

The Effects of Resource Limitation
on the fitness of *Daphnia pulex*

Carol Blanchette
University of Notre Dame, (219) 283-2964

Abstract

The intrinsic growth rate of *Daphnia pulex*, based on lifespan and fecundity, was measured in a lifetable experiment carried out on Peter Lake at the University of Notre Dame Environmental Research Center (UNDERC). The experiment was designed to track the effects of resource limitation on *Daphnia* as food levels in large enclosures varied due to the inclusion and exclusion of planktivorous fish, and due to a regime whereby certain treatments received supplemental food in the form of *Chlamydomonas reinhardtii* "spikes." The r-values for the lifetable daphnids were calculated and the zooplankton densities in the enclosures as well as chlorophyll *a* levels were monitored. The supplemented treatments resulted in *Daphnia* having the greatest r-values, suggesting that resource limitation was critical for daphnid fitness. Although the presence of planktivorous fish in some enclosures resulted in an increased algal biomass over the fishless bags, the presence of other small herbivorous zooplankton and possible competition for food with these zooplankton resulted in slightly lower r-values for the daphnids in these lifetable treatments. The results of the lifetable experiment suggest that *Daphnia* are also temperature restricted, since the *Daphnia* in the cold hypolimnetic water all had extremely low r-values, and that daphnid overall fitness is restricted by food availability.

Introduction

The fitness level of *Daphnia pulex* can be affected by a number of factors, but is ultimately affected by food availability. The trophic interactions in a lake determine the composition and abundance of phytoplankton (food). Predation on zooplankton by fish can reduce the abundance of certain size classes, and thereby provide more resources for individuals in the less preferred class (Vanni, 1987 b). Grazing by zooplankton can also influence the phytoplankton abundance and community structure. However, large species such as *Daphnia*, affect phytoplankton abundance more than smaller species (Lynch and Shapiro, 1981). The benefits and costs of attaining a particular body size also vary with food concentration. *Daphnia* respond to food limitation by curtailing growth and reproduction (Taylor, 1985).

In this experiment, varying food concentrations were established by introducing a predator in enclosed treatments, restricting predators in other enclosed treatments, and providing *Chlamydomonas* supplemented and non-supplemented conditions to both treatments as well as an open-water control site. The effects of the resource limitation were measured as r -values, representing the intrinsic growth rate, based on the Euler equation (Threlkeld, 1980) in a life table experiment.

Methods

EXPERIMENTAL DESIGN:

The life table experiment was carried out on Peter Lake, a meso-oligotrophic lake located at the University of Notre Dame Environmental Research Center (UNDERC), in Gogebic Co., MI. The experiment ran from June 29, 1987 to July 29, 1987 under normal summer weather conditions. There were four treatments: shallow enclosures (4 m deep) with no fish, shallow enclosures with fish, deep enclosures (10 m deep) with no fish, and deep enclosures with fish. Each enclosure type was triplicated. There were also two non-enclosed control sites in the lake. Sets of ten life table jars were suspended in each of several mesh bags within large enclosures of clear polyethylene sheeting which took the form of tubes when filled with water. The deep enclosures with fish contained two largemouth bass (*Micropterus salmoides*) and the shallow enclosures contained one bass, which purportedly reflected the ambient density of the 1985 year class in Peter Lake. The enclosures were filled with water pumped from 1 m and filtered through a 150 μm mesh plankton net. The enclosures were stocked with the ambient density of zooplankton normally found in the upper 6 m of the lake during daytime, with the exception of second through fourth instar chaoborid larvae which were excluded.

One enclosure from each of the four treatments was randomly chosen to receive an extra set of life table jars whose algal assemblages were supplemented with *Chlamydomonas reinhardi*. Another set, suspended in the lake, served as a control for this group. Cultures of *Chlamydomonas* were grown under continuous fluorescent lighting in synthetic pond water (Dini, personal communication). The flasks containing the cultures were kept on a continuous shaker table at a speed of 150 RPM to prevent the algae from entering the palmeloid stage.

