PHYSIOLOGICAL RESPONSES OF DAPHNIA CATAWBA, COKER TO MANIPULATIONS OF NATURALLY OCCURRING SESTON IN IN SITU ENCLOSURES

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PHYSIOLOGICAL RESPONSES OF *DAPHNIA CATAWBA*, COKER TO MANIPULATIONS OF NATURALLY OCCURRING SESTON IN *IN SITU* ENCLOSURES

BY

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ABSTRACT

Filtering rate, length-specific weight, and fecundity were used as measures of short, medium and long-term responses of individual *Daphnia catawba* to manipulation of the natural food supply and to seasonal fluctuations in water temperature. Natural lake seston concentration was manipulated to encompass a realistic range of food concentrations in *in situ* enclosures and six, four or eight day experiments were conducted over a two month period to take advantage of seasonal variability in lake water temperature. Based on prior laboratory studies, both filtering rate and length-specific weight were expected to respond to changes in food supply and water temperature and a threshold food concentration was expected above which weight gain would occur. The results of this study are in general agreement with predictions from earlier laboratory studies. The general seston components (chl *a*, POC, PON) were not significantly correlated with filtering rate and net weight change in *Daphnia catawba*. Only the plankton biomass component and water temperature variables explained significant levels of variation in filtering rate and net weight change. Filtering rates were weakly, negatively correlated with inedible (>30 μm) plankton biomass and positively correlated with an edible (5-10 μm) plankton biomass component. Multiple linear regression analysis indicated that these variables explained 14, 20 and 13% of the variance in filtering rate of small, medium and large animals respectively. Net weight change, calculated as the difference between the final and initial dry body weights, was found to be significantly positively correlated with the smallest plankton component (≤ 2 μm) and significantly negatively correlated with water temperature, with multiple regression models explaining 35, 37 and 58% of the variance in net weight change for small, medium and large animals respectively. Net weight change in large animals was also significantly negatively correlated with initial animal weight ($r=-.782$). Fecundity responses to seston manipulation and water temperature fluctuation were minimal; adult females appeared to allocate excess energy to growth rather than reproduction.

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INTRODUCTION

Cladocerans play a prominent role as primary consumers in many freshwater systems and additional insight into their physiological response to changing environmental conditions should further our understanding of trophic interactions in these ecosystems. Large-bodied cladocerans are particularly important in freshwater ecosystems because of the major grazing impact they have on plankton particles due to their high filtering rates (Knoechel and Holtby 1986a,b). The large body size and high visibility of these zooplankters makes them particularly susceptible to predation by fish (Brooks and Dodson 1965) and consequently they play a significant role in energy flow between trophic levels.

In insular Newfoundland lakes and ponds, *Daphnia catawba* and *Holopedium gibberum* are commonly the dominant large-bodied cladocerans (Chengalath *et al.* 1984).

Previous investigation (Page 1983) has shown that an approximate 50% dry body weight loss and near zero egg ratios during mid-summer in *Daphnia catawba* were correlated with minimum food availability and maximum water temperature in Quidi Vidi Lake. Similarly, Cooper (1985) observed low summer egg ratios in both *Daphnia catawba* and *Holopedium gibberum* suggesting that these animals were food limited in the lake during summer.

The present study is an investigation of the physiological responses of *Daphnia catawba* to the manipulation of natural seston and to seasonal water temperature fluctuations in *in situ* enclosures.

1.1. Historical perspective

1.1.1. Trophic interactions

The study of trophic interactions in freshwater plankton communities has been approached from a number of perspectives during this century. The earliest studies of zooplankton taxonomic composition, gross anatomy, and feeding appendage morphology cultivated the initial interest in zooplankton food sources. Gellis and Clarke (1935) discussed the controversy of dissolved organic matter versus particulate organic matter as a source of nutrition for zooplankton, summarizing the earlier studies of Knörrich, Wolff, and Kerb conducted at the turn of the century. They demonstrated unequivocally that particulate organic matter and not dissolved organic matter was of primary importance as a food source for cladocerans. Hasler (1935,1937) identified digestive enzymes in the cladoceran gut that he interpreted as indications that particulate organic matter was the food source for cladocerans. The establishment of the trophic relationship between phytoplankton and zooplankton spawned an interest in the study of cladoceran population dynamics in terms of the influence of food supply and temperature on growth and reproduction (Ingle *et al.* 1937, Pratt 1943, Slobodkin 1954). The inhibitory effects of various algal species on cladoceran growth also received some attention (Ryther 1954).

While the primary role of food supply in zooplankton dynamics was widely accepted, few studies attempted to quantify accurately the food available to be ingested by zooplankton. Food supply was typically characterized as "low", "high" or "in excess" of that required for good growth in cladocerans. Edmondson (1957) recognized the need for quantification of the food supply and he suggested that investigations of trophic interaction and general physiology needed to be augmented by detailed feeding rate studies conducted under simplified laboratory conditions to allow the direct measurement of food supply and feeding rates. He believed that the information provided by such studies would aid the interpretation of observations in natural plankton species.

Subsequently, the effects of diet species, food concentration and temperature on cladoceran feeding rates, filtering rates, metabolic rates, growth and reproduction were studied in ever greater detail in the laboratory (Richman 1958, Rigler 1961, McMahon and Rigler 1963, Hall 1964, McMahon 1965 and 1968, Burns and Rigler 1967, Burns 1968a, Schindler 1968). Further laboratory investigations of cladoceran filtering rates, respiration rates, growth and reproduction continued to become increasingly more sophisticated (Heisey and Porter 1977, Richman and Dodson 1983, Lampert and Bohrer 1984, Korpelainen 1986).

The challenge to develop field techniques for verifying laboratory observations was met by Haney's (1971) introduction of an *in situ* grazing chamber for the determination of individual and community filtering rates in natural zooplankton populations. This resulted in subsequent studies of species' filtering rates (Haney 1973), diurnal and seasonal filtering rate fluctuations (Haney and Hall 1974, Thompson *et al.* 1982, Haney 1985, Lampert and Taylor 1985) and general models of body-length-based filtering rate relationships (Knoechel and Holtby 1986a,b) for cladoceran communities.

1.1.2. Predation

Simultaneous to the above-noted laboratory studies, field researchers were emphasizing the role of predators as a factor responsible for structuring zooplankton communities in terms of zooplankton size and total abundance. Size selective predation, both visual and non-visual, was recognized as a force capable of structuring zooplankton communities (Brooks and Dodson 1965), and the effects of both have been studied in the field (Wells 1970, Hutchinson 1971, Archibald 1975, Confer *et al.* 1978). Visual detection of zooplankton by planktivores has been studied in the laboratory (Vinyard and O'Brien 1975, O'Brien *et al.* 1979) and analyses of fish gut contents (Zaret 1971, Costa and Cummins 1972, Stenson 1976) have confirmed the size selectivity of vertebrate predation on zooplankton in the field.

1.1.3. Food limitation

1.1.3.1. Effects of food availability on net production

The effects of food limitation on the structure of natural zooplankton communities have been less frequently studied (Hrbáčková-Esslová 1963, Weglenska 1971). This may indicate that food limitation is less important in determining zooplankton community structure or, alternately, it may reflect the greater difficulty of monitoring the effects of food limitation under natural conditions. Arnold (1971) and Porter (1975) demonstrated in the laboratory and field that food quality, in terms of nutritional adequacy, is important to cladoceran physiological condition; Dumont *et al.* (1975) reported the utility of the length:weight regression relationship as a monitor of cladoceran physiological condition.

In the laboratory, food concentration and water temperature have been shown to affect cladoceran metabolic rates and net production. Lampert (1977a,b,c,d) measured respiration and assimilation rates of *Daphnia pulex* fed monospecific algal diets over a range of food concentrations and water temperatures in order to elucidate the effects of food supply and temperature on cladoceran metabolism and net production. He found that both specific assimilation relative to body size and the percentage of assimilated carbon lost by respiration increased as functions of increasing water temperature and decreasing animal body size. Assimilation rate increased faster than respiration rate with increasing temperature up to 20°C above which Lampert observed declining assimilation rates as respiration continued to increase. Lampert also determined threshold food concentrations for basic metabolic maintenance by offering *Daphnia pulex* a range of food concentrations over a range of experimental temperatures. The threshold concentration, defined as that level below which weight loss was observed, varied considerably with diet species and was found to be positively correlated with animal size. This lead Lampert to conclude that net production under conditions of low food concentration and high water temperature may be negative for large animals and positive for small animals.

In the field, food concentration and water temperature have recently been shown to influence cladoceran physiological condition. Page (1983) presented evidence of food limitation in a natural population of *Daphnia catawba* in which fluctuations in cladoceran length-specific weight were found to be significantly negatively correlated with water temperature and positively correlated with the edible component of the natural phytoplankton assemblage. A mid-summer weight loss of nearly 50% in all individuals was attributed to conditions of low food availability combined with increased water temperature. Weight loss was proportionately greater in large *Daphnia catawba* coincident with increased water temperature and a decline in edible phytoplankton to a level well below Lampert's (1977d) lab-estimated threshold concentration.

Tessier and Goulden (1982) monitored body lipid levels in natural *Daphnia* populations and attributed temporal fluctuations in body lipid content to the seasonal variability in the natural food supply. Body lipid levels declined in both *Daphnia catawba* and *Daphnia pulex* over a one month period in response to declining food abundance. Tessier (1986) also monitored body lipid content and fecundity as physiological responses of *Daphnia catawba* to food availability in lake enclosures. Animals raised in control enclosures on a natural lake seston diet were compared to animals raised in experimental enclosures on a diet supplemented with an edible algal species (*Ankistrodesmus falcatus*). Significantly increased lipid levels were observed in the food-supplemented animals as compared to the control animals, indicating that both control animals and lake animals were food limited. Increased fecundity in the food supplemented animals was observed in four of the six, six-day experiments.

1.1.3.2. Effects of food availability on feeding rate

The effects of food quantity and water temperature on cladoceran feeding rates have also been intensively studied in the laboratory. Negative relationships between filtering rate and both total food concentration and inedible particle concentration have been observed.

The feeding rate of a filter-feeding animal is a measure of the mass of food eaten per unit time, while the filtering rate or clearing rate is a measure of the volume of water cleared of particulate matter per unit time. Laboratory studies typically report an "incipient limiting level" (ILL) as the food concentration above which the feeding rate is constant and the filtering rate declines. Rigler (1961) and McMahon (1965) studied the effect of food concentration on the feeding rate of *Daphnia magna* and they both reported increased feeding rate with increasing food supply up to an ILL of 10^5 cells ml^{-1}. Above the ILL further increases in food concentration did not increase the feeding rate, suggesting that filtering activity decreased. McMahon (1965) also reported increased feeding rate with increased water temperature in *Daphnia magna* up to 24°C beyond which feeding rate declined.

The effect of food quality, in terms of the ingestibility of food particles by cladocerans, has also been studied in the laboratory and in the field. The rate of rejection of food from the food groove increases when larger, difficult to ingest particles are abundant, resulting in overall reduced ingestion due to loss of filtering time (Edmondson 1957). Webster and Peters (1978) have shown that rejection rate increases as the concentration of filamentous algae increases and Chow-Fraser and Sprules (1986) reported depressed *Daphnia* filtering rates in the presence of *Anabaena* filaments.

1.2. Present study

The present study was designed to investigate further the short, medium and long-term responses of *Daphnia catawba*, monitored as individual filtering rate, length-specific weight and fecundity, to natural seston manipulation and seasonal water temperature fluctuation.

