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MASTER

DETERMINATION OF CLADOCERA PRODUCTION

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OF

TORGNY J. VIGERSTAD

APPROVED:

Dissertation Committee:

Major Professor ______________________

University Advisor ______________________

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Dean of the Graduate School

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UNIVERSITY OF RHODE ISLAND

1980

DETERMINATION OF PRODUCTION BIOLOGY OF CLADOCERA IN A RESERVOIR RECEIVING HYPERTHERMAL EFFLUENTS FROM A NUCLEAR PRODUCTION REACTOR

BY

TORGNY J. VIGERSTAD

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
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IN
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ABSTRACT

The purpose of this study was to examine the effects on zooplankton of residence in a cooling reservoir receiving hyperthermal effluents directly from a nuclear-production-reactor. The design of the study was to compare rates of cladoceran population production at two stations in the winter and summer of 1976 on Par Pond, the cooling reservoir located on the Savannah River Plant, Aiken, S. C. One station was located in an area of the reservoir directly receiving hyperthermal effluent (Station MAS) and the second was located about 4 km away in an area where surface temperatures were normal for reservoirs in the general geographical region (Station CAS). The statistical properties of the Edmondson egg ratio model (Edmondson, 1960) were examined to determine if it would be a suitable method for calculating cladoceran production rates for comparison between stations. Based on an examination of the variance associated with standing stock and fecundity measurements, disagreement in the literature on the exact formulations to use for calculations, a relatively large number of published negative death rate calculations (58% in one study), the inability to choose among three different methods of assigning confidence intervals to egg ratio parameters, and the sensitivity of the model to uncertainty in instar duration calculations over the range of temperatures encountered in Par Pond, the use of the egg ratio model was abandoned.

Instead, a non-parametric comparison between stations of standing stock and fecundity data for Bosmina longirostris, taken for the egg

ratio model, were used to observe potential hyperthermal effluent effects. There was a statistically higher incidence of deformed eggs in the *Bosmina* population at Station MAS in the summer. *Bosmina* standing stock underwent two large oscillations in the winter and three large oscillations in the summer at Station MAS compared with two in the winter and one in the summer at Station CAS. These results are consistent with almost all other Par Pond studies which have found the two stations to be essentially similar in species composition but with some statistically significant differences in various aspects of the biology of the species.

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This thesis is dedicated to my parents, Alice and Josef Vigerstad, who supported me as only truly loving parents can.

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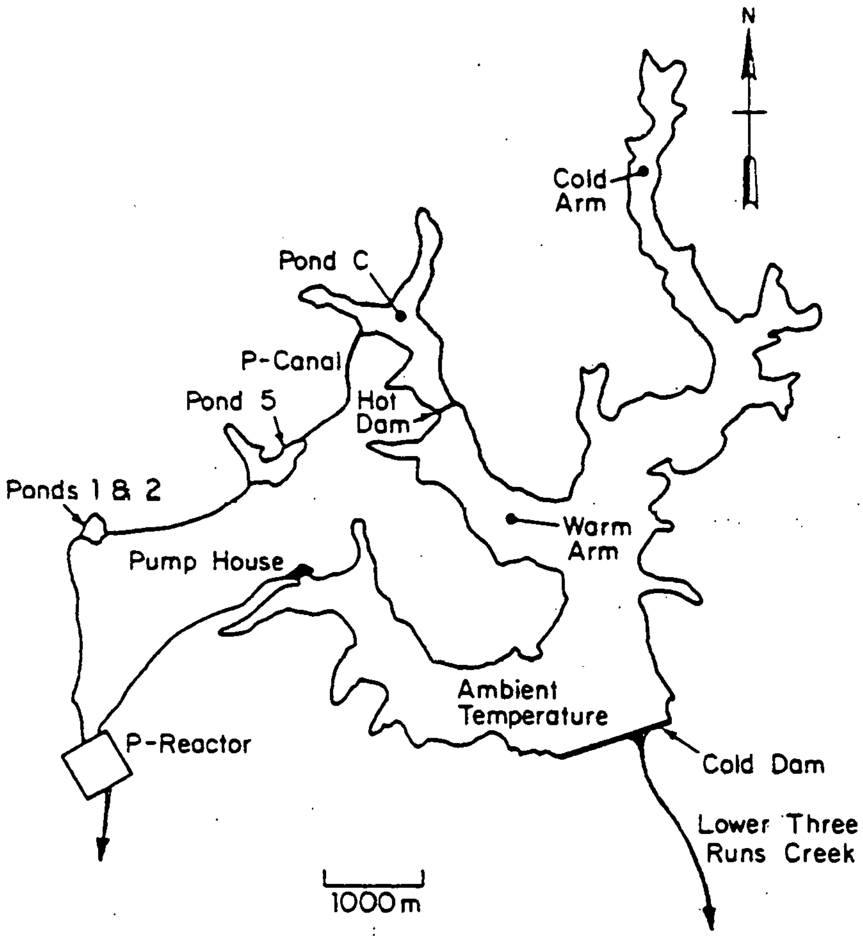
INTRODUCTION

The purpose of this study was to examine the effects on zooplankton of residence in a cooling reservoir receiving hyperthermal effluents directly from a nuclear-production-reactor. The study had two objectives: to determine whether zooplankton biology, especially production rates, was affected by hyperthermal effluents, and to examine in detail the method used to measure production rates so that the study could be useful in discovering future directions for research on zooplankton in the study area and elsewhere.

This study was carried out on Par Pond, the reactor cooling reservoir on the Savannah River Plant (Fig. 1), built in 1958 by damming of Lower Three Runs Creek, in Barnwell County, South Carolina. Par Pond was originally filled with heated Savannah River water effluent from the P and R production reactors on the Savannah River Plant. R reactor was removed from service in 1964, leaving the P reactor as the only current source of effluent into Par Pond. Most of the published information on Par Pond concerns the effects of the operation of the P reactor on the Par Pond system. The Par Pond system consists of 7.1 km of cooling canals, six precooler ponds, and Par Pond itself (Neill and Babcock, 1971, Fig. 1). "Reactor operations" involve flow, heat, and the addition of water from the Savannah River used to compensate for losses to evaporation of the recirculating Par Pond water.

This reservoir has been the object of impact studies since its

Figure 1 - The Par Pond cooling system.


N
Cold Arm
Pond C
P-Canal
Pond 5
Hot Dam
Ponds 1 & 2
Pump House
Warm Arm
Ambient Temperature
P-Reactor
Cold Dam
Lower Three Runs Creek
1000 m

construction by the Savannah River Laboratory (Department of Energy), the Savannah River Ecology Laboratory (University of Georgia) and independent investigators from colleges and universities throughout the United States (Murphy and Gibbons, 1977). Publications on Par Pond invertebrate consumers are represented by Bowen (1976) alone. There are none on zooplankton. Although there is some suggestion that the zooplankton are a minor component of the total carbon budget of lakes in general (Wetzel, 1976), they are included as a major source of secondary production in most of the major lake system models (Park, 1975). Regardless of their contribution to the carbon budget of a lake, their intermediate position in the food chain may cause them, at least to some extent, to regulate events at lower levels (Brooks, 1969; Porter, 1973; Fliwicz, 1975) and reflect events at higher levels (Dodson, 1974; Brooks, 1969). Their study, therefore, fulfills a need for information necessary to understand the Par Pond reservoir system and could be useful in other similar cooling system operations.

The scope of the project narrowed from a general examination of zooplankton biology to a more detailed study of the population dynamics of a particular group representative of the type of response expected from most zooplankton species. Cladocerans were chosen for several reasons. There are no distinct or particular juvenile stages which make identification difficult, as in the Copepoda. The adults carry their eggs until molting so that fecundity can be readily observed. They have been the subject of several population studies in the recent ecological literature (Keen, 1973; George and Edwards, 1974; DeBernardi and Guissani, 1975; Kerfoot, 1975) and are therefore reasonably well known. A method of estimating their population parameters such as birth rate and death rate also appeared readily available (Edmondson, 1971).

The original design of the study, therefore, was to calculate the rates of cladoceran population production using the Edmondson egg ratio methods (Edmondson, 1960). Calculations made from samples taken at a station directly receiving hyperthermal effluents were to be compared with those made from samples at an ambient station. Because there were no published accounts of how to calculate confidence intervals for the egg ratio model, several approaches were examined and compared using data collected from Par Pond and from other investigators.

Zooplankton population measurements are assumed to have an interval value and the statistical methodology recommended usually depends on the use of normal statistics (Edmondson and Wineberg, 1971).

The standing stock data for Bosmina were used to calculate several population dispersion indices which, when compared with values in the literature, might indicate if the observed sampling variance was unusually high. The variance, along with the number of samples taken, determines the power of the measurements to detect differences. The power was used to determine the number of samples that a sampling program would need to answer a specific question about population production and to estimate the costs of sampling programs. Results of previous Par Pond studies have received national attention in scientific journals (e.g., Gibbons and Sharitz, 1974) and have been cited during drafting by the Environmental Protection Agency of water quality legislation for the nuclear power industry (Lauer, 1974). While a great deal of money, time and effort has been expended on these studies, the assigning of statistical power to the sampling programs and actual costs involved had yet to be addressed. Standing stock sampling variance, egg production sampling variance and potential variance around instar duration measurements were also evaluated for their effect on the precision of the Edmondson egg ratio model in general and in this study in particular.

Other aspects of the Edmondson egg ratio model were also examined. An important parameter in Edmondson's model for birth rate is the rate of egg development, which is usually strongly correlated with environmental temperatures for Cladocera (Hall, 1964). Most investigators applying Edmondson's model to cladoceran populations (Hall, 1964; Wright, 1965; Kerfoot, 1975) have used mean water column temperature as the estimate of the temperature to which the population is exposed. However, Edmondson (1974) suggested that vertical migration may result in a thermal history for a population different from the mean water column temperature. Based on laboratory experiments and a review of the literature, Gehrs (1974) hypothesized that heated wastewater effluents could restrict the vertical migration of zooplankton.

Edmondson's suggestion would be particularly relevant for a reservoir receiving hyperthermal effluents, if the laboratory results of Gehrs (1974) proved to also occur in the field. If thermal effluents interfere with the migration of cladocera, the result would be a lower or higher thermal history for a population than would be predicted from average water column temperatures. Using average water column temperatures might lead to erroneous conclusions about the effects of hyperthermal effluents. In Appendix B is a test of Gehr's (1974) hypothesis that thermal effluents interfere with cladoceran migration and a calculation of the thermal history of the population to to determine if any bias would result from the use of average water column temperature in the calculation of birth rates.

Edmondson's model (1960) assumes a stable age distribution for the animals in the population. In examining this assumption, the possibility of synchronous egg hatching was discovered and evidence for this phenomenon was gathered from as many of the different sampling programs as was possible (Appendix C). Besides being of general biological interest, a report of synchronic egg production is important to investigators studying population dynamics, because synchrony of egg hatching can cause serious errors to be made in the estimation of standing egg stocks from samples taken only once during a day (Gophen, 1978).

Based on the examination of the variance associated with standing stock and fecundity measurements, a careful review of the published applications of the egg ratio model (reported in the Review of the Literature chapter), the inability to choose among three different methods of assigning confidence intervals to the egg ratio parameters, and the mathematical properties of the model, the use of the Edmondson egg ratio model was abandoned. Instead, a non-parametric comparison between stations of standing stock and egg production data for Bosmina longirostris taken for the egg ratio model was used to evaluate hyperthermal effluent effects.

All of the published values on thermal tolerance are based upon laboratory experiments (Goss & Bunting, 1976). While the regular sampling program for population dynamics was underway, an opportunity arose to attempt to measure thermal tolerance in a field situation and this study is reported in Appendix D.

REVIEW OF THE LITERATURE

The environmental effects of effluents from electrical power generating plants have attracted considerable attention during recent years (Coutant, 1970, and et seq.; Gallagher, 1977; Gibbons and Sharitz, 1974a; Esch and McFarlane, 1976). Undoubtedly, this is in response to an increased density of conventional (fossil fuel) power plants and anticipated shifts to nuclear power plants which often require 50% more colling water than comparable conventional plants (Cairns, 1971). According to Schurr (1971), the U. S. Federal Power Commission estimated that power station waste heat would increase from the 1970 value of 5.3 x 10^{15} Btu/year to 12.8 x 10^{15} Btu/year in 1980 and 28.4 x 10^{15} Btu/year in 1990. In 1990, cooling requirements would be 470 billion gallons per year which is 39% of the national average stream flow. This would appear to be a reasonable rationale for concern about the environmental effects of thermal effluents.

Available methods of providing for cooling capacity of water at a power station include: once-through-cooling, cooling lakes, and cooling towers. Once-through-cooling refers to the practice of taking water from the upstream area of a power plant, and discharging the heated water downstream of the plant. When cooling lakes are used, water is recycled after being cooled in a reservoir. Cooling towers provide a larger surface area where cooling water has contact with the surrounding atmosphere before being reused or released to any body of water (Schurr, 1971).

Once-through-cooling is the least expensive (cost per megawatt) method of cooling a power plant. However, if once-through-cooling is not possible or permissible, then cooling ponds (reservoirs) provide the second least expensive method, especially where land is cheap (Schurr, 1971; Mullenbach, 1974). An added attraction of cooling reservoirs is their potential use as recreational areas (Harmsworth, 1974). The multiple uses of cooling reservoirs necessitate management of the reservoir to ensure water quality that is compatible with the public interest. It was perceived by the U.S. Atomic Energy Commission that there would be two diverging opinions on the definition of public interest (Wash. 1169, 1971). One opinion held that the management of desirable species was the most important goal of management. Others considered the goal to be to preserve an unaltered system. According to the AEC in 1971, the goal of research was to determine the thermal receiving capacity of each unique aquatic system which would determine the amount of heated water that could be safely introduced (Wash. 1169, 1971). This statement appeared to favor the "assimilative-capacity-of-ecosystems" concept (Cairns, 1974, 1976). According to Cairns, most freshwater environments are capable of assimilating a certain amount of heated wastewater discharges (and other types as well) without being seriously degraded (Cairns, 1974).

In 1969, the Division of Biology and Medicine of the Atomic Energy Commission (now incorporated into the Department of Energy) began funding ecological studies on Par Pond, a cooling reservoir located on the Savannah River Plant (SRP) near Aiken, S.C. At that time it was hoped that the information gathered from Par Pond studies would be useful to the siting and regulation of commercial nuclear power plants.

Par Pond is today the most extensively studied reservoir in South Carolina.

Lewis (1974a) has described, in some detail, the pattern of temperatures created by addition of the hyperthermal effluents to Par Pond. He also described surface slicks (Lewis, 1974b) and the possible addition of nutrients to the reservoir resulting from the controlled burning of nearby pine plantations (Lewis, 1974c). Marshall and Leroy (1971) and Tilly (1975) have described the general and unique water characteristics of the cooling reservoir. Marshall and Tilly (1971) and Tilly (1973, 1974, 1975) have observed the changes in planktonic primary productivity due to reactor operations. Boyd (1970a, b, c) was primarily interested in the chemical composition of the dominant emergent vegetation. Parker, et al. (1973) and Grace and Tilly (1975) have characterized the emergent and submerged macrophyte communities. Bowen (1976) examined the distribution and abundance of the Ostracoda found in Par Pond. Mosquito fish (_Gambusia affinis_) reproduction and fat content have been described by Ferens and Murphy (1974) and Falke and Smith (1974). Mosquitofish parasite loads were examined by Aho, et al. (1976). Largemouth bass (_Micropterus salmoides_) have been the subject of the largest number of published accounts. Several aspects of their biology, including catchability (Gibbons and Bennett, 1973; Gibbons, et al., 1972), body temperature (Bennett, 1971), food habits of adults (Bennett and Gibbons, 1972) reproductive cycles (Bennett and Gibbons, 1975), growth rates of juveniles (Bennett and Gibbons, 1974), thermal tolerance and biochemical polymorphism of juveniles (Smith and Scott, 1975) and dominant endoparasites (Eure and Esch, 1974) and exoparasites (Fliermans, et al., 1977), have been studied. Bluegill

(*Lepomis machrochirus*) thermal tolerance (Murphy, et al., 1976; Holland, et al., 1974) and its relationship to the biochemical genetics of the species (Yardley, et al., 1974; Avise and Smith, 1974) has received attention. Gibbons (1970) and Christy, et al. (1974) have reported on the biology of a population of *Pseudemys scripta* (yellow bellied turtle) inhabiting the reservoir and Bourque and Esch (1974) have described the parasite load of that population. An alligator population in Par Pond has been followed by Murphy and Brisbin (1974) and Brisbin (1974) has reported on the winter waterfowl community. The results of most of these studies have been included in general reviews of thermal effects research on the SRP by Beyers (1974), Gibbons and Sharitz (1974b) and Gibbons, et al. (1975).

