ice-out in April 2005 were much more abundant than those in April 2004. 2) The large over-wintering *Daphnia* population coincided with low algal abundance and very clear water (> 7 m) in April of 2005, but the *Daphnia* population decreased in size by late May and the clear-water state did not persist. 3) Average water clarity and algal concentrations did not differ significantly between the ice-free seasons of 2004 and 2005, and a demographic analysis based on sonar estimates of *Daphnia* and trout population sizes implies that trout predation had very little impact on the population dynamics of *D. pulicaria*.

Project Results Use and Dissemination

Project results were presented to the members of the Square Lake Clean Water Partnership, the Minnesota Department of Natural Resources, and the local community at a meeting sponsored by the Marine-on-St. Croix Water Management Organization and Square Lake Association on June 29, 2006. A second presentation is scheduled for September 17, 2006 for the members of the Square Lake Association.

In addition, project results are being distributed to local and state decision-makers and citizens interested in the protection and sound management of Square Lake. Study results will also be available on the Web through L. Hembre's Hamline University faculty web page.

Aspects of this research have also been presented at two national meetings and are the subject an undergraduate honors thesis by Rachel McAlpine, a 2006 graduate of Hamline University (see citations below). The research presented at these scientific meetings is also the basis for a manuscript (co-authored by Maria Spitael, Miki Hondzo, and Robert Megard of the University of Minnesota and Rachel McAlpine of Hamline University) that is in preparation for submission to a peer reviewed journal.

"Spatial ecology of predator and prey in a Minnesota lake." **Hembre, L.K.**, McAlpine, R.J., Spitael, M.S., Megard, R.O., and Hondzo, M. Poster presentation given at the Ecological Society of America meeting in August 2005, Montreal, Canada.

"Diel patterns of patchiness in lake zooplankton." Spitael, M.S., **Hembre, L.K.**, McAlpine, R.J., Megard, R.O., and Hondzo, M. Oral presentation to be given at the American Society of Limnology & Oceanography meeting in February 2005, Salt Lake City, Utah.

McAlpine, Rachel. 2006. Day in the life of *Daphnia*: An intensive acoustic study assessing the patchiness of zooplankton in Square Lake. Undergraduate honors thesis, Department of Biology, Hamline University.

In addition, this project was featured in two newspaper articles in 2004 (see descriptions below):

Country Messenger, Scandia; Weekly circulation: 1,800; May 12, 2004
"Stocked trout suspected in Square Lake water-quality decline"
Professor Leif Hembre commented on the declining water quality in Square Lake. Two photos that accompanied the story showed Hembre taking water quality tests.

St. Paul Pioneer Press; July 11, 2004
"Algae eater to the rescue"
Article on the front page of the Local News section that describes the LCMR project on Square Lake. Four photos accompany the story in which Hembre and Project Manager Dean Tharp are quoted.

30 June, 2006
LCMR Final Work Program Report

FINAL REPORT
8/4/2006

I. PROJECT TITLE: Maintaining Zooplankton (*Daphnia*) for Water Quality: Square Lake

PROJECT MANAGER: Dr. Leif K. Hembre.
ORGANIZATION: Marine-on-St. Croix Water Management Organization
ADDRESS: c/o Dean I. Tharp, 14089 Oakland Road N.
Stillwater, MN 55082
WEB PAGE ADDRESS: NA
FUND:

TOTAL BIENNIAL LCMR PROJECT BUDGET:

LCMR Appropriation:	$32,000.
Minus Amount Spent:	$29,331
Equal Balance:	$ 2,669

LEGAL CITATION: ML 2003, [Chap.128], Sec. [9], Subd.07f.

APPROPRIATION LANGUAGE:
7 (f) Maintaining Zooplankton (*Daphnia)* for Water Quality: Square Lake. $16,000 the first year and $16,000 the second year are from the trust fund to the commissioner of natural resources fro an agreement with the Marine-on-St. Croix Water Management Organization to determine whether trout predation on *Daphnia* significantly affects *Daphnia* abundance and water quality on Square Lake, Washington County. This appropriation is available until June 2006, at which time the project must be completed and final products delivered, unless an earlier date is specified in the work program.

II. & III. FINAL PROJECT SUMMARY:

High frequency sonar and conventional sampling methods were used to assess whether rainbow trout, stocked by the Minnesota DNR, adversely affected the water clarity of Square Lake via an ecological domino effect whereby trout consume *Daphnia*, algae are released from grazing pressure by *Daphnia*, and high algal concentrations result in low water clarity. Square Lake is one of many lakes in Minnesota stocked with rainbow trout. These lakes are valued both for the aesthetic appeal their clear water, and for the recreational opportunity provided by the trout fishery. Understanding the impact of trout stocking on water quality is therefore relevant for the proper management of these popular recreational lakes. The fisheries management of the lake was altered between the first (2004) and second year (2005) of this study to assess whether a respite from trout predation over winter would allow a larger "seed" population of *Daphnia pulicaria* to survive into spring. The "seed" population was expected to grow and create clearer water in the spring and summer of 2005 than in 2004. *Major findings*: 1) Stomach content analyses showed that trout preferentially preyed on *Daphnia pulicaria* over the winter of 2004 and during the ice-free season, but to a lesser extent. When not subject to predation by autumn-stocked trout, *Daphnia* present at ice-out in April 2005 were much more abundant than those in April 2004. 2) The large over-wintering *Daphnia* population coincided with low algal abundance and very clear water (> 7 m) in April of 2005, but the clear-water state did not persist. 3) Average water clarity and algal concentrations did not differ significantly between the ice-free seasons of 2004 and 2005, and a demographic analysis based on sonar estimates of *Daphnia* and trout population sizes implies that trout predation had very little impact on the population dynamics of *D. pulicaria*.

IV. OUTLINE OF PROJECT RESULTS:

Result 1: Acoustic (Sonar) Data Collection and Net Sampling of *Daphnia.*

Description: A high frequency sonar system will be employed to map the distribution of zooplankton and locate aggregations of the animals for net sampling. The relationship between the number of *Daphnia* in the net samples and the recorded sonar information will enable us to estimate the size of the *Daphnia* population on each collection date.

Summary Budget Information for Result 1: **LCMR Budget $ 9,810**
Balance $ 170

(Unspent $170 in travel expense)

Personnel: $9,090

Other Supplies $560

Other: $160 (mileage)

To evaluate the distribution and abundance of zooplankton in Square Lake, sonar data were collected during daylight while traveling slowly (~ 5 km h^{-1}) along a transect of the lake's long axis from the northwest to the southeast end of the lake (Fig. 1). These data were collected monthly between and April and October of 2004 and 2005. In addition to acoustic sampling, net samples of zooplankton were collected from depth increments at a sampling site in the center of the lake (Fig. 1) using a closing plankton net. To assess the zooplankton community during winter, net samples were taken through the ice at three locations (Fig. 1).

The sonar system (described in detail in Megard et al., 1997) consists of a Lowrance X-16 high-frequency (192 kHz) single-beam echosounder and a Loran-C navigation receiver connected to an IBM personal computer. An analog converter digitizes voltage variation due to backscattered sound from zooplankton aggregations and other sound-scatterers. The system software uses the sonar equations (e.g. Urick, 1983) to transform the digitized echo strengths to volume scattering strengths, which are displayed instantaneously on the computer monitor as echograms. Echograms were used to locate zooplankton aggregations and to select depth increments for net sampling. In addition to providing instantaneous information to guide sampling with plankton nets, sonar data were saved on the computer's hard disk and later analyzed to estimate total *Daphnia* abundance in the lake.

Daphnia pulicaria are considerably larger than other zooplankton in Square Lake and they are the dominant contributor to volume backscattering at depths where they occur. A previous study (Hembre & Megard, 2003) found the target strength of *D. pulicaria* to be -120 dB, and this value was used to estimate densities of *D. pulicaria* in terms of "*Daphnia* equivalents" by dividing mean volume backscattering strengths in depth increments inhabited by *D. pulicaria* by the target strength. Whole lake population densities were estimated by multiplying *Daphnia* equivalent concentrations by the water volumes of the relevant depth intervals, summing those products, and dividing by the lake volume.

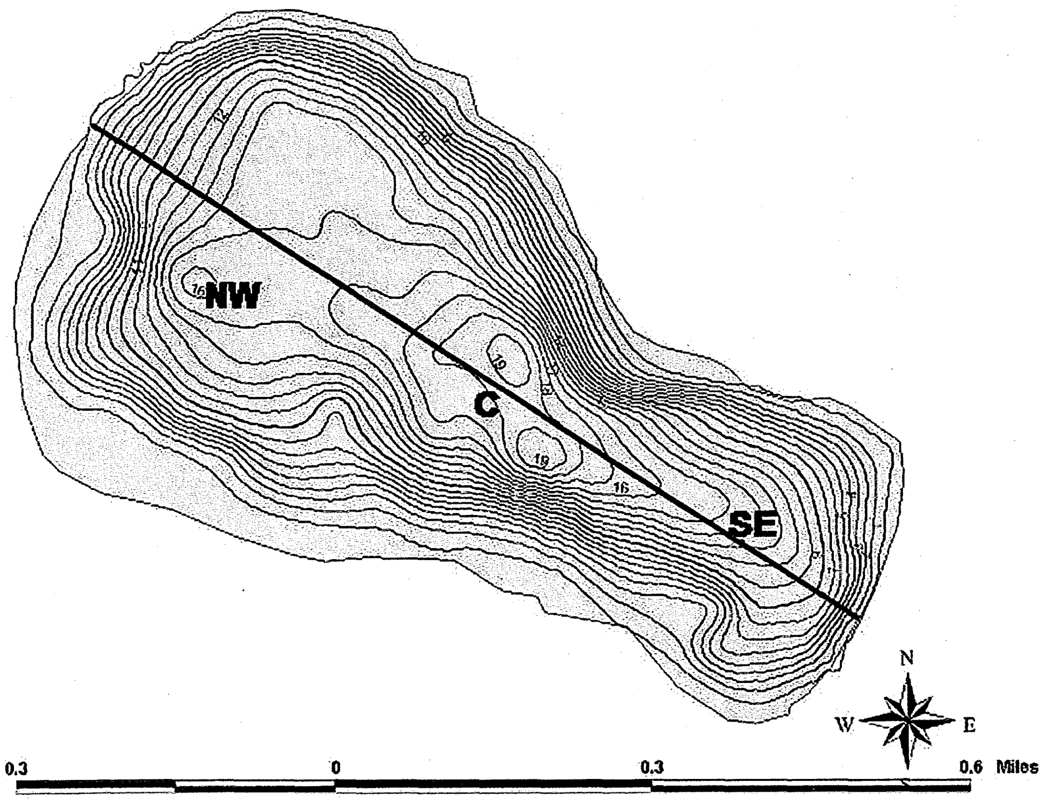

Figure 1. Map of Square Lake (courtesy of Ray Valley, MDNR Fisheries) showing the locations of the sonar transect (solid black line) and the locations where zooplankton samples were collected. During the ice-free season zooplankton were collected from the central (C) location. During winter, samples were collected from the central location and deep locations in the northwest (NW) and southeast (SE) ends of the lake.

Acoustic survey of zooplankton

The series of long-axis transect echograms illustrates the spatial distribution and abundance of zooplankton during the ice-free months of 2004 (Fig. 2) and 2005 (Fig. 3).

2004 results

The April sampling date in 2004 occurred two days after ice-out, and two days before the MDNR stocked the lake with 3000 yearling rainbow trout (2.15 fish lb^{-1}). On this date the lake had not yet completely mixed following winter stratification. At the sampling station, water temperatures were 5.5 °C to a depth of 10 m, and decreased to nearly 4 °C at the bottom (Fig. 4). Zooplankton backscattering was generally low, with the exception of a patch (green-yellow on echogram) at the west end of the lake (Fig. 2a). The conspicuous sloping layer of backscattering from deep water at the east end to shallow water at the west end of the lake suggests that upwelling was occurring.

On May 8, the highest backscattering was seen in the thermocline (6-12 m) where *Daphnia pulicaria* were most abundant. There was very little backscattering in the hypolimnion (below 12 m).

By June 4, the strongest backscattering had shifted to deep water (12-14 m) where oxygen concentrations were between 1-3 mg L^{-1} (Fig. 4). Net samples indicate high concentrations of *D. pulicaria* at these depths. Below 14 m oxygen concentrations were less than 1 mg L^{-1}. This level of oxygen tends to be the lower limit for *D. pulicaria*.