Natural lake seston was manipulated in order to obtain a realistic range of natural food supply concentrations in *in situ* enclosures. Seston was either diluted or concentrated in transparent polycarbonate carboys fitted with 1 μm mesh-covered openings that allowed the transfer of water and solutes between the lake and the enclosures while retaining most of the seston within the enclosures. Experiments were conducted over a two month period to take advantage of the temporal variability in lake water temperature. Experimental durations of four and eight days were chosen based on the results of Tessier's (1981) study in which changes in fecundity were not always observed despite significant increases in lipid in *Daphnia catawba* incubated in food-supplemented lake enclosures for six days. Multiple linear regression analysis was used to examine the physiological responses of *Daphnia catawba* to the variability in food supply and water temperature.

Although laboratory conditions do not directly parallel conditions encountered in the field, laboratory studies may be used to predict field results. Predictions relating food limitation and water temperature to the physiology of *Daphnia catawba* to be evaluated in the present study included the following:

1) Filtering rate should increase with increasing water temperature. Filtering rate should decrease with increasing total food supply and increasing inedible food supply.

2) Cladoceran length-specific body weight should increase with increasing food supply and with decreasing water temperature.

3) Relative weight gain should increase with decreasing body size, and

4) Fecundity should increase with increasing food supply and with decreasing water temperature.

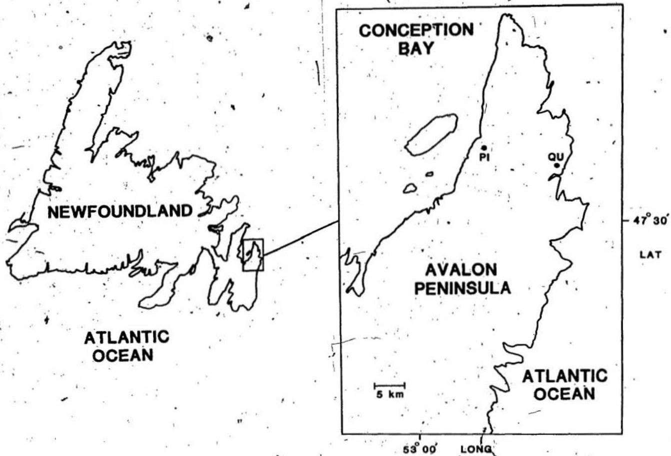
METHODS

2.1. Study sites

Field experiments were conducted in two small lakes, Piccos Pond South and Quidi Vidi Lake (Fig. 2-1), which differ markedly in drainage area and land use. Piccos Pond South (47°36'30"N, 52°51'15"W) is a small lake located 11 km west of St. John's, Newfoundland. Total surface area of the lake is 27.7 ha, maximum depth is 9 m and the drainage area is 2.3 km^2. There is a single inflow from Western Round Pond into Piccos Pond South from the southeast and a single outflow empties to the north. Land to the north and east of the lake is residential with the remainder being primarily forested area. Recreational use of the lake is minimal.

Quidi Vidi Lake (47°35'00"N, 52°41'15"W) is a larger lake located within the St. John's city limits. The total lake surface area is 42.9 ha, the maximum depth is 13.5 m and the drainage area is 49.6 km^2. The primary inlet is Rennie's Mill River which drains a large, heavily populated watershed within which much of the light industry of St. John's is located. The river flows into the lake from the southwest and the lake empties into Quidi Vidi Gut to the northeast. There is also inflow from Virginia Lake via Virginia River to the north. Land surrounding the lake is primarily residential and the lake is heavily used for rowing, sailing and fishing; power boating is restricted.

Figure 2-1: Piccos Pond South (PI) and Quidi Vidi Lake (QU), two small lakes located on the northeast tip of Newfoundland's Avalon Peninsula.


NEWFOUNDLAND
ATLANTIC OCEAN
CONCEPTION BAY
PI
QU
AVALON PENINSULA
ATLANTIC OCEAN
5 km
47° 30′
LAT
53° 00′
LONG

2.2. Field procedures

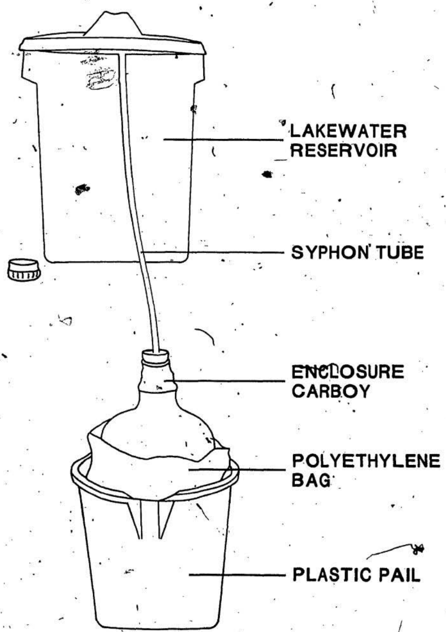
The physiological response of *Daphnia catawba* to *in situ* manipulation of the natural particulate food supply and seasonal water temperature fluctuations was monitored as changes in filtering rate, length-specific weight and fecundity.

2.2.1. Enclosure preparation

The enclosures used in this study were 9 l polycarbonate carboys. In each carboy, four openings measuring 15 cm by 6 cm were fitted with 1 μm nylon-mesh (**Nitex**) to allow the passage of water and solutes between the lake and the enclosures while preventing the exchange of most seston.

Ten enclosures were prepared for each initial experiment. Natural lake seston was manipulated by either diluting (0.25x,0.50x) the ambient lake seston with filtered (**Whatman #1** qualitative filter paper) lakewater or by concentrating the lake (2.00x,4.00x) seston by slowly syphoning additional lakewater into the respective carboys in order to establish a realistic range of seston concentrations within the enclosures. An unmanipulated (1.00x) seston concentration was also established. No attempt was made to replicate the food levels in the enclosures between experiments. The goal was to introduce variability into the seston parameter; the establishment of specific food levels was not critical. Each enclosure was placed in a polyethylene bag and a plastic pail before filling. The bag surrounding the enclosure was then filled with lakewater so that the water pressure inside the enclosure was counteracted by the water pressure inside the bag thus reducing the risk of dislodging the meshes or of forcing very small food particles through the mesh. The lakewater used to fill the enclosures was first poured through a screen to remove the larger zooplankton. To concentrate the seston within the 2.00x and 4.00x enclosures, screened lakewater (18 l and 36 l respectively) was slowly syphoned from containers positioned above the enclosures (Fig. 2-2). Excess water escaped through the meshes, which retained most of the seston. The 1.00x enclosure was filled directly with 9 l of screened lakewater. The dilution enclosures were filled directly with

Figure 2-2: Syphon apparatus used to concentrate lake seston within the 2.00x and 4.00x seston manipulation enclosures.


LAKEWATER RESERVOIR
SYPHON TUBE
ENCLOSURE CARBOY
POLYETHYLENE BAG
PLASTIC PAIL

2.25 l of screened lakewater plus 6.75 l of filtered lakewater (0.25x) or 4.50 l of screened lakewater plus 4.50 l of filtered lakewater (0.50x). When all enclosures were filled they were capped and then suspended just below the lake surface in a sheltered location of the lake (Figs. 2-3 and 2-4). Zooplankton was collected by vertical hauls made from approximately 1 m above the sediment in the deepest part of the lake using a 25 cm diameter zooplankton net fitted with 100 μm mesh. A roughly equivalent number of animals (within an experiment) was then added to each suspended enclosure using a wide bore pipette to minimize handling damage. Animal concentrations at the termination of the experiments varied between approximately 3-90 l^{-1}, a density range consistent with that naturally occurring in the lake during summer. A sub-sample of the zooplankton was retained on 100 μm mesh and frozen upon returning to the laboratory for subsequent initial length and weight determinations.

The cladoceran lipid index and fecundity observations of Tessier (1981) suggested that responses to changes in food supply should be detectable within four to eight days. Consequently, one set of five enclosures was incubated in the lake for four days while the second set was incubated for eight days. Chlorophyll fluoresence of the enclosure water was measured daily using a **Turner Designs Model 10** fluorometer. Daily samples of the enclosure water were taken for particulate organic carbon (POC) and particulate organic nitrogen (PON) determination and for the preparation of permanent plankton slides in the laboratory. Plankton samples were preserved with Lugol's acid iodine. The component of the total food supply measured by microscopic examination was comprised mainly of bacterioplankton and phytoplankton. Because the majority of the biomass was contributed by the phytoplankton this measurement of food supply will be referred to as "plankton". Seston refers to the total organic content of the enclosures and is more accurately represented by particulate organic carbon and particulate organic nitrogen levels.

Figure 2-3: Floating ring from which the seston manipulation enclosures were suspended below the lake surface.

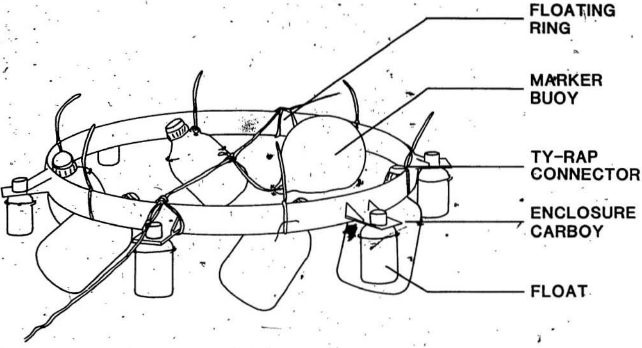
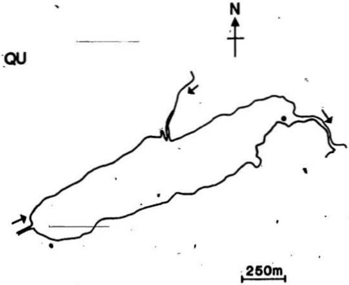
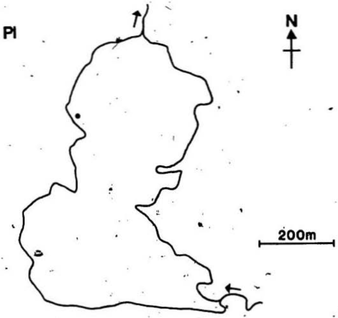

FLOATING RING
MARKER BUOY
TY-RAP CONNECTOR
ENCLOSURE CARBOY
FLOAT

Figure 2-4: Quidi Vidi Lake (QU) and Piccos Pond South (PI) indicating the location of the seston manipulation enclosures during incubation (●). Note that scales differ.


QU
N
250m


PI
N
200m

2.2.2. Grazing experiments

Filtering rates of individual animals were determined at the termination of each four or eight day experiment using the radiotracer technique of Holtby and Knoechel (1981). Three milliliters (approx. $9*10^6$ cells) of ^{14}C labelled *Scenedesmus quadricauda* were added to each enclosure and the animals were allowed to feed for 10 minutes, a period short enough to preclude defecation of labelled cells. Gut clearance time has been shown to be a negative function of both water temperature (Bjørnsen *et al.* 1986) and food concentration (Geller 1975). Haney (1971) reported a gut clearance time of greater than 15 minutes for *Daphnia* feeding at 17°C and Geller (1975) reported 17 minutes as the time required to fill the daphnid gut at a water temperature of 15°C and a food concentration of 0.9 mg C l^{-1} (approximately $9*10^3$ mg wet weight m^{-3}, which is well above the highest total plankton concentration encountered in the present study). After 10 minutes a 2 ml sample of the radioactive "food water" was collected for the determination of available radioactive food levels and the zooplankton was then strained onto squares of 100 μm nylon mesh. Animals were immediately heat killed by immersion in scalding water which also served to remove any radioactive cells that may have adhered to the carapace. The meshes were placed in individually labelled plastic bags and stored in a freezer upon return to the laboratory. Animals were heat killed and frozen to avoid the loss of radioactive label observed with chemical preservation (Holtby and Knoechel 1981).