The published literature up until 1964 on cladoceran biology has been reviewed by Hutchinson (1967). Since then, the two most active areas of research on cladoceran population biology have been on the effect of selective predation by planktivorous fish on the size, shape, color, and even survival of cladoveran species (see review by Hall, et al., 1976) and studies on population dynamics using the Edmondson egg ratio model and other methods (reviewed later on in this section). There have been relatively few published studies of hyperthermal effluent effects on cladoceran population biology in freshwater lakes. Patalas (1970) concluded that a 6° increase in a heated lake did not affect the standing crop of herbivorous zooplankton, but based on values taken from the literature, he speculated that production was probably double that of a nearby unheated lake. Fenlon, et al. (1971) observed large increases in standing crops of *Bosmina* and *Daphnia* near the hyperthermal discharge of a nuclear power plant on Lake Ontario.

Measurements of the realized rate of increase (r) could not, however, support the hypothesis of enhanced reproductive rate in these species. Brauer, et al. (1974), attributed similar increases in standing crop of total zooplankton in the vicinity of a nuclear power plant outfall on Lake Monona, Wisconsin, to the circulation of reactor cooling water that transported zooplankton-rich limnetic water into the littoral zone.

Whitehouse (1971) examined the cycles of abundance and number of *Bosmina obtusirostris*, *Daphnia longispina*, *Diaphanosoma brachyurum* and *Ceriodaphnia quadrangula* in Lake Trawsfyndd, a lake receiving discharge of warmed water from a power station in Wales. He could not observe any changes in the composition, abundance, or in the timing of periods of increase and decrease of populations that could be directly attributed to the discharge of the hyperthermal effluent.

Cladoceran spatial distributions have also received relatively little attention (see Hutchinson, 1967) despite Hutchinson's (1953) discussion of the importance of pattern to process in ecological systems. The general approach has been to fit a series of samples to a known mathematical density function (Cassie, 1961). Several investigations have employed various indices of dispersion and related correlation techniques in an attempt to understand the significance of pattern formation in cladocerans (Dumont, 1967; George, 1974; Whiteside, 1974). All these studies have found cladoceran populations to have been distributed in a non-random pattern. Cassie (1961) and Whiteside (1974) used a negative binomial distribution to describe the copepod and cladoceran spatial distributions. Stavn (1971) has explored the behavioral mechanism that may be the cause of observed clumping in *Daphnia* populations. Extreme clumping, described as swarming, has been observed by Ratzlaff (1974) in *Moina micrura* populations. Because of this clumping, which results in non-normal or skewed sampling distributions, general methods texts such as Eliot (1977) often suggest that zooplankton population estimates be mathematically transformed to meet the assumptions of normal statistics.

As stated in the introduction, the original intent of research for this thesis was to use the Edmondson egg ratio model (Edmondson, 1960, 1968) to estimate the birth rate, death rate, and realized rate of increase for *Bosmina longirostris* in Par Pond. When Edmondson first proposed the egg ratio model, he began with the notion that the finite death rate per individual (P) of an existing population (N_o) during an interval of time (T) could be estimated by the difference between the expected population size at time T ($N_{T\ calc}$) and the observed population size at time T (N_T) by the equation

$$P = \frac{N_{T\ calc} - N_T}{TN_o} \qquad (1)$$

where

$$N_{T\ calc} = (B \cdot T \cdot N_o) + N_o \qquad (1a)$$

B in equation 1a is the rate of egg laying as eggs per female per day derived by dividing the number of eggs per female by the time it takes for the eggs to hatch.

The rotifers with which Edmondson was working were convenient for this model because they carried their eggs until hatching and the eggs could be easily counted. Edmondson noted that in most routine limnological work, the time between collections is fairly long relative to the generation time of the rotifers. Equation 1 assumes linear growth and, therefore, it would underestimate population growth by ignoring the reproduction of animals produced early in the interval. Thus, it seemed more useful to Edmondson to adopt Lotka's formulation of population growth which accounts for the tendency of populations to grow geometrically:

$$N_T = N_0 e^{rt} \tag{2}$$

Using equation 2, the death rate, d, would be calculated as the difference between the realized rate of increase $\underline{r}$ and the expected rate of increase assuming no mortality, $\underline{b}$:

$$d = b - r \tag{3}$$

r was estimated by the formula

$$\underline{r}' = \ln(N_t/N_0)/t \tag{4}$$

and b from the formula

$$\underline{b}' = \ln\left(\frac{E/F}{D} + 1\right) \tag{5}$$

where E was the number of eggs in the population, F the number of females, and D the duration of development to the time of hatching of an egg.

The use of Lotka's exponential growth model makes to important assumptions. First, reproduction and death rates must be continuous over the interval measured and, secondly, there must be a stable age structure to the population. If there were a large difference in egg ratio at the beginning and the end of the sampling period, the second assumption might be questioned. As an alternative, Edmondson suggested averaging the values of $\underline{b}'$ (equation 5) at the beginning and the end of the period which would assume an even change during the period. Since his *r*, *b*, and *d* parameters were calculated from samples (and were therefore based on discrete events separated by finite intervals), he placed a prime designation (*r*', *b*', *d*') behind his parameters to denote the fact they were based on measurements and not derived from age specific mortality tables; thus, he emphasized that they were an average rate for an interval. Despite these criteria, Edmondson argued that his rotifer population did meet the assumptions of the Lotka model during the times when "the environmental conditions were not sharply changing" (Edmondson, 1960). He added a statistical caution, noting that all the parameters would be subject to field sampling error, especially the *d*' determination because it was calculated from values, both of which had errors associated with them.

Hall (1964) was the first to use the egg ratio method on Cladocera. Cladocera reproduce primarily by parthenogenesis, although in some species, males are produced at certain times of the year, after which resting or overwintering eggs are laid. The life cycle includes egg, immature, and adult stages. Cladocerans brood their eggs and release their young during molt (Hutchinson, 1967). The trait of parthenogenesis, which eliminates the need for consideration of time between fertilizations and the brooding of eggs in a transparent pouch, makes cladoverans a suitable animal for the egg ratio. Hall chose to study *Daphnia galeata mendotae* in Base Line Lake, Michigan. He determined from laboratory experiments that temperature was the dominant factor affecting the rate of instar development as compared to the effect of a sixty-four fold increase in food level. (Instar development was measured because egg development itself may or may not coincide with molt, making molting or hatching rate the critical 'D' (equation 5) measurement for Cladocera). After measuring instar durations at various temperatures in the laboratory, he used the average water column temperature of the lake to determine the rate of hatching of eggs. The $\underline{b}'$ and $\underline{d}'$ he calculated showed him that birth rate and death rate were highest in the summer months and he could correlate the high $\underline{d}'$ with a large increase in an invertebrate predator population (*Leptodora kindtii*: Cladocera).

Weight (1965) repeated this study on a population of *Daphnia schödleri* in Canyon Ferry Reservoir, Montana. He also observed a large increase in death rate concurrent with a large increase in *Leptodora* population.

In a 1968 paper, Edmondson discussed his method in further detail, this time with reference to the Cladocera. He again noted that the values of $\underline{b}$ and $\underline{d}$ calculated from his model were based on discrete events separated by finite intervals that would differ from those originating from pure mathematical theory. Commenting on other methods such as the Leslie matrix, he stated that instar analysis appeared to be impractical with many species because of the small increments in size with each molt. Size frequency analysis, with the larger Cladocera, he thought possible to accomplish (see Hairston and Pastorak, 1975). He also noted a problem with his method of calculating $\underline{b}'$. He thought a bias would be introduced by the fact that the egg age distribution would not be uniform in a growing population. He proposed a new formula (equation 6) which, he stated, involved no assumptions about the age distribution of the eggs.

$$\underline{b}' = \frac{\ln(1 + (\frac{E}{F}))}{D}$$

Cummins, et al. (1969) applied the egg ratio model to five species of Cladocera (the predaceous _Leptodora_ and four possible cladoceran prey species) in Pymatuning Reservoir, Pennsylvania. They did not use Edmondson's change in the birth rate formula (equation 6). Amassing 74 determinations of death rate on each of the four prey species and 195 estimates of death rate for _Leptodora_, they then calculated Pearson's-r-correlation-coefficients of _Leptodora_ _d'_ with each individual prey species _d'_ for different combinations of dates. They concluded that the predator-prey relationships were complex and that _Leptodora_ usually depended upon at least two prey species at any given time. The only discussion of the results of the _b'_, _r'_, and _d'_ determination for the prey species involved mention of the ranges observed.

In a 1971 publication of IBP (International Biological Program) methodology, Edmondson (1971) presented equation 5. Dodson (1972) calculated _b'_ also using equation 5 for a population of _Daphnia rosea_ in Leechemere Pond, Colorado. He compared the _b'_ using the egg ratio model to a linear model he was able to develop because _Daphnia_ in his alpine pond hatched from ephippial eggs as a cohort which he could follow. He found that the estimates of _b'_, as he had calculated them from the egg ratio model, were significantly higher (sign test, n = 11) than those of a linear model of which he was more confident. He therefore discarded the egg ratio model in favor of the linear model and used it to show that 90% of the mortality of _Daphnia_ population could be explained by predation by the invertebrate predator _Chaoborus_.

Keen (1973) noted that Edmondson's formulation (equation 5, not 6) determined _b'_ independent of the time interval of sampling (t). He made what he termed a "consistent and probably more realistic" formulation

$$b' = [\ln (N_0 + \frac{E \cdot t}{D}) - \ln N_0]/t \qquad (7)$$

where N_0 is the initial population size, E is the eggs per female, D is the instar duration, and t is the sampling period. His equation assumed that the eggs-embryos have a uniform age distribution, that all eggs have an equal time of replacement, and that brooding females are not recruited to increase E during time t. He argued that using the time interval between samples made b' time dependent and avoided the assumption of exponential growth because it predicted the number of eggs being hatched over time. However, the argument seems to be correct only if t is short relative to D.

Edmondson (1974) in a further discussion of his method noted that even if the rate of egg laying were quite uniform, the age distribution would be skewed in a growing or declining population. The size of the effect would be related to D and the rate of change, r, but he stated that the error would only be on the order of 10%. Noting that this had happened, Paloheimo (1974) rederived the b' formula in a way that accounted for the dependence of b on r. His formula was exactly the same as Edmondson (1968) had prepared before (equation 6). When Paloheimo compared the results of the two methods of calculation, he found them to be different by as much as 30%, depending on the size of D. This alone may account for the difference that Dodson (1972) observed between his linear model and the egg ratio model (calculated using equation 5, but sufficient data are not available in Dodson's paper to recalculate his birth rates using equation 6). Paloheimo, while stating that his reformulation cleared up the mathematical difficulties of calculating b', acknowledged that he did not know how any violation in the assumption of steady state conditions would affect the calculations.

De Bernardi and Guissani (1975) used Edmondson's method to determine that the largest b' and d' values for *Daphnia hyalina* in Lake Maggiore, Italy, occurred in the summer. These high d' values were correlated with large population increases of two predaceous cladocerans, *Bythotrephes longimanus* and *Laptodora kindtii*. Using a compartmental analysis model, the death rates for *Daphnia* population as a whole were divided between young and adults. The young were seen to suffer the largest impact at the time the invertebrate predators were present, which supported the notion that the invertebrate predators (as opposed to vertebrate predators) were responsible for the major share of mortality. Exactly which of Edmondson's equations were used by Bernardi and Guissani was not mentioned.

Kerfoot (1975), working with Dr. Edmondson, compared b' and d' for *Bosmina longirostris* at two stations in Lake Washington. He used the d' values to support the hypothesis that morphological differences between the populations at the two stations altered the effects of predation on the population dynamics. Kerfoot did incorporate the formulation for b' suggested by Paloheimo.

Examination of the results of the calculation of death rates (d'_ in published results revealed what appeared to be a problem in the use of the Edmondson model. Theoretically, death rate can only have a minimum value of zero. However, Hall (1966) obtained negative values of d' twice out of 29 observations. Wright (1965) calculated negative d' twice out of 22 observations; Cummins, et al., (1969) had 245 negative results out of a total of 420 observations on 5 species (58%); Keen (1973) had 40 out of 318 on 4 species; and Kerfoot (1975a) calculated 6 negative d' values out of his 36 observations. Furthermore, in the case of Cummins, et al., Keen, and Kerfoot, the negative d' values were the greatest or nearly the greatest in absolute value of all the d' values for each species.

This observation, the literature which showed cladoceran populations to sometimes have a non-normal distribution, and a lack of discussion about the statistics of the model in the literature in general, led me to be concerned about the amount of quantitative precision I could achieve using the Edmondson egg ratio model with the population data I collected. The use of the Edmondson model has been extremely useful in making several important qualitative observations. For example, it was used by Hall (1969), Wright (1965) and others to show that predators are often primarily responsible for low cladoceran standing stocks in the summer. However, I needed a way to compare production rates at two stations over a relatively short period of time (one year or less), which might require a fairly high level of quantitative precision.

METHODS AND METHODS DEVELOPMENT

Description of the study area

The Par Pond cooling water, a mixture of 17/18 Par Pond water and 1/18 water taken from the Savannah River, leaves P reactor at about 80°C in the summer months and passes through 7.1 km. of canals and six precooler ponds (Neill and Babcock, 1971). The heated effluent enters Par Pond about 10°C higher than natural water temperature. The waste heat is horizontally distributed with little mixing near the discharge and is principally restricted to the upper two meters (Lewis, 1974a). This distribution pattern causes a secondary, artificial thermocline during periods of natural stratification. Lewis (1974a) presented data and arguments to show that hypolimnetic temperatures are approximately 5°C warmer than for a comparable lake not receiving an effluent. Unpublished studies by Dr. L. J. Tilly, Savannah River Laboratory, (personal communication) indicate that surface temperatures are similar to comparable lakes in the area.

According to Tilly (personal communication), reactor operations include several processes which may affect water quality:

1. The Savannah River water added to Par Pond waters before the water passes through the reactor is equal to the annual rainfall run-off to Par Pond and is higher in nutrients than local drainage because of its Piedmont and industrial-urban area origin.
2. Water pumped from Par Pond for cooling is taken 6 m beneath the surface and is likely to be higher in nutrient levels than the surface waters with which it is eventually mixed.

3. The death of organisms which are entrained in the cooling water, as well as the physical and chemical action of the temperatures in the heat exchange system (80°C) are likely to cause changes in both nutrient quality and quantity as well as increases in amounts of suspended material.
4. As water passes through the precooling canals and ponds its quality is potentially influenced by the resident populations of algae and bacteria.

Par Pond is generally described in Table 1.

Pond C, the last of the precooler ponds, is 56.7 ha (140 acres) in area and has a mean depth of 3 m. The entire water column of Pond C may be as much as 20°C above the normal Par Pond temperature. It is separated from the body of Par Pond by a dam and culvert (Fig. 2).