PROCEDURE:

On the afternoon of June 28, *Daphnia pulex* were collected from Peter Lake at 9 m by vertical and horizontal tows and transported to the laboratory. Approximately 500 adults were picked, supplemented with *Chlamydomonas* and incubated in GF/F filtered lakewater overnight at 19^o C. On the morning of July 29, water was pumped separately from the hypolimnion at 8 m and epilimnion at 2 m and filtered through an 80 μ m mesh plankton net for use in the life table jars. 280 daphnid neonates were then picked from the incubated culture, and 1 daphnid was added to each jar along with 120 mL of water from the appropriate depth. 60 of the jars received *Chlamydomonas* supplements which brought their *Chlamydomonas* concentration to a mean value of 4.1×10^7 cells mL⁻¹. The *Chlamydomonas* concentrations were determined using Utermohl's technique. The jars were labelled with a grease pencil and were suspended in the enclosures that same evening.

Every other day, the jars were retrieved from the enclosures by a pair of researchers working from a rowboat. The contents of the jar were poured into an enamel pan, and if alive, each daphnid was removed from the pan by pipette. The number of neonates present was recorded, and the water from the pan, including dead adults(if present), and neonates, was discarded. Water from inside the appropriate enclosure at the hypolimnetic (8 m) or epilimnetic(2 m) depth was then collected using a vanDorn water sampler and filtered through an 80 μ m filter to eliminate other large zooplankton, but allow the passage of phytoplankton. This water was added to each 120 mL jar, and the original daphnid was then replaced and the jar returned to the appropriate depth.

ANALYSIS:

Based on the survivorship and fecundity of *Daphnia* in the life table jars, fitness was calculated using the Euler equation:

$$(1) \quad l = e^{-rt} \sum l(t) m(t)$$

Where $l(t)$ represents the proportion of daphnids surviving to day (t), and $m(t)$ represents the number of neonates born on day (t). The r-values represent the overall daphnid fitness and were calculated using a computer program developed specifically for this purpose by Dr. Stephen R. Carpenter. The daphnid density in each bag was also determined on a weekly basis at 2200 hours using an 80 μm mesh plankton net. Two vertical hauls were taken from each bag, pooled, counted at 60 X on a Wild model M5-72651 microscope and preserved in 4% sugared formalin (Dini, personal communication). Weekly zooplankton samples from each of the enclosures were taken back to the laboratory, preserved and counted. The densities were based on two vertical hauls from the bottom of each enclosure with a plankton net efficiency of 21% for daphnids. The densities of other zooplankton were based on the same net efficiency as the daphnids. Chlorophyll a concentrations of each enclosure were also determined on a weekly basis from the mid-epilimnion in the shallow enclosures and from the mid-epilimnion and mid-metalimnion in the deep enclosures. The chlorophyll a contents were determined using the modified fluorescence method of Strickland and Parsons (1968) after Carpenter et al. (1987, in press).

Results

r-VALUES:

The r-values, representing intrinsic rate of increase were calculated for each of the treatment replicates (Table 1). Each treatment was triplicated, and mean values for each treatment were also recorded. The mean values for the shallow fish and fishless enclosures were .112 and .105 respectively. The mean values for the deep fish and fishless enclosures were .039 and .085 respectively. The mean r-value for the control groups was .094. Where values of $-\infty$ were recorded in the hypolimnetic data, means were not calculated. A statistical analysis based on two-tailed T-tests and one way ANOVA's, shows that there was no significant difference between treatments including and excluding fish, or between control groups. The r-values from each of the four treatment types were also pooled (meaning that each treatment type was treated as a group) and recorded (Fig. 1). There were significant differences between supplemented and non-supplemented groups, with the supplemented groups having a greater overall fitness, as expected (Taylor, 1985). Values of $-\infty$ were obtained for several treatments because no neonates were produced. The r-values represent the intrinsic growth rate of the population: a measure of the fitness of *Daphnia*. These r-values are based on fecundity and survivorship of *Daphnia*. The hypolimnetic *Daphnia* suffered negative r-values and $-\infty$ r-values due mainly to low or zero fecundity rather than high mortality. The hypolimnetic daphnids were also much smaller and slower than the epilimnetic daphnids (personal observation); water at 8 m was also at least 10°C colder than water at 2 m. Fecundity was greatest among daphnids in epilimnetic, supplemented lifetable jars and in epilimnetic, non-supplemented lifetable jars.