On July 8, there was relatively weak and uniform backscattering at depths shallower than 10 m, a layer of more intense backscattering in the lower part of the metalimnion between 10-12 m, and very high backscattering in the hypolimnion at depths below 12 m. Net sampling showed that the backscattering from 10-12 m was caused by crustacean zooplankton (mostly *D. pulicaria* and calanoid copepods). The backscattering from depths below 12 m, however, was from *Chaoborus* (a dipteran midge) larvae. *Chaoborus* are able to tolerate anoxic water (Fig. 4), and typically migrate into deep anoxic water or sediments during the daytime to avoid fish predators, and then ascend into shallow water at night to feed on zooplankton.

The August 16 data are similar to those from July 8 and show the following features: 1) a relatively uniform distribution of zooplankton at depths above 9 m, 2) a layer of slightly stronger backscattering in the lower part of the metalimnion between 9-12 m where D. pulicaria were most abundant, and 3) strong backscattering in the anoxic hypolimnion below 12 m by *Chaoborus.*

In September and October 2004, there was a layer of backscattering from *Daphnia pulicaria* in the oxic part of the metalimnion (7-9 m on September 19, and 10-11 m on October 17). Also, as in July and August, a deeper layer of backscattering from Chaoborus was evident in the hypoxic (< 1 mg/L) water below 13 m (Fig. 4).

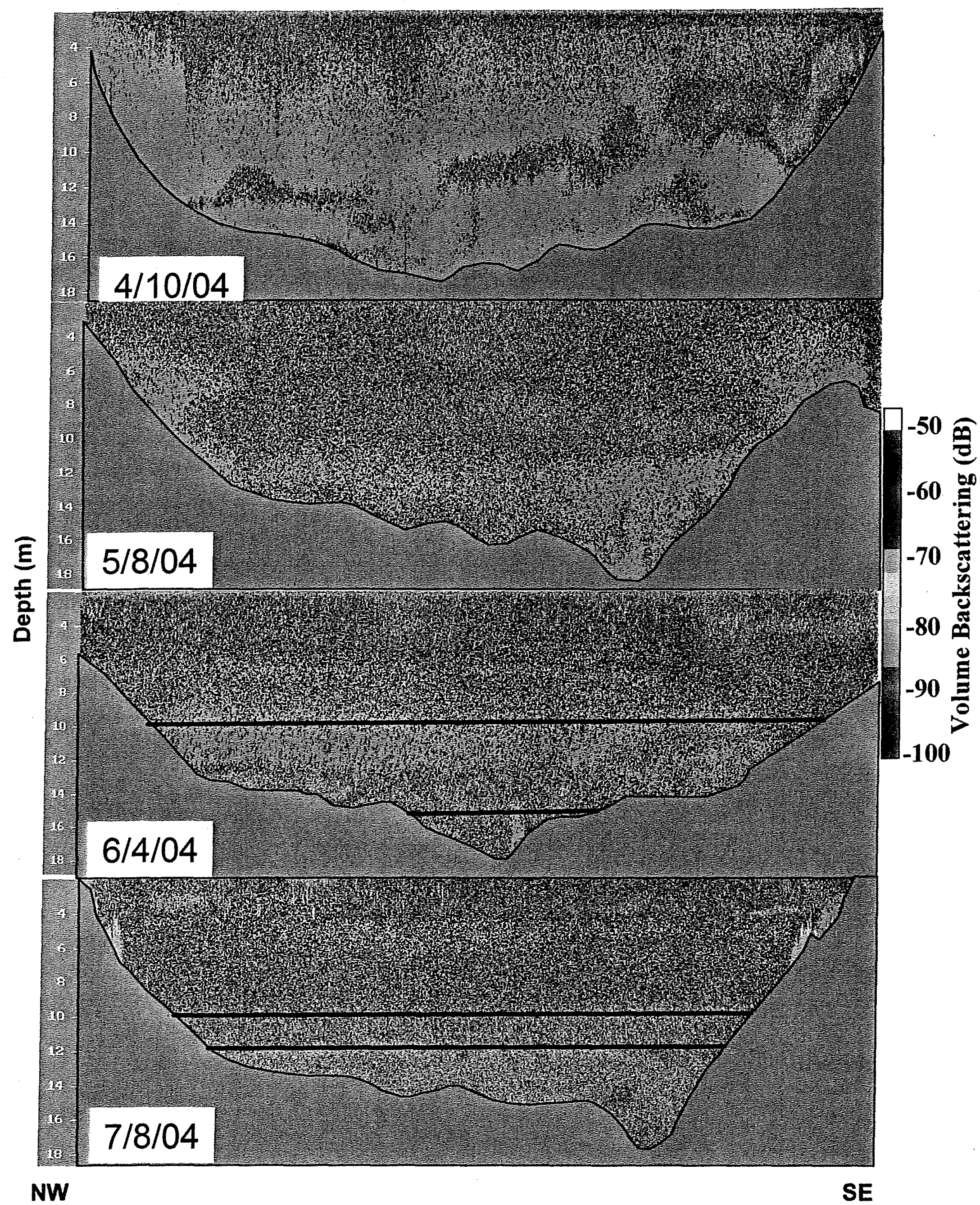

Figure 2a. Long-axis transect echograms for April-July 2004. The scale on the left indicates depth (m) and the color scale on the right corresponds to volume backscattering strength (dB). The gray shaded area indicates the lake bottom. In April and May D. pulicaria were found throughout the water column. Black horizontal lines delimit depths at which *D. pulicaria* occurred in June and July. Backscattering below the lowest black line on July 8 is from *Chaoborus* larvae.

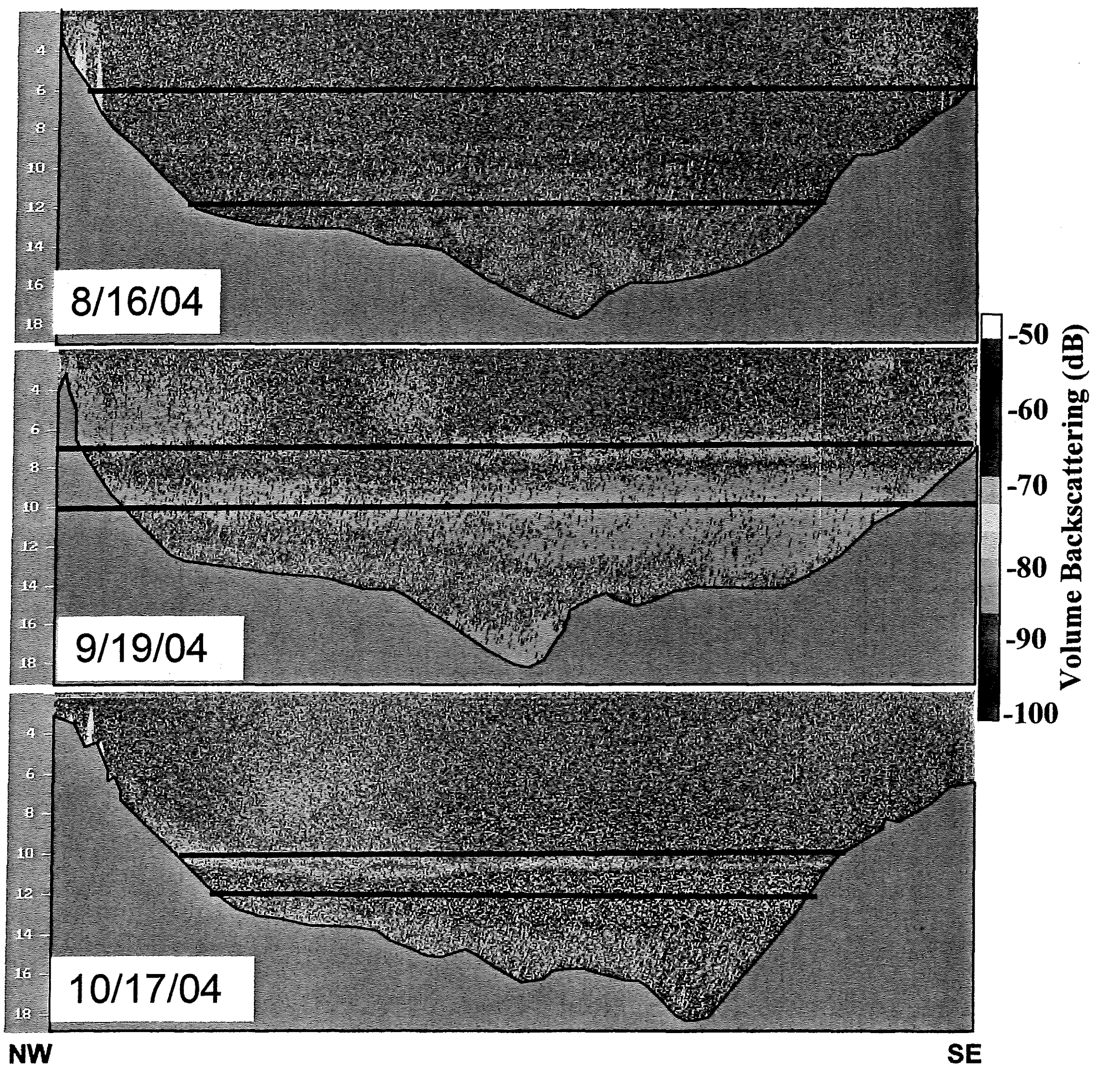

Figure 2b. Long-axis transect echograms for August-October 2004. Black horizontal lines delimit depths at which *D. pulicaria* occurred. Backscattering below the lowest black line is from *Chaoborus* larvae.

2005 results

Trout were not stocked in the autumn of 2004 so the *Daphnia pulicaria* population was subject to less predation over winter in 2005 than it was in the winter of 2004. The first sampling date in the ice-free season of 2005 was on April 17, about 3 weeks before the MDNR stocked the lake with 3000 trout (3 fish lb^{-1}). On this date, the eastern third of the lake at depths above 10 m had strong acoustic backscattering (Fig. 3a), which was markedly stronger than in April of 2004. In addition, net samples showed that *D. pulicaria* were abundant, and that they had relatively large brood sizes (3.16 ± 0.46). This finding supported the hypothesis that the over-wintering *Daphnia pulicaria* population would be larger and have high reproductive potential when trout were not stocked in autumn (Hembre & Megard, 2005).

By May 20, we expected that the large, fertile *D. pulicaria* population that over-wintered would have grown substantially. However, contrary to that expectation, backscattering from zooplankton diminished substantially between April 17 and May 20 (Fig. 3a), and *D. pulicaria* were less abundant in net samples. There is no obvious explanation for this decline in the *D. pulicaria* population. One possibility, though, is that *Daphnia* reproductive rates may have been slowed by the unusually cool weather between the April and May sampling dates (surface water temperatures only increased by 2.5 ^{o}C). This could account for diminished reproduction of *Daphnia*, but does not explain the high mortality that was observed (for more discussion of this topic see Result 4 ("Evaluate predation on *Daphnia* by trout").

On June 7, *D. pulicaria* was most abundant below 10 m (Fig. 3a), and the population had grown modestly since May. Oxygen concentrations in deep water were higher than in June of 2004 which enabled *D. pulicaria* to be present in the very deep water.

In July (Fig. 3a) and August (Fig. 3b) the pattern of acoustic backscattering from *D. pulicaria* was similar with the strongest backscattering at depths in the lower part of the metalimnion and the hypolimnion (Fig. 4) and the *D. pulicaria* population grew considerably from June.

In September and October oxygen concentrations below 11 m were less than 1 mg L^{-1} (Fig. 4) and *D. pulicaria* were restricted to shallower depths. Echograms from September and October (Fig. 3b) show much weaker backscattering at the depths inhabited by *D. pulicaria* and patches of very strong backscattering in the hypolimnion where oxygen levels were very low (Fig. 4). As in 2004, the strong backscattering at these depths was caused by *Chaoborus* larvae.