Sampling stations

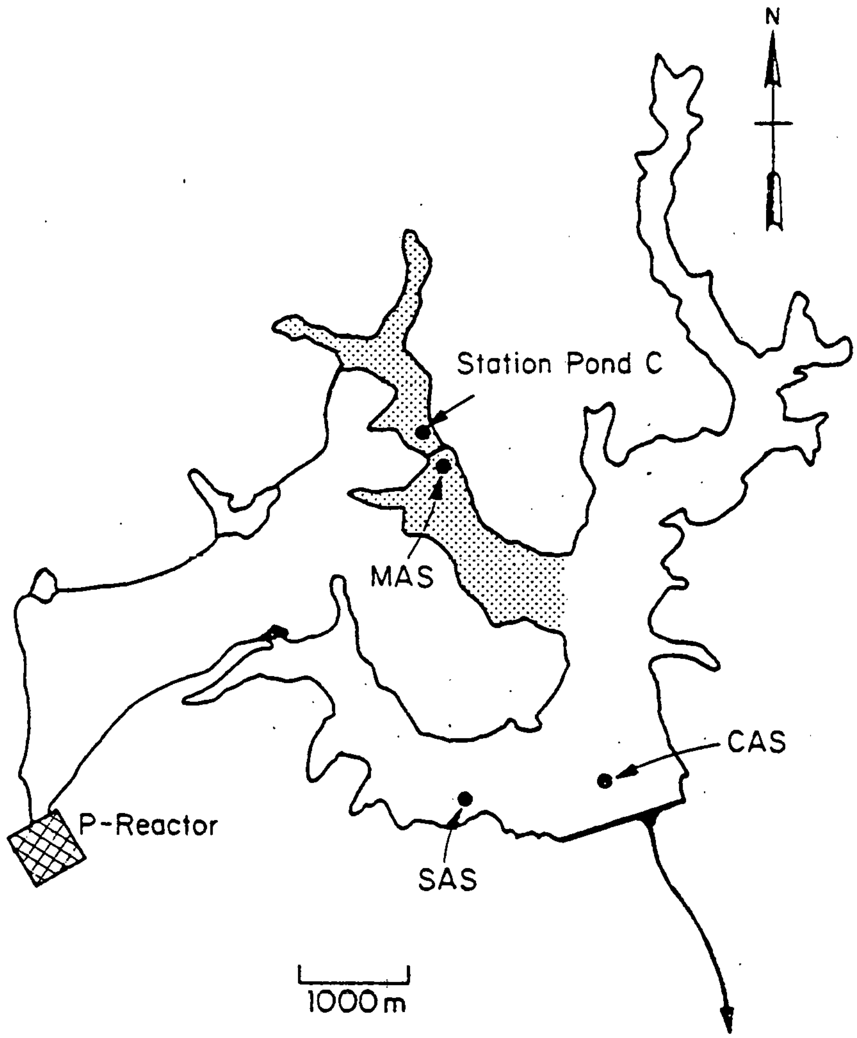
Most of the data in this study of reactor operations effects on zooplankton come from the comparison of samples taken at two stations on Par Pond, MAS and CAS (Fig. 2). Station MAS is located in the central position of the arm of Par Pond which receives hyperthermal effluents. The depth of the station is 8 m. Surface current-meter measurements, there dye studies and 10 years of temperature measurements ahve shown that station MAS is in the area of the middle arm least affected by flow (Vigerstad and Kiser, 1977; Appendix E). Station CAS is in the deepest portion of Par Pond, 16 m; no prevailing current has been detected there (Tilly, personal communication), and the station has normal temperatures.

Table 1. General features of Par Pond[1]

Area	$1.0 \times 10^7 m^2$
Mean Depth	6.2 m
Euphotic Zone	6 m
Secchi Transparency	2.5 m
Turbidity	1.2 JTU
Total Alkalinity	15.0 ppm $CaCO_3$
Total Dissolved Solids	40 ppm
pH	7.7
Dissolved Oxygen	7.1 ppm

1. Mean values are from Tilly (1973) and based on samples taken from the unheated portion of Par Pond during June - August 1967. Water quality data are from the euphotic zone in the normal temperature portion of the reservoir.

Figure 2 - Map of Par Pond system showing sampling locations.


N
Station Pond C
MAS
CAS
P-Reactor
SAS
1000 m

These two stations were chosen for comparison because of the long history of data recorded from them. The Savannah River Laboratory has used them as primary water quality monitoring stations since studies on Par Pond were begun. Tilly (1976) has reported on annual net integral phytoplankton productivity measured at Station CAS since 1965 and measured simultaneously at CAS and MAS stations from 1971 to 1974 (unpublished data). Tilly (unpublished manuscript) has also compared the net productivity and species composition of periphyton collected on diatometers suspended at the surface of the two stations from 1972 to 1975. Siler (1975) collected fish in gill nets at the two stations. Appendix A presents water quality data collected by the Savannah River Laboratory during the course of the study and primary productivity data done by graduate students under the direction of Dr. L. J. Tilly during this study.

Two other stations were used for parts of this study. Station SAS (Fig. 2), located in the ambient portion of Par Pond was chosen because of its similar depth to Station MAS. Station PC (Fig. 2) in Pond C was chosen because it was in the deepest portion of Pond C far enough distant from the culvert between Par Pond and Pond C to be weakly affected by culvert flow. The studies conducted at these stations will be described in Appendices B and D.

Routine field sampling

Standing stocks of zooplankton were sampled at Stations MAS and CAS between 1 and 3 PM approximately weekly from September 1975 to May 1976 and at intervals of 3 to 6 days from June 1, 1976, to August 31, 1976. From September 1975 to June 1976 vertical hauls extended from the bottom to the top of the water column at each station. In June, the vertical distribution of the Cladocera was monitored and the depth of the hauls was steadily decreased during the summer to a minimum of 10 meters at Station CAS and 7m at Station MAS by August 1976 following the exclusion of Cladocera from the hypolimnion.

Plankton samples were made using a one-half meter diameter number 10 nylon plankton net. Mesh size of a number 10 net was measured under a microscope to be 153μ which is 13μ smaller than the smallest Bosmina individuals reported by Kerfoot (1972) in Frains Lake, Michigan. The net was lowered to the desired depth and retrieved by a hand winch attached to the side of a Boston Whaler boat. The whaler was anchored to a buoy which marked the station. Care was taken to be sure the net line was perpendicular to the surface of the water before the retrieval was begun. The net was raised through the water slowly and carefully at a speed of between 5 and 10 cm. per second (Schwoerbel, 1970).

After retrieving the net to the surface, it was partially resuspended in the surface water several times to wash the animals into the bottom bucket. The bucket was emptied by opening a valve at the bottom into a 1 liter-wide-mouth plastic bottle. The bottle was immediately capped, the net washed in the surface water and the bucket drained, and the next sample taken. Four samples were taken for the determination of standing stock and fecundity parameters.

Upon return to the laboratory, the samples to be counted were gently poured through a number 10 net diaphragm and washed from the diaphragm through a glass funnel into 150 ml plastic bottles with 95% ethyl alcohol. About 20 ml of 95% ethyl alcohol was then added to the sample bottle and the bottles labeled with the date, sampling station, and sample number.

Species identification

Bosminidae were identified using Deevey and Deevey (1971). Daphnidae were identified from Brooks (1957). All other species were identified from Brooks (1959).

Cladocera standing stock

From a sample taken every other week during the course of the study of stations CAS and MAS from September 1975 to September 1976, 1 ml subsamples were counted for all species of Cladocera. These were used as a general description of the presence and relative abundance of Cladocera species.

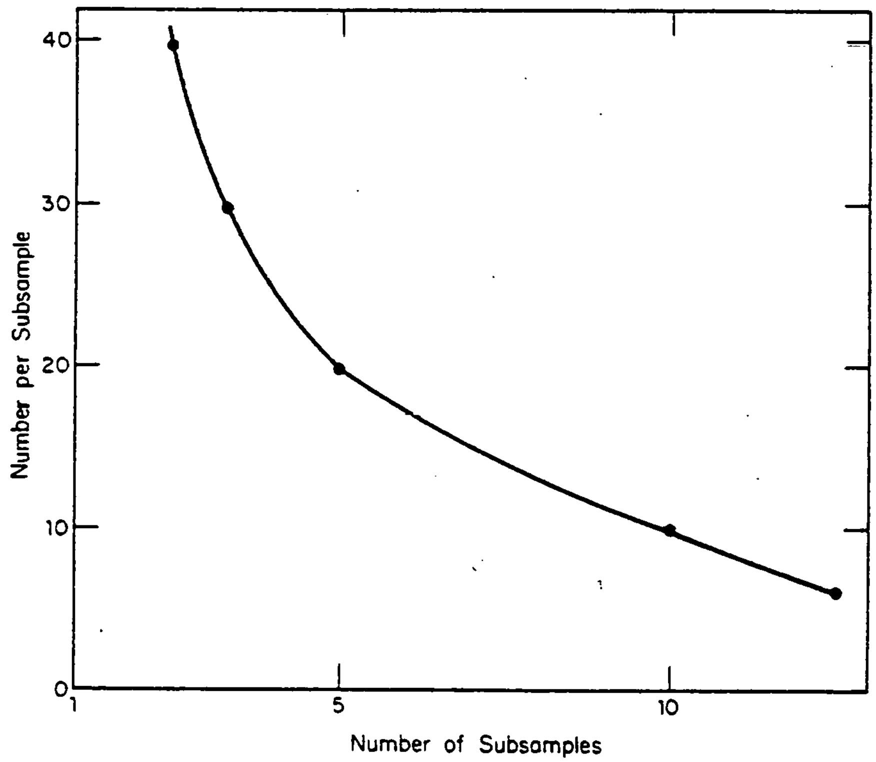
Bosmina longirostris was chosen for detailed study because it is a species of worldwide distribution (Brooks, 1969) that had been the subject of several recently published studies (Kerfoot, 1974, 1975a, 1975b; Zaret and Kerfoot, 1975). Samples from January, February, and March were chosen to represent the "winter months" in Par Pond and samples taken in June, July and August were used to represent the "summer months." October, November, April, and May were excluded originally to avoid periods of environmental instability that might bias the use of the Edmondson egg ratio method (see the section on Edmondson egg ratio in this chapter).

Several sets of samples taken in March of 1976 were used to determine a subsampling regime for *Bosmina* standing stock estimation. The sampling mixture was made by diluting a preserved sample to 150 ml in a graduated beaker, pouring the diluted sample into a flat bottomed culture dish and swirling the sample with a Hensen-Stemple pipette. One ml subsamples were taken with the Hensen-Stemple pipette resting on the bottom of the dish. The sample was approximately one and one-half inches deep. Out of 24 samples from which 10 subsamples were taken, 23 had variance to mean ratios for the subsamples not significantly different from 1.0, suggesting a Poisson distribution was being sampled (Elliot, 1971).

Figure 3 shows the number of subsamples necessary to have a 95% confidence interval of ± 20% of the mean. Twenty percent would keep the error under 10 animals, given the fact that the subsample count was usually under 40 animals. Ten subsamples were chosen as a maximum number for low counts (< 5 per subsample) because of the large increase of subsamples necessary to maintain a ± 20% confidence on the mean. The error around these estimates would be larger but less than ± 50% and therefore less than 10 animals.

To estimate the total error in a sampling regime, it is recommended that an analysis of variance be performed with the total mean square between samples being the best estimate of error (Snedecor and Cochran, 1967). However, this assumes that both subsamples and samples come from a normal distribution. The subsamples can be transformed with a square root transformation but the samples themselves probably come from other than a Poisson distribution. Therefore, it was unclear exactly how to account for the subsampling error. Conversations with Dr. Robert Keen (Michigan Technology University) and an extensive search of statistical textbooks led to the conclusion that no one had satisfactorily addressed this problem.

Figure 3 - The number of subsamples needed to have a 95% confidence of $\pm$ 20% error on the mean.


40
30
20
10
0
Number per Subsample
1
5
10
Number of Subsamples

The solution proposed was to use a machine analogy. For example, when weighing a sample, it is usually known to what decimal place the scale reading is accurate. The last digits of the reading are rounded off to the nearest significant figure. If the counting procedure is considered to be done by machine, then the average number per subsample should be accurate to the tens place. A computer program was used to round off all subsample means to the nearest ten using the method of 5 or above to be rounded to the next highest value (i.e., 44 = 40; 45 = 50). These rounded values were then multiplied by 150 ml to give the estimated number of animals in a sample. Standard statistics could then be applied to these estimates to determine sampling error at a station. The only major difficulty with this method was with average subsample sizes less than 5. These were considered zero. Average subsample sizes less than 5 occurred 7 times during the study.

Table 2 compares the mean and variance calculated from the rounded subsample averages and from the averages if subsampling error had been ignored (unrounded subsample averages) from the samples at Station MAS in the summer of 1976. Ten of the calculated means from rounded averages were higher than unrounded averages. The difference was never more than 400 animals. Fifteen of the 22 variance estimates using rounded averages were higher than those using unrounded averages. This indicates that rounding will give a generally more conservative result when statistical testing is done. This conversion is the desired result of the rounding procedure as it reflects the error in subsampling that should be included in the testing of differences. One problem that can be discerned from Table 1 was that when sample sizes were small (less than 15 animals per subsample), there was a tendency for all subsample

Table 2. Mean and variance of rounded and unrounded subsample means from Station MAS in the summer of 1976 (June - August).

DATE	MEANS		VARIANCE	
	Rounded	Unrounded	Rounded	Unrounded
6-1	6750	6533	1687503	1601767
4	11625	11824	6046847	6290815
9	2625	3024	421875	257367
15	2250	2310	562499	198531
20	7500	7482	1124999	1426003
25	14250	14086	6187455	7478591
30	9000	9361	5624959	5098239
7-6	9375	9424	7171839	5519807
10	9750	9702	2812479	4887743
14	11250	11692	562495	948223
19	2625	2936	421875	319835
23	3750	3892	562499	155727
28	3750	3604	562499	67247
8-1	9000	9087	1124927	1614143
5	14250	14041	19687408	19480752
9	7125	7157	13921883	12848871
13	0	1425	0	138038
17	3000	2877	1124999	835091
20	2250	2379	562499	361343
22	3000	2916	1124999	758855
27	4125	4511	1546883	1901503
31	4500	4349	3374999	3078871

averages to be rounded to the same value, leaving no variance around the estimated mean. This was not considered to be a problem in the comparison of stations because the meaning of the dynamics of such low standing stocks would be difficult to interpret for the plankton community as a whole. These dates (8 of them) were not included when variance was analyzed.

To determine if the standing stock estimates should be used as they were or could be transformed to meet assumptions associated with parametric statistical methods, homogeneity of variance was examined.

Examination of homogeneity of variances using the F-max test for the times series within Stations CAS and MAS for the two seasons (Sokal and Rohlf, 1969) yielded curious results (Table 3). The untransformed variance was significantly heterogeneous only at Station CAS in the winter, suggesting that only the data from this station would need to be transformed. The correlation of a $\log_{10}$ mean regression, however, was significant for both stations in the summer (Table 3) while it was not significant for either station in the winter. According to Southwood (1966) a significant correlation suggests that a transformation is necessary. The appropriate transformation (X^P) is also determined from the same $\log_{10}$-$\log_{10}$ regression by the formula $p = 1 - 1/2 \cdot b$ where b is the slope of the regression. The transformation (1) was calculated to be 0.32 for both stations in the summer. After the transformation the variances were again checked for homogeneity. At Station CAS (Table 3), the F-max was reduced; for Station MAS, the variances were now significantly heterogeneous. The 0.32 transformation was also applied to the winter data. For both stations the F-mas statistic was increased. Because the $\log_{10}$ transformation is often recommended for

Table 3. Correlation (r) of $\log_{10}$ mean and $\log_{10}$ variance and analysis of homogeneity of variance (F-max) using untransformed and transformed data at Station CAS and MAS, winter and summer, 1976.

STATION/DATE	r	F-MAX OF TRANSFORMATIONS		
		None	.32	$\log_{10}$
CAS - winter	0.02*	131.64	578.37	1218.9
CAS - summer	0.812	43.66*	13.45*	12.72*
MAS - winter	0.204*	27.66*	97.94	182.58
MAS - summer	0.715	46.67*	75.22	140.77

* = not significant at the 0.05 alpha level.

plankton data (Cassie, 1971; Elliot, 1977), it was also used for all stations with the same results as the 0.32 transformation (Table 3). In summary, neither transformation reduced the F-max statistic at Station CAS in the winter (where it was needed), both transformations increased the F-max statistic for Station MAS in both summer and winter (making them heterogeneous when before transformation they were homogeneous) and both transformations reduced the F-max statistic for the summer data from Station CAS (which was homogeneous before transformation). To calculate standard descriptive statistics, therefore, the untransformed data were used.

The effect of time of day and station locality on mean Bosmina standing crop estimates was cursorily examined using a one-way analysis of variance design. On August 19 and 30 four tows were taken at each station at 0900, 1300, 1500, and 1800 hours. On September 1 four tows were taken at four locations; one at each of the four major compass points surrounding each station at least 50 yards distant. There was a significant added variance due to time of day on one of the two days sampled at each station and a significant added variance due to location at both stations on the day sampled for that factor (Table 4).

Table 5 is an example of the sampling error associated with the standing stock estimates. Confidence intervals for these measurements varied between 20% and 40%. Sample sizes are small and subsampling error is not directly accounted for in the variance estimates. The true distribution from which the samples were taken is unknown. Furthermore, the sampling variance associated with sampling several stations within an area of the reservoir would probably be greater than the within-station variance and sampling the middle arm is further confounded by

Table 4. Results of analysis of variance sampling design at stations CAS and MAS in the summer of 1976. (See text for complete explanation of this experiment.) Significance between treatments was assigned using the Kruskal-Wallis Test (Sokal and Rohlf, 1969).