DAPHNID DENSITY:

Daphnid densities for each of the treatment replicates are reported with their means (Table 2). A statistical analysis based on two-tailed T-tests showed that

there was a significant difference in the mean densities only between the fish and the fishless enclosures in each of the treatments. The concentration of *Daphnia* present in the enclosures was determined on a weekly basis for each of the enclosures. In the shallow enclosures, the daphnid densities declined steadily over time in the presence of fish (Fig. 2). *Daphnia* in the shallow enclosures without fish were free from predation and their densities sharply increased over time until reaching their carrying capacity. The daphnid densities in the deep enclosures followed the same pattern as those in the shallow enclosures (Fig. 3).

CHLOROPHYLL *a* CONCENTRATIONS:

The chlorophyll *a* concentrations were used as a rough estimate of algal biomass. Chlorophyll *a* concentrations for each of the triplicated treatments and their means are reported (Table 3). A statistical analysis based on two-tailed T-tests shows that there were no significant differences between any of the treatments. Mid-epilimnetic chlorophyll *a* densities were calculated over time for each of the treatment types (Fig. 4). In the deep enclosures the chlorophyll *a* concentration in the presence of fish remained consistently greater than the chlorophyll *a* concentrations in the absence of fish. The peak in chlorophyll *a* concentration in the deep enclosures containing fish occurred on the same date as the low point in daphnid density in the same enclosures. In the enclosures without fish, the chlorophyll *a* concentrations started out high and declined in proportion to the daphnid density, which started out low and increased over time. The chlorophyll *a* concentrations in the shallow enclosures followed a similar pattern (Fig. 5). The chlorophyll *a* concentrations in the fishless enclosures reached their lowest point at the same time that the daphnid density was at a high level. The chlorophyll *a* concentrations in the enclosures containing fish were consistently higher than those in the enclosures without fish. The inverse relationship between daphnid density and chlorophyll *a* concentration can be expected on the basis of the cascading trophic interactions hypothesis (Carpenter et al. 1985).

OTHER HERBIVOROUS ZOOPLANKTON:

The weekly zooplankton samples consisted mainly of herbivorous zooplankton such as *Diaphanosoma* sp., *Ophrioxus*, *Chydorus*, and calanoid copepods (Tables 4 & 5). In the enclosures containing fish, the daphnid densities were low, as expected, while the concentrations of other small herbivorous zooplankton remained high. In the enclosures without fish, the concentrations of both *Daphnia* and other herbivorous zooplankton remained high.

Discussion

Daphnid fitness is represented by r -values which increase as a function of a daphnid's lifespan and fecundity. The mean r -values for the non-supplemented lifetable daphnids indicated that there were no significant differences between the r -values of the fish enclosure and fishless enclosure treatments. However, based on the cascading trophic interactions hypothesis (Carpenter and Kitchell, 1984; Carpenter et al. 1985), this would not be the expected situation.

The cascading trophic interactions hypothesis predicts that the presence of fish in the enclosures should yield a decrease in daphnid density in these enclosures, since *Daphnia pulex* are relatively large zooplankton and are selected as food items by the bass (Dini, personal communication). Since the daphnids feed on phytoplankton, the chlorophyll a levels, which measure algal biomass, should increase due to the decreased grazing pressure by *Daphnia*. The high phytoplankton content, indicated by increased chlorophyll a , should benefit the lifetable daphnids in these enclosures, resulting in higher r -values.