After the completion of a four-day experiment at one site, the enclosures were cleaned (and repaired if necessary) and then deployed at the other experimental site for a similar four day period. In total, six experiments were conducted at the two sites in 1985. One four day and one eight day experiment was begun at Piccos Pond South on July 18 (water temperature 19.8°C). Upon completion of the four day experiment at Piccos Pond South a four day experiment was begun at Quidi Vidi Lake on July 25 (water temperature 17.9°C). In August, a similar series of experiments was conducted beginning with simultaneous four and eight day experiments in Quidi Vidi Lake on August 26 (water temperature 16.0°C). The final four day experiment was begun on August 31 at Piccos Pond South (water temperature 14.8°C).

2.3. Laboratory procedures

2.3.1. Seston

The potential food value of the seston was characterized by determining chlorophyll *a*, particulate organic carbon, particulate organic nitrogen, and plankton biomass levels in the enclosures.

2.3.1.1. Fluorometric determination of chlorophyll *a*

Fluorometric measurements of chlorophyll *a* in "fresh" water samples were obtained daily for each enclosure using a **Turner Designs Model 10** fluorometer. Chlorophyll *a* was calculated from a regression equation relating fluorometry readings to chlorophyll *a* concentrations measured by acetone extraction, using phaeophytin-corrected spectrophotometric techniques as outlined by Strickland and Parsons (1968).

2.3.1.2. POC and PON

A 50 ml aliquot of the unpreserved water sample from each enclosure was filtered onto a precombusted (1 h at 450°C) **Whatman GF/A** glass microfibre filter (2.54 cm diameter, 1.6 μm pore size). The particulate organic carbon (POC) and particulate organic nitrogen (PON) content of initial and final day seston samples were measured using a **Perkin-Elmer Model 240 Elemental Analyzer** calibrated with acetanilide standards.

2.3.1.3. Plankton biomass

Permanent slides of the preserved plankton, made using plexiglass settling columns (Knoechel and Kalff 1976), were scanned at 480x using phase contrast microscopy. The cells were enumerated and sized using an ocular micrometer. Plankton biomass (mg wet weight m^{-3}) was calculated using formulae for simple geometric solids, assuming a specific gravity of 1.00. These data were accumulated into size categories for cells with maximum dimensions of <2 μm, 2-5 μm, 5-10 μm, 10-30 μm and >30 μm. The biomass of colonial forms was calculated from the dimensions of the individual cells and then placed in the appropriate size category based on colony dimensions.

2.3.2. Zooplankton

Body lengths, number of eggs or embryos present in the brood pouch, dry weights and filtering rates were determined for individual *Daphnia catawba* from each enclosure to allow the comparison of filtering rates, length-specific weight relationships and fecundity between enclosure concentrations.

2.3.2.1. Length and weight determination

The concentration of animals observed in each 9 l enclosure at the termination of each experiment ranged from approximately 3-90 l^{-1}; a density range consistent with that occurring in the lakes during summer (Page 1983, unpub. data).

From each enclosure, 48 *Daphnia catawba* were selected (except when fewer were present) for length and dry weight determination. Body length was measured at 250x using a **Wild M5A** stereoscope and an ocular micrometer, to the nearest eyepiece scale unit, and was defined as the distance from the anterior margin of the head to the base of the tail spine. Unit lengths were converted to millimeters (1 unit= 0.038 mm) and rounded to the nearest 0.01 mm. The number of eggs or embryos present in the brood pouch was recorded for each animal. Animals were dried individually on small pieces of aluminum foil at 50°C for at least 24 h and weighed to the nearest 0.1 μg using a **Cahn Model 21** digital microelectrobalance. Animals were first weighed on the foils, then separated from the foils which were reweighed. Animal weight was calculated as the difference between the weight of the foil plus the animal and the weight of the foil alone. Weights and tares were determined for paired samples (two weights followed by two tares) in order to minimize the influence of electronic balance drift on the accuracy of the dry weight measurements. Animals were placed individually in labelled polyethylene scintillation vials for subsequent determination of radioactive food content.

2.3.2.2. Filtering rate determination

Filtering rates were calculated from the radioactivity of the labelled *Scenedesmus quadricauda* accumulated by each animal during the 10 minute feeding experiments. Individual *Daphnia catawba* from each enclosure were digested overnight at 25°C in polyethylene scintillation vials with 0.5 ml of tissue solubilizer (**Protosol, NEN**) and were subsequently counted following the addition of 5 ml of scintillation solution (**Econofluor-2, NEN**). Polyethylene scintillation vials containing 2 ml radioactive "food water" samples were left standing overnight following the addition of 0.1 ml of 0.5 N HCl to rid the samples of any unincorporated dissolved inorganic ^{14}C. Samples were then counted following the addition of 10 ml of scintillation cocktail (**Aquassure LSC, NEN**).

Radioactivity was determined by liquid scintillation counting (**Beckman Model LS-3150 T** Liquid Scintillation Counter). Triplicate 10 minute counts were obtained for each of the animal and "food water" samples. Sample counting efficiency was monitored by the external standard channel ratio technique calibrated by internal standardization with ^{14}C-toluene. Background levels of radioactivity were determined by preparing and counting five samples containing non-radioactive animals. Individual daily filtering rates were calculated in the following manner:

$$\mathrm{FR(ml/d)} = \frac{Animal(dpm) - Background(dpm)}{Food(dpm/ml) - Background(dpm/ml)} \times \frac{1440(mins/d)}{10(mins)}$$

2.4. Statistical analysis

Data were analyzed using least squares regression and multiple linear regression routines available through the SPSSX statistical package (SPSS Inc. 1983) implemented at Memorial University Computing Services on a VAX 8800 computer operating under VMS.

2.4.1. Regression analysis

Relationships between filtering rate, weight and length were examined by multiple linear regression following $\log_{10}$ transformation to reduce the dependence of the sample variance on the mean and to normalize the distribution of the data.

Traditionally, filtering rate has been expressed as a function of cladoceran body length (Burns and Rigler 1967, Burns 1968b, Haney 1973, Chow-Fraser and Knoechel 1985, Knoechel and Holtby 1986a,b). However in this study multiple linear regression analysis indicated that dry weight was the better predictor of filtering rate (r^2=.261 for weight vs. r^2=.159 for length). Length, in turn, was the best predictor of cladoceran body weight.

Individual cladoceran filtering rates, dry weights, and body lengths were used to construct filtering rate:weight and weight:length regression relationships for each enclosure of the form:

$$\log Y = a' + b \log X \qquad \text{Eq. 1}$$

where (X) is the independent variable (weight or length), (Y) is the dependent variable (filtering rate or weight), (b) is the regression coefficient and (a') is the intercept. This relationship may also be expressed as a power function:

$$Y = aX^b \qquad \text{Eq. 2}$$

where (a) is the antilog of (a').

Ricker (1973) argued that geometric mean, or "functional", regressions are better representations of the underlying relationships than are "predictive" (least square) regressions because the least square regression coefficients (slopes) are strongly affected by the strength of the correlation (r). Jensen (1986) concurred that the functional regression is a better representation of the underlying relationship between two variables but he suggests that the functional relationship should not be used for predictive purposes. Following these recommendations, functional regressions were employed throughout this study to compare relationships between enclosures. The slope of the functional regression was calculated as the least squares regression slope divided by the correlation coefficient (Ricker 1973). The intercept was calculated by solving the resulting equation for the means of X and Y.

The regression intercepts (a) and exponents (b) can be used to quantify the differences between the filtering rate:weight and weight:length relationships calculated for each experimental enclosure. A shift in the intercept alone indicates a filtering rate or weight change of equal proportion in animals of all sizes while a change in the exponent indicates proportionately different degrees of filtering rate or weight change in different-sized animals. The heterogeneity in the exponents of the length-specific weight and weight-specific filtering rate relationships (cf. APPENDIX A) suggested body-size-related weight and filtering rate responses. Weights and filtering rates were therefore calculated from each regression relationship for three animal body sizes representative of the range of animal lengths observed in the lake: small (0.75 mm), medium (1.00 mm) and large (1.50 mm). These estimates were subsequently used as dependent variables in multiple linear regression analyses to investigate the influence of environmental factors on the physiological condition of *Daphnia catawba*.

RESULTS

The effects of manipulating the natural food supply on the growth and physiological condition of *Daphnia catawba* were monitored in terms of individual filtering rate, length-specific weight and fecundity.

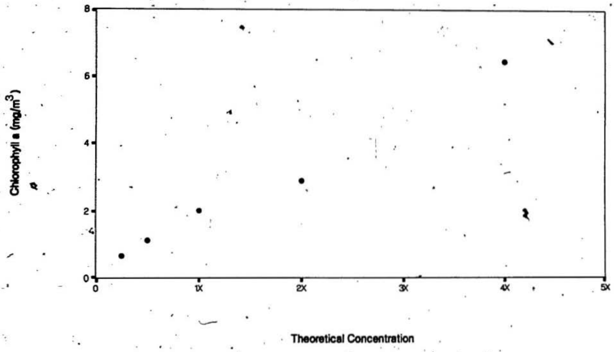
3.1. Manipulation and characterization of the natural food supply

Chlorophyll *a*, particulate organic carbon (POC), particulate organic nitrogen (PON) and plankton biomass levels were monitored in order to characterize the potential food value of the natural particulate food supply in the enclosures and to determine the effectiveness of the seston manipulations.

Enclosure manipulations were prepared as previously described to theoretically encompass a 16-fold range from 0.25 to 4.00 times the ambient lake seston for each experiment. Inspection of chlorophyll *a*, POC, PON and total plankton biomass levels permits evaluation of the general success of establishing a range of food concentrations within the experimental enclosures. For each experiment, concentration ratios were calculated to relate the initial chlorophyll *a*, POC, PON and total plankton biomass levels in the 0.25x enclosure to the corresponding levels in the 4.00x enclosure. The ratios calculated for chlorophyll *a* ranged from 3.59 to 9.79, with a mean ratio of 6.90 across the six experiments. The maximum ratio established was in the enclosures of the four day experiment begun at Piccos Pond South on July 18 (Fig. 3-1). POC manipulation ratios ranged from 1.17 to 4.57 with a mean 3.14-fold range in POC levels within enclosures across all experiments. The expected range of food concentrations was best maintained during the four day experiment begun in Quidi Vidi Lake on July

Figure 3-1: Range of chlorophyll *a* concentrations established in 4-day enclosures in Piccos Pond South, 18 July 1985.

Range of Chlorophyll a Concentrations
Piccos Pond South, 18 July 1985



25 as indicated by the chlorophyll a and POC levels in the enclosures. A general increase in food levels was noted within each enclosure over the duration of the experiment; the order of enclosure food concentrations was therefore maintained (Figs. 3-2 and 3-3). Manipulations of total plankton biomass (microscopy estimates) and PON levels achieved narrower concentration ratios. Total plankton biomass ranged from 2.68 to 5.87 (mean 4.83) and PON from 1.98 to 4.83 (mean 2.92). More detailed data are provided in APPENDIX B.