Date	Factor	CAS Sign.	MAS Sign.
8-19	Times hour	n.s.[1]	$p < .05$
8-30	Times hour	$p < .05$	n.s.
9-1	Locations	$p < .05$	$p < .05$

1. n.s. - not significant at alpha of 0.05.

Table 5. Mean standing stocks per net samples and 95% confidence limits at Stations CAS and MAS during Winter, 1976. See text for full discussion of methods.

Date	$\bar{X}$	S^2	95% CL
		STATION MAS	
6-1	6750	2250005	4363 to 9136
6-4	11625	8062464	7107 to 16142
6-9	2625	562501	1431 to 3818
6-15	2250	750000	872 to 3627
6-20	7500	1500000	5551 to 9448
6-25	14250	8249941	9680 to 18819
6-30	9000	7499946	4642 to 13357

Date	$\bar{X}$	S^2	95% CL
		STATION CAS	
6-1	4875	2062512	2590 to 7159
6-4	5625	562501	4431 to 6818
6-9	7500	1500000	5551 to 9448
6-15	15375	6562474	11299 to 19450
6-20	25125	24562512	17239 to 33010
6-25	18375	11062613	13083 to 23666
6-30	14625	8062378	10107 to 19142

the variable flow pattern (Vigerstad and Kiser, 1977, Appendix E). Therefore the sample means were considered to be of ordinal value and nonparametric methods were used to compare stations and/or seasons whenever possible. Standard errors are not presented in the figures to discourage comparison between single dates or between two stations on a single date because the stocks are of ordinal value.

Fecundity

To estimate egg and embryo standing stocks for Bosmina longirostris the animals subsampled for standing stock were replaced in the culture dish. The sample was mixed again and a small portion poured back into the counting dish. Each sample was checked for loose eggs. Bosmina were not found to be broken or to have been deformed during preservation so that their eggs could fall out. No loose eggs were found in the samples.

Samples were tabulated into the categories; bearing no eggs, bearing eggs-embryos, or bearing eyed embryos. The number of eggs and embryos in each female was also recorded. If a female bore a deformed egg(s), the female was placed in the category, no embryos. Deformed eggs were eggs that were twisted, flattened and greatly elongated, or concave in shape. From these data, for each sample, it was possible to calculate the percent of the females that were gravid, the average number of eggs per female (called by Edmondson (1971), the eggs per female ratio, E/F), and average clutch size.

Based upon Edmondson's (1971, 1977) affirmation of the feasibility of making the eggs per female determination from qualitative samples, the original design for calculation E/F was to combine the results from all samples into one number. For the winter samples, the first 100 females encountered in each subsample from each sample were tabulated. This would have made the sample size for a station 400 in total. However, the variance and confidence intervals around the mean E/F using the four samples as independent estimates were much higher than expected based on Edmondson's statements (Table 6). To ensure that this was not due to subsampling error, the determination of E/F was repeated from one sample by increments of approximately 50 animals:

EGGS AND EMBRYOS	TOTAL FEMALES COUNTED	E/F
41	63	.651
63	100	.630
84	155	.542
104	201	.517
131	252	.520
156	301	.518
184	356	.517

The text table shows that 100 animals was too small a subsample size and that by 200 animals E/F leveled off, at least in the second decimal place. Therefore, for the summer data the subsample size was increased to 200 animals per sample. Again, however, the variance and confidence intervals remained fairly substantial (95% confidence intervals up to 40% of the mean in some cases, Table 7). The variance for the estimation was therefore considered to be unavoidable and needed to be calculated using the results from each sample. Subsampling error was considered to be so small that it could be ignored.

Table 6. Mean, variance, and 95% confidence limits for the egg per female ratio at Station CAS in the winter of 1976.

Date	E/F	σ^2	95% C.L.
1-5	.69	.0031	.60 to .78
1-12	.91	.0013	.85 to .97
1-19	.69	.0033	.60 to .78
1-26	.99	.0068	.86 to 1.13
2-16	.68	.0044	.57 to .78
2-23	.54	.0024	.46 to .62
3-2	.49	.0013	.43 to .55
3-8	.67	.0135	.49 to .85
3-15	.73	.0008	.69 to .78
3-21	.48	.0082	.33 to .62

Table 7. Mean eggs per female variance and 95% confidence limits for June. Samples at Stations CAS and MAS.

Date	$\bar{X}$	S^2	95% CL
		STATION MAS	
6-1	.66	.023	.42 to .90
6-4	.73	.005	.62 to .85
6-9	.76	.001	.70 to .82
6-15	.70	.002	.63 to .76
6-20	.59	.002	.51 to .67
6-25	.55	.002	.46 to .63
6-30	.42	.001	.36 to .48

Date	$\bar{X}$	S^2	95% CL
		STATION CAS	
6-1	.74	.010	.58 to .90
6-4	.77	.008	.62 to .91
6-9	1.22	.012	1.05 to 1.40
6-15	.98	.004	.88 to 1.08
6-20	.59	.001	.53 to .64
6-25	.40	.004	.30 to .49
6-30	.39	.001	.34 to .44

The 95% confidence intervals around the means for E/F varied between 10% and 40% of the mean at the two stations. Therefore, for the same reasons listed for the standing stock estimates, the sample means at a station were considered to be of ordinal value and non-parametric methods were used to compare stations and/or seasons whenever possible.

Bosmina body size

Twenty egg bearing females from each station were measured from three sampling dates in the winter and seven sampling dates in the summer. Measurements were of total length following Kerfoot (1972) and were made using an ocular micrometer mounted in a Wild dissecting microscope.

Spatial pattern

For the dates Bosmina standing stocks were estimated, mean ($\overline{X}$), variance (s^2), Lloyd's mean crowding index ($\overset{*}{X}$), Lloyd's index of patchiness ($\overset{*}{X}/\overline{X}$), a dispersion coefficient ('C') and the variance to mean ratio ($s^2/\overline{X}$) were calculated.

The computational formulae for the indices are in Table 8. All standard statistical formulae appear in Sokal and Rohlf (1969). Lloyd's mean crowding index is a measure of the mean number, per individual, of other individuals in the sampling unit. The ratio of mean crowding to mean density is Lloyd's index of patchiness (Pielou, 1974). The variance to mean ratio is a commonly recommended measure of spatial heterogeneity (Elliot, 1977; Pielou, 1974). The coefficient of dispersion, 'C', derived from the negative binominal distribution, is used

Table 8. Formulae used to calculate indices of dispersion for winter and summer samples of 1976. All formulae correct for bias due to small sample size.

Variance to mean ratio (Elliot, 1977)	$S^2/\overline{X}$
Lloyd's mean crowding (Pielou, 1974)	$\overset{*}{X} = \overline{X} + (S^2/\overline{X} - 1)$
	$(1 + S^2/q\ \overline{X}^2)$
	where q = sample size
Lloyd's index of patchiness (Pielou, 1974)	$\overset{*}{X}/\overline{X}$
Coefficient of dispersion (Cassie, 1961)	"C" = $(S^2 - \overline{X})$
	$(1 + S^2/q\ \overline{X}^2)/\overline{X}^2$

to describe the variance of the mean according to the relationship $s^2 = \overline{X} + \overline{X}^2 \cdot C$. Low values of 'C' would indicate small departures from the Poisson or random distribution. The negative binominal distribution has been commonly applied to plankton population studies (Cassie, 1971). Both the variance to mean ratio and the index of patchiness have the property that populations with a random distribution will have values of these indices equal to 1.0 while values greater than 1.0 indicate a clumped distribution.

Two cautions are noteworthy. First, as noted by Pielou (1974), a variance to mean equal to 1.0 or index of patchiness equal to 1.0 does not necessarily imply that the dispersion of the population is truly random. Because these indices depend on the size (area) of the sample being taken, randomness may be encountered within a series of patches. Secondly, according to George (1974), the variance to mean ratio is not a good measure of the relative amount of contagion as its value is dependent on the size of the mean. This is not true for the index of patchiness, or the coefficient of dispersion. Also, because estimates are considered to be non-parametric, the mean of a series of standard statistics of indices of dispersion taken over a discrete time interval is considered the "best measure" of the condition of the population.

Estimation of sampling effort requirements

The "power" of a statistical test is the ability of the test to show that two population means are different, when, in the real world, they actually are different. When the power of a test is known, the number of samples that will be necessary to detect a difference between population the investigator believes to be important, can be determined. Because it is often difficult to assign power to non-parametric statistical methods (Siegel, 1956), an analysis of variance model suggested by McCaughran (1977) was used to examine the question of "how many samples?" To use the Pearson-Hartley charts for power requires the calculation of a parameter ϕ according to the formula

$$\phi = \frac{N^{\frac{1}{2}}}{2T} \cdot \frac{\Delta}{\sigma}$$

where N is the number of samples necessary to detect a minimum difference, Δ, between T stations given a sampling standard deviation σ. The square root of the median variance from the summer and winter samples was used as estimates of σ and the Δ's were varied from the mean population size for those periods at both stations.

Estimation of confidence limits for the Edmondson egg-ratio model

There are two possible sources of error in the application of Edmondson's egg ratio. As in any model, the assumptions upon which the model is based could be incorrect for the population being observed. If the model is robust enough, however, it may be able to tolerate small deviations from these assumptions. Edmondson (1960) himself suggested that it be applied only under reasonably stable environmental conditions. He also suggested a formula for b' which avoids the problems of a stable age distribution of eggs (Equation 6, Review of Literature).

The second source of error is in the estimation of the parameters from field samples. Edmondson (1971, 1974, 1977) stated that the b' estimates can be made from qualitative samples. However, many of the authors used the estimates of population sizes in their calculations of eggs per female. Hall (1965), for example, used the formula

$$E/F = \frac{N_A \cdot \bar{E}}{N_O} \qquad (9)$$

where N_A is the estimated number of adults, $\bar{E}$ is the estimated clutch size, and N_O, the estimated total population size. Cummins, et al. (1969), Dodson (1972), and Wright (1965) used the formula

$$E/F = \frac{E'}{N_O} \qquad (10)$$

where E' is the estimated total number of eggs in the population. Keen (1973) also used the estimated population size in his formula for birth rate (Equation 7). Dodson (1972) used Equation 9 because his daphnids "ballooned" upon preservation and he had a large number of loose eggs in his samples, but the other authors presented no reasons for their using population sizes.

The use of Equations 8 and 9 means that any error in the measurement of population size would be visited upon both the b' and r' values. This was avoided by using equation 4 (Literature Review) for realized rate of increase and equation 6 (Literature Review) for birth rate. These formulae still have several parameters that have sampling error. Hall (1964) stated that sampling errors could produce changes in b' values. Keen (1973) attributed his negative d' values to sampling error. Kerfoot (1975a) noted that his observed fluctuations in d' in the winter were probably greatly modified by sampling error. Wright (1965) and Cummins et al. (1969) made no mention of the problem of negative d' values.

All formulae in the Edmondson model have several parameters in them that have sampling error. Based on discussions with other researchers three methods were developed for making error estimations around r', b', and d'. The first was suggested by Drs. Arnett and Suich of the Savannah River Laboratory. According to them, sample sizes of such large magnitudes (1000 animals per sample) which meet the criteria of homogeneity of variance can be considered to be variates from a normal distribution. The variance (σ_z^2) of the result of any calculation [z = f (x,y)] using the means of the variates (x,y) can be determined from the equation

$$\sigma_z^2 = \frac{\sigma z}{\sigma x} \sigma_x^2 + \frac{\sigma z}{\sigma x} \sigma_y^2 \qquad (11)$$

Each of these derivatives, depending on the function f (x,y), will have a covariance term which can be dropped if x and y vary independently.

In the case of the r' determination, the formula for the variance or r' would be

$$\sigma_r^2 = \left(\frac{1}{\Delta t}\right)^2 \left(\frac{\sigma_x^2}{(x)^2} + \frac{\sigma_y^2}{(\bar{Y})^2}\right) \qquad (12)$$

where $(x,y) = \frac{\ln\left(\frac{x}{y}\right)}{t}$ and x,y are the standing stock estimates at the beginning and the end of the sampling period.

The second method was suggested by Dr. R. Keen of Michigan Technological University. According to Dr. Keen, r' values can be used as normally distributed variates and standard descriptive statistics would describe the variance around r' values using the pairs of samples.

The third method is the author's developed in consultation with Dr. L. J. Tilly of the Savannah River Laboratory. In it, the means and 95% confidence intervals determine the reliability of an estimate on any sampling date. The values of the 95% confidence limits can then be used to determine a range of values. The upper limit of the range is calculated by pairing the lowest standing stock confidence limit on the first sampling date with the highest standing stock confidence limit on the second sampling date. The lower limit of the range is determined by reversing the confidence limits used. This range is called the 95% confidence limits range (95% C.L.R.).

The formula for estimating variance of b' for use in the Arnett-Suich method derived from Equation 11 was

$$\sigma^2_{b'} = \frac{1}{\bar{y}^2} \cdot \sigma^2 x + \frac{\bar{X}^2}{\bar{y}^4} \cdot \sigma^2 y \qquad (13)$$

where $f(x,y) = \frac{x}{y} = \frac{\log(1 + E/F)}{D}$ and

$x = \log(1 + E/F)$ and y is the instar duration (D).

The Keen method does not provide for incorporation of the variance associated with instar duration. Therefore, the mean and variance of b' using his method were generated by calculating four separate b' using the E/F ratio from the individual samples and the predicted instar duration at a specific temperature.

The author's method determines the 95% CLR by pairing the lower 95% C.L. for instar duration (i.e. the shortest instar duration time) with the upper 95% confidence limit for E/F and the lower 95% confidence limit for instar duration with the lower 95% confidence limit for E/F.

Instar duration was not measured for *Bosmina longirostris* in Par Pond. However, Dr. Charles Kerfoot of Dartmouth College has generously allowed the use of his data from three different lakes, one in Michigan, one in Washington, and one in Utah, for this analysis.

The data in Table 9 are 24 means of observations of instar duration at 15 temperatures ranging from 8°C to 32°C. When transformed to common logarithms ($\log_{10}$), the data can be fitted to a straight line of the form

$$\log_{10} D = -1.510 \log_{10} (T) + 2.282 \quad (14)$$

where D is the instar duration and T is the temperature in degrees centigrade.

To estimate the variance around any instar duration for use in the Arnett-Suich and the author's methods, an analysis of variance was made on Kerfoot's untransformed data,

	df	SS	MS
$Y - \bar{Y}$ Among groups	13	127.47	9.800
$Y - \bar{Y}$ Within groups	10	1.01	0.101

The within groups mean square was used as the estimate of variance of D at a specific temperature.

Temperatures for use in Equation 14 were taken from the routine limnological survey conducted by the Savannah River Laboratory at Stations CAS and MAS. The predicted temperature for a two-week interval was derived from the average of the water column averages at the beginning and the end of the period between surveys. In the following test table are the calculated water column temperature averages and the predicted instar durations for June 1976 and Station CAS.

DATE	$\bar{X}$ TEMP	INSTAR DURATION
5-20 to 6-3	23.25	1.65
6-3 to 6-17	23.90	1.59
6-17 to 7-1	24.45	1.53

Table 9. Instar duration in days for Bosmina longirostris at 14 different temperatures. Data are from Kerfoot (personal communication; see the text for complete discussion).

Temperature (°C)	Instar duration (DAYS)
8	8.00, 8.00
9	7.30, 7.00
10	7.38, 6.14
14	3.60
15	3.40, 3.00, 2.94, 2.92
16	3.00, 2.80
16.5	2.50
20	2.03
21	1.93
21.5	1.75, 1.84
22	1.70, 1.80
25	1.40, 1.40
29	1.20
30	1.30

The ratio of eggs per female (E/F) was determined from the fecundity data already described. Death rate is calculated by difference (Equation 3, Literature review). For the Arnett-Suich method the variance would be calculated from the formula

$$\sigma^2_{d'} = \sigma^2_x + \sigma^2_y \quad (15)$$

where f (x,y) = x - y; x = b' and y = r'. The Keen method calculates the mean d' from the differences of the mean birth rate and rate of increase. Variance of d' is the sum of the variances of birth rate and rate of change. The author's methods pairs the largest b' and the smallest r' from the respective 95% CLR's to determine the limits of the 95% for d'.

Calculation of confidence intervals for the Keen and Arnett-Suich methods require the appropriate degrees of freedom (df) be determined. For the Keen method df always equals 3. For the Arnett-Suich method, df was calculated from the formula

$$(n_1 + n_2 - 2) \quad (16)$$

where n_1 and n_2 are the samples sizes for the r' and b' determinations and the degrees of freedom associated with r' and b' for the death rate determinations. For the b' determinations, the sample size for instar duration was considered to be 2. To compute standard errors a sample size of 4 was used for the r' and d' parameters and 3 for the b' parameter (the average of 4 E/F determinations and 2 instar duration determinations).