The inconsistencies between the actual and predicted results are explainable in part, yet problematic too. In order to determine the effect of the trophic interactions between the fish, *Daphnia* and phytoplankton, it is important to determine whether *Daphnia* are food-limited. If *Daphnia* are not food-limited, then the lack of any significant difference in r -values between the fish and fishless treatments will have no meaning in terms of the trophic interactions that have been described.

The expected results for *Daphnia* that are resource-limited are large r -values for supplemented treatments and lower r -values for non-supplemented treatments, since an increase in food should increase survivorship and fecundity (Taylor, 1985). The actual results show this to have been the case. The r -values for the supplemented lifetable daphnids were significantly higher than those for the non-supplemented treatments.

Since a quantifiable increase in food in the supplemented treatments yielded significantly higher r-values, the lack of a significant difference in r-values between the fish and fishless enclosures in the non-supplemented treatments indicates that the food levels in both treatment's jars must have been similar. The mid-epilimnetic chlorophyll a values showed no significant differences between any of the treatments. Although the values were not statistically different, the chlorophyll a levels in the six enclosures with fish were consistently higher than for the six fishless treatments. This result is predictable based on the cascading trophic interactions hypothesis, since daphnids in the enclosures with fish are heavily preyed on by the fish, therefore greatly reducing the grazing pressure on the phytoplankton.

A comparison between the chlorophyll a values in the deep fish enclosures with daphnid density shows that the chlorophyll a values peaked at the same time that the daphnid population crashed-an expected result. However, chlorophyll a concentration dropped off sharply even at an extremely low concentration of *Daphnia*. This was not expected. However, as the concentration of daphnids in these enclosures declined, the levels of other small herbivorous zooplankton, too small to be seen by fish, remained high. These other small herbivorous zooplankton such as calanoid copepods and copepodites, as well as other cladocerans, *Diaphanosoma* sp., and chydorids ate the same algal species that form the bulk of the diet of *Daphnia*. These other zooplankton were freed from competition with *Daphnia* for food, and utilized much of the ingestible phytoplankton, leaving the lifetable daphnids with inadequate food and resulting in their lower than expected r-values.

The daphnid densities in the deep fishless enclosures were significantly higher than in the deep fish enclosures due to the absence of predation. As the densities of daphnids increased in these enclosures, the chlorophyll a levels also declined due to grazing pressure. Predation on *Daphnia* in the fish enclosures drove the levels of *Daphnia* to near-extinction, however, the abundance of *Daphnia* in the fishless

enclosures did not entirely abolish the chlorophyll *a* concentration, indicating that there were certain algal species present in the enclosures which were inedible to *Daphnia*. This might also explain why *Daphnia* in the fish and fishless deep enclosures had similar r-values even though the chlorophyll *a* levels were much higher (though not significantly higher) in the deep fish enclosures. Most of the small algal species that *Daphnia* prefer were probably eaten by the other herbivorous zooplankton, leaving only large inedible algal species for the lifetable daphnids (Dini, personal communication). Grazing can selectively reduce the abundance of small or naked algae that are vulnerable to zooplankton, favoring the survival of large phytoplankton species, or those with gelatinous sheaths or other structures that reduce vulnerability to grazing (Vanni, 1987 a). A preliminary analysis of the final phytoplankton content of the enclosure water indicated that *Aphanacapsa* sp., a sheathed, colonial blue-green alga, with diameter usually greater than 25 μm , was present in great abundance, almost to the exclusion of other species. This alga is too large for all but the largest daphnids to consume, thus lifetable daphnids in the deep fish enclosures were food-limited even though chlorophyll *a* levels were clearly higher.