Strong correlations between final chlorophyll a, POC, and PON suggested that these variables were manipulated consistently among experiments (Table 3-1). Correlations between these general seston estimates and subcomponents of the plankton biomass determined by microscopy were considerably weaker. Some of the increased variation is undoubtedly due to counting error. For example, slightly weaker correlations with the intermediate plankton biomass components (10-30 μm and 5-10 μm) might result from increased counting error as the categories became narrower and hence the number of particles counted declined. The very weak correlations between general seston variables and the smallest (2-5 μm and <2 μm) plankton components should not result from counting error, however, because these categories have the largest number of particles. The >30 μm category, which consisted largely of gelatinous green algae, also had reasonably high item counts. Overall, the smallest and largest plankton particle categories did not seem to be manipulated predictably in the enclosures. However, the manipulations did establish a wide range of variation in the concentrations of these categories (APPENDIX B) to which the animals could subsequently respond (see below).

3.2. Filtering rates

Based on the literature from field and laboratory investigations of cladoceran filtering rates (Burns and Rigler 1967, Haney 1985, Paffenhöfer and Orcutt 1986), it was expected that filtering rates should have responded positively to changes in water temperature and negatively to changes in total food concentration within the enclosures.

Figure 3-2: Daily chlorophyll *a* concentrations in the 4-day enclosures in Quidi Vidi Lake, 25-29 July 1985.

Daily Chlorophyll a Concentrations
Quidi Vidi Lake, 25-29 July 1985

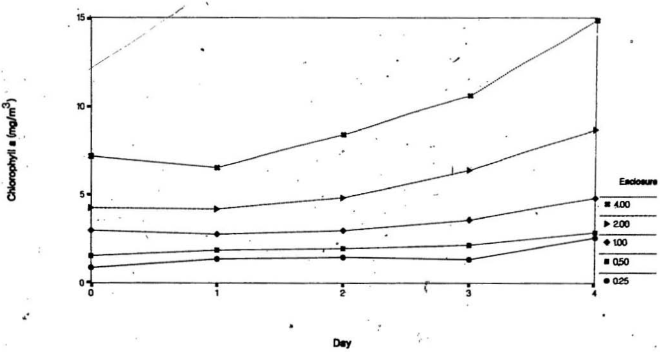


Figure 3-3: Initial and final day particulate organic carbon (POC) concentrations in the 4-day enclosures in Quidi Vidi Lake, 25-29 July 1985.

Initial and Final Day POC Concentrations Quidi Vidi Lake, 25-29 July 1985

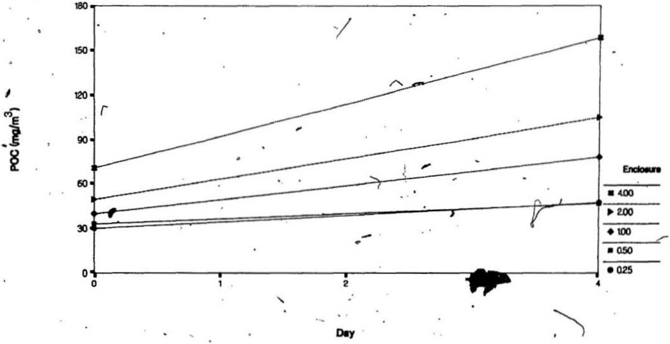


Table 3-1: Simple correlation matrix of final day POC, PON, chlorophyll *a* and plankton biomass concentrations in the seston manipulation enclosures. Significant r=.367, α=.05 and r=.470, α=.01; n=29.

				Plankton Biomass Size Categories						
Variable	POC	PON	Chl*a*	Total	>30µm	<30µm	10-30µm	5-10µm	2-5µm	<2 µm
POC	—	.860	.927	.553	.133	.704	.613	.542	.196	.044
PON	—	—	.849	.464	.346	.430	.361	.374	.068	-.101
Chl*a*	—	—	—	.671	.292	.667	.523	.554	.256	.191

Final day food levels were expected to be of greater importance in predicting *Daphnia catawba* filtering rate variability than initial day levels because cladocerans have been noted to be capable of altering their filtering rates rapidly in response to changing food conditions (McMahon and Rigler 1963). This expectation was confirmed by the regression analyses which always indicated stronger relationships with final day concentrations.

Multiple linear regression analysis was used to investigate the relationships of small, medium and large animal filtering rates with the independent variables: water temperature, number of animals per enclosure, and final day $\log_{10}$ transformed chlorophyll *a*, POC, PON and plankton biomass components. Initial and final concentrations of the general seston variables (chl *a*, POC, PON), which were manipulated most consistently in the enclosures (see above), were not significantly correlated with animal filtering rates. Only the plankton biomass component variables explained significant levels of variation in filtering rates. Initial analysis revealed that the filtering rates of small and medium individuals were weakly, positively correlated with the plankton biomass component representing cells of 5-10 μm maximum dimension (Figs. 3-4, 3-5) while the filtering rates of large individuals were weakly positively correlated with water temperature (Fig. 3-6). Inspection of the filtering rate plots revealed two probable outliers in all three relationships. Animals in these two enclosures (one in Piccos Pond South and one in Quidi Vidi Lake) had extremely low filtering rates which could not, however, be attributed to any procedural or analytical error. Removal of these outliers permitted additional significant variables to enter the multiple regression analyses (Table 3-2). The largest plankton particle category (spheres >30 μm) had a negative influence on the filtering rate of all animals (e.g. Figs. 3-7 and 3-8) while the second largest plankton particle category (10-30 μm) had a negative influence on the filtering rate of medium and large sized animals (Table 3-2). The 5-10 μm plankton biomass component still had a positive effect on the filtering rate of all animals (Figs. 3-4, 3-5 and 3-9), while the response to water temperature varied with animal size as did the order of importance of the plankton biomass variables.

Figure 3-4: Daily filtering rates of small sized *Daphnia catawba* as a function of log 5-10 μm plankton biomass (r=.344, p=.068). Possible outliers indicated as (●).

Daphnia catawba Daily Filtering Rate as a Function of Log 5-10 µm Plankton Biomass

Small Animals

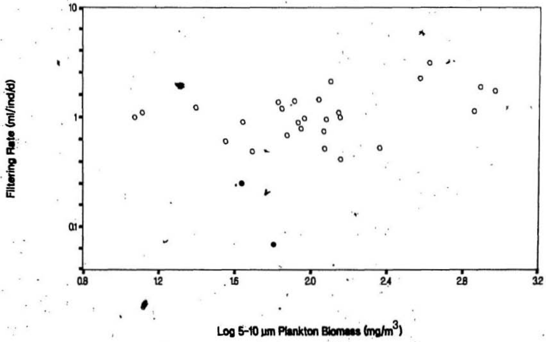


Figure 3-5: Daily filtering rates of medium sized *Daphnia catawba* as a function of log 5-10 μm plankton biomass (r=.377, p=.044). Possible outliers indicated as (●).

Daphnia catawba Daily Filtering Rate as a Function of Log 5-10 μm Plankton Biomass

Medium Animals

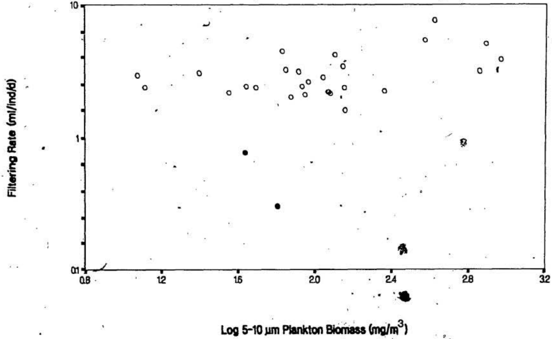


Figure 3-6: Daily filtering rates of large sized *Daphnia catawba* as a function of water temperature (r=.371, p=.047). Possible outliers indicated as (●).(*) indicates four overlapping points.

Daphnia catawba Daily Filtering Rate as a Function of Water Temperature

Large Animals

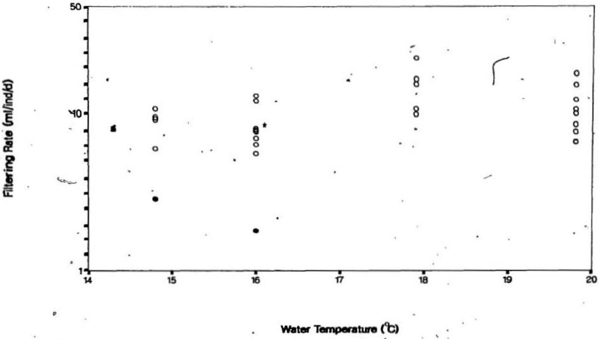


Table 3-2: Stepwise multiple regression models of daily filtering rate as a function of plankton biomass and water temperature for small, medium and large *Daphnia catawba*. Regression coefficients (b), explained variance (r^2), stepwise increase in explained variance (Δr^2), *F* statistic and significance level (p). Variables listed in order of entry into regression equation. Variable names explained in the text.

Variable	b	r^2	Δr^2	*F*	p
		Small Animals			
LogSph. >30μm	-0.137	0.141	0.141	4.105	0.0536
Log 5-10μm	0.218	0.269	0.128	4.411	0.0234
Temp	-0.049	0.454	0.185	6.369	0.0027
Constant	0.741				
		Medium Animals			
LogSph. >30μm	-0.114	0.195	0.195	6.060	0.0211
Log 5-10μm	0.203	0.381	0.186	7.371	0.0032
Log 10-30μm	-0.057	0.457	0.076	6.442	0.0025
Constant	0.431				
		Large Animals			
Log 5-10μm	0.191	0.131	0.131	3.776	0.0633
LogSph. >30μm	-0.093	0.249	0.118	3.986	0.0320
Log 10-30μm	-0.097	0.342	0.093	3.980	0.0202
Temp	0.030	0.437	0.095	4.276	0.0104
Constant	0.513				

Figure 3-7: Daily filtering rates of small sized *Daphnia catawba* as a function of log spherical cell colonies >30 μm plankton biomass.

<u>Daphnia</u> <u>catawba</u> Daily Filtering Rate as a Function of Log Spherical >30 µm Plankton Biomass

Small Animals

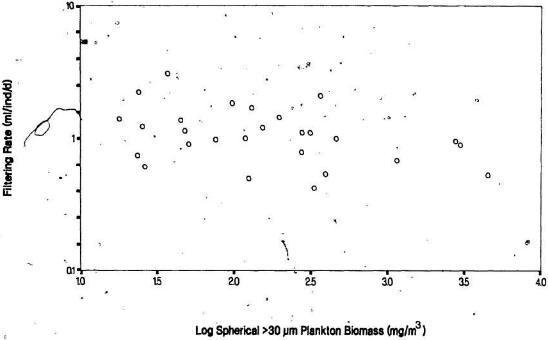


Figure 3-8: Daily filtering rates of medium sized *Daphnia catawba* as a function of log spherical cell colonies >30 μm plankton biomass.