For purposes of comparison all three methods were applied to the data on standing stock and fecundity from Station CAS in June 1976.

General Cladocera Survey

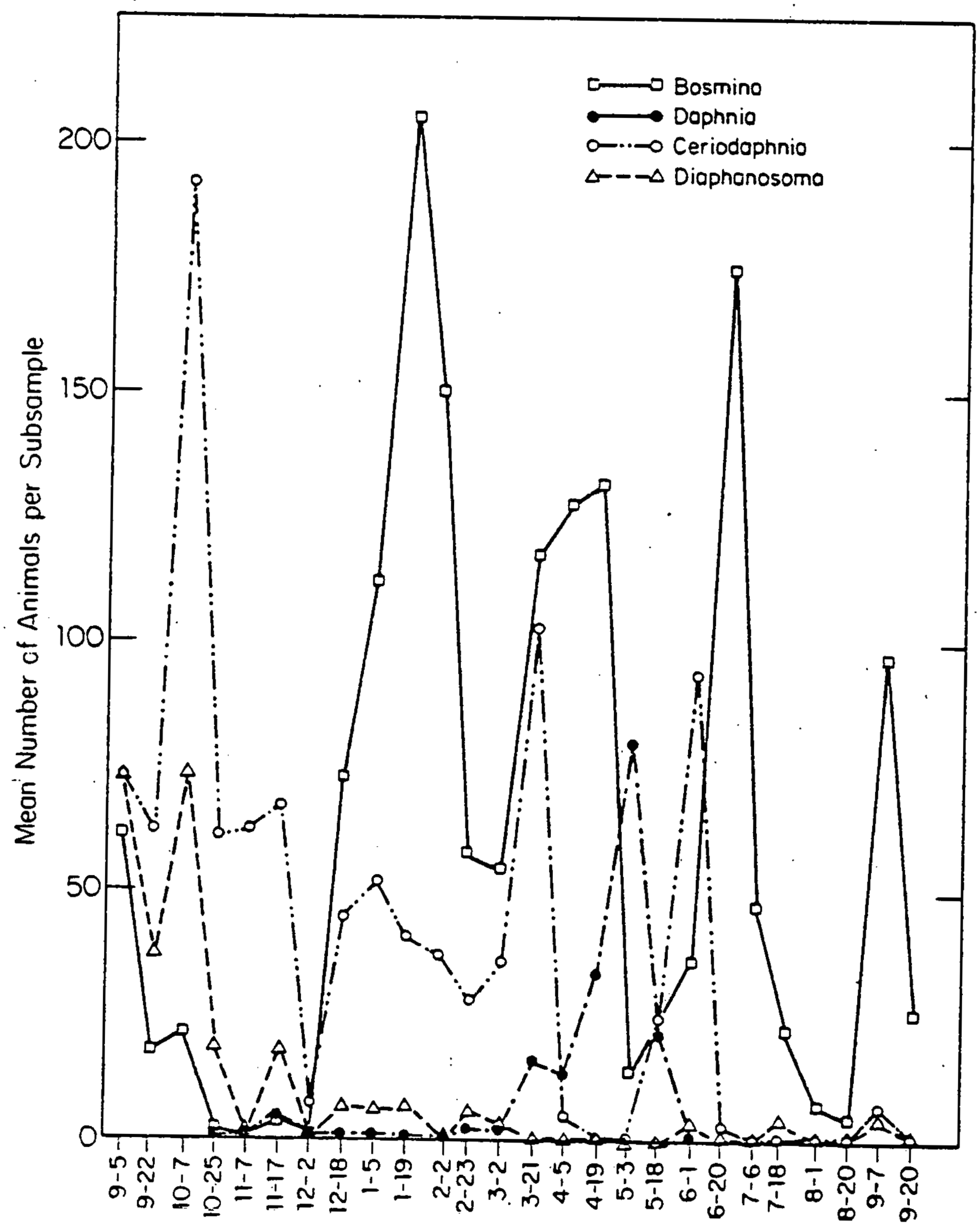
Figures 4 and 5 present the average counts per 1 ml subsample for each sample date from September 1975 to September 1976 for the four species found most regularly in the samples (> 50%). *Bosmina longirostris*, *Ceriodaphnia lacustris*, *Diaphanosoma brachyurum* and *Daphnia parvula*.

According to a sign test *Bosmina* had an overall higher standing stock at CAS than at MAS for this time period ($p < .05$). There were no significant differences between the stations for the other species. According to a Friedmans one way analysis of variance there were significant differences ($p < .05$) between the standing stocks of the species. At both stations *Bosmina* had the highest rank total (see Siegel, 1957) and is, therefore, regarded as the dominant cladoceran species in the limnetic portion of Par Pond. Much of the results reported here will focus on *Bosmina longirostris*.

Spatial pattern - *Bosmina longirostris*

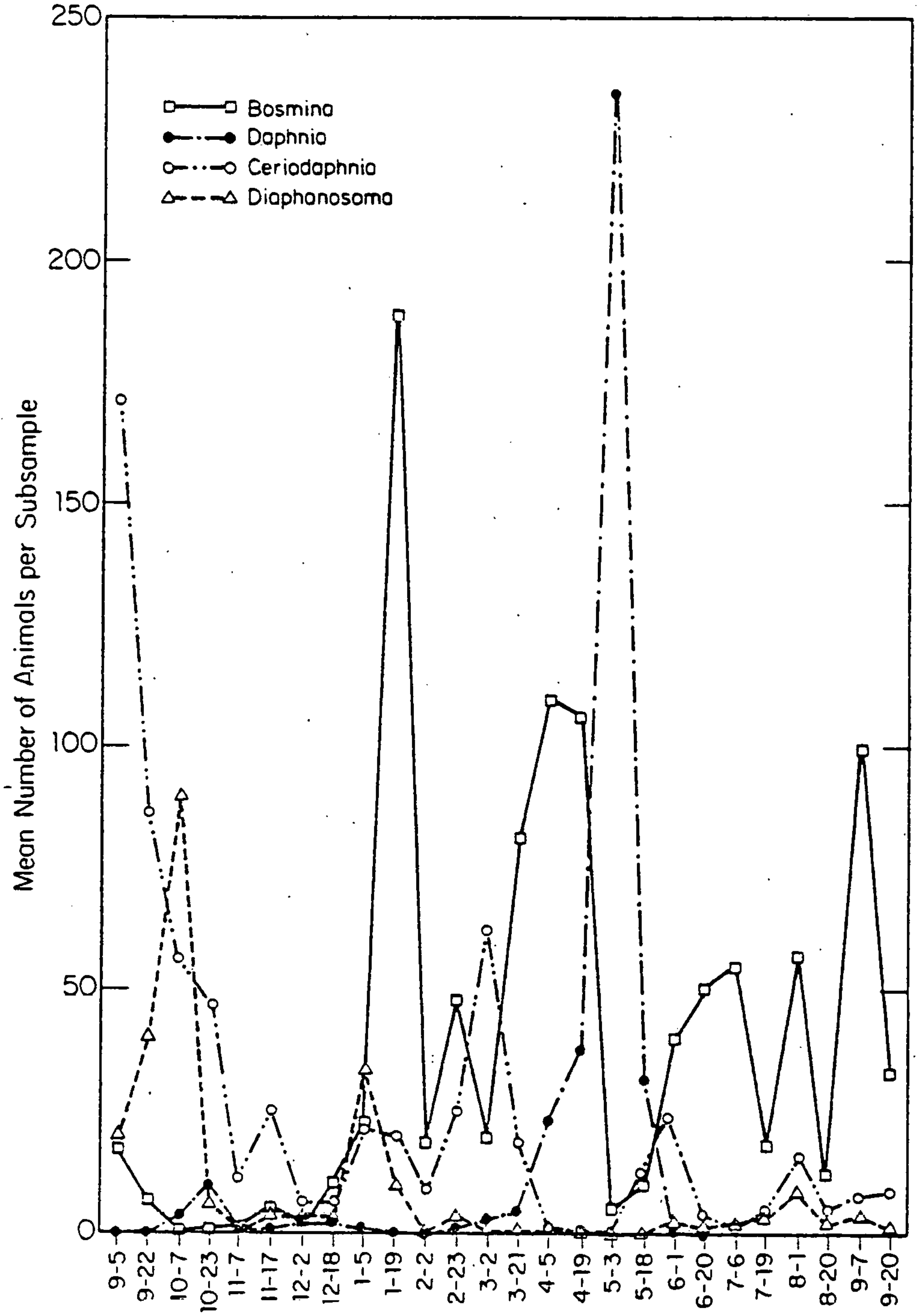
Tables 10 and 11 show the standard statistics and indices calculated from standing stock estimates of *Bosmina longirostris* at stations CAS and MAS in the winter and summer of 1976. Because the rounding procedure in some cases caused all the subsample averages to be equal, several sampling dates had no variance associated with the means. These dates were excluded from further analysis. Table 12 is a summary of the means of each series of indices. The Wilcoxon-Pairs-Signed-Ranks-Test was used to compare the series of pairs of indices taken at the two stations during the same time period, and the Wilcoxon-Two-Sample-Test to compare series taken at the same

Figure 4 - Estimated numbers of major species of Cladocera in samples taken at Station CAS from September 1975 to September 1976.



Bosmina
Daphnia
Ceriodaphnia
Diaphanosoma
Mean Number of Animals per Subsample
200
150
100
50
0
9-5
9-22
10-7
10-25
11-7
11-17
12-2
12-18
1-5
1-19
2-2
2-23
3-2
3-21
4-5
4-19
5-3
5-18
6-1
6-20
7-6
7-18
8-1
8-20
9-7
9-20

Figure 5 - Estimated numbers of major species of Cladocera in samples taken at Station MAS from September 1975 to September 1976.



250
200
150
100
50
0
Mean Number of Animals per Subsample
Bosmina
Daphnia
Ceriodaphnia
Diaphanosoma
9-5
9-22
10-7
10-23
11-7
11-17
12-2
12-18
1-5
1-19
2-2
2-23
3-2
3-21
4-5
4-19
5-3
5-18
6-1
6-20
7-6
7-19
8-1
8-20
9-7
9-20

Table 10. Mean ($\overline{X}$), variance (S^2), variance to mean ratio ($S^2/\overline{X}$), mean crowding ($\overset{*}{X}$), coefficient of patchiness ($\overset{*}{X}/\overline{X}$), and coefficient of dispersion ("C") calculated from standing stock estimates of <u>Bosmina longirostris</u> for sampling dates at Stations CAS and MAS in the winter of 1976.

<u>Cold Arm Station (CAS)</u>

Date	$\overline{X}$	S^2	$S^2/\overline{X}$	$\overset{*}{X}$	$\overset{*}{X}/\overline{X}$	"C"
1/15/76	14625	8062378	551.2	15318.2	1.047	0.038
1/12/76	13875	74062496	5337.8	21059.5	1.518	0.422
1/19/76	22125	562602	25.4	22155.7	1.001	0.001
1/26/76	15750	33750016	2142.8	18500.4	1.175	0.141
2/02/76	25500	17999872	705.8	26386.2	1.035	0.028
2/16/76	22125	9562538	432.2	22666.3	1.024	0.020
2/23/76	12375	11062442	893.9	13507.5	1.092	0.073
3/02/76	10500	10500010	1000.0	11772.7	1.121	0.097
3/08/76	6375	2062501	323.5	6782.4	1.064	0.051
3/15/76	13500	67499936	4999.9	20211.8	1.497	0.405
3/21/76	21000	14999978	714.2	21897.9	1.043	0.034
3/28/76	21000	6000042	285.7	21357.0	1.017	0.014

<u>Middle Arm Station (MAS)</u>

Date	$\overline{X}$	S^2	S^2/X	$\overset{*}{X}$	$\overset{*}{X}/\overline{X}$	"C"
1/05/76	3000	0	0.0	2999.0	1.000	0.000
1/12/76	9750	8250026	846.1	10825.0	1.110	0.089
1/19/76	21375	15562410	728.0	22290.2	1.043	0.034
1/26/76	12000	2999978	249.9	12312.7	1.026	0.021
2/02/76	7125	15562512	2184.2	10021.5	1.407	0.330
2/16/76	27750	6750122	243.2	28053.5	1.011	0.009
2/23/76	12750	3749973	294.1	13118.3	1.029	0.023
3/02/76	8625	2062506	239.1	8924.5	1.035	0.028
3/08/76	12000	4499968	374.9	12470.6	1.039	0.031
3/15/76	10875	562517	51.7	10938.7	1.006	0.005
3/21/76	13500	4499968	333.3	13917.7	1.031	0.025

Table 11. Mean ($\bar{X}$), variance (S), variance to mean ratio ($S/\bar{X}$), mean crowding ($\overset{*}{X}$), coefficient of patchiness ($\overset{*}{X}/\bar{X}$) and coefficient of dispersion ('C'), calculated from standing stock estimate of <u>Bosmina longirostris</u>, for sampling dates at Stations CAS and MAS in the summer of 1976.

			Cold Arm Station (CAS)			
Date	$\bar{X}$	S^2	$S^2/\bar{X}$	$\overset{*}{X}$	$\overset{*}{X}/\bar{X}$	'C'
6-1-76	4875.0	2062512.0	423.0	5412.0	1.11	0.088
6-4-76	5625.0	562501.3	100.0	5749.4	1.02	0.018
6-9-76	7500.0	1500000.0	200.0	7750.3	1.03	0.027
6-15-76	15375.0	6562474.0	426.8	15910.4	1.03	0.028
6-20-76	25125.0	24562512.0	977.6	26355.5	1.04	0.039
6-25-76	18375.0	11062613.0	602.0	19131.4	1.04	0.033
6-30-76	14625.0	8062378.0	551.2	15318.2	1.04	0.038
7-6-76	5250.0	750005.3	142.8	5428.5	1.03	0.027
7-10-76	5250.0	3750005.0	714.2	6166.1	1.17	0.140
7-14-76	3000.0	1500000.0	500.0	3644.7	1.21	0.173
7-19-76	4875.0	562512.0	115.3	5018.9	1.03	0.024
7-23-76	3000.0	0.0	0.0	2999.0	1.00	0.000
7-28-76	0.0	0.0	0.0	0.0	0.00	0.000
8-1-76	0.0	0.0	0.0	0.0	0.00	0.000
8-5-76	0.0	0.0	0.0	0.0	0.00	0.000
8-9-76	0.0	0.0	0.0	0.0	0.00	0.000
8-13-76	2250.0	750000.0	333.3	2677.9	1.19	0.153
8-17-76	1875.0	562500.0	300.0	2260.9	1.20	0.166
8-20-76	0.0	0.0	0.0	0.0	0.00	0.000
8-22-76	0.0	0.0	0.0	0.0	0.00	0.000
8-27-76	6375.0	9562501.0	1500.0	8337.1	1.30	0.249
8-31-76	7125.0	5062512.0	710.5	8029.8	1.12	0.102

Table 11 (cont'd)

	Middle Arm Station (MAS)					
Date	$\bar{X}$	S^2	$S^2/\bar{X}$	$\overset{*}{X}$	$\overset{*}{X}/\bar{X}$	'C'
6-1-76	6750.0	2250005.0	333.3	7169.7	1.06	0.050
6-4-76	11625.0	8062464.0	693.5	12501.2	1.07	0.060
6-9-76	2625.0	562501.3	214.2	2896.2	1.10	0.083
6-15-76	2250.0	750000.0	333.3	2677.9	1.19	0.153
6-20-76	7500.0	1500000.0	200.0	7750.3	1.03	0.027
6-25-76	14250.0	8249941.0	578.9	14978.5	1.05	0.041
6-30-76	9000.0	7499946.0	833.3	10059.9	1.11	0.095
7-6-76	9375.0	9562453.0	1019.9	10676.7	1.13	0.112
7-10-76	9750.0	3749973.0	384.6	10233.5	1.05	0.040
7-14-76	11250.0	749994.6	66.6	11332.4	1.00	0.006
7-19-76	2625.0	562501.3	214.2	2896.2	1.10	0.083
7-23-76	3750.0	750000.0	200.0	4001.6	1.06	0.054
7-28-76	3750.0	750000.0	200.0	4001.6	1.06	0.054
8-1-76	9000.0	1499904.0	166.6	9208.0	1.02	0.018
8-5-76	14250.0	26249888.0	1842.0	16611.1	1.16	0.133
8-9-76	7125.0	18562512.0	2605.2	10618.6	1.49	0.399
8-13-76	0.0	0.0	0.0	0.0	0.00	0.000
8-17-76	3000.0	1500000.0	500.0	3644.7	1.21	0.173
8-20-76	2250.0	750000.0	333.3	2677.9	1.19	0.153
8-22-76	3000.0	1500000.0	500.0	3644.7	1.21	0.173
8-27-76	4125.0	2062512.0	500.0	4764.1	1.15	0.125
8-31-76	4500.0	4500000.0	1000.0	5804.4	1.29	0.234

Table 12. Mean values of variance to mean ($S^2/\overline{X}$), mean crowding ($\overset{*}{X}$), coefficient of patchiness ($\overset{*}{X}/\overline{X}$), and coefficient of dispersion ('C') for Stations CAS and MAS.