The similar fitness levels of the lifetable daphnids in the fish and fishless enclosures is important when comparing daphnid mortality in the enclosures. Any significant differences in mortality between daphnids in the fish enclosures versus those in the fishless enclosures were not the result of differences in food availability that were the indirect result of the fish treatment. Mortality in the fish enclosures was primarily a function of fish predation, and not starvation, since the lifetable daphnids were fed the same water, and had the same r-values as lifetable daphnids in fishless treatments.

The r-values for the shallow fish and fishless enclosure treatments were nearly identical. The chlorophyll *a* levels in the fish and fishless shallow enclosures were also much closer to each other than the values for the deep treatments. These similar values suggest that in the smaller volume, nutrient mixing may have been

more important than the presence or absence of fish, since the shallow enclosures were almost wholly in the mixed layer which might have prevented nutrients from getting trapped below the thermocline as the experiment proceeded (Dini, personal communication).

The hypolimnetic daphnids have been virtually ignored so far in this discussion since their extremely low and $-\infty$ r-values in supplemented and non-supplemented fish and fishless enclosures make them a special case. The hypolimnetic water was at least 10° colder than the epilimnetic water and had a tremendous effect on the daphnids' fitness level. Temperature effects on *Daphnia* were not studied as such in this experiment and no other temperature changes were noted. In all cases where they occurred, the $-\infty$ r-values were caused by zero fecundity. Since the r-values for the supplemented hypolimnetic treatments were also $-\infty$, the hypolimnetic *Daphnia* were apparently not food limited, and their fitness levels were primarily a function of their cold environment.

Due to the nature of this experiment, and working with live animals, certain problems arose which were possible sources of error in generating the r-values. At the start of the experiment there was a very high mortality rate in all the treatments because of "bottle shock", the reaction of some of the animals to being handled and/or confined in a jar. Errors also occurred in handling and transferring the animals with pipettes, when the daphnids would sometimes become irretrievably stuck to the upper wall of the pipette or marooned on the wall of the enamel picking pan and lost. One set of enclosure jars was also mistakenly dropped to the bottom of the enclosure, never to be recovered.