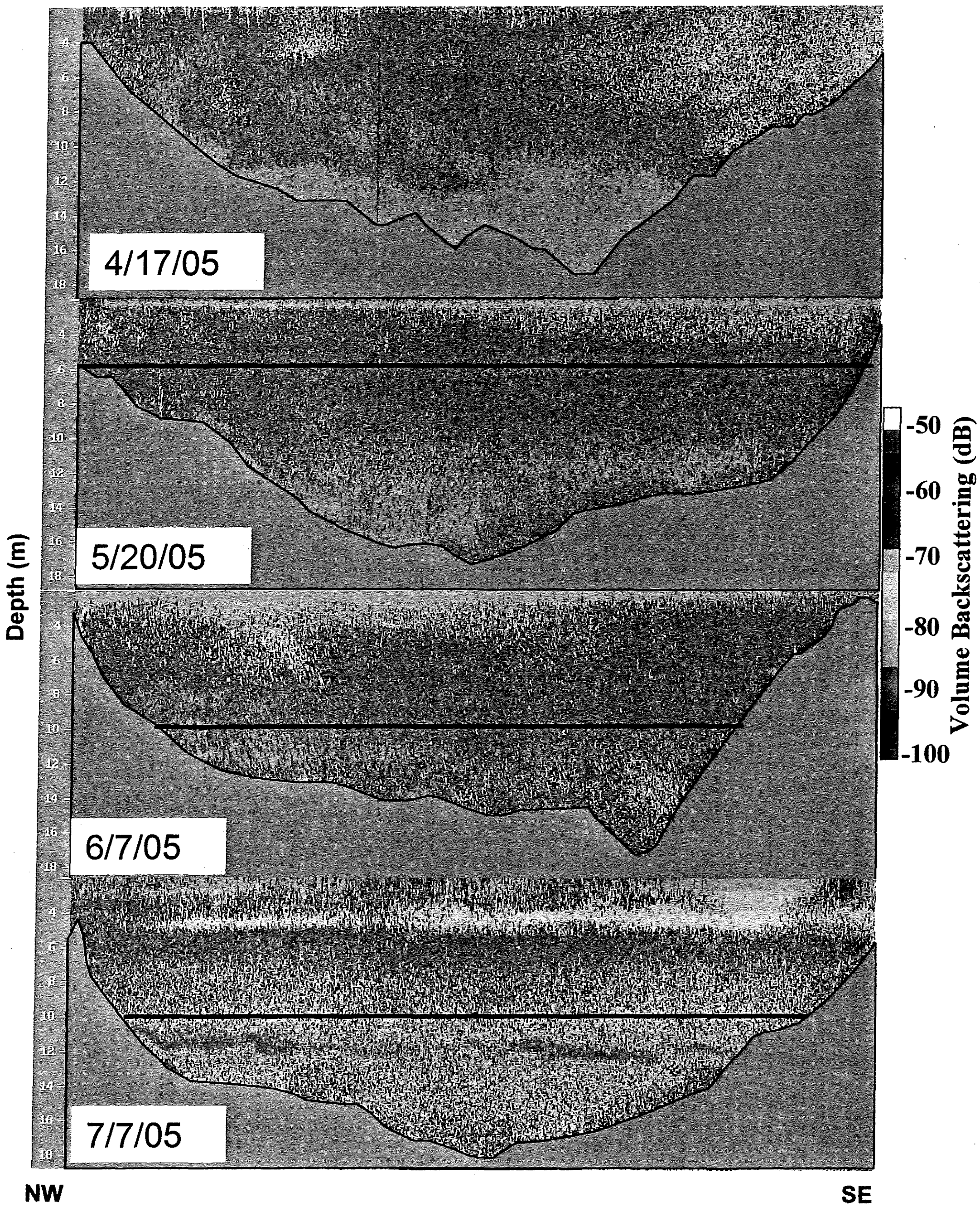

Figure 3a. Long-axis transect echograms for April-July 2005. In April *D. pulicaria* were found throughout the water column. In May, June, and July *D. pulicaria* occurred at depths below the black horizontal lines.

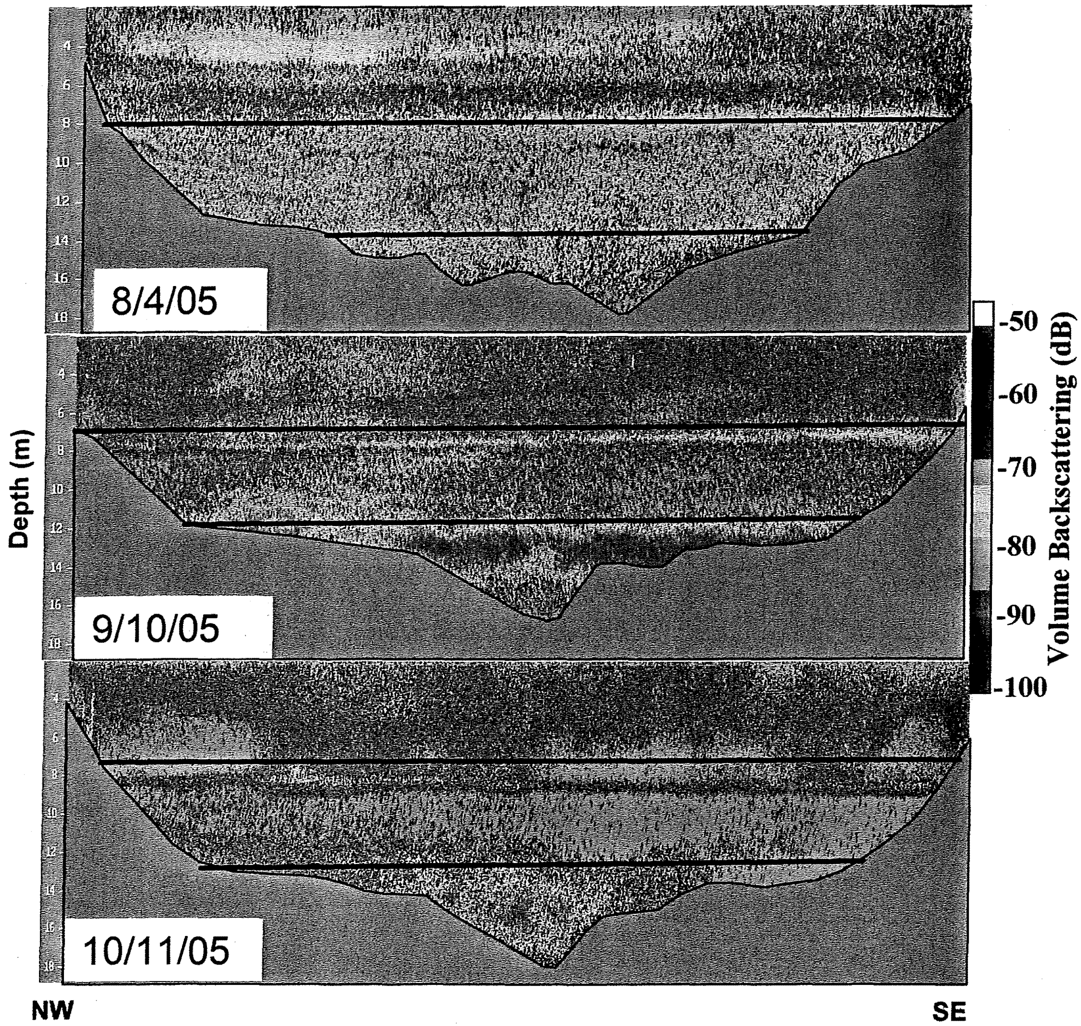

Figure 3b. Long-axis transect echograms for August-October 2005. Horizontal black lines delimit the depths where *D. pulicaria* occurred. Scattering below the lowest black line in September and October was from *Chaoborus* larvae.

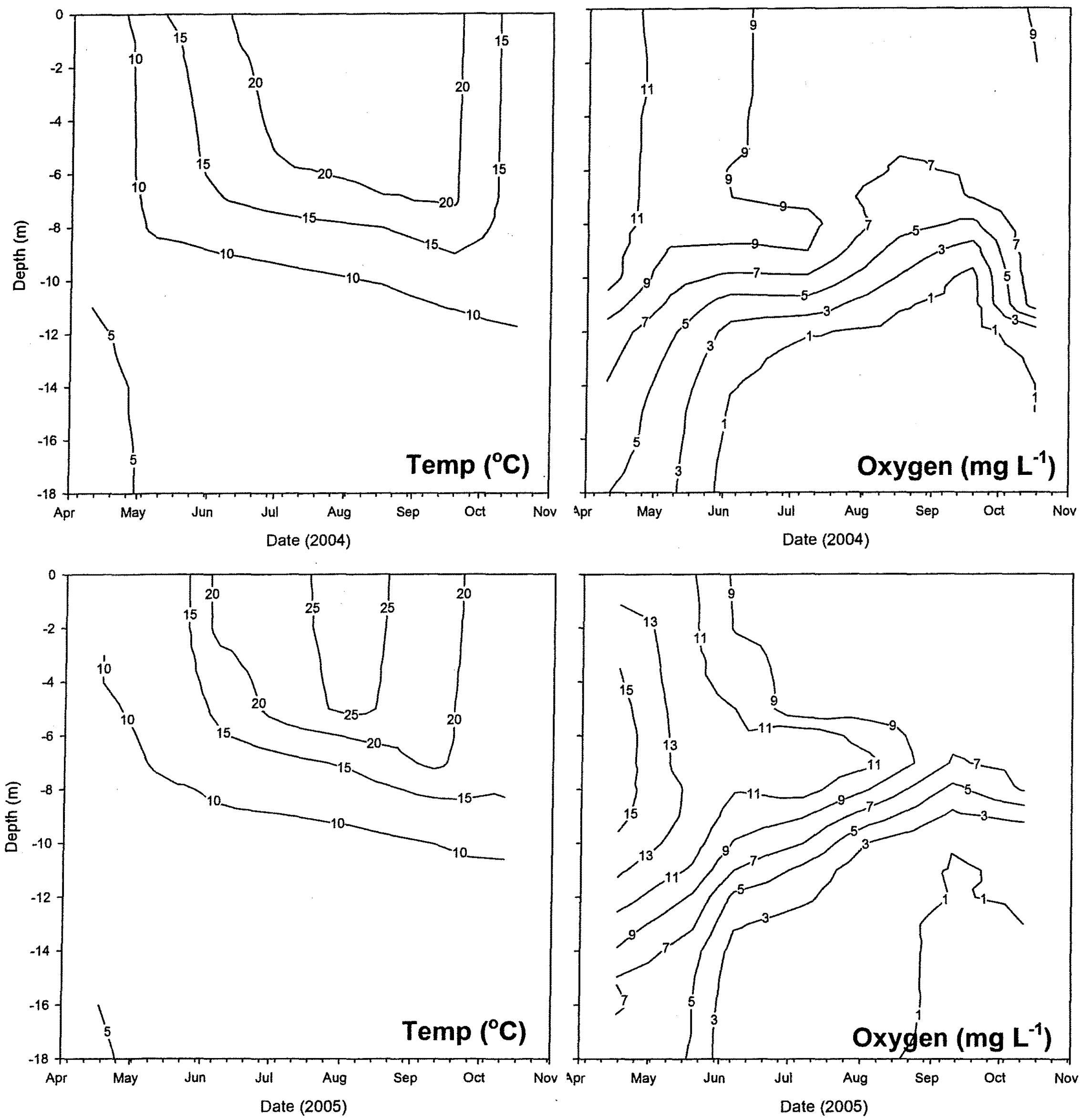

Figure 4. Contour plots for temperature and oxygen for 2004 and 2005. Note that hypoxia (< 1 mg L^{-1}) in deep water occurred earlier in 2004 and extended into shallower water than in 2005.

With knowledge of the target strength of *D. pulicaria* and the depths where *D. pulicaria* occurred on each date, the sonar data from these long-axis transect were used to calculate whole lake population densities (Fig. 5). *Daphnia pulicaria* concentrations from net samples collected during the winter of 2004 and 2005 were also included to show the size of the over-wintering populations.