Dates	Station	$S/\overline{X}$	$\overset{*}{X}$	$\overset{*}{X}/\overline{X}$	'C'
Winter 1976	CAS	1451	18467	1.136	0.110
	MAS	504	12988	1.067	0.060
Summer 1976	CAS	506	9146	1.108	0.087
	MAS	639	7601	1.134	0.108

station but at different times of the year. The Kruskal-Wallis Test was used to compare the times and locations in the ANOVA sampling model (Sokal and Rohlf, 1974). The alpha level for test of significance used in all comparisons was 0.05.

The values of $S^2/\overline{X}$ were always greater than 1.0 on all dates at both stations (Tables 10 and 11). The $\overset{*}{X}/\overline{X}$'s for both stations in both seasons were also greater than 1.0 but only in the second decimal. The largest range of values (1.001 to 1.497) was seen at Station CAS in the winter. The largest range of "C" values (0.001 to 0.422) was again in the winter at Station CAS.

Because the magnitudes of $S^2/\overline{X}$ and $\overset{*}{X}$ depend on the sample size, they were not compared between stations. There were no significant differences between the stations either in summer or winter for the remaining indices of dispersion ($\overset{*}{X}/\overline{X}$ and "C"). Comparisons between summer and winter for both stations also showed no significant differences in Bosmina spatial pattern.

Sampling Requirements

Table 13 presents the estimated sampling effort necessary to statistically detect a difference in mean population sizes between our two sampling stations on any one date (or between any two dates at one station). The power assigned to the sampling effort shows very little chance of detecting differences less than 40%.

The realized rate of increase, calculated by the method suggested by Edmondson (1960, 1971), can be used to compare the results with other studies. Table 13 indicates the calculation of r' based on a three-day interval between sampling periods. Sampling effort can now

Table 13. Estimation of the total number of samples (TS) necessary to detect a difference-between-means (Δ) and its corresponding realized rate of increase (r') at Stations CAS and MAS at an alpha level of 0.05 and a power of 0.95. Also included is the calculated power (P) of the sampling design described to detect Δ. Median (σ) and mean expected population size (N_ε) are all observations at both stations for a particular time series (winter and summer 1976) were used.

		Winter 1976 σ = 3817 N_ε = 15190		Summer 1976 σ = 2014 N_ε = 2497	
Δ(%)	r'	TS	P	TS	P
50	.14	8	.92	10	.85
40	.11	14	.67	14	.66
30	.09	24	.42	28	.41
20	.06	53	.22	60	.22
10	.03	212	< .20	244	< .20

be interpreted as being likely to detect an increase of 0.14 individuals per individual per day (based on the definiation of r').

Edmondson Egg Ratio

Tables 14, 15 and 16 show the variance and confidence intervals of r', b', and d' calculated by three different methods applied to data from station CAS in June 1976 (from Table 7 for fecundity and Table 11 for standing stock). The author's method does not calculate a variance, only the 95% confidence limits range (95% CLR).

Standing Stock Comparison - *Bosmina*

Bosmina longirostris standing stock (Figure 6) was generally higher at Station CAS than at MAS in the winter (January-March, 1976) although the differences were not significant according to a sign-test. While fluctuations in standing stock were generally larger and more rapid at Station MAS in January and early February than as CAS, both stations had a large decrease in standing stock after February 16. Station CAS returned to its pre-February 23 level by March 21, but Station MAS did not. The February decrease was coincident with a reactor shutdown period (February 22 to March 16).

The standing stock (Figure 6) was again more variable at Station MAS in the summer (June - August 1976). Station CAS had one large peak in population in June (ca. 25,000 animals per sample) and then decreased to a minimum detectable level during July and most of August. Station MAS underwent several increases and decreases in population size throughout the summer, decreasing between 36 and 86% each time the population climbed above 11,000 animals per sample.

Table 14 - Variance and 95% confidence intervals or 95% confidence limits range for the r' parameter calculated using three methods. Data is from Station CAS in June 1976. Methods are explained in the text.

Method	Dates	r'	$\sigma^2_{r'}$	95% C.L.
Arnett-Suich	6- 1 to 6- 4	.05	.012	-.08 to .18
	6- 4 to 6- 9	.06	.002	.00 to .11
	6- 9 to 6-15	.12	.002	.07 to .17
	6-15 to 6-20	.10	.003	.03 to .17
	6-20 to 6-25	-.06	.003	-.13 to .01
	6-25 to 6-30	-.05	.003	-.12 to .02
	6-30 to 7- 6	-.17	.002	-.22 to -.12
Keen	6- 1 to 6- 4	.05	.019	-.09 to .22
	6- 4 to 6- 9	.06	.003	-.01 to .13
	6- 9 to 6-15	.12	.001	.07 to .18
	6-15 to 6-20	.10	.004	.03 to .19
	6-20 to 6-25	-.06	.001	-.11 to .03
	6-25 to 6-30	-.05	.004	-.13 to .02
	6-30 to 7- 6	-.17	.003	-.23 to .13
Vigerstad				95% C.L.R.
	6- 1 to 6- 4	.05		-.16 to .32
	6- 4 to 6- 9	.06		-.03 to .16
	6- 9 to 6-15	.12		.04 to .22
	6-15 to 6-20	.10		-.01 to .22
	6-20 to 6-25	-.06		-.19 to .05
	6-25 to 6-30	-.05		-.17 to .07
	6-30 to 7- 6	-.17		-.29 to -.10

Table 15. Variance and 95% confidence intervals or 95% confidence limits range for the b' parameter calculated using three methods. Data is from Station CAS in June 1976. Methods are explained in the text.

Method	Dates	b'	$\sigma^2_{b'}$	95% C.L.
Arnett-Suich	6- 1	.33	.004	.23 to .43
	6- 5	.36	.005	.25 to .47
	6- 9	.50	.007	.37 to .63
	6-15	.43	.005	.32 to .54
	6-20	.30	.004	.20 to .40
	6-25	.22	.003	.12 to .31
	6-30	.22	.003	.13 to .31
Keen	6- 1	.33	.002	
	6- 5	.36	.001	
	6- 9	.50	.001	
	6-15	.43	.0003	
	6-20	.31	.00006	
	6-25	.22	.001	
	6-30	.22	.0002	
				95% C.L.R.
Vigerstad	6- 1	.33		.22 to .56
	6- 5	.36		.23 to .60
	6- 9	.50		.34 to .81
	6-15	.43		.30 to .69
	6-20	.30		.21 to .49
	6-25	.22		.13 to .40
	6-30	.22		.14 to .36

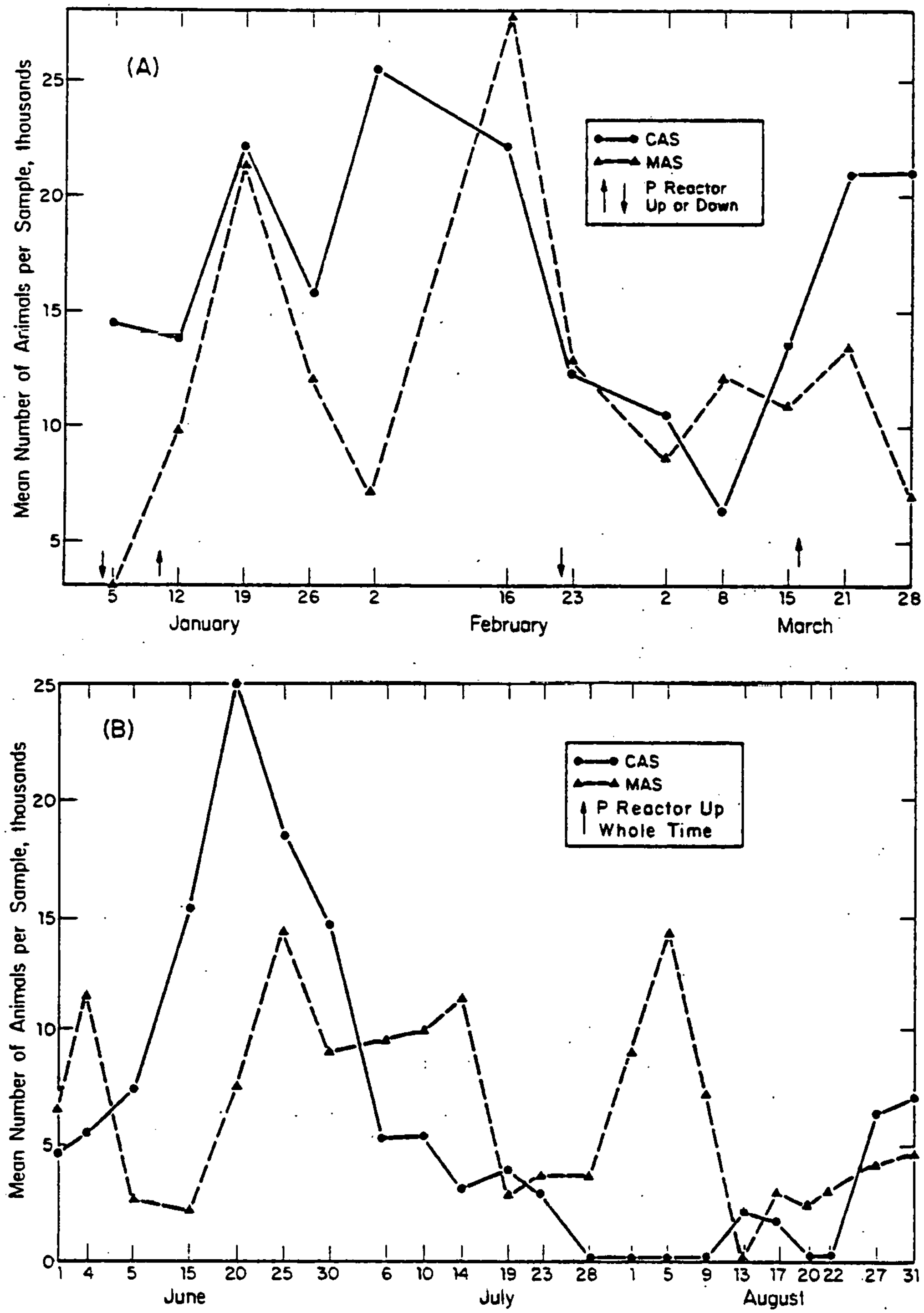
Table 16. Variance and 95% confidence intervals or 95% confidence limits range for the d' parameter calculated using three methods. Data is from Station CAS in June 1976. Methods are explained in the text.

Method	Dates	d'	$\sigma^2_{d'}$	95% C.L.
Arnett-Suich	6- 1 to 6- 5	.29	.017	.13 to .43
	6- 5 to 6- 9	.30	.007	.20 to .40
	6- 9 to 6-15	.38	.009	.27 to .49
	6-15 to 6-20	.33	.008	.23 to .43
	6-20 to 6-25	.34	.007	.24 to .44
	6-25 to 6-30	.27	.006	.18 to .36
	6-30 to 7- 6	.39	.005	.31 to .47
Keen	6- 1 to 6- 5	.29	.021	.05 to .51
	6- 5 to 6- 9	.30	.004	.20 to .40
	6- 9 to 6-15	.38	.002	.31 to .45
	6-15 to 6-20	.33	.004	.27 to .39
	6-20 to 6-25	.37	.001	.32 to .42
	6-25 to 6-30	.27	.005	.16 to .38
	6-30 to 7- 6	.39	.003	.30 to .48
				95% C.L.R.
Vigerstad	6- 1 to 6- 5	.29		-.10 to .72
	6- 5 to 6- 9	.30		.08 to .64
	6- 9 to 6-15	.38		.13 to .78
	6-15 to 6-20	.33		.09 to .67
	6-20 to 6-25	.34		.15 to .68
	6-25 to 6-30	.27		.06 to .57
	6-30 to 7- 6	.39		.21 to .63

Figure 6 - Mean number _Bosmina longirostris_ per sample at Stations CAS and MAS

A. Winter (January through March) 1976

B. Summer (June through August) 1976


(A)
CAS
MAS
P Reactor Up or Down
Mean Number of Animals per Sample, thousands
5
10
15
20
25
5 12 19 26 2 16 23 2 8 15 21 28
January
February
March
(B)
CAS
MAS
P Reactor Up Whole Time
Mean Number of Animals per Sample, thousands
0
5
10
15
20
25
1 4 5 15 20 25 30 6 10 14 19 23 28 1 5 9 13 17 20 22 27 31
June
July
August

Comparing the stations between seasons, standing stock was generally higher in the winter than in the summer at Station CAS. For example, the lowest mean estimate of standing stock at this station in the winter was greater than 65% of all the summer estimates. At Station MAS, average standing stock appeared very similar between summer and winter, but the maximum standing stock in the winter was almost 100% higher than the summer maximum.

Fecundity - *Bosmina longirostris*

Fecundity results from the winter, 1976 are summarized in Table 17 and plotted over the sampling period in Figure 7. There were no significant differences according to the Wilcoxon-Matched-Pairs-Signed-Rank-Test between the two stations for any of the parameters measured. Eggs per female (Figure 7A) tended to be lower after February 2 at both stations, but two periods were not significantly different according to the Mann-Whitney U Test. There was no difference in the percent gravid (Figure 7B) at either station between these periods, but clutch size (Figure 7C) was significanly lower ($p < .05$) after February 2 at both stations. The percentage of the females carrying deformed eggs (Figure 7D) was small, always less than 10% at both stations.

The results of the fecundity estimates for the summer, 1976, are summarized in Table 18 and Figure 8. According to the Wilcoxon-Matched-Pairs-Signed-Rank-Test the eggs per female (Figure 8A) and the percentage gravid (Figure 8B) were higher at CAS than MAS for the summer ($p < .05$). Clutch size was significantly higher ($p < .05$) at station CAS (Wilcoxon-Matched-Pairs-Signed-Rank-Test) although, again, the difference was small (0.07 eggs per clutch) (Table 18).

Table 17. Mean of means and range of means for Bosmina egg production parameters at Stations CAS and MAS during winter 1976. See text for full discussion of methods.

	Station CAS $\bar{X}$	Range
Clutch size	1.5	1.3 - 2.1
% gravid	44.9	36.30 - 56.5
Eggs per female	.69	.49 - .99
% females with deformed eggs	2.4	0.75 - 5.5

	Station MAS $\bar{X}$	Range
Clutch size	1.6	1.4 - 1.9
% gravid	46.1	28.2 - 78.9
Eggs per female	.76	.44 - 1.46
% females with deformed eggs	2.7	0.5 - 7.2

Figure 7 - Means of *Bosmina* egg production parameters during the winter, 1976 at Station CAS and MAS.