<u>Daphnia</u> <u>catawba</u> Daily Filtering Rate as a Function of Log Spherical >30 µm Plankton Biomass

Medium Animals

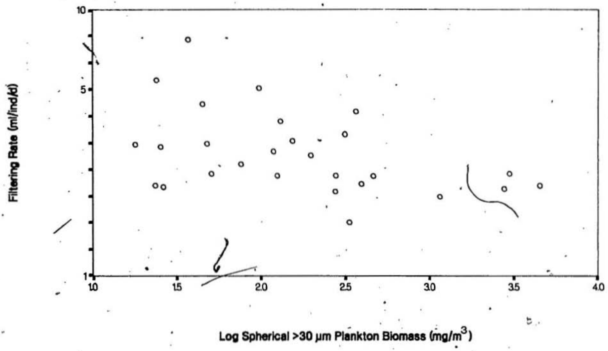
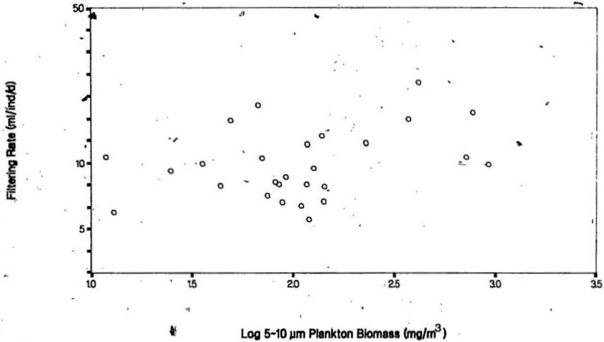


Figure 3-9: Daily filtering rates of large sized *Daphnia catawba* as a function of log 5-10 μm plankton biomass.

Daphnia catawba Daily Filtering Rate as a Function of Log 5-10 µm Plankton Biomass

Large Animals



3.3. Length-specific weight

The weights calculated for small, medium and large animals represented the dry weights of animals at the end of each experiment. Initial dry weights were calculated from length-specific weight relationships constructed for the lake animals collected at the beginning of each experiment (APPENDIX A). Net weight change was then calculated for small, medium and large animals as the difference between the final and initial dry weights.

Based on results from the literature regarding cladoceran feeding rates, growth rates and physiology (Rigler 1961, McMahon 1965, Burns and Rigler 1967, Lampert 1977, Tessier and Goulden 1982, Page 1983), it was expected that net weight change in *Daphnia catawba* should have responded to both natural food supply manipulation and water temperature within the enclosures.

It was expected that initial day food levels might better predict weight change than final day food levels because a time lag might exist between fluctuations in food supply and subsequent weight responses. However, stepwise multiple linear regression analysis of small, medium and large net weight change with the independent variables: water temperature, number of animals per enclosure, initial and final day $\log_{10}$ transformed POC, PON, chlorophyll *a*, and plankton biomass components indicated that final day plankton biomass and initial day water temperature were the factors best correlated with net weight change in *Daphnia catawba* (Table 3-3). Simple correlations with the general seston variables (chl *a*, POC, PON) were all non-significant. The net weight change of all animals responded positively to the smallest plankton biomass component while medium and large animal weight change also responded negatively to water temperature. Small and medium sized animals responded most strongly to increases in the smallest plankton biomass component (Figs. 3-10 and 3-11) while large animal net weight change was most strongly influenced by water temperature (Fig. 3-12). Initial day water temperature was a better indicator of weight change than final day water temperature even though water temperature over the duration of each experiment was fairly uniform.

Table 3-3: Stepwise multiple regression models of net weight change as a function of Log <2 μm edible plankton and water temperature for small, medium and large *Daphnia catawba* and as a function of initial weight for large *Daphnia catawba*. Regression coefficients (b), explained variance (r^2), stepwise increase in explained variance (Δr^2), *F* statistic and significance level (p). Variables listed in order of entry into regression equation. Variable names explained in the text.

Variable	b	r^2	Δr^2	*F*	p
			Small Animals		
Log <2μm	0.769	0.353	0.353	14.712	0.0007
Constant	-0.769				
			Medium Animals		
Log <2μm	1.315	0.334	0.334	13.519	0.0010
Temp	-0.134	0.526	0.192	14.425	0.0001
Constant	1.017				
			Large Animals		
Temp	-0.634	0.580	0.580	37.355	<0.0001
Log <2μm	1.694	0.646	0.066	23.719	
Constant	9.407				
			Large Animals		
Initial Wt.	-0.752	0.611	0.611	47.478	<0.0001
Constant	6.261				

Figure 3-10: Net weight change in small sized *Daphnia catawba* as a function of log <2 μm plankton biomass.

Net Weight Change in <u>Daphnia</u> <u>catawba</u> as a Function of Log <2 µm Plankton Biomass

Small Animals

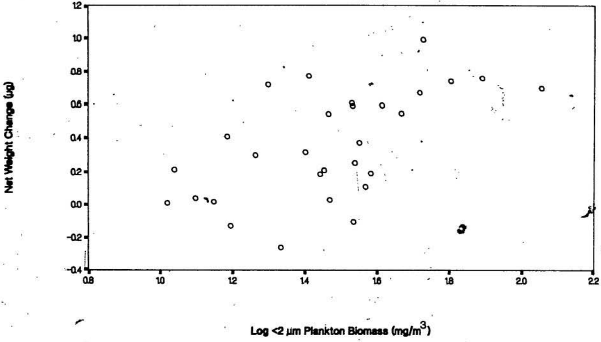


Figure 3-11: Net weight change in medium sized *Daphnia catawba* as a function of log <2 μm plankton biomass.

Net Weight Change in Daphnia catawba as a Function of Log <2 µm Plankton Biomass

Medium Animals

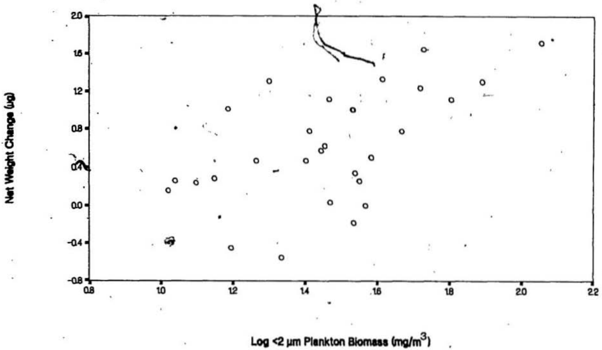
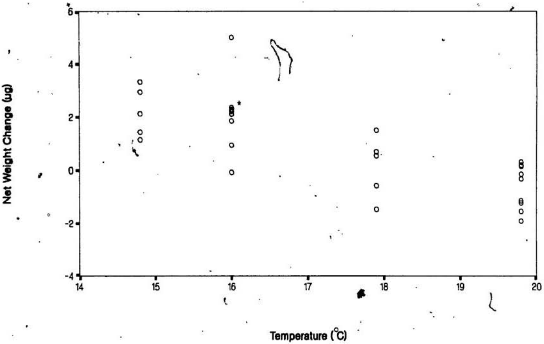


Figure 3-12: Net weight change in large sized *Daphnia catawba* as a function of water temperature.(*) indicates six overlapping data points.

Net Weight Change in <u>Daphnia</u> <u>catawba</u> as a Function of Water Temperature

Large Animals



It was also expected that the initial weight of the animals could influence the direction and magnitude of the body weight response. It seemed likely that upper and lower weight boundaries existed for a given sized animal and thus the weight of an animal at the beginning of an experiment relative to these limits would limit the magnitude of net weight change in at least one direction. The influence of initial dry weight on small, medium and large animals was therefore examined, in the presence of all other independent variables, in a multiple linear regression analysis. The results indicated that the variability in net weight change in large *Daphnia catawba* could be explained as well by the initial weight of the animals as by the water temperature and food supply (see above). A significant negative relationship between net weight change and initial weight (Fig. 3-13, Table 3-3) suggested that initially "very heavy" animals tended to lose weight and initially "light" animals tended to gain weight over the course of an experiment. A high positive correlation between large initial body weight and water temperature (r= .953) precluded the entry of initial weight into the original large net weight change regression equation once temperature had been entered.

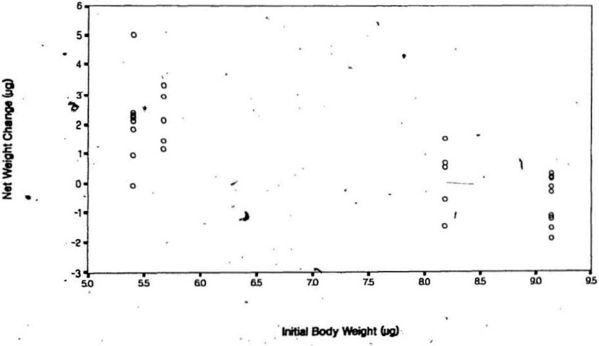
3.4. Fecundity

Fecundity, measured as the number of eggs and embryos present in the brood pouch of individual *Daphnia catawba* and as percent ovigerous females and eggs per 100 adult females, was low throughout the study period from July 18 to September 4, 1985 (APPENDIX C). This was consistent with previous summer fecundity observations in the two study lakes (Page 1983, unpub. data). The percentage of adult females carrying eggs and the mean number of eggs per adult female are typically higher in early summer and in fall in Avalon lakes (Page 1983, Cooper 1985, Davis pers. comm.). Ninety-five percent binomial confidence limits indicated that changes in the percentage of ovigerous females and changes in the number of eggs per hundred adult females were generally insignificant over the duration of the experiments; further analysis of the fecundity data was therefore not warranted.

Figure 3-13: Net weight change in large sized *Daphnia catawba* as a function of initial body weight.(*) indicates six overlapping data points.

Net Weight Change in <u>Daphnia</u> <u>catawba</u> as a Function of Initial Body Weight

Large Animals



DISCUSSION

Field investigations of the physiological responses of primary consumers to fluctuating environmental factors are an important step toward the understanding of the dynamics of these organisms and trophic interactions in natural freshwater ecosystems. The present study addressed the effects of seston manipulation and water temperature fluctuation on the filtering rate, length-specific weight and fecundity of *Daphnia catawba* maintained in *in situ* enclosures.

The ingestion and assimilation of energy in excess of the level required for basic metabolic maintenance by cladocerans is necessary for growth and reproduction. The dependence of cladoceran ingestion and assimilation rates on food supply, water temperature and body size, demonstrated in the laboratory, suggested that *Daphnia catawba* filtering rates and net production should have responded to seston manipulation and water temperature fluctuations in *in situ* enclosures. The results of the current field study generally confirm laboratory observations of the responses of Cladocera to manipulations of food supply and temperature.

4.1. Filtering rate responses

Direct comparison of the observed magnitude of filtering rate response to changes in food concentration and water temperature with the results of previous laboratory studies is not possible because regression coefficients and confidence intervals have not usually been reported in the literature. It is possible however, to compare the signs of the filtering rate responses observed in the current study with the results of laboratory investigations.

Negative relationships between cladoceran filtering rates and the concentration of both inedible plankton and total food supply have been reported. Webster and Peters (1978) observed increased rates of food rejection for *Daphnia* with increasing inedible plankton concentration and Chow-Fraser and Sprules (1986) observed reduced *Daphnia* filtering rates in the presence of *Anabaena* filaments. Increased rejection of food groove contents presumably results in reduced ingestion due to decreased filtering time (Edmondson 1957). These results were corroborated by the negative relationships observed between the inedible plankton component (spherical colonies >30 μm) and the filtering rates of small and medium sized *Daphnia catawba* in the present study. A negative relationship was also observed between filtering rate and inedible plankton biomass for large animals. However, spherical colonies were of secondary importance to edible plankton biomass in influencing filtering rate.