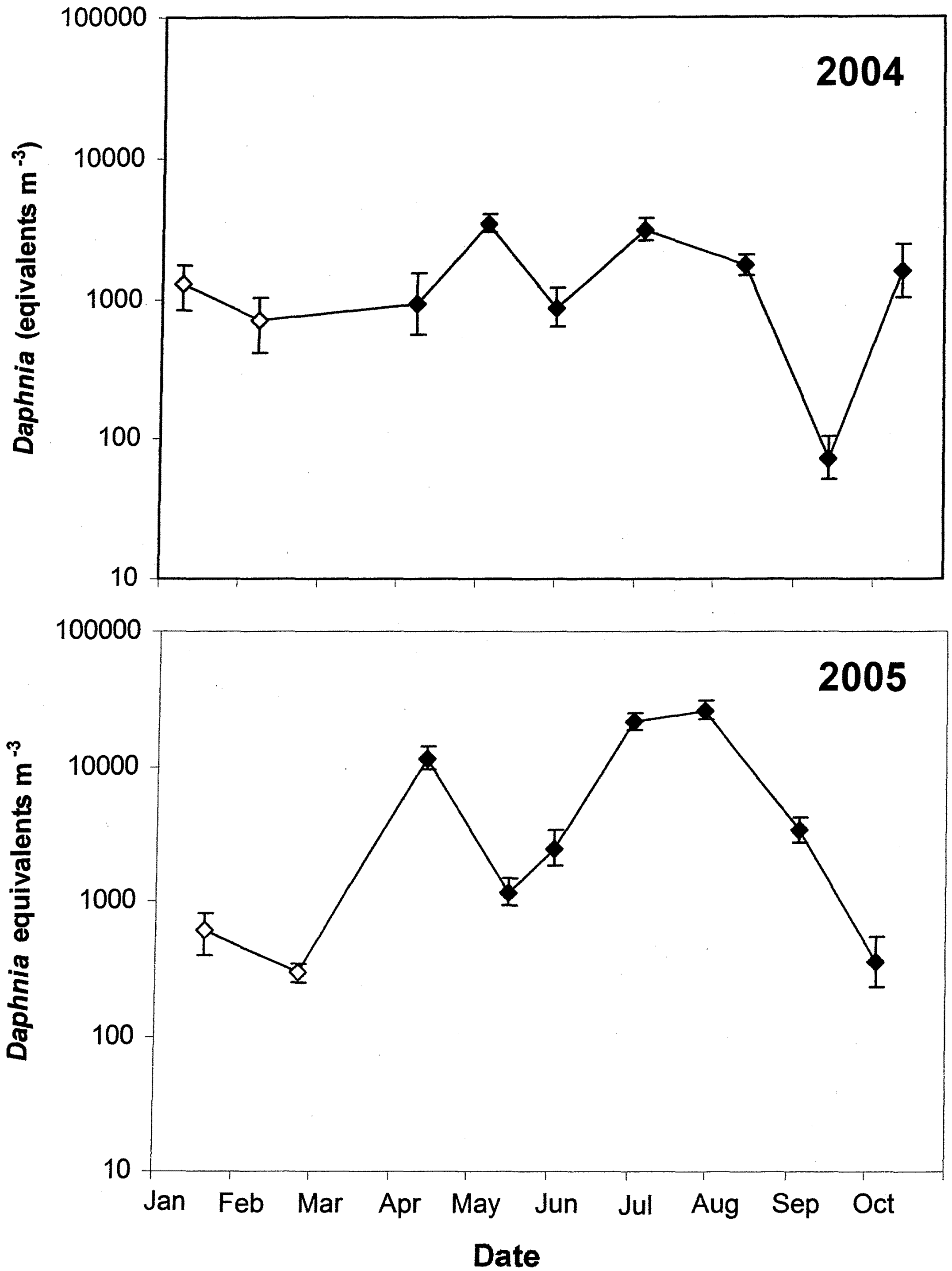

Figure 5. Population dynamics of *D. pulicaria* for 2004-2005. Sonar data were used to estimate population density (*Daphnia* equivalents m^{-3}) on all but the four winter dates (open symbols). On those dates, population density was estimated from conventional net sampling. Error bars represent ± s.e.

Our expectation was that suspension of autumn stocking of trout in 2004 would have alleviated predation pressure on *D. pulicaria* over winter and allowed for the build-up of a larger "seed" population in the spring population 2005 than was present in the spring of 2004 when the population was subject to predation by trout stocked in the autumn of 2003. Consistent with this expectation, the April 2005 population was about an order of magnitude larger than the April 2004 population (Fig. 5). However, counter to our expectation, the large April population declined in May and June instead of increasing as seen in a previous study (Hembre & Megard, 2005). Ramifications of this population decline in late spring-early summer on phytoplankton biomass and water clarity are discussed in Result 3 ("Water quality monitoring and angler creel survey"). One other notable difference between the two years is that the size of the *D. pulicaria* population increased substantially in July and August of 2005 and was much larger than in 2004 (Fig. 5).

References

Hembre L.K. & Megard R.O. (2003) Seasonal and diel patchiness of a *Daphnia* population: An acoustic analysis. *Limnology & Oceanography* **48**, 2221-2233.

Hembre L.K. & Megard R.O. (2005) Timing of predation by rainbow trout controls *Daphnia* demography and the trophic status of a Minnesota Lake. *Freshwater Biology* **50**, 1064-1080.

Megard R. O., Kuns M.M., Whiteside M.C. & Downing J.A. (1997) Spatial distributions of zooplankton during coastal upwelling in western Lake Superior. *Limnology & Oceanography* **42**, 827-840.

Urick R. J. (1983) Principles of underwater sound., 3rd ed. McGraw-Hill. Boston, USA.

Result 2: Acoustic (Sonar) Data Collection

Description: High frequency sonar system using a wide-beam transducer will be used to obtain information about the abundance of rainbow trout. Sonar surveys from before and after trout are stocked to the lake will be compared to estimate the total trout in the lake

Summary Budget Information for Result 2:	**LCMR Budget**	$ 1,750
	Balance	$ 150

(Unspent $150 in Equipment)

Personnel:	$1,600
Equipment:	$150

The Minnesota Department of Natural Resources (MDNR) stocked Square Lake with rainbow trout three times during the course of this study. 2000 trout were stocked in the fall of 2003 (October 12), and 3000 trout were stocked in the spring of 2004 (April 12) and 2005 (May 9).

Estimation of trout abundance

Rainbow trout abundance was estimated from acoustic data from long axis transects (Fig. 1). Fish echoes were enumerated from depths within the bounds of the rainbow trout habitat (temperature $\leq 21^\circ$ C and oxygen ≥ 5 mg L^{-1}, Wang et al., 1996), and a range-weighted approach (Yule, 2000) was employed to account for the increased volume of water insonified as the acoustic beam spreads. For clarity, weaker echoes (< -60 dB) from zooplankton scattering were excluded from this analysis. Fish echoes detected were weighted to a 1-m wide swath at the surface using the formula:

$$F_w = 1/[2 \cdot R \cdot \tan(\theta)],$$

where F_w is the number of weighted fish, R is the range from transducer (m) and θ is the half angle of the transducer beam. Backscattered sound from a near-field blind zone within 1.5 m of the transducer was ignored.

On three occasions (October 2003, April-May 2004, and April-May 2005) we had the opportunity to acoustically assess the size of the trout population before and after the lake was stocked with a known number of trout. By comparing the increase in our acoustic estimate with the known number of fish added to the lake we established a conversion factor to be used to extrapolate trout population size from sonar data. Our post-stocking attempt on 16 October, 2003 was unsuccessful because the newly stocked trout were aggregated in a large school near the boat landing at the southeast end of the lake. Dense schooling prevents the resolution of individual trout echoes, and thus made it impossible to relate the sonar data from 16 October to the number of trout stocked to the lake. Fortunately the April-May comparisons in 2004 and 2005 were successful and allowed us to establish a relationship between the sonar count and the known number of fish added to the lake. In 2004, the number of "weighted fish" increased from by 52.6 between the pre-stocking (April 10) and post-stocking (May 8) sampling date. In 2005 the number of "weighted fish" increased by 44.3 between the pre-stocking (April 17) and post-stocking (May 20) sampling date. Relating the average of those two estimates (48.5) to the number of trout stocked (3000) provides a conversion factor of 61.9 trout per weighted fish.

The April 2004 population was larger than the population present in April 2005 (Fig. 6), which is consistent with the fact that trout were stocked in autumn of 2003, but not in 2004. In both years the largest populations were observed in May after the addition of 3000 trout by the MDNR. In 2004 the trout population appeared to decline more steeply from May to July than in 2005, but overall, the population dynamics of trout were similar between the two years.

For results of trout diet analysis and for discussion of the effect of trout predation on *D. pulicaria* see Result 4 (Evaluate predation on *Daphnia* by trout)

References

Wang L., Zimmer K., Diedrich P & Williams S. (1996) The two-story rainbow trout Fishery and its effect on the zooplankton community in a Minnesota lake. *Journal of Freshwater Biology* **11**, 67-80.

Yule, D.L. 2000. Comparison of horizontal acoustic and purse-seine estimates of salmonid densities and sizes in eleven Wyoming waters. *North American Journal of Fisheries Management.* 20: 759-775.

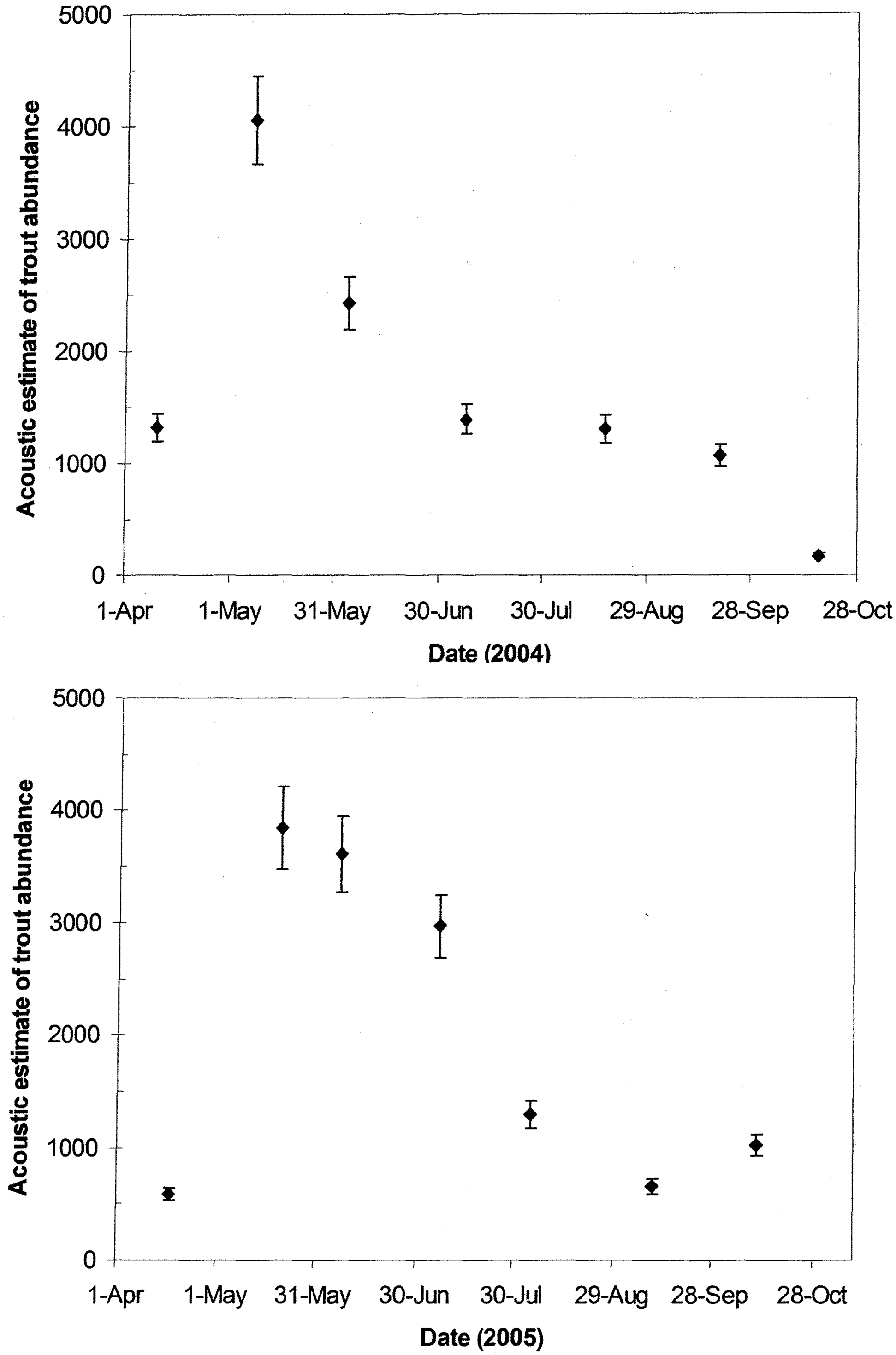

Figure 6. Sonar estimates of trout abundance for 2004 and 2005 (error bars indicate ± s.d.).