A. Eggs per female

B. Percentage gravid

C. Clutch size

D. Percentage of females bearing deformed eggs

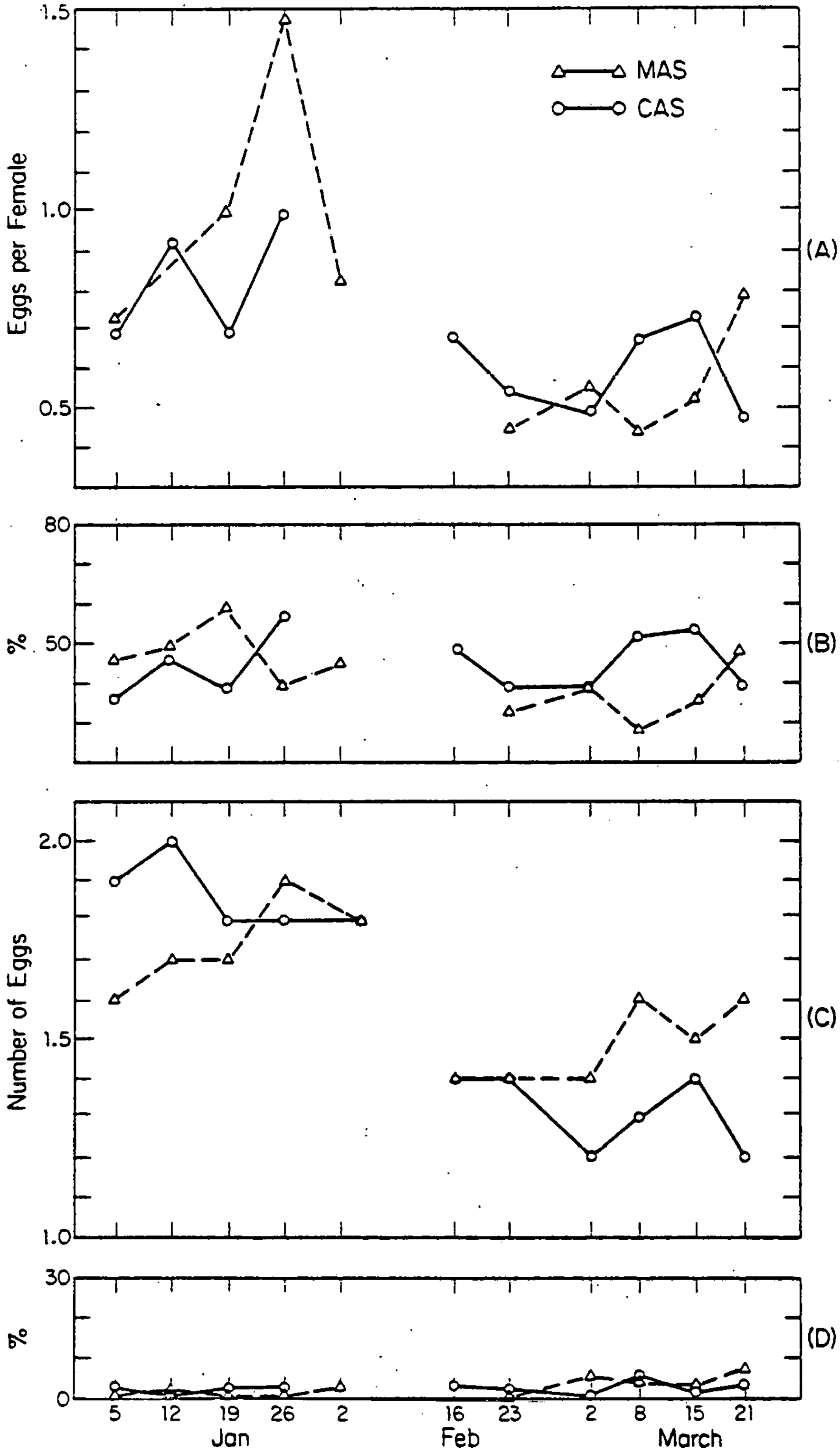

MAS
CAS
Eggs per Female
(A)
%
(B)
Number of Eggs
(C)
%
(D)
5
12
19
26
2
16
23
2
8
15
21
Jan
Feb
March

Table 18. Mean of means and range of means for *Bosmina* egg production parameters at Stations CAS and MAS during summer 1976. See text for discussion of methods.

	Station CAS $\bar{X}$	Range
Clutch size	1.28	1.01 - 1.62
% gravid	59.0	27.9 - 77.1
Eggs per female	.77	.32 - 1.25
% females with deformed eggs	3.9	0.7 - 9.1
	Station MAS $\bar{X}$	**Range**
Clutch size	1.21	1.04 - 1.48
% gravid	53.6	30.5 - 67.6
Eggs per female	.65	.42 - .94
% females with deformed eggs	7.2	1.4 - 17.6

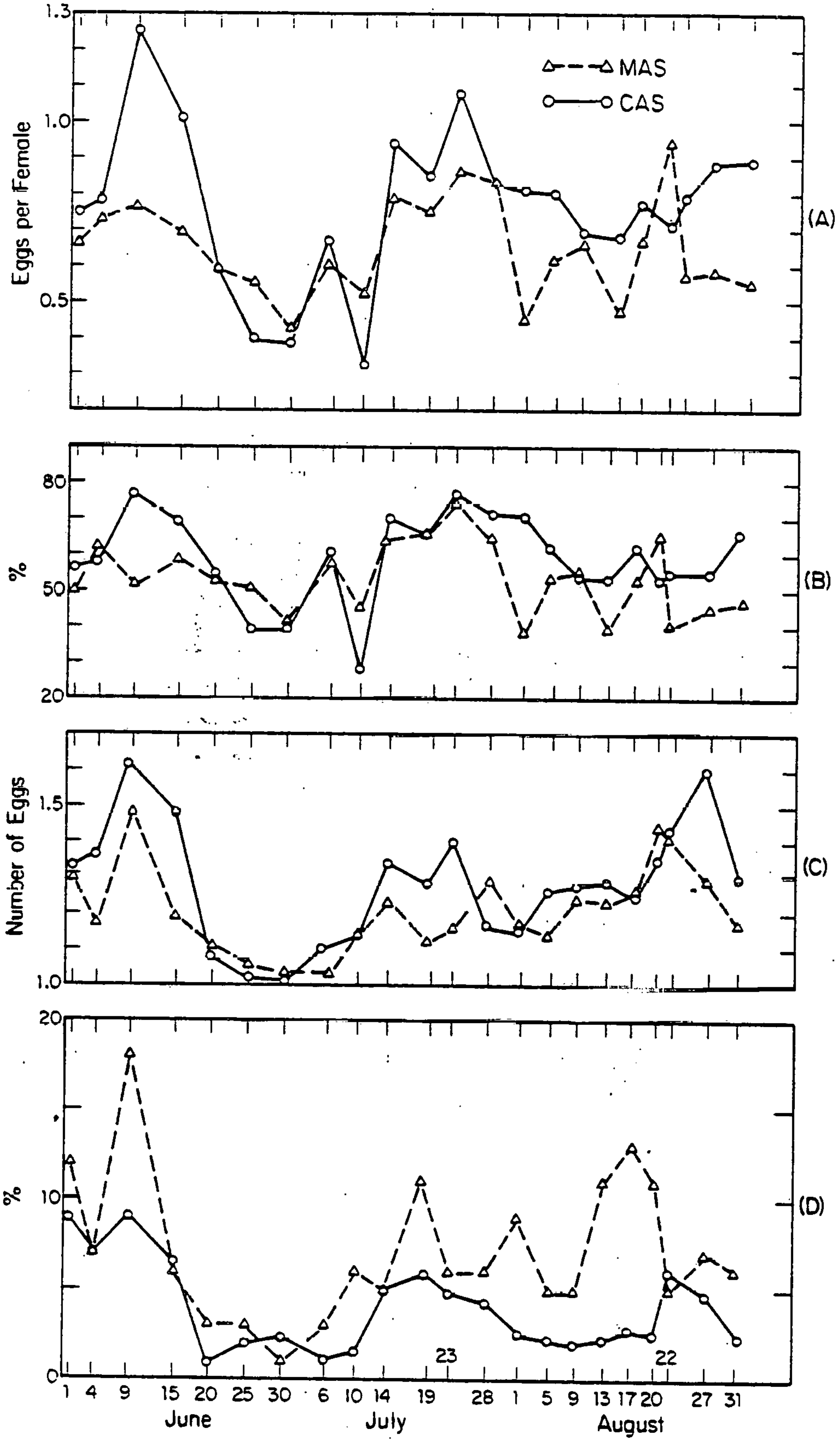
Figure 8 - Means of *Bosmina* egg production parameters during the summer, 1976 at Stations CAS and MAS.

A. Eggs per female

B. Percentage gravid

C. Clutch size

D. Percentage of females bearing deformed eggs


MAS
CAS
Eggs per Female
1.3
1.0
0.5
(A)
%
80
50
20
(B)
Number of Eggs
1.5
1.0
(C)
%
20
10
0
(D)
23
22
1 4 9 15 20 25 30 6 10 14 19 28 1 5 9 13 17 20 27 31
June
July
August

Station MAS had a significantly higher percentage ($p \leq .05$, Wilcoxon-Matched-Pairs-Signed-Rank-Test), of females bearing deformed eggs, reaching above 10% on six different sampling dates.

There was no significant difference for either station in eggs per female between seasons. Average clutch size was higher in the winter at both stations, but this was offset by a significantly lower percentage of gravid females in the winter ($p < .05$, Mann-Whitney-U-Test).

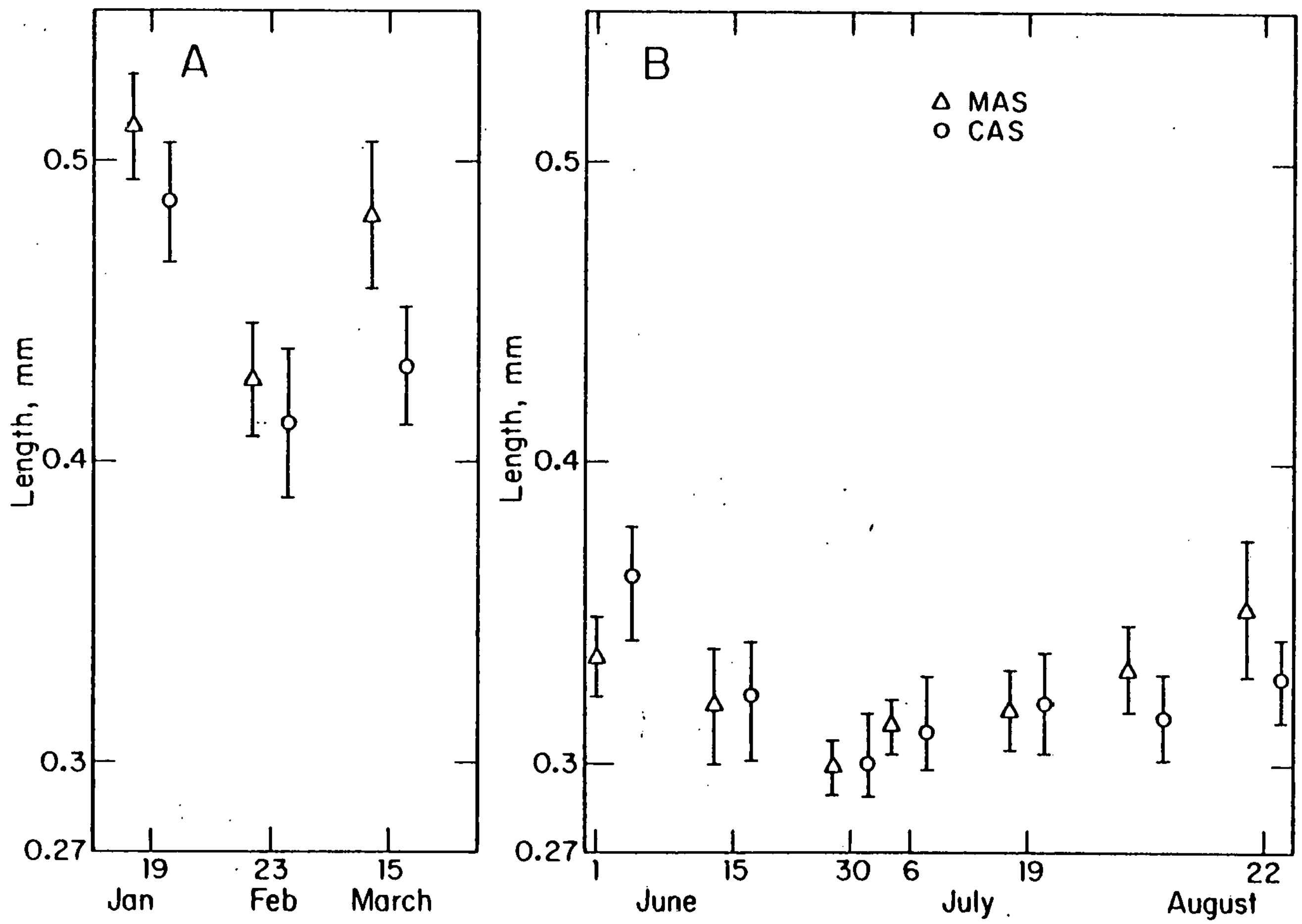
<u>Size of reproductive females</u>

The average size of reproductive females (Figures 9A and B) was larger in the winter than in the summer. The two stations differed significantly on only one date, March 15, 1976. On that date the body size of reproductive females was higher at MAS than CAS. Both stations in the summer had smallest average size in late June and early July and their largest average size in early June and late August.

Figure 9 - Mean and 95% confidence limits of size of reproductive Bosmina females at Stations CAS and MAS.

A. Winter, 1976

B. Summer, 1976


A
B
△ MAS
○ CAS
Length, mm
0.5
0.4
0.3
0.27
19
Jan
23
Feb
15
March
1
15
30
6
19
22
June
July
August

DISCUSSION

Edmondson Egg Ratio Model

Edmondson's egg ratio model is very attractive to the field ecologist because it requires only routine counting of individuals which can easily and rapidly be accomplished during a short period of time. Aging or sizing of individuals, for the Leslie Matrix, by contrast, is extremely tedious and time consuming. Although the exponential growth equation may be simplistic, other growth models, such as the logistic growth equation, require detailed knowledge of the biology of the organisms and often include concepts, carrying capacity, for example, which have no operational definition outside the behavior of the organism and which will certainly vary over time. The general consensus in the literature seems to have been that the theoretical underpinnings of the Edmondson model are reasonable if the precautions discussed in the literature review are taken.

As has been noted, most of the currently popular statistical methodology for zooplankton sampling depends upon the use of normal distribution statistics which require very specific assumptions. To do the estimation of power in this study, for example, normality was assumed. Statisticians seem to be divided into two camps, those who stress the importance of meeting all assumptions and those who emphasize the "robustness" of normal statistics. These uncertainties are reflected in the use here of three different methods for estimating confidence intervals around the Edmondson model results.

The variance for r' and d' between the Arnett-Suich and Keen methods are homogeneous (F-max = 19.0, Table 14, and 21.0, Table 16). The principal difference is in the 95% confidence intervals, caused by the differences in degrees of freedom between the two methods. The similarity in *d'* variances is curious because the Keen method does not include the variance associated with instar duration (Table 15). The Arnett-Suich method assumes from the start that Gaussian derived statistical methods can be applied. If this assumption is incorrect, the confidence limits will be narrower than they should be if a skewed sampling distribution was in reality more appropriate. The major difficulty with the Keen method is that it emphasizes the distribution of the calculations of *r'* and *b'*, and *d'* which Keen and Nassor (unpublished manuscript) have determined will be normally distributed, even if the samples are drawn from two populations with negative binomial (skewed) distributions. While the mathematical properties of these calculations may be distributed in a Gaussian manner, it is difficult to reconcile that population samples which came from non-normal distributions can be combined to produce a complex calculation that has greater precision than can be derived by taking into account the actual error as the author's method does.

The 95 percent confidence interval range is always greater than the confidence intervals of the other two methods. For the *r'* determination, the author's method utilized the variance associated with the samples themselves, while the Arnett-Suich method and the Keen method calculate the variance utilizing the natural log values $[\ln(\frac{x}{y})]$. In the *d'* determinations, the same is true with a further difference. It can be seen from the second partial derivitive of Equation 6,

$$\frac{\partial b'^2}{\partial D \partial (E+1)} = \frac{-1}{D^2(E+1)}$$

that birth rate is much more sensitive to changes in instar duration than eggs per female. Furthermore, the degree of sensitivity of b' to changes in instar duration varies depending upon the value of D. Figure 10 is an example of this function when E/F is held constant at 1.0 and D varies in increments of 0.5 days. When instar duration is short (<6 days), b' is very sensitive to changes in D. The author's method directly reflects this relationship while in the Keen and Arnett-Suich methods the variance of birth rate is assumed to be insignificantly affected by variance in instar duration or assumed to be homogeneous. The difference in the methods for d' between the Arnett-Suich and the author's method can be illustrated by the following inequality:

$$\sqrt{\sigma^2_{r'}} + \sqrt{\sigma^2_{b'}} > \sqrt{\sigma^2_{r'} + \sigma^2_{b'}}$$

There seems to be no resolution at this time as to which of the three methods most accurately estimate the uncertainty around the egg ratio parameters. All three methods have their strengths and weaknesses.

In addition to there being no readily available method to determine confidence intervals, there appeared to be no agreement in the literature on which form of Edmondson's (1960) equations to use.