Acknowledgements

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POOLED LIFE TABLE RESULTS

L-values

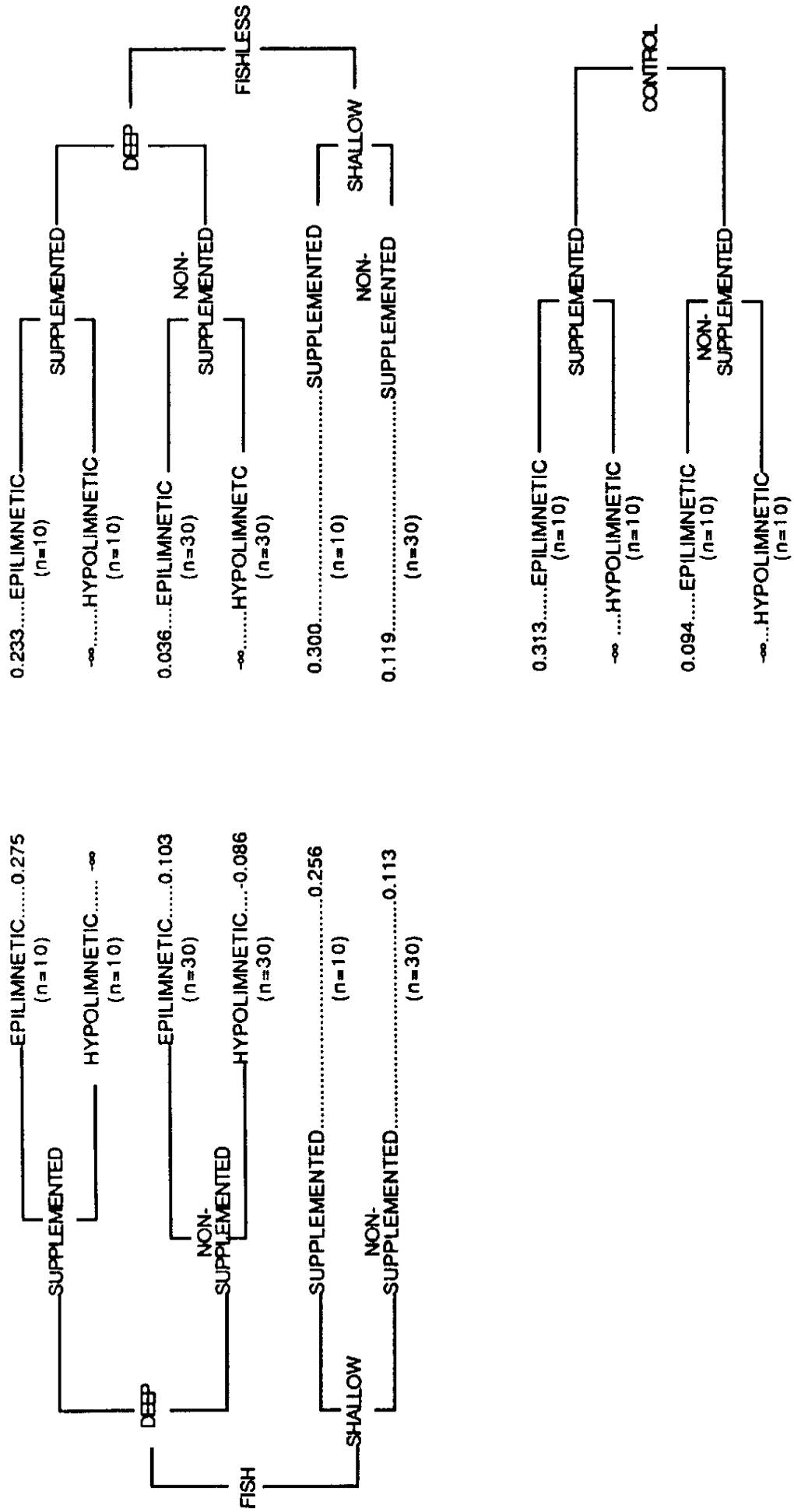


Fig. 1 Resultant r-values for the Daphnia in the different treatments, based on survivorship and fecundity and calculated using the Euler equation (1).

Daphnid Density over time: Shallow Enclosures

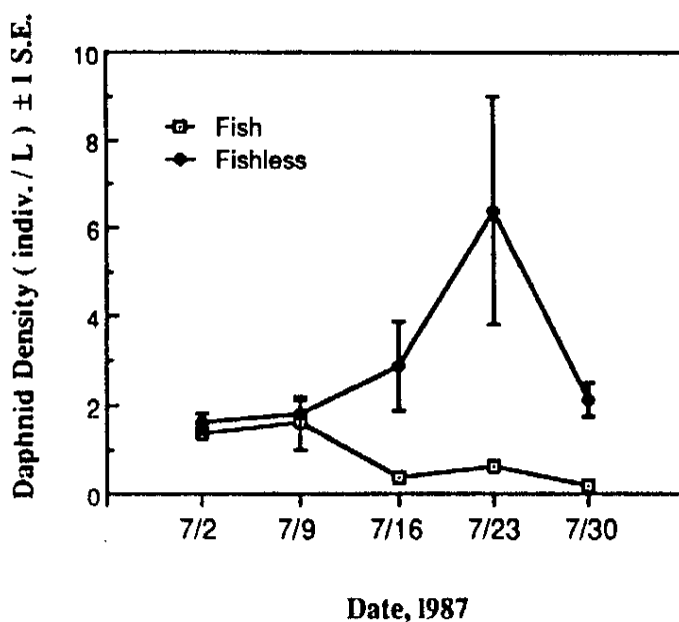


Fig. 2 Daphnid densities in the shallow enclosures, based on weekly vertical hauls with an 80 μ m mesh plankton net.