Result 3: Water Quality Monitoring and Angler Creel Survey

Description: Periodic sampling and analysis of water quality will include measurements of temperature/dissolved oxygen, phosphorus concentrations, and Chlorophyll *a* concentrations. Secchi disk/light meter measurements will be used to measure water clarity. A Creel Survey of anglers on Square Lake using the "complete trip" method will be conducted in order to estimate angle harvest, fishing pressure, and contribution of trout to the creel relative to other species. This data would be used for reviewing the fisheries management plans for Square Lake.

LCMR Budget:	$10,700
Balance:	$ 1,760

(Unspent $1,760: The second year of the creel survey was funded by the Marine on St. Croix Water Management Organization, Square Lake Association, and Hamline Unversity.)

Personnel:	$10,700
Other:	

Secchi transparency, vertical profiles of temperature and oxygen, and water samples for chlorophyll a analyses were collected at the central sampling site on all dates (Fig. 1). Temperature and dissolved oxygen were measured at 1-m intervals with a YSI dissolved oxygen meter. Water samples for chlorophyll a analyses were collected from the epilimnion with a Van Dorn water sampler. Samples were filtered through a glass fiber filter and pigments were extracted with methanol (Holm-Hansen & Reiman, 1978). If chlorophyll analyses were not done immediately, the filters were frozen for future analysis. Supplemental water transparency data were obtained from the MPCA and Metropolitan Council Citizen Assisted Monitoring Program (CAMP). Data for total phosphorus concentrations were also obtained from the Met Council's CAMP program (Anhorn, 2004; 2005).

Water quality of Square Lake in 2004 and 2005

The water clarity of Square Lake in 2004 did not differ significantly from that in 2005 (Fig. 7). In both years water clarity was highest in the spring, and generally decreased through the summer and into autumn. One subtle difference was that the lake was exceptionally clear (secchi depth = 6.7-7.6 m) in April of 2005, and more transparent than in April 2004 (secchi depth = 4.6-5.75). As with water clarity, chlorophyll a concentrations (a measure of phytoplankton abundance) in surface water were also very similar between 2004 and 2005 (Fig. 8), and were inversely related to water clarity. A logarithmic regression shows that epilimnetic chlorophyll a concentration was a significant predictor ($p = 0.0003$) and explained 67% of the variation in secchi depth (Fig. 9). Phytoplankton biomass (Chl a) was positively correlated with total phosphorus concentrations in both 2004 and 2005, but the relationship was not statistically significant (Fig. 10). However, phytoplankton biomass was significantly related to *D. pulicaria* density (Fig. 11). While this regression was significant ($p = 0.04$) *D. pulicaria* density explained only a modest amount of the variation (31%) in Chl a.

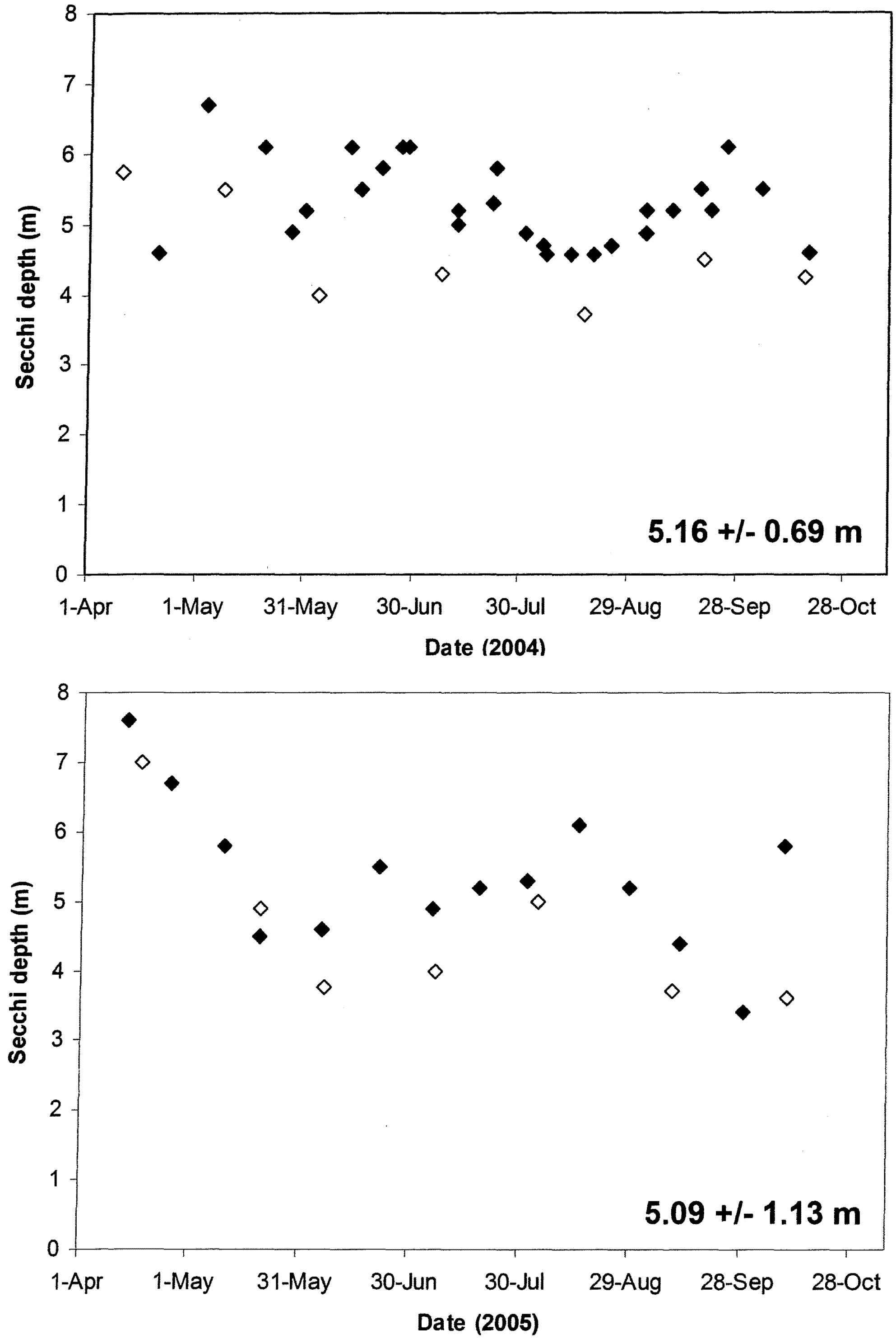

Figure 7. Water clarity (secchi depth) of Square Lake for 2004 and 2005. Open symbols indicate measurements we made and black symbols indicate data from the MPCA and Met Council.

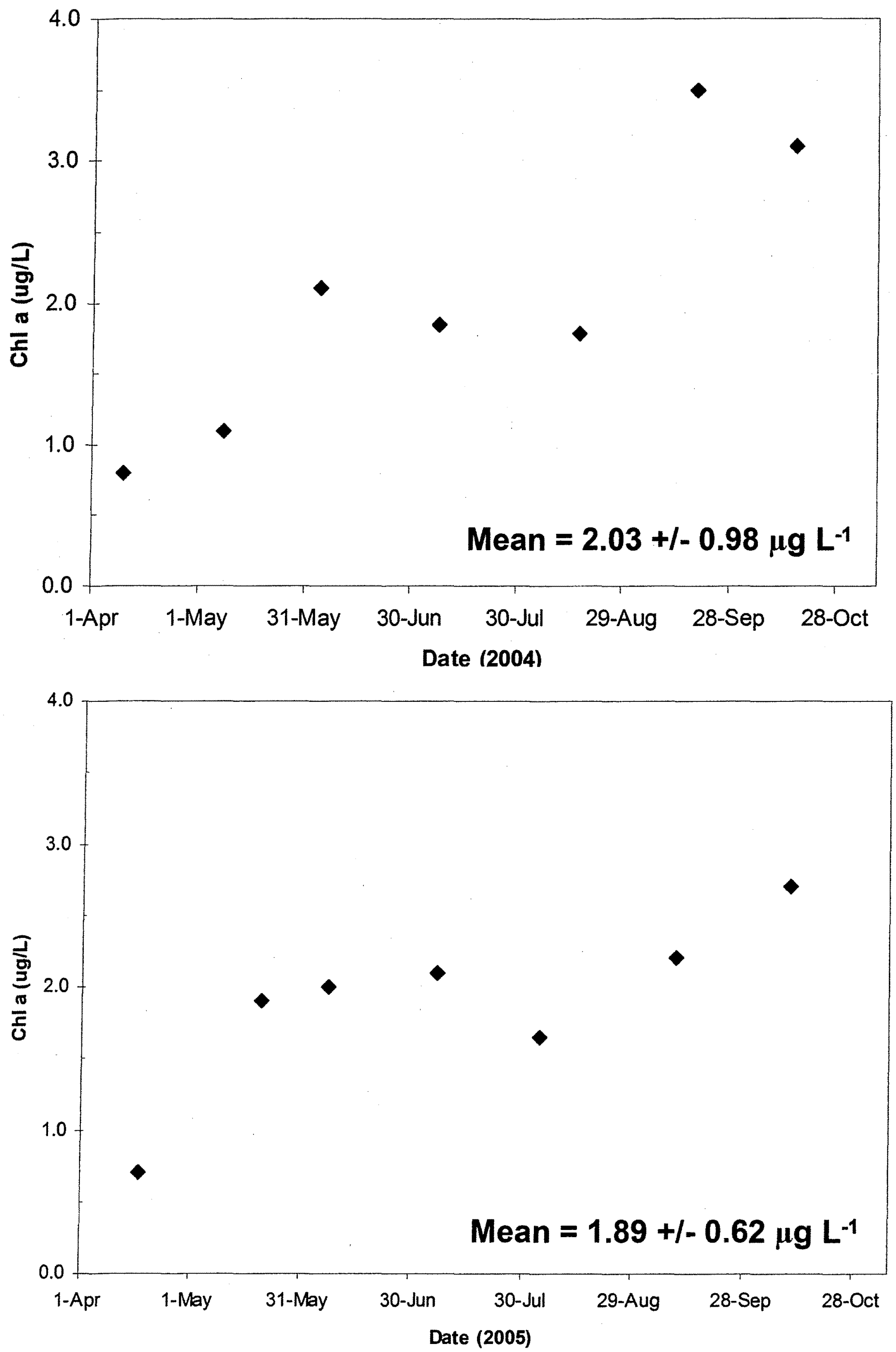

Figure 8. Surface water chlorophyll a concentrations in Square Lake in 2004 and 2005.

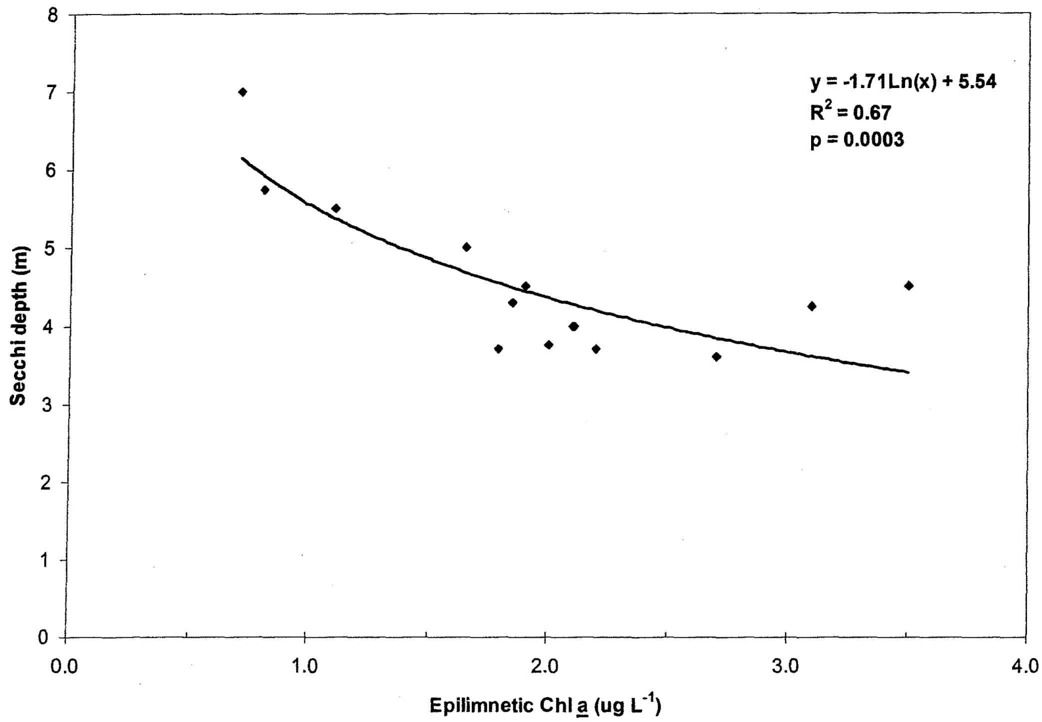

Figure 9. Logarithmic regression of secchi depth versus epilimnetic chlorophyll a (Chl a) concentration.