It has been commonly reported that cladoceran feeding rates are proportional to food concentration up to an incipient limiting level (ILL), above which feeding rate plateaus. Filtering rate, consequently, is reported to be constant below the ILL and decreases above the ILL (as reviewed by Wetzel 1983). In this context, the positive relationships observed between the 5-10 μm plankton component and the filtering rates of all sized *Daphnia catawba*, appear to contradict the negative filtering rate relationships observed in the laboratory.

Porter *et al.* (1982) reported an ILL organic carbon concentration of 0.2 mg C l^{-1} which is approximately 2000 mg wet weight biomass m^{-3} assuming carbon content to be 50% of dry weight which in turn is 20% of wet weight. This ILL is well above the highest 5-10 μm biomass level observed in this study; consequently a constant filtering rate might be expected at all field food concentrations. However, closer inspection of Burns' results (1968a, Fig. 3) for *Daphnia rosea* thoracic appendage activity in various concentrations of yeast, reveals increasing filtering activity with increasing food concentration up to an ILL of 10^4 *Rhodotorula glutinis* cells ml^{-1}. This would be consistent with the positive correlation between the 5-10 μm plankton component and filtering rates observed in the present study. Care must be exercised when interpreting Burns' data, however, because no confidence limits were included with her observations. Porter *et al.* (1982) also pointed out that the limitations of the ^{14}C method for determining filtering rates at food concentrations below 10^3 cells ml^{-1} make the interpretation of their results, and the results of others, equivocal at low food concentrations. In order to ensure that enough labelled food cells are ingested to yield accurate filtering rate estimates during the short 10 minute feeding periods used in most filtering rate studies, approximately 10^3 labelled cells ml^{-1} must be added. This will greatly elevate the total food concentration when added to unlabelled suspensions of $<10^3$ cells ml^{-1} and even if the total food supply is labelled, accurate filtering rate estimates in food concentrations $<10^3$ cells ml^{-1} will be susceptible to error due to particle encounter probability. Similar problems with encounter probability arise even at higher food concentrations ~~for~~ very small animals with low individual filtering rates (Chow-Fraser and Knoechel 1985).

The negative relationships observed between the 10-30 μm plankton component and the filtering rates of medium and large sized animals are not consistent with earlier laboratory observations of declining filtering rates with increasing food concentration because the biomass levels observed in the 10-30 μm plankton component were usually well below the accepted ILL. The variability in the biomass estimates of this component (APPENDIX B) suggests that its inclusion in the multiple regression equation may have been spurious. The "noise" in the 10-30 μm component may be attributed to the relative scarcity of particles (usually <10) encountered in this category while sizing and enumerating the plankton. A similar problem was not expected in other plankton components because particles were more abundant in all other size categories.

Both McMahon (1965) and Burns and Rigler (1967) reported positive relationships between *Daphnia* filtering rate and water temperature up to a critical temperature. The filtering rate response of large *Daphnia catawba* to fluctuating water temperature was consistent with these earlier laboratory observations but the negative response of small animals was not. The reason for the negative filtering rate response to increasing water temperature observed for small animals is unclear.

4.2. Net production responses

The effects of food supply and water temperature on the physiological condition of *Daphnia catawba* observed in this study are consistent with the results of earlier field studies (Tessier and Goulden 1982, Page 1983, Tessier 1986) and support the view that natural zooplankton communities can be food limited.

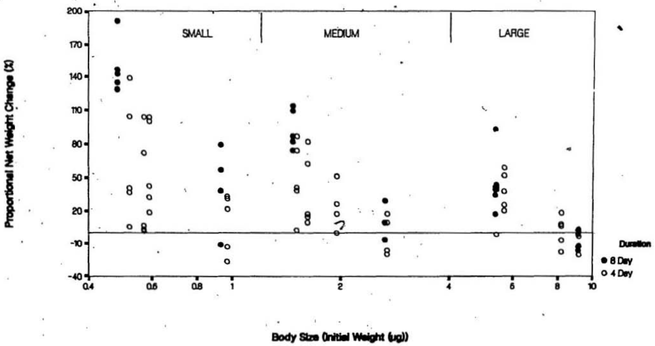
Positive net production in cladocerans is only possible when the energy assimilated by the animals is greater than the energy expended for metabolic requirements. Excess energy may be allocated to growth and/or reproduction and both the quantity of food available and water temperature have been invoked as environmental factors capable of regulating net production in cladocerans.

The observation of increasing body weight with increasing food supply and decreasing water temperature in the field agrees well with Lampert's (1977) laboratory studies of the carbon balance in *Daphnia*. Lampert also noted that net production relative to body size was greater in small individuals especially under conditions of low food supply and increased water temperature because the threshold food concentration, above which net production was positive, increased as a function of both increasing body size and increasing water temperature. Consistent with Lampert's predictions, higher net weight gain proportional to body size was observed in small *Daphnia catawba* as compared to medium and large sized individuals (Fig. 4-1). Significantly higher proportional net weight gain was also observed in small animals incubated in the lake enclosures for eight days as compared to those incubated for four days (*t*-test, $p < .05$). The possible influence of experimental duration was tested by coding duration as a dummy binary variable in all multiple regression analyses. The duration variable was non-significant ($p > 0.05$) in all cases; the higher proportional net weight gain was attributable to higher edible plankton levels in the enclosures at the end of the eight day experiments rather than to the length of the experiment.

Proportional net weight change within a size category (small, medium or large animals) also exhibited a negative relationship with initial body weight

Figure 4-1: Proportional net weight change as a function of body size (initial body weight) in *Daphnia catawba*.

Proportional Net Weight Change in <u>Daphnia</u> <u>catawba</u> as a Function of Body Size (Initial Weight)



suggesting that initially "light" animals possessed a greater potential or scope for growth than did initially "heavy" animals.

The greater proportional net weight gain observed in smaller *Daphnia catawba* may be related to greater feeding efficiencies, or to either higher relative assimilation rates or lower specific respiration rates under conditions of increased water temperature and low edible food levels as suggested by Lampert (1977). The lower proportional weight change observed in the larger animals might be accounted for by adults molting and releasing young just prior to the termination of each experiment, but this seems improbable because the few eggs observed in the adults at the beginning of the experiments suggested low reproductive output (in terms of neonates) during the experiments. Likewise, adults molting to a larger carapace length without a corresponding gain in weight or production of young immediately prior to the termination of each experiment seems unlikely. The most probable explanation for the lower proportional net weight change observed in large animals was the greater effects of food limitation and increased water temperature on the net production of these animals. Lampert's laboratory data indicated maximum weight gain in excess of 80% per day for small *Daphnia pulex* (equivalent in mass to large *Daphnia catawba*) under optimal food and temperature conditions (Fig. 6 in Lampert 1977c). In contrast, the maximum daily rate for large animals in the present study was approximately 15% (60% weight gain over 4 days, Fig. 4-1) and for small animals about 24% (190% over 8 days, Fig. 4-1) suggesting that all animals were strongly food limited even in the food enhanced enclosures, and obviously even more so in the lake.

4.3. Life history characteristics

Differential energy allocation relative to cladoceran developmental stage may influence proportional net weight change. Juveniles allocate excess energy to growth while adults may allocate excess energy to growth or reproduction (Lynch 1977). Increased fecundity was not observed in the present study suggesting that excess energy was allocated entirely to growth and storage products. The failure to detect increased fecundity probably cannot be attributed to egg loss by reproductively mature individuals because few stray eggs were observed in the samples. Lynch (1977) stated that while reproduction is necessary for the survival of genes and species, individuals may remain in a non-reproductive state throughout their entire life-cycle if food concentrations remain sufficiently low. Allocation of excess energy primarily to growth and storage products by adult *Daphnia* may be a reasonable life history strategy under conditions of food limitation and low vertebrate predation because the allocation of energy reserves to reproduction and the release of neonates into a food limited environment could result in the starvation of all individuals. If vertebrate predation is low, a large adult body size may be of no disadvantage to *Daphnia*; neonate production may actually be disadvantageous during periods of high invertebrate predation on small animals. Campbell and Knoechel (1987) have reported low vertebrate predator density in Piccos Pond South and Quidi Vidi Lake (0.0 and 0.6 *Gasterosteus aculeatus* per trap, respectively) and relatively high invertebrate predator density in Piccos Pond South (139 *Chaoborus* larvae m^{-3}) and Quidi Vidi Lake (71 *Leptodora* m^{-3}), compared to nine other Avalon lakes, during late summer. Under these conditions *Daphnia catawba* appears to allocate excess energy to adult growth and maintenance and the production of a few, relatively large offspring (neonate length $\simeq$1/3 adult length (Page, pers. obs.)).

Future studies including measurements of assimilation and respiration rates in natural cladoceran populations under field conditions would allow the comparison of predicted and observed net production in the field.

4.4. Implications for lake trophic structure

The results of this study generally confirm the conclusions of earlier laboratory and field studies, that food availability and water temperature are key factors influencing cladoceran physiology and net production. An abundance of easily ingested and assimilated food is required for growth and reproduction in cladocerans and in the field this food supply is represented by the nannoplankton.

Gelin and Ripl (1978) have redefined the nannoplankton as the plankton size fraction that is ingestible by zooplankton, in recognition of its functional role.

A positive relationship between zooplankton biomass and phytoplankton biomass has been reported for a variety of lakes (McCauley and Kalff 1981). The slope of the relationship (<1) suggests that zooplankton biomass increases at a slower rate than phytoplankton biomass with increasing lake trophic status. This is presumably due to the fact that not all of the total phytoplankton assemblage is available to zooplankton as edible food. A positive relationship between nannoplankton biomass and phytoplankton biomass with a slope <1 reported by Watson and Kalff (1981) similarly suggests a lower rate of increase of the edible component of the total phytoplankton assemblage with increasing lake trophic status. A positive relationship between zooplankton biomass and nannoplankton biomass, with a slope approximating 1, has been interpreted by McCauley and Kalff as suggesting that zooplankton biomass responds directly to edible nannoplankton biomass.

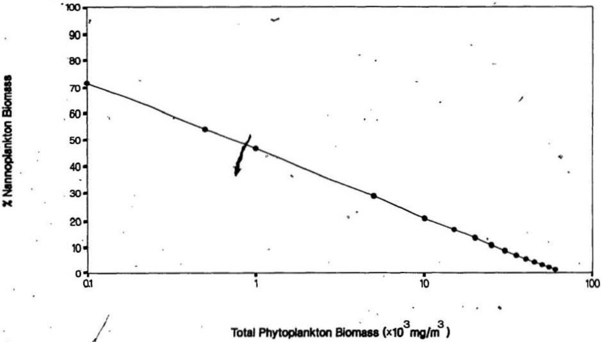
Because percent nannoplankton biomass decreases with increasing lake trophic status (Watson and Kalff 1981), and because zooplankton biomass responds directly to nannoplankton biomass, one could speculate that a critical total phytoplankton concentration should exist above which the negative interference effects of the inedible phytoplankton fraction would outweigh the positive resource effects of the nannoplankton. This possibility can be evaluated by combining the literature regression relationships with the physiological observations of the present study.

Watson and Kalff's percent nannoplankton:log total phytoplankton biomass relationship (Fig. 4-2) was used to estimate the biomass contributions of the nannoplankton (edible) and netplankton (inedible) components for a range of total phytoplankton concentrations (10^2 to $6*10^4$ mg wet weight m^{-3}). Multiple linear regression equations from the present study were then used to estimate cladoceran filtering rates by substituting the calculated nannoplankton and netplankton

Figure 4-2: Percent nannoplankton biomass as a function of total phytoplankton biomass (increasing lake trophic status).