Daphnid Density over time: Deep Enclosures

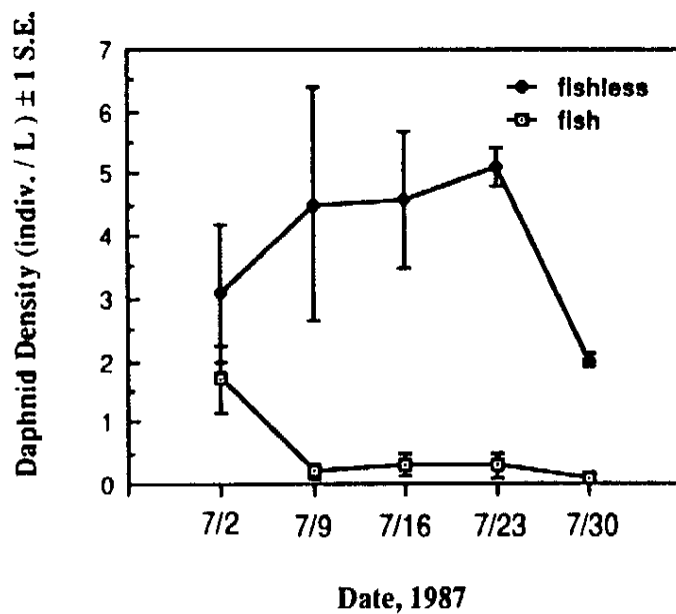


Fig. 3 Daphnid densities in the deep enclosures based on weekly vertical hauls with an 80 μ m mesh plankton net.

Mid-epilimnetic Chlorophyll a over time: Deep Enclosures

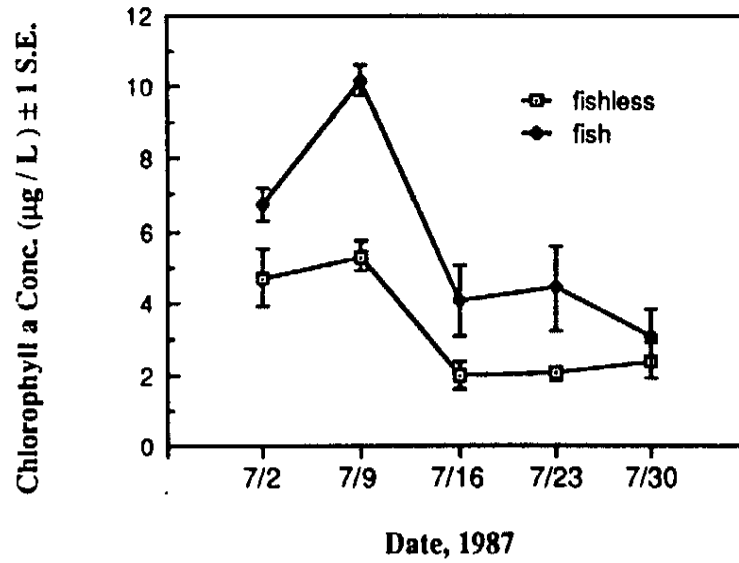


Fig. 4 Mid-epilimnetic chlorophyll a levels in the deep enclosures, determined on a weekly basis.