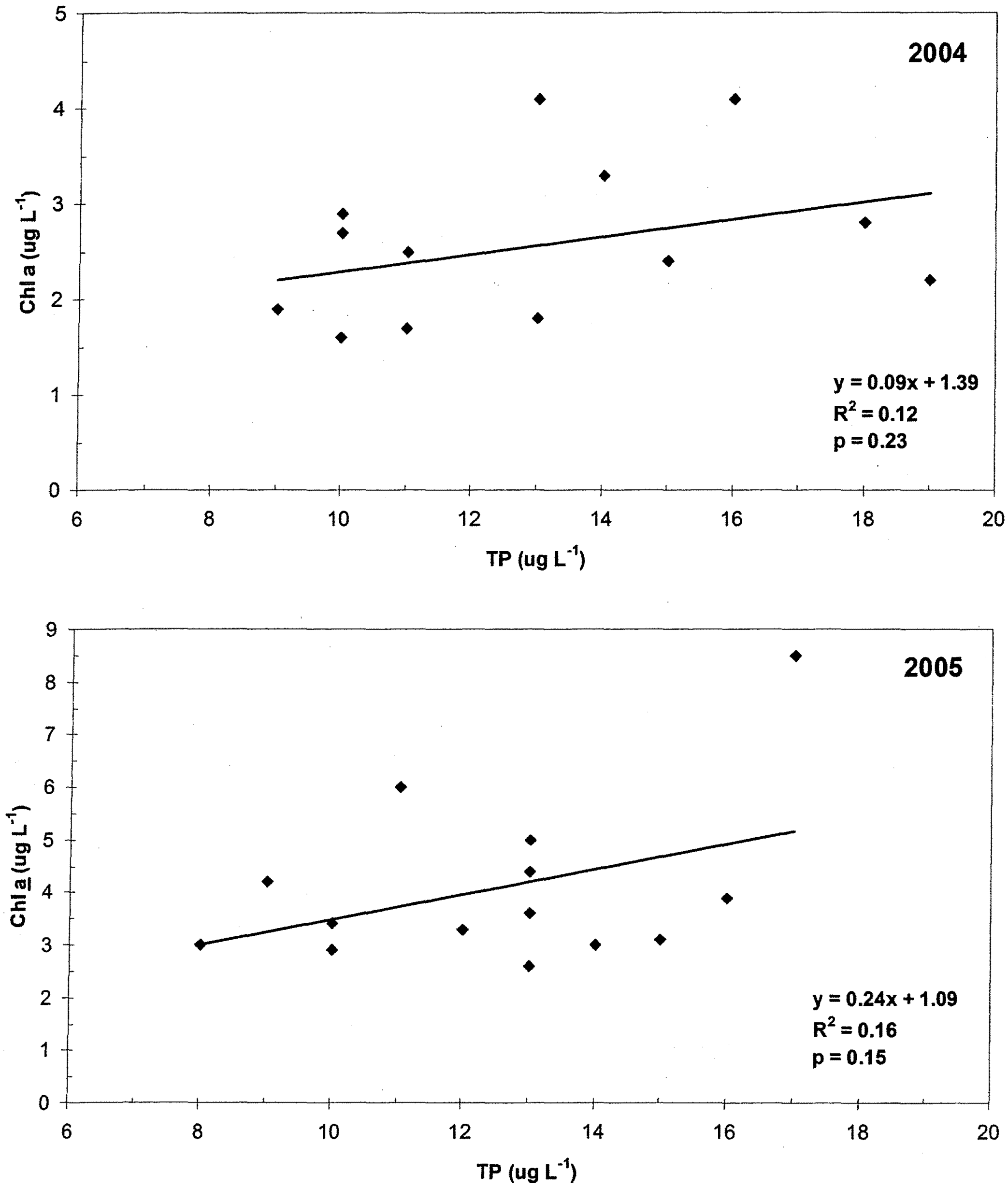

Figure 10. Linear regressions of chlorophyll a (Chl a) versus total phosphorus (TP) in surface water in 2004 and 2005. In both years there was a weak positive correlation, but the relationships were not statistically significant. Data are from Metropolitan Council CAMP program (Anhorn, 2005; 2006).

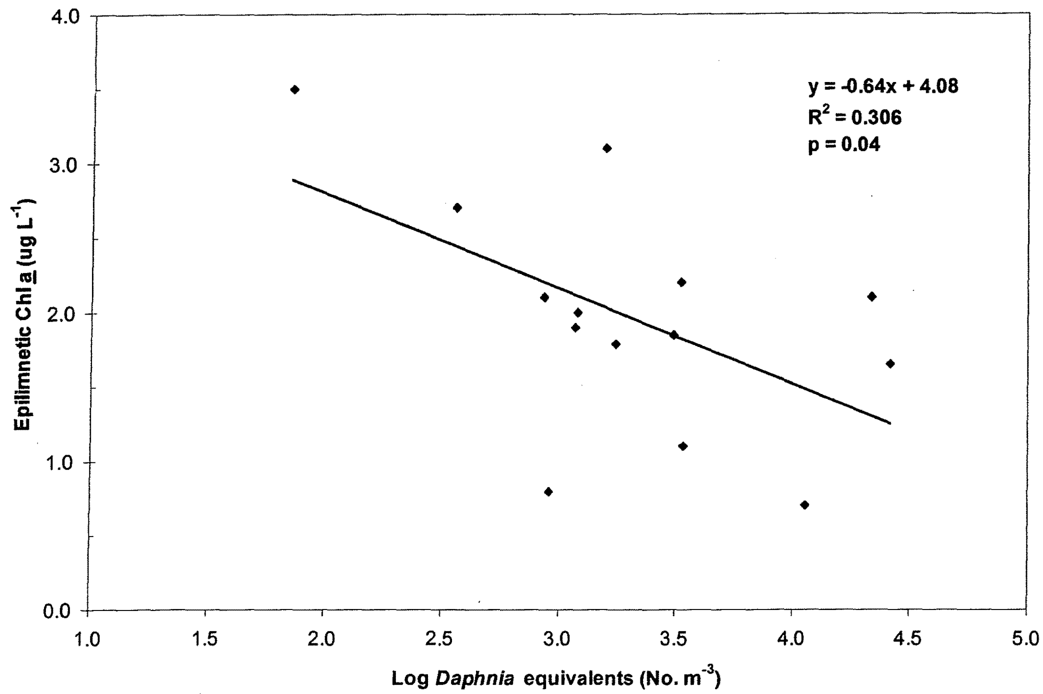

Figure 11. Simple linear regression of epilimnetic chlorophyll a (Chl a) concentration versus Log *Daphnia* concentration determined from acoustic analyses in 2004 and 2005.

To summarize, the 2004-2005 water quality data for Square Lake indicate the following:

1) There is no evidence that the change in fisheries management between the two years (i.e., autumn and spring stocking in 2003-2004, and only spring stocking in 2005) significantly affected the water quality, in terms of average water clarity (Fig. 7) and average surface water phytoplankton concentrations (Fig. 8). The large over-wintering population of *D. pulicaria* in April 2005 did coincide with very high measurements of water clarity and low levels of phytoplankton, but the decline in *D. pulicaria* abundance in May and June of 2005 prevented the clear-water state from persisting.

2) *Daphnia pulicaria* population size was significantly correlated to phytoplankton concentrations (Fig. 11), but total phosphorus concentrations were not (Fig. 10). This supports the view that food chain effect of grazing by *Daphnia* on planktonic algae plays a larger role in governing the trophic status of Square Lake than phosphorus levels.

3) Hypoxia (oxygen < 1 mg L^{-1}) in deep water occurred earlier and affected a greater portion of the water column in 2004 than in 2005 (Fig. 4). This allowed for a larger refuge zone for *Daphnia* later into the summer of 2005 and likely was responsible for the resurgence of the *D. pulicaria* population July and August of 2005 (Figs. 3 & 5).

Creel surveys

The 2004 creel surveys were coordinated by David Zappetillo and Jeffrey Gorton, MDNR East Metro Area Fisheries. The winter survey began at the start of the trout ice-fishing season (18 January 2004) and continued until the end of the season (16 March 2004). The creel clerk (Garret Vail) interviewed angler and collected information such as the species of fish sought and the number and size of fishes caught. Stomach samples of trout were also collected for gut content analyses when possible. Jodie Hirsch, MPCA Ecological Services, processed the stomach samples collected by Mr. Vail. The summer creel survey in 2004 began on May 14 and lasted until October 31. Square Lake has a catch and release regulation for rainbow trout from the fishing opener in mid-May to June 10. As in the winter survey, the creel clerk (Garret Vail or Ben Saunders) interviewed anglers to determine the number and sizes of fishes caught. Jeffrey Gorton compiled the data and produced a report on the results of the 2004 creel surveys. In 2005, there was not a winter creel survey because trout were not stocked in the autumn of 2004.

To obtain additional information about angling pressure during summer and to collect trout stomachs for diet analyses a "mini" creel survey was conducted in the summer of 2005 (this survey was not part of the LCMR project work program). The 2005 survey began on June 11 (the first day trout could be harvested by anglers) and lasted until the end of August. The survey was coordinated by Leif Hembre (Hamline University), and funding for creel clerks was provided by the Square Lake Association (Gracia Tharp) and Hamline University (Thomas Haycraft). The methodology for this survey was modeled after the methods employed by the MDNR in their 2004 surveys.

Summary of creel survey results

The winter (January 17-March 15) and open-water season (May 15-October 31) creel surveys found that fishing pressure, catch, and harvest estimates were considerably lower in 2004 than in previous years when creel surveys were done (Gorton, 2004).

During the winter survey, 75% of anglers interviewed stated that they were primarily fishing for trout. A total of 532 fish were caught and 143 were harvested. Rainbow trout accounted for 128 of the catch, and 103 of the fish harvested (Table 1). During the open-water fishing season, 59% of anglers interviewed were primarily fishing for trout and 23% were fishing for panfish (e.g., crappies and sunfish). A total of 2,552 fish were caught and 746 fish were harvested. Most of the fish caught (1,705) were panfish, but relatively few were harvested (363). Rainbow trout represented a significant portion of the catch (439) and the harvest (322).

One of the interesting findings of the creel surveys was that of the 5,000 rainbow trout stocked by the MDNR in the autumn of 2003 and spring of 2004 only 9.3% of these fish were estimated as harvested by anglers. The low harvest rate could be a result of either 1) the relatively low fishing pressure documented by the creel surveys, 2) poor survival of the stocked trout, or both of these. Poor survival of the trout could have been caused by early mortality near the time of stocking, predation by other fish (e.g., northern pike), or a lack of food. Gorton notes that the trout harvested were typically "similar to the size of the stocked fish". This indicates that trout growth rates were low, and implies that the fish may have been food-limited.

Sonar estimates of trout population size (Fig. 6) show a steep decrease in trout abundance from early May to early June, a time when harvesting of trout by anglers is prohibited. This finding supports the idea that trout experienced mortality from a source other than angling during that time period. Mortality from angling was more substantial between June and September 2004. The trout population was estimated to have decreased by about 1400 (Fig. 6) between June and September 2004. During that time period the creel survey harvest estimate was 388 (Table 1), accounting for 28% of the reduction in the trout population. Between September and October, however, angler harvest only accounted for only about 2% of the decrease in the trout population size.

Table 1. Observed and estimated catch and harvest of rainbow trout in 2004 by anglers (Gorton, 2004).