Percent Nannoplankton Biomass as a Function of Total Phytoplankton Biomass

Calculated from Watson and Kalff (1981)



biomass concentrations for the 5-10 μm and >30 μm plankton variables respectively. Filtering rates for small, medium and large *Daphnia catawba* all show negative responses to increasing total phytoplankton biomass (Fig. 4-3).

The ration actually obtained by the filter feeding animals was subsequently calculated as the product of the individual filtering rate and the nannoplankton concentration (Fig. 4-4). Ration increases with increasing total phytoplankton biomass up to a critical level of $2.5 * 10^4$ mg wet weight m^{-3} for all sized individuals after which it declines. The initial increase is due to the increased absolute concentration of nannoplankton while the subsequent decline results from declining nannoplankton biomass combined with declining filtering rate due to the negative influence of inedible algae. This overall relationship suggests that mesotrophic lakes should thus be better nutritional environments for *Daphnia* than either oligotrophic or ultra-eutrophic lakes. The classification of lakes as oligotrophic, mesotrophic or eutrophic is based on a variety of criteria and the transition between levels of lake trophic status is often poorly defined. It is generally agreed however, that lakes with total phytoplankton biomass levels below 10^3 mg wet weight m^{-3} are oligotrophic, while plankton biomass levels well in excess of 10^4 mg wet weight m^{-3} are characteristic of eutrophic lakes (cf. Carlson 1977 and Wetzel 1983); mesotrophy occupies an intermediate position between oligotrophy and eutrophy.

A shift in the zooplankton community to smaller sized species with increasing lake trophic status may result in a bottleneck to energy flow from the primary consumer level because small sized zooplankton are less efficiently preyed upon by planktivorous fish. It seems clear that a thorough understanding of the relationships between *Daphnia* physiology and environmental variables would be of utility in designing a freshwater quality management programme.

Figure 4-3: Filtering rates of small, medium and large *Daphnia catawba* as functions of total phytoplankton biomass (increasing lake trophic status).

Filtering Rates of Small, Medium and Large *Daphnia catawba* as Functions of Total Phytoplankton Biomass

Estimated from multiple linear regressions from this study

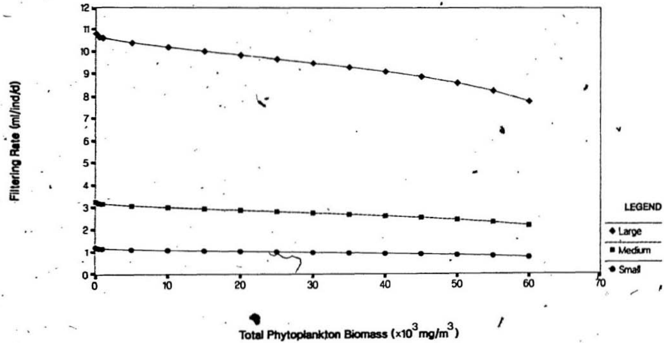
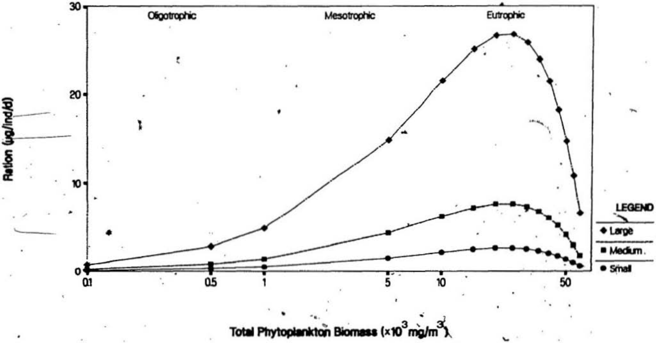


Figure 4-4: Predicted nannoplankton ration as a function of total phytoplankton biomass (increasing lake-trophic status).

Predicted Ration as a Function of Total Phytoplankton Biomass

Small, Medium and Large <u>Daphnia catawba</u>



4.5. Summary

The present study investigated the short, medium and long-term responses of *Daphnia catawba* to the manipulation of natural seston concentration and to seasonal variability in water temperature. Lake seston was either concentrated or diluted in *in situ* transparent polycarbonate carboys; six experiments of either four or eight days duration were conducted over a two month period in order to take advantage of seasonal lake water temperature fluctuations. Multiple linear regression analysis was used to examine the relationships between filtering rate, length-specific weight and fecundity of *Daphnia catawba* and variability in the food supply and water temperature.

Both filtering rate and net weight change in *Daphnia catawba* responded to the variability in food supply and water temperature; the order of importance of these variables was observed to be body size-dependent. The general seston components (chl *a*, POC, PON), which were most consistently manipulated in the enclosures, were not significantly correlated with either filtering rate or net weight change in *Daphnia catawba*. Only plankton biomass components, measured by microscopy, and water temperature explained significant levels of variation in the physiological variables. Fecundity responses to seston manipulation and fluctuating water temperature were minimal; adult *Daphnia catawba* appeared to allocate excess energy to growth rather than reproduction, a strategy possibly influenced by food limitation, low vertebrate predation on adults and relatively high invertebrate predation on juveniles in both Piccos Pond South and Quidi Vidi Lake. The low fecundity and low relative production rates observed for *Daphnia catawba* under conditions of food enhancement further supports the view that these animals are food limited during mid-summer in the lake due to conditions of minimum food availability and maximum water temperature.

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APPENDIX A

Zooplankton data

Log Dry Weight:Log Body Length and Log Daily Filtering Rate:Log Dry Weight functional regression relationships for *Daphnia catawba* from the seston manipulation enclosures in Piccos Pond South (PI) and Quidi Vidi Lake (QU) where (a)= intercept, (b)= functional slope and r= correlation coefficient. No data are provided for the 0.25x enclosure in Piccos Pond South, 18-26 July because only 10 *Daphnia* were present.

Date	Site	Encl	LogW:LogL			LogFR:LogW		
			a	b±95%CI	r	a	b±95%CI	r
July 18- July 22	PI	0.25	-2.53	2.64±0.321	0.92	0.65	1.29±0.351	0.45
		0.50	-2.65	2.83±0.538	0.77	0.34	1.30±0.306	0.60
		1.00	-2.50	2.60±0.437	0.83	0.42	1.10±0.279	0.52
		2.00	-2.67	3.18±0.595	0.85	0.20	1.21±0.334	0.62
		4.00	-2.50	2.64±0.624	0.84	0.38	0.95±0.343	0.55
July 18- July 26	PI	0.50	-2.46	2.39±0.739	0.75	0.38	1.90±0.768	0.50
		1.00	-2.46	1.99±0.323	0.84	0.51	1.44±0.412	0.27
		2.00	-2.60	3.08±0.947	0.77	0.35	1.34±0.617	0.29
		4.00	-2.53	2.28±0.546	0.76	0.32	1.23±0.429	0.30
		Lake	-2.57	2.95±0.438	0.86	-	- -	-
July 25- July 29	QU	0.25	-2.53	2.61±0.359	0.89	0.88	1.01±0.163	0.84
		0.50	-2.64	2.89±0.454	0.85	0.70	1.01±0.192	0.77
		1.00	-2.61	3.31±0.439	0.89	0.73	0.78±0.146	0.77
		2.00	-2.71	2.98±0.365	0.91	0.50	0.99±0.214	0.70
		4.00	-2.53	2.65±0.352	0.89	0.58	0.87±0.161	0.78
		Lake	-2.71	3.46±0.616	0.80	-	- -	-
Aug 26- Aug 30	QU	0.25	-2.58	2.61±0.355	0.89	0.49	0.89±0.263	0.07
		0.50	-2.81	2.98±0.447	0.87	-0.51	1.45±0.433	0.11
		1.00	-2.68	3.12±0.393	0.90	0.34	1.00±0.250	0.54
		2.00	-2.67	3.09±0.343	0.93	0.38	0.92±0.218	0.60
		4.00	-2.55	2.37±0.233	0.94	0.45	0.83±0.208	0.54
Aug 26- Sept 3	QU	0.25	-2.49	2.82±0.750	0.71	0.53	1.16±0.409	0.38
		0.50	-2.55	2.28±0.406	0.80	0.30	1.35±0.367	0.40
		1.00	-2.58	2.12±0.398	0.78	0.33	1.09±0.305	0.34
		2.00	-2.56	2.42±0.305	0.91	0.38	1.19±0.348	0.16
		4.00	-2.50	2.13±0.332	0.85	0.34	1.94±0.574	0.18
		Lake	-2.82	3.07±0.353	0.92	-	- -	-
Aug 31- Sept 4	PI	0.25	-2.58	2.86±0.321	0.92	0.47	1.09±0.245	0.66
		0.50	-2.75	3.24±0.333	0.94	0.48	0.82±0.160	0.76
		1.00	-2.53	2.69±0.501	0.81	0.62	0.74±0.199	0.54
		2.00	-2.73	3.23±0.288	0.95	0.38	0.69±0.137	0.74
		4.00	-2.72	3.40±0.378	0.93	-0.12	0.94±0.235	0.54
		Lake	-2.79	3.02±0.474	0.85	-	- -	-

APPENDIX B

Seston data

a. Daily chlorophyll *a* concentrations (mg m^{-3}) in the seston manipulation enclosures in Piccos Pond South (PI) and Quidi Vidi Lake (QU).

b. Initial and final day POC (mg m^{-3}) and PON (mg m^{-3}) concentrations in the seston manipulation enclosures in Piccos Pond South (PI) and Quidi Vidi Lake (QU). (*) indicates values below level of detection.

c. Initial day plankton biomass (mg wet wt m^{-3}) in the seston manipulation enclosures in Piccos Pond South (PI) and Quidi Vidi Lake (QU).

d. Final day plankton biomass (mg wet wt m^{-3}) in the seston manipulation enclosures in Piccos Pond South (PI) and Quidi Vidi Lake (QU).