Mid-epilimnetic Chlorophyll a over time: Shallow Enclosures

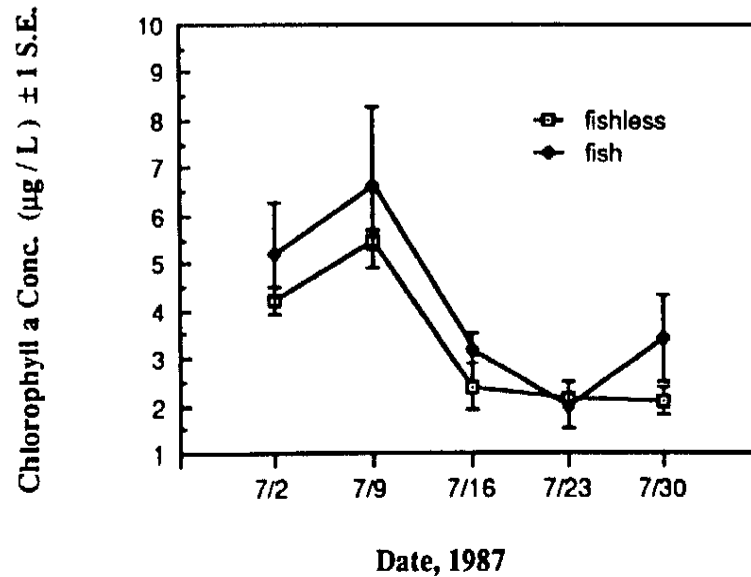


Fig. 5 Mid-epilimnetic chlorophyll a levels in the shallow enclosures, determined on a weekly basis.

r-Values

TREATMENTS:	A	B	C	MEAN
SHALLOW FISHLESS	.161	.107	.067	.112
SHALLOW FISH	.103	.108	.105	.105
DEEP EPILIMNETIC FISHLESS	.067	.016	.034	.039
DEEP EPILIMNETIC FISH	.021	.091	.143	.085
DEEP HYPOLIMNETIC FISHLESS	- ∞	-.042	LOST	NO MEAN
DEEP HYPOLIMNETIC FISH	-.088	-.083	- ∞	NO MEAN
CONTROL EPILIMNETIC	ONLY ONE TREATMENT			.094
CONTROL HYPOLIMNETIC	ONLY ONE TREATMENT			- ∞

Table 1 The intrinsic rate of increase (r-values) for each of the individual treatments and the mean values for the three treatments.

Mid-Epilimnetic Daphnid Densities

TREATMENTS:	A	B	C	MEAN
SHALLOW FISHLESS	21.0	7.40	15.9	14.7
SHALLOW FISH	1.70	2.50	4.50	2.90
DEEP EPILIMNETIC FISHLESS	26.3	10.5	23.5	20.1
DEEP EPILIMNETIC FISH	3.90	0.80	4.10	3.00

Table 2 Final Mid-epilimnetic daphnid densities (individuals / L) for each of the three treatment replicates, as well as the mean for the three replicates.

Mid-Epilimnetic Chlorophyll a Values

TREATMENTS:	A	B	C	MEAN
SHALLOW FISHLESS	3.67	3.58	3.50	3.58
SHALLOW FISH	3.75	3.67	5.34	4.25
DEEP EPILIMNETIC FISHLESS	3.75	3.62	3.09	3.48
DEEP EPILIMNETIC FISH	3.91	7.45	5.74	5.70

Table 3 Final Mid-epilimnetic Chlorophyll a values (μg / L) for each of the three treatment replicates, as well as a mean value for the three replicates.

Deep Fish Enclosure Zooplankton Concentrations

Date	Other indiv. / L	<i>Daphnia</i> indiv. / L
7/9	7.02	0.02
7/16	5.83	0.03
7/23	5.97	0.03
7/30	8.75	0.01

Table 4. Concentration of other herbivorous zooplankton and *Daphnia* (in individuals per L of lakewater) from deep fish enclosures, calculated weekly.

Deep Fishless Enclosure Zooplankton Concentrations

Date	Other indiv. / L	<i>Daphnia</i> indiv. / L
7/9	7.46	4.5
7/16	7.40	4.6
7/23	6.95	5.1
7/30	4.78	2.0

Table 5. Concentration of other herbivorous zooplankton and *Daphnia* (in individuals per L of lakewater) from deep fishless enclosures, calculated weekly.