Month (2004)	Caught (obs)	Harvest (obs)	Caught (est)	Harvest (est)
January	12	9	31	23
February	10	8	60	48
March	5	2	37	32
Ice fish subtotal	**27**	**19**	**128**	**103**
May	3	0	36	0
June	3	1	45	16
July	36	34	214	185
August	15	15	66	66
September	8	7	64	55
October	3	0	15	0
Open-water subtotal	**68**	**57**	**439**	**322**
2004 TOTAL	**95**	**76**	**567**	**425**

During the mini-creel survey in the summer of 2005 (June 11-August 29), 50% of anglers stated that they were fishing for trout, 21% for panfish, and the remainder were pursuing either northern pike, largemouth bass, or had no particular target species. Catch and harvest levels observed were considerably higher in June of 2005 than in 2004, but very low in August (Table 2). This finding is consistent with the sonar estimates of trout population size during 2005. The trout stocked in the spring of 2005 appeared to have had better survival into June and July than in 2004, but decreased precipitously between July and August (Fig. 6).

Table 2. Observed catch and harvest levels of rainbow trout by anglers in 2005.

Month	Caught (obs)	Harvest (obs)
June	32	27
July	24	24
August	5	5
Total	**61**	**56**

The low angling harvest rates observed during the creel surveys imply that the special regulation restricting angling to catch-and-release between mid-May and June 10 may not be necessary. The aim of this regulation is to prevent the rapid removal of trout by anglers and to enable the trout to grow for an extra month so that anglers can catch larger fish (Doneux, 2002). It appears that trout are primarily incurring mortality from sources other than anglers. Allowing harvest of trout during the spring would also lessen predation on *Daphnia* and enable *Daphnia* to become more abundant and exert greater grazing pressure on phytoplankton.

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Result 4: Evaluate Predation on *Daphnia* by Trout

Description: To evaluate the diet of trout and other *Daphnia* predators, we will obtain fish stomachs from anglers. The stomachs will be dissected and the contents will be identified and enumerated. This will allow us to directly determine whether the trout are selectively feeding on *Daphnia,* and the nature of their diet at different times of year.

Egg ratio analysis will be used to determine the reproductive rate of the *Daphnia* population each year. This information will be compared to the mortality imposed by trout to assess the impact of trout predation on the dynamics of the *Daphnia* population.

The mortality of *Daphnia* due to the trout population will be estimated through analysis of sonar information and consumption rates of *Daphnia* by trout based on previous research.

LCMR Budget:	**$4,160**
Balance:	**$ 0**
Personnel:	*$4,160*

Trout stomach contents

Trout stomachs obtained from anglers were preserved with 95% ethanol until analyzed. Stomachs were dissected and their contents were gently rinsed over a sieve (230 µm mesh) with distilled water. Larger invertebrates (e.g., insect larvae and pupae) were individually picked from the samples and counted, and the abundance of zooplankton (e.g., *D. pulicaria*) was calculated by subsampling. Body lengths of *D. pulicaria* in trout stomachs were also recorded.

The diet of trout consisted of *Daphnia pulicaria* and larval and pupal aquatic insects (Table 3). Stomach samples analyzed during the winter of 2004 (by Jodi Hirsch, MPCA) indicate that *Daphnia pulicaria* comprised the majority of their diet (2331 ± 373 individuals, 76 ± 11% of stomach content wet weight). In addition, *Daphnia* consumed by trout were larger (2.06 ± 0.02 mm), on average, than those collected from the water column in January and February 2004 (1.85 ± 0.03).

The summer diet of trout in 2005 was less specialized than that of the trout present over the winter in 2004 (Table 3). *Daphnia pulicaria* still comprised a substantial portion of the diet of trout (370 ± 92), but were less abundant in stomachs than in the winter of 2004. As in winter, trout predation on *Daphnia* was size-selective. The mean body size of *D. pulicaria* in trout stomachs (mean = 1.96 ± 0.03 mm) was greater than that of *Daphnia* in the water column (June-August mean = 1.74 ± 0.07 mm). Chironomid and *Chaoborus* larvae and pupae were a component of the trout diet on all but one date that stomach samples were obtained. On that date (August 6, 2005) the stomachs were empty (Table 3). The relative abundance of chironomids and *Chaoborus* in the trout stomachs collected in the summer of 2005 implies that trout were foraging in the sediments where these immature insects occur as well as the water column where Daphnia occurs. Chironomids are obligately benthic (present in sediments) while *Chaoborus* is typically benthic during the day and becomes planktonic at night. Though *Chaoborus* can be found suspended in the water column during the day (see echograms from July-October 2004 and September and October 2005, Figs. 2 & 3) they are found at depths with extremely low oxygen concentrations (Fig. 4) where trout would not occur. Therefore, this implies that trout were preying on *Chaoborus* in sediments at oxic depths or in the water column at night.

Table 3. Stomach contents of rainbow trout collected during winter 2004 and summer 2005. Values are mean number (s.e.) of each taxon per trout stomach.

Date	n	*Daphnia pulicaria*	Chironomid larvae/pupae	*Chaoborus* larvae/pupae	Trichoptera	Other larval/pupal insects
1/25/04	3	3330 (704)	0	0	2.0 (1)	0.67 (0.67)
1/31/04	1	4200 (NA)	0	0	0	0
2/9/04	1	2120 (NA)	0	0	14 (NA)	0
2/?/04	1	2480 (NA)	0	0	3 (NA)	1 (NA)
2/14/04	1	2960 (NA)	0	0	0	0
2/21/04	1	0	0	0	2 (NA)	0
2/23/04	2	2320 (560)	0	0	0	2.0(1)
2/27/04	1	600 (NA)	0	0	0	0
Winter mean		**2331 (373)**	**0**	**0**	**2.27 (1.24)**	**0.64 (1.31)**
6/26/05*	5	595 (308)	8.2 (5.5)	2.2 (1.2)	0	0
7/2/05*	8	314 (63)	7.4 (4.1)	1.1 (0.6)	0	0
7/16/05*	2	424 (188)	7.5 (4.5)	11.5 (6.5)	0	0
7/21/05*	2	534 (270)	3.0 (3.0)	3.0 (3.0)	0	0
8/6/05	3	0	0	0	0	0
Summer mean		**370 (92)**	**6.1 (2.2)**	**2.5 (0.96)**	**0**	**0**

NA, not applicable (due to sample size of 1). Asterisks indicate that stomachs also contained material used by anglers as bait (e.g., corn kernels, earthworms).

Though summer data imply that the trout-*Daphnia* coupling is not obligate during summer, to assess the potential affect of trout predation on *Daphnia* demography, the assumption is that trout are specialist predators on *Daphnia* year-round.

Demographic analysis

Egg ratio analyses (Paloheimo et al., 1982) were used to determine the reproductive rate of *Daphnia* for comparison with mortality due to trout predation. Egg development time (D, days) was calculated from temperature (T, °C) with:

$$\ln(D) = \ln(3.3956) + \ln(T) - 0.3414(\ln(T))^2$$

(Bottrell et al., 1976; Sterner, 1998). The average temperature experienced by the population over 24 h was estimated for each date. To account for different temperatures encountered by *Daphnia* between day and night as a result of diel vertical migration it was assumed that *Daphnia* experienced the mean temperature below the epilimnion during the day (excluding depths where oxygen concentrations were < 1 mg/L), and the mean eplimnetic temperature at night. The average temperature in these environments, adjusted for day length, was used to calculate D. Birth rate (b, day^{-1}) was calculated with

$$b = \ln (E/D + 1),$$

where E is the mean brood size per individual. Birth rate multiplied by population size is the reproductive rate of the population (R, births day^{-1}).

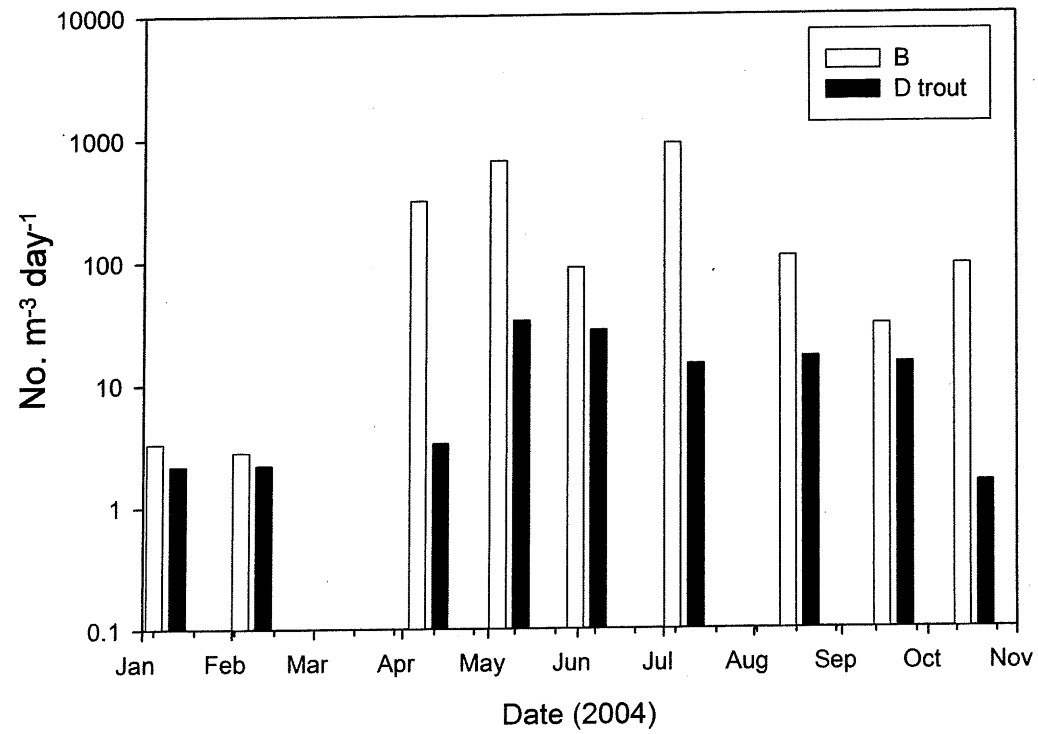
A bioenergetics analysis of rainbow trout in another Minnesota lake (Hirsch & Negus, 2000) concluded that the mean daily per capita consumption of *Daphnia* by over-wintering (late November and mid-May) rainbow trout was 12,000 (*Daphnia* $trout^{-1}day^{-1}$). The mean water temperature in that study was 5.1 °C. Because consumption rates increase with temperature, mean water temperature in the trout habitat zone (temperature $\leq 21°$ C, oxygen ≥ 5 mg L^{-1}) was determined for each date, and per capita consumption rates were scaled using the consumption algorithm for cold-water fish species from the model (Fish Bioenergetics 3.0, Hanson et al., 1997) used in study by Hirsch & Negus (2000). The product of this value and the size of the trout population provided an estimate of the daily mortality imposed by trout on *Daphnia.*

Egg ratio analysis results

Over the winter of 2004, *Daphnia* reproductive rates were nearly balanced by mortality due to trout (Fig. 12) and may have been responsible for the relatively small population of *Daphnia* present in April of 2004 (Figs. 2 & 5). Over the winter of 2005 reproductive rates exceeded mortality due to trout by about an order of magnitude and a large population of *Daphnia* was present in April of 2005 (Figs. 3 & 5). The average body size of *Daphnia* (Fig. 13) was also larger in January-April in 2005 than in 2004 ($p = 0.04$, one-tail T-test). Rainbow trout are size-selective predators on *Daphnia*, so this result is consistent with the fact that more trout were present in Square Lake in the winter of 2004 following autumn stocking than in the winter of 2005 when trout were not stocked the previous autumn. Body sizes between May and October did not differ significantly between 2004 (1.84 ± 0.03) and 2005 (1.82 ± 0.02).

While *Daphnia* reproductive rates and potential loss rates due to trout predation were nearly balanced over the winter of 2004, the comparison of these rates on other dates illustrates that reproductive rates were typically considerably higher than mortality rates due to trout predation (Fig. 12). A study (Hembre & Megard, 2005) of another Minnesota lake (Long Lake, Clearwater County, MN) that is stocked with rainbow trout found that the *Daphnia* population grew when reproductive rates exceeded loss rates from trout predation. That same phenomenon was not observed in this study. On several occasions when reproductive rates exceeded trout predation rates the *Daphnia* population size *decreased* in size instead of increasing. One particularly notable instance of this was between April and May of 2005 when the large, fecund population of *Daphnia* that was present in early spring decreased dramatically (Fig. 5) in spite of the large surplus in reproduction versus mortality due to trout (Fig. 12). This implies that other sources of mortality were more relevant to the *Daphnia* population. Other sources of mortality could include predation by other fish species (e.g., minnows, sunfish), or invertebrates (e.g., *Chaoborus*).