a

Date	Site	Encl	DAY 0	1	2	3	4	5	6	7	8
July 18- July 22	PI	0.25	0.66	0.66	0.32	0.78	0.56	-	-	-	-
		0.50	1.12	0.96	0.87	1.21	1.55	-	-	-	-
		1.00	2.01	1.49	1.27	2.01	2.66	-	-	-	-
		2.00	2.88	2.72	2.07	3.86	4.20	-	-	-	-
		4.00	6.46	3.74	4.42	5.84	6.05	-	-	-	-
July 18- July 26	PI	0.25	0.69	0.69	0.50	0.81	0.56	1.27	1.80	2.66	2.44
		0.50	1.55	0.96	0.69	1.12	0.93	2.01	2.51	3.74	3.77
		1.00	1.86	1.64	1.33	2.60	2.60	3.34	3.15	4.54	4.73
		2.00	3.71	2.63	2.26	3.19	4.51	6.18	5.75	7.75	7.01
		4.00	6.18	4.91	4.54	7.29	6.98	8.18	7.87	9.60	9.45
July 25- July 29	QU	0.25	0.90	1.37	1.46	1.33	2.54	-	-	-	-
		0.50	1.55	1.86	1.95	2.14	2.85	-	-	-	-
		1.00	2.97	2.75	2.97	3.59	4.79	-	-	-	-
		2.00	4.23	4.17	4.82	6.42	8.74	-	-	-	-
		4.00	7.20	6.55	8.46	10.68	14.85	-	-	-	-
Aug 26- Aug 30	QU	0.25	0.56	0.25	0.22	0.25	0.41	-	-	-	-
		0.50	0.69	0.44	0.22	0.44	0.53	-	-	-	-
		1.00	0.90	0.84	0.59	0.84	1.27	-	-	-	-
		2.00	1.46	1.18	0.93	1.09	1.58	-	-	-	-
		4.00	2.01	[illegible]	1.61	1.70	1.95	-	-	-	-
Aug 26- Sept 3	QU	0.25	0.41	[illegible]	0.13	0.22	0.35	0.66	0.81	1.58	2.20
		0.50	0.72	0.62	0.32	0.44	0.72	1.06	1.21	1.92	2.51
		1.00	1.06	0.84	0.59	1.03	1.21	1.77	2.38	3.06	3.22
		2.00	1.40	1.12	0.81	1.18	1.18	2.35	2.48	3.09	3.43
		4.00	2.17	1.58	1.27	1.33	2.01	3.77	3.59	5.13	5.44
Aug 31- Sept 4	PI	0.25	0.72	0.69	0.56	0.44	0.50	-	-	-	-
		0.50	1.03	0.81	0.87	0.87	0.96	-	-	-	-
		1.00	1.58	1.33	1.27	1.30	1.64	-	-	-	-
		2.00	2.60	2.32	2.54	2.38	2.82	-	-	-	-
		4.00	4.14	4.30	4.23	3.83	5.10	-	-	-	-

b

Date	Site	Encl	POC (mg m^{-3})		PON (mg m^{-3})	
			Initial Day	Final Day	Initial Day	Final Day
July 18- July 22	PI	0.25	17.66	37.61	2.26	6.33
		0.50	21.08	35.33	3.16	2.86
		1.00	32.48	42.73	5.42	5.42
		2.00	46.72	63.76	5.42	5.93
		4.00	80.62	99.16	5.02	7.60
July 18- July 26	PI	0.25	23.31	50.84	0.00	4.26
		0.50	35.67	60.39	1.22	2.58
		1.00	40.73	73.31	3.19	4.41
		2.00	54.78	99.72	6.84	8.51
		4.00	90.17	118.26	10.64	8.36
July 25- July 29	QU	0.25	30.06	46.91	3.04	3.65
		0.50	32.86	46.35	2.74	3.04
		1.00	39.40	77.85	1.85	4.33
		2.00	49.17	104.82	2.78	6.49
		4.00	70.40	158.18	6.03	11.90
Aug 26- Aug 30	QU	0.25	9.01	4.99	0.00	0.93
		0.50	35.40	45.73	0.00	0.00
		1.00	19.91	14.74	0.46	0.15
		2.00	15.89	19.33	0.62	1.55
		4.00	32.53	25.07	2.63	2.16
Aug 26- Sept 3	QU	0.25	27.70	19.91	0.80	2.78
		0.50	15.32	21.06	1.55	2.01
		1.00	12.45	27.94	1.55	3.40
		2.00	22.20	33.68	3.09	3.86
		4.00	32.50	47.45	3.86	5.56
Aug 31- Sept 4	PI	0.25	16.47	21.06	2.63	3.25
		0.50	22.20	15.89	3.40	2.63
		1.00	19.33	16.47	2.16	2.94
		2.00	37.12	35.40	5.10	5.41
		4.00	54.91	47.75	6.96	5.10

c

Date	Site	Encl	Initial Plankton (mg wet wt m^{-3})						
			<2µm	2-5µm	5-10µm	10-30µm	<30µm	≥30µm	Total
July 18- July 22	PI	0.25	17.2	48.4	61.8	3.2	130.6	103.5	234.1
		0.50	39.4	57.5	35.6	91.5	224.0	151.8	375.8
		1.00	44.6	104.0	234.5	1448.7	1831.8	566.2	2398.0
		2.00	19.1	141.0	174.6	382.8	717.4	146.9	864.4
		4.00	20.5	77.1	122.5	379.4	599.5	707.2	1306.6
July 18- July 26	PI	0.25	26.8	97.5	96.8	300.4	521.5	16.3	537.8
		0.50	25.4	81.2	243.4	637.0	986.9	150.0	1136.9
		1.00	37.7	207.7	183.0	173.1	601.4	169.2	770.6
		2.00	16.1	57.6	68.4	69.8	211.9	184.8	396.6
		4.00	33.0	190.5	335.1	907.2	1465.9	809.7	2275.5
July 25- July 29	QU	0.25	43.1	91.4	162.9	79.2	376.6	272.3	648.9
		0.50	33.7	189.9	269.7	482.0	975.3	640.5	1615.8
		1.00	82.1	133.1	213.2	204.4	632.8	2908.5	3541.3
		2.00	38.5	180.4	298.4	320.3	837.7	2992.0	3829.7
		4.00	38.7	166.6	267.6	158.3	631.2	3176.8	3808.0
Aug 26- Aug 30	QU	0.25	29.8	191.0	141.7	0.0	363.5	53.7	417.2
		0.50	46.8	126.7	141.5	182.3	497.3	109.1	606.4
		1.00	37.5	90.7	81.7	125.1	335.0	120.1	455.1
		2.00	38.3	118.2	170.9	299.7	627.1	171.7	798.8
		4.00	37.3	118.8	180.0	507.9	844.0	275.2	1119.2
Aug 26- Sept 3	QU	0.25	40.1	176.0	66.0	0.0	283.1	37.2	320.3
		0.50	31.4	79.7	178.1	37.6	326.8	62.9	389.7
		1.00	42.6	149.7	99.3	150.7	442.4	313.2	755.6
		2.00	57.5	173.9	87.6	148.9	467.9	629.1	1097.0
		4.00	61.0	168.2	89.7	122.9	441.9	1246.4	1688.3
Aug 31- Sept 4	PI	0.25	30.5	48.2	86.0	50.1	214.7	3.7	218.4
		0.50	34.9	40.6	54.6	35.9	166.1	30.0	196.1
		1.00	16.6	36.5	16.0	80.8	149.8	277.7	427.6
		2.00	30.6	76.2	51.3	78.5	236.6	245.5	482.1
		4.00	54.8	103.3	127.5	244.8	530.3	638.6	1168.9

d

Date	Site	Encl	Final Plankton (mg wet wt m^{-3})						
			<2μm	2-5μm	5-10μm	10-30μm	<30μm	>30μm	Total
July 18- July 22	PI	0.25	11.0	62.2	67.3	65.0	205.5	145.8	351.3
		0.50	15.7	56.8	35.6	151.5	259.5	41.0	300.5
		1.00	25.2	69.3	92.1	233.1	419.7	259.3	679.0
		2.00	21.6	85.4	142.9	329.4	579.3	649.8	1229.1
		4.00	18.4	135.4	141.9	1392.1	1687.7	691.5	2379.1
July 18- July 26	PI	0.25	36.0	48.4	76.9	770.5	931.8	156.9	1088.7
		0.50	46.5	77.1	49.2	1304.9	1477.6	163.8	1641.3
		1.00	25.8	88.3	70.2	916.0	1100.3	263.6	1363.9
		2.00	34.3	137.0	229.1	1834.7	2235.1	769.1	3004.2
		4.00	35.6	123.9	88.5	1721.6	1969.5	373.4	2342.9
July 25- July 29	QU	0.25	33.9	86.4	415.8	1196.6	1732.7	97.3	1830.0
		0.50	34.6	131.4	771.1	1787.1	2724.0	107.2	2831.2
		1.00	38.3	86.8	371.3	536.6	1033.0	51.6	1084.5
		2.00	36.2	65.1	715.9	1652.1	2470.1	59.5	2529.6
		4.00	34.1	102.9	922.2	4399.1	5458.2	309.3	5767.6
Aug 26- Aug 30	QU	0.25	29.2	95.8	81.8	166.2	373.1	23.6	398.6
		0.50	29.5	86.8	63.9	171.6	351.7	65.9	417.6
		1.00	27.8	110.6	116.7	493.1	748.1	51.5	799.6
		2.00	28.5	88.9	43.8	491.5	652.7	65.6	718.3
		4.00	19.9	62.9	109.6	1077.9	1270.3	201.2	1471.5
Aug 26- Sept 3	QU	0.25	113.2	194.4	139.0	0.0	447.7	570.7	1018.3
		0.50	77.6	189.7	74.8	46.4	388.5	1480.4	1868.9
		1.00	63.6	171.6	119.8	132.2	487.2	3676.4	4163.6
		2.00	52.1	98.2	85.3	29.7	265.3	3022.0	3287.3
		4.00	53.3	81.7	117.6	29.7	282.3	5097.3	5379.6
Aug 31- Sept 4	PI	0.25	15.3	31.9	11.9	0.0	60.1	119.4	179.5
		0.50	10.5	29.5	24.9	11.9	76.7	26.4	103.0
		1.00	41.0	80.8	126.3	220.1	468.3	374.1	842.4
		2.00	12.5	40.7	13.0	114.1	180.3	277.8	458.1
		4.00	14.1	67.9	43.2	120.6	245.8	611.8	857.5

APPENDIX C

Fecundity data

Fecundity data for *Daphnia catawba* raised in seston manipulation enclosures in Piccos Pond South (PI) and Quidi Vidi Lake (QU). N= number of females, N_{mat}= number of mature (>1.1 mm) females, Ovig.= number of ovigerous females, Total Eggs= total number of eggs carried by all ovigerous females, %Ovig.= percentage of mature females bearing eggs and, Eggs/100= number of eggs per 100 mature females. No data are provided for the 0.25x enclosure in Piccos Pond South, 18-26 July because only 10 *Daphnia* were present.

Date	Site	Encl	N	N_{mat}	Ovig	Total Eggs	%Ovig	Eggs/100.
July 18- July 22	PI	0.25	46	31	0	0	0.00	0.00
		0.50	48	41	1	1	2.44	2.44
		1.00	48	38	0	0	0.00	0.00
		2.00	35	24	3	3	12.50	12.50
		4.00	25	17	0	0	0.00	0.00
July 18- July 26	PI	0.50	22	19	1	1	5.26	5.26
		1.00	48	40	1	1	2.50	2.50
		2.00	21	14	0	0	0.00	0.00
		4.00	33	20	0	0	0.00	0.00
		Lake	48	38	0	0	0.00	0.00
July 25- July 29	QU	0.25	48	37	1	1	2.70	2.70
		0.50	48	43	3	5	6.98	11.63
		1.00	48	39	3	4	7.69	10.26
		2.00	48	43	0	0	0.00	0.00
		4.00	48	39	1	1	2.56	2.56
		Lake	48	40	1	1	2.50	2.50
Aug 26- Aug 30	QU	0.25	48	31	1	1	3.23	3.23
		0.50	48	30	1	1	3.33	3.33
		1.00	48	30	1	1	3.33	3.33
		2.00	48	30	1	2	3.33	6.66
		4.00	48	30	1	1	3.33	3.33
Aug 26- Sept 3	QU	0.25	31	8	0	0	0.00	0.00
		0.50	48	26	0	0	0.00	0.00
		1.00	48	26	0	0	0.00	0.00
		2.00	48	30	0	0	0.00	0.00
		4.00	47	21	0	0	0.00	0.00
		Lake	48	40	1	1	2.50	2.50
Aug 31- Sept 4	PI	0.25	48	31	0	0	0.00	0.00
		0.50	48	34	1	1	2.94	2.94
		1.00	42	22	0	0	0.00	0.00
		2.00	48	35	1	2	2.86	5.71
		4.00	48	39	2	3	5.13	7.69
		Lake	48	47	0	0	0.00	0.00