Chaoborus were observed in deep water in daytime sonar echograms from July-October in 2004 (Fig. 2) and in September-October 2005 (Fig. 3). A 24 hour sonar survey of Square Lake on July 8-9, 2004 (McAlpine, 2006), performed as an offshoot of this study, found that *Chaoborus* became very abundant in the water column at night and as they migrated into surface water. While *Chaoborus* migrated into surface water, this study found that the backscattering layer between 10-12 m that was dominated by *D. pulicaria* on that date (Fig. 2), did not appear to migrate. This is relevant because if *D. pulicaria* are not migrating into surface water they are not grazing on phytoplankton in surface water and clearing the water. If this is a seasonal pattern it could help to explain why the water clarity in July and August of 2005 (Fig. 7) was lower than expected given the very large population size of *D. pulicaria* on those dates (Figs. 3 & 5). Additional research would need to be done to determine whether this is a consistent behavior in by *D. pulicaria* in Square Lake.



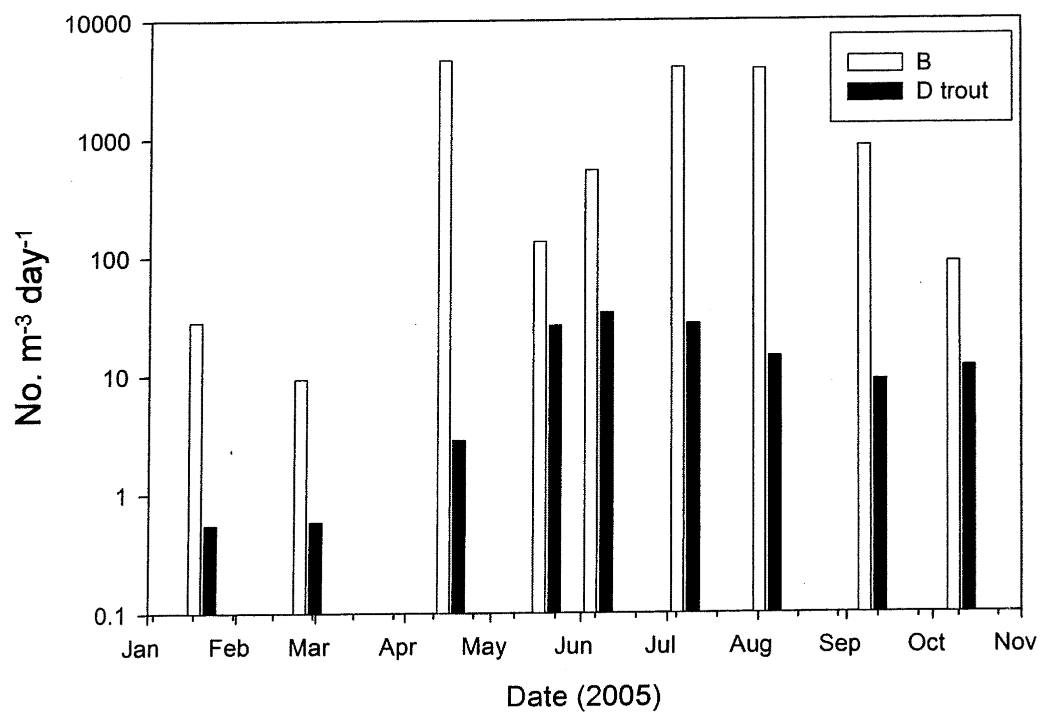
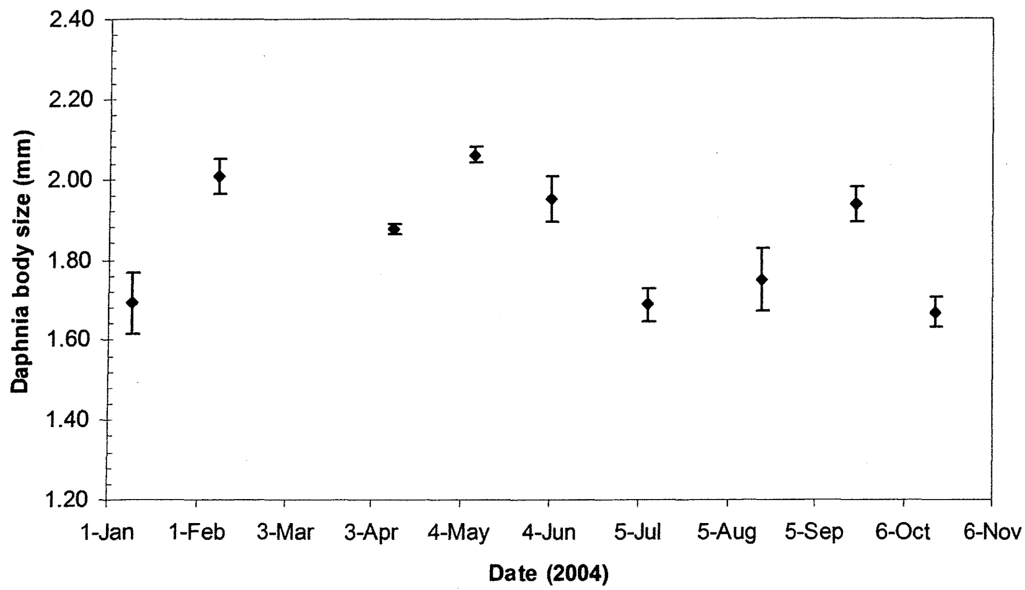


Figure 12. Comparison of *Daphnia* reproductive rates (white bars) and potential mortality rates due to trout predation (black bars) for 2004 and 2005.



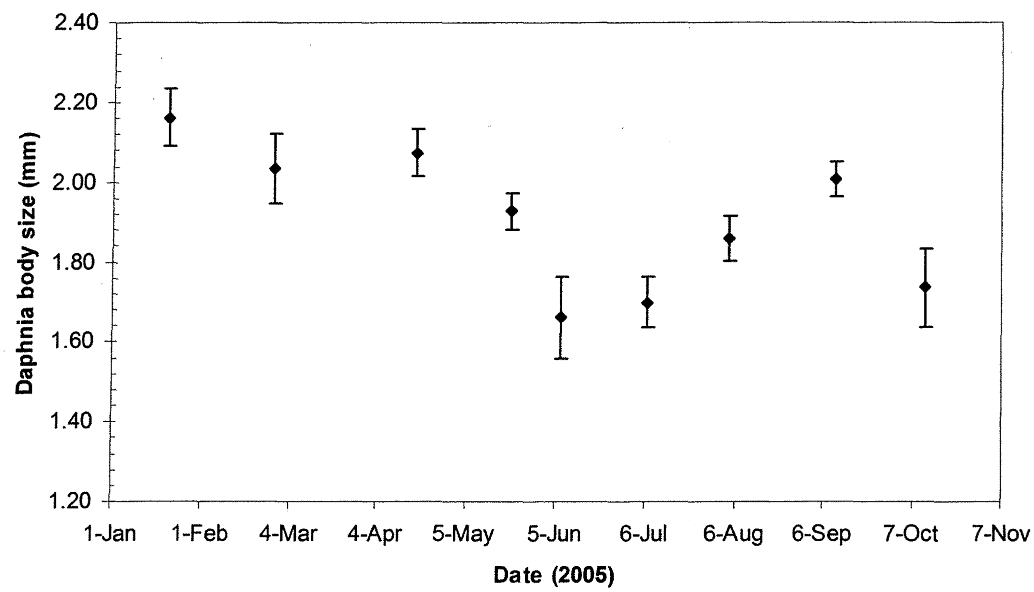


Figure 13. Mean *D. pulicaria* body size (± s.e.) on all sampling dates in 2004 and 2005.

References

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Result 5: Final Report and Presentation

Description: Project results will be printed and distributed to local and state decision-makers and citizens interested in the protection and sound management of Square Lake. The results will be presented to the members of the Square Lake Clean Water Partnership and the Minnesota Department of Natural Resources. Marine-on-St. Croix Water Management Organization and Square Lake Association will sponsor a meeting presenting the project results to the local community. The study results will also be available on the Internet for use by the scientific community.

LCMR Budget: $5,580
Balance: $ 0

Personnel: $5,000
Other: $580 (Printing/Postage/Supplies)

V. TOTAL LCMR PROJECT BUDGET

All Results: Personnel: $30,550
All Results: Other Supplies $ 550
All Results: Other: $ 900

Printing/Postage/Supplies:$580
Mileage: $320

TOTAL LCMR PROJECT BUDGET: $ 32,000

VI. PAST, PRESENT, AND FUTURE SPENDING

A. Past Spending:
Marine WMO 1992-2002 (water monitoring, net sampling) $12,525
B. Current Spending:
Marine WMO (water monitoring, net sampling, and Creel Survey) $8,950

C. Required Match (if applicable)*:* Not applicable.

D. Future Spending: Local funds will be used to continue water quality monitoring and net sampling for *Daphnia* beyond the project completion date.

VII. PROJECT PARTNERS

A. Partners Receiving LCMR Funds: None.

B. Project Cooperators:

VIII. DISSEMINATION

Project results will be printed and distributed to local and state decision-makers and citizens interested in the protection and sound management of Square Lake. The results will be presented to the members of the Square Lake Clean Water Partnership and the Minnesota Department of Natural Resources. Marine-on-St. Croix Water Management Organization and Square Lake Association will sponsor a meeting presenting the project results to the local community. The study results will also be available on the Web through Hamline University search out links. Project results will be submitted to peer review journals for publication.

IX. LOCATION

Square Lake is located in Washington County, Town of May, 55082.

X. REPORTING REQUIREMENTS

Periodic work program progress reports will be submitted not later than Result Status as of:

Feb. 28, 2004
August 31, 2004
February 28, 2005
February 28, 2006.

XI. RESEARCH PROJECTS

See Attachment B

- *Update and check for accuracy of ATTACHMENT A spreadsheet. Make sure calculations in the spreadsheet coordinate and are the same as in your final work program report.*

Reimbursement Request – Invoice Summary Spreadsheet

Instructions:

1. Enter your budget from your current approved work program (Attachment A)
2. Update the beginning balances with the ending balance from your previous Invoice Summary Spreadsheet.
3. Insert the amounts of your current invoice by category and provide the total.
4. Calculate the ending balances for this invoice.
5. Attach copies of invoices, checks and time cards.
6. Fill out and submit the Reimbursement Request Form
7. Send completed documentation to the authorized state contact person.

Project Title: Maintaining Zooplankton (Daphnia) for Water Quality- Square Lake -Agreement #A52753

Legal Citation: Laws of Minnesota 2005, Chapter ___128______, Section __1_______, Subdivision 7(f)_________. From Nov. 2003 to June 29, 2006

Budget for Results from Work Program

Budget Item	Result 1: Sonar Collection Net Sampling Budget	Beginning Balance	Current Invoice	Ending Balance	Result 2: Acoustic Sonar Data Collection Budget	Beginning Balance	Current Invoice	Ending Balance
Use information from Attachment A from Work Program								
Professional Services Hamline/Hembre	9090	0	0	0	1600	0	0	0
Professional Services DNR								
Equipment Tools Lab	550	550	550	0	0	0		0
Postage								
Office Suplies								
Travel in Minnesota	180	180		180	150	150		150
Printing								
Column Total	9350	730	550	180	1750	150	0	150

Budget Item	Result 3: Water Monitoring/Creel Survey Budget	Beginning Balance	Current Invoice	Ending Balance	Result 4: Evaluate Predation Budget	Beginning Balance	Current Invoice	Ending Balance
Use information from Attachment A from Work Program								
Professional Services Hamline/Hembre					4160	788	788	0
Professional Services DNR	10700	1769	0	1769				
Equipment Tools Lab	0	0		0				
Postage								
Office Suplies								
Travel in Minnesota								
Printing								
Column Total	10700	1769	0	1769	4160	788	788	0

Budget Item	Result 5: Final Report Budget	Beginning Balance	Current Invoice	Ending Balance	Project Total Budget	Beginning Balance	Current Invoice	Ending Balance
Use information from Attachment A from Work Program								
Professional Services Hamline/Hembre	5000	5000	5000	0	19850	5788	5788	0
Professional Services DNR					10700	1769	0	1769
Equipment Tools Lab					550	550	550	0
Postage	150	150		150	150	150		150
Office Suplies	80	80		80	80	80		80
Travel in Minnesota					320	320		320
Printing	350	350		350	350	350		350
Column Total	5580	5580	5000	580	32000	9007	6338	2669