In this thesis the following equations were used:

$$r' = \frac{\ln (Nt/No)}{t} \qquad \text{(equation 4, literature review)}$$

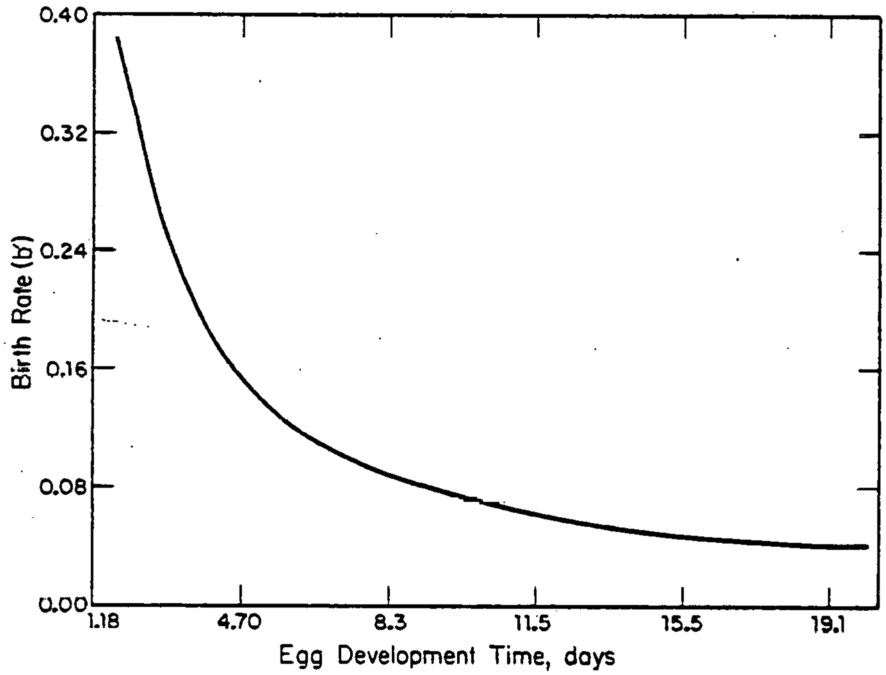
$$b' = \frac{\ln (E+1)}{D} \qquad \text{(equation 6, literature review)}$$

$$d' = b' - r' \qquad \text{(equation 3, literature review)}$$

Equation 4 has no controversy as to its form. Equation 6 was chosen on the strength of Palaheimo's (1974) arguments and sample calculations and because it was recommended by Edmondson as well (1968). The method for determining eggs per female (E, equation 6; see Methods and

Figure 10 - The change in birth rate, $\underline{b}'$, as a function of the change in instar duration (D) when eggs per female (E/F) is held constant at 1.0 and instar duration is varied in increments of 0.5 days.

The equation for $\underline{b}'$ is $\underline{b}' = \frac{[(E/F) + 1)]}{D}$


0.40
0.32
0.24
0.16
0.08
0.00
Birth Rate (b')
1.18
4.70
8.3
11.5
15.5
19.1
Egg Development Time, days

Methods Development) did not use the estimated population size and thus compounding errors in population and egg number estimates was avoided. Equation 3 also is very straightforward. These equations are the most likely to yield reasonable results, again assuming that the underlying assumptions of the model are met. The examination of potential synchronous egg hatching (Appendix C) is an example of a problem that might arise in trying to meet the assumptions.

The primary problem in working with the Edmondson egg ratio model in this and, according to Prepas and Rigler (1978), all other studies is that population estimates cannot easily be made without substantial sampling error or uncertainty. For the determination of $\underline{r}'$, for example, every aspect of the measurement of how many Bosmina were in a given area of Par Pond had measurement errors (see Methods and Methods Development). Subsampling for abundance estimates had a 20 percent error (Figure 3). No straightforward method appeared available to incorporate that error into a total error statement. Transformation of sample estimates using two different methods yielded spurious results (Table 3). Ignoring the subsampling error, the variances that were calculated were substantial and examination of the power of the sampling program showed that only differences of 40 percent or more between means were able to be detected at the standard error level of 0.05.

In the examination of spatial pattern of Bosmina in Par Pond, the observation that the $s^2/\bar{X}$'s (variance to mean ratios) were all greater than 1.0 (Tables 10 and 11) suggests that the distribution pattern of Bosmina at both stations was clumped. However, while the $\overset{*}{X}/\bar{X}$'s (index of patchiness) were also always greater than 1.0, the magnitude of difference over 1.0 was quite small which suggests a random pattern.

The relatively small 'c' values indicate that departures from a Poisson distribution would be small. These conflicting results may be explained by the possibility that the animals could be distributed randomly within patches which are themselves clumped in distribution (George, 1974). The special sampling experiments to examine time of day and station locality on mean Bosmina standing stock estimate lend support to this hypothesis (Table 4). There was a significant added variance due to time of day on one of the two days sampled at each station and a significant added variance due to location at both stations on the day sampled for that factor. The added variance due to time of day and station locality (Table 4) suggests that point variance estimates, despite their size, may be underestimates of area variability.

These results are not unique to Par Pond. Dumont (1967), for example, took single samples of Bosmina in Lake Donk, Belgium, at 36 stations on each of five consecutive days. His indices of patchiness ($\overset{*}{X}/\bar{X}$) ranged from 1.30 to 2.53 and 'c' values calculated from his data ranged from 0.30 to 1.54. Both these indices exceeded the upper range of the observed Par Pond Bosmina values (Tables 10 and 11). George (1974) reported a yearly mean index of patchiness for Bosmina longirostris of 1.19 ± .06 in 1970 and 1.21 ± .06 in 1971 in Eglwys Nynydd, a water supply reservoir in South Wales. He concluded that the distribution pattern for Bosmina was not randum; i.e., clumped. Whiteside reported index of patchiness values of 1.37 to 2.61 for the more benthic dwelling chydorid cladocerans he sampled in Elk Lake, Minnesota. For some species of zooplankton, Lewis (1978) has demonstrated that spatial variation may be as great as temporal variation over a 5-month period.

Prepas and Rigler (1978) recommended that population estimates be smoothed before use in the Edmondson egg ratio model because of bias in net sampling. However, even if completely unbiased net samples could be made, large natural spatial heterogeneity as documented by Dumont (1967), George (1974), Whiteside (1974), Lewis (1978) and by the indices reported in this study can also cause over or under-estimation of true population sizes when means are calculated from small sample sizes. The expression of this potential is large confidence intervals.

The only way to reduce the effect of large variance on confidence intervals and maintain statistical rigor is to increase sample size. We can estimate the level of effort we might have to make if we wished to increase our precision by examining the results of Kerfoot (1975). He made 37 measurements of $\underline{r}'$ for Bosmina in Lake Washington, Washington. If his estimates were generally accurate and ignoring the direction of change, sixty-four percent of his $\underline{r}'$ measurements were equal to or less than 0.09 and forty-six percent were less than 0.06. Therefore, according to Table 13, unless enough samples were taken to detect less than a twenty percent difference between means, almost half of the probable changes in population size would be missed. A three-month study in the summer, sampling at three-day intervals, would require that at least 930 samples per station be processed for that period (Table 13). From experience, one sample requires, at minimum, one man-hour to process. (Included in this estimate is time for sampling, sample preparation, and counting of subsamples). Assuming an average work day to include six hours of actual working time and a five-day work week, one 930 sample station would require 32 man-weeks to complete, and this for only a three-month study.

There were uncertainties and difficulties with determination of birth rate as well. While the study of vertical migration in August (Appendix B) did not conclusively show that Bosmina had a population thermal history that was significantly different from the mean water temperature at Station MAS, the evidence was enough to conclude that a calculation of birth rate, even interpreted non-parametrically, could lead to misleading results. Average temperatures in the summer ranged from 23°C to 30°C (Figure 2A, Appendix A) at the two stations. Instar duration would, therefore, have varied between 1.8 and 1.3 days (Table 9) and this range is in the most sensitive portion of the birth rate curve as a function of change in instar duration (Figure 10). The sensitivity of birth rate to plankton thermal history is also emphasized by Prepas and Rigler (1978). In addition, the estimates of eggs per female had substantial confidence intervals (as much as ± 40%) associated with them, and uncertainty was even higher with the winter samples when subsampling error was potentially higher (see Methods and Methods Development).

Taken together, the number of negative death rate determinations already published, the range of potential measurement errors, problems in the mathematical properties of the model and the number of different formulations used in the literature, and the uncertainties in determining the confidence intervals, lead to the conclusion that, at best, Edmondson egg ratio model calculations yield ordinal results and should be interpreted using a non-parametric frame of reference. Although not stated in this way, this was also the conclusion reached by Prepas and Rigler (1978). For this study, because of the uncertainties in measurement, the lack of instar development data from Par Pond, the difficulties already mentioned with uncertain thermal histories, and the large estimated confidence intervals (sometimes greater than twice the mean, Table 16) for the $\underline{r}'$, $\underline{b}'$, and $\underline{d}'$ estimates, it was decided that more reliable information on reactor operation effects could be determined by direct non-parametric comparison of data between stations.

The problems associated with the egg ratio model are by no means unique. Whenever multiple measurements are utilized, such as in productivity to biomass ratios or diversity indices, variance will be additive, increasing the possibility of spurious or uncertain results like death rates less than 0. Science, for example, has published arguments over the structure of planktonic niches (Lane, 1978; Makarewicz and Likens, 1978). The authors do not reveal the actual confidence that can be placed in the measurements of productivity or competition coefficients. The number of samples taken in this study at the Par Pond stations is similar to most reported sampling programs in the literature. The conclusion that the population estimates were, at best, ordinal in value suggests real difficulties for plankton biologists doing quantitative research on the behavior of populations. Prepas and Rigler (1978) while they point to several general observations that were made using the Edmondson egg ratio model, conclude that this is not a truly quantitative method and the results of this study support that conclusion. It would not be surprising to discover that the same result was true of competition coefficients, diversity indices, and productivity to biomass ratios.

A truly rigorous sampling program should include a preliminary estimation of the variance expected and what that suggests for the type of questions to be asked. This task can be expensive and time-consuming as is illustrated by the work of Platt (1975) and Platt, et al. (1970). Our estimates of sampling effort suggest that small changes in population sizes may be difficult or too expensive to detect. Egg numbers and ratios such as egg to embryo ratios are somewhat less variable from sample to sample and provide a lot of useful information. It would have been more profitable to spend more time on egg or instar development times and egg numbers and sizes and less on population sizes. More information about the future course of the populations and the mechanisms of population change would have been learned for the same effort. If population estimates are absolutely necessary, such as for competition coefficient calculations (Lane, 1978), a non-parametric experimental design should be considered.

For the biologist doing a monitoring study for an industry where the importance of an observation is decided using only a statistical method of assigning level of significance, an interesting but sometimes vexing political situation is created. If the intensity of studies is not regulated, a utility might be tempted to spend as little money as possible on data collection, which could result in insufficient samples to detect statistical differences. Then the utility could demonstrate or conclude that there was no environmental effect. On the other hand, opponents of power plants can insist that great effort is expended on sampling, that small differences are found statistically significant irregardless of their biological importance, and then claim that unacceptable damage to the environment had occurred (McCaughran, 1977).

In this situation, what should occur is an 'a priori' determination of the quantitative change caused by industry operation that would result in an unacceptable biological event. A preliminary estimation of natural variability should then be made to determine the sampling effort required to detect that amount of change.

Standing stock and fecundity - *Bosmina longirostris*

The *Bosmina* standing stock at CAS and MAS were quite similar in all respects in the winter (Figure 6A). The only demonstrable differences were the peak in eggs per female at Station MAS on January 26 (Figure 7A) and the fact that MAS showed two large oscillations of standing stock during the period compared to one at CAS.

In the summer, the population at Station CAS seems to have undergone a classical life history cycle in June. A peak for the summer in eggs per female was followed by a peak in standing stock (Figures 6B and 8A). Eggs per female then decreased continuously until early July and standing stock showed a similar decline until the middle of July. For the rest of the summer standing stock remained low (10,000 animals per sample) even though the eggs per female has recovered to early June levels. This suggests that mortality was high from July through August. This pattern of standing stock and eggs per female changes over time is similar to that reported by Hall (1964) for *Daphnia galeata mendotae* in Michigan.

At Station MAS, the population underwent three large oscillations (> 70%) in standing stock during the summer. While statistically different, the means of eggs per female appear quite similar (a difference of .12 eggs per female) at the two stations and the range smaller at MAS (Table 18). Thus, we might conclude that mortality was irregular in intensity at Station MAS. "Mortality" at MAS must include loss of animals due to "downstream" currents in the areas of the reservoir nearest the littoral zones (Appendix E). However, it is difficult to imagine how this factor would be highly irregular. Reactor operations were suspended for only seven days during the summer (July 20 to 28), a time when standing stock was near its lowest level. Furthermore, increases and decreases were usually continuous over at least two sampling periods. The only exception was between July 14 and 19, but this occurred before reactor operations were suspended. Immigration of Bosmina into the area from the precooler reservoir (Figure 2) during the summer can also be discounted as a factor causing observed increases because of the lethally high temperatures in the precooler pond (40°C).

A second demonstrable difference in the summer between the two stations was the greater percentage of deformed eggs at Station MAS. The temperature in the upper meter may reach as high as 34°C which is only 2°C below the upper thermal tolerance limit estimated in this study for Bosmina (Appendix D). The vertical migration data shows that some of the Bosmina population is in the upper meter at all times (Figure B2, Appendix B). Other factors which might influence this phenomena might be the generally lower O_2 levels (Appendix A), higher bacterial populations (Fliermans, et al., 1977), toxic chemicals or heavy metals released by the reactor in low levels, or a synergism of all these factors. Hall (1964) reported the presence of degenerate eggs in Daphnia galeata mendotae and while it is not possible to directly compare the two studies because he reported his results as the number of degenerate eggs and used color and texture to make his observations, he also did not find any degenerate eggs in January or February.

Both populations were demonstrably different between summer and winter in clutch size, percentage of females gravid, percentage of females bearing deformed eggs and body size. Summer animals were smaller (Figure 9) and bore smaller clutch sizes on the average. The seasonal cycling of body size has been reported by Kerfoot (1974) for Bosmina longirostris in Frains Lake. While the average clutch sizes reported by Kerfoot for the summer months fall within the range observed in Par Pond, his two observations in February (one in 1968 and one in 1969) are below the range of means in the winter from Par Pond. Mean body size of reproductive females was also higher in Par Pond as compared to Frains Lake in both summer and winter. Kerfoot's range of means for the summer months was 254μ to 291μ (five observations over two years) while in Par Pond the range was 300.0μ to 362.0μ (seven observations at each station). For all other months of the year, Kerfoot reported a range of means of 290.2μ to 389.8μ (17 observations over two years) compared to the range in winter in Par Pond of 412.0μ to 810.0μ (three observations at each station, Figure 9).

The lowest percentages of females gravid in the winter at both stations might be due to a combination of lower productivity (Marshall and Tilly, 1971) and higher standing crops of Bosmina. These higher population sizes coupled with the slower development times due to lower water column temperatures in the winter indicate that turnover is much slower in the winter months, as it is in lakes of northern latitudes.

It cannot be concluded, as did Patalas (1970) for the Polish lakes that he studied, that the addition of heated effluents has caused a doubling of the secondary productivity. Nor is there evidence to support a conclusion of reduced productivity due to reactor operations. Bosmina longirostris has been reported from Par Pond since 1965 (J. S. Marshall and L. J. Tilly, unpublished data), so reactor operations have not created conditions that have influenced the survival of Bosmina as a population.

However, I did find a difference between Stations CAS and MAS Bosmina populations in egg mortality and there was a difference in the course of standing crop changes in the summer. These results are consistent with other Par Pond studies which have found these stations to be essentially similar with some statistically significant differences. For example, none of the yearly averages of integral productivity from 1970 to 1974 differed between the two stations. However, P-mas was always higher and the depth of P-max in the winter column was always higher at MAS (Tilly, 1974). Diatometers in the surface water at MAS usually reveal similar species composition to those at CAS but standing crops have been significantly higher at MAS (Tilly, unpublished data). The same fish species have been reported for both stations during reactor operations with the notable exception of the obligate planktivore, Alosa aestivalis (Siler, 1975) which appears at MAS when the reactor is not operating (Vigerstad, field observations). There are large numbers of largemouth bass (Micropterus salmoides) at both stations (Gibbons and Bennett, 1973) but the bass captured near MAS have statistically lower body conditions, higher incidence of infection by red-sore disease caused by the bacterium, Aeromonas hydrophila and the ciliate, Epistylis sp. (Esch and Hazen, 1978), and higher population sizes of the intestinal parasite Neoechinorhyncus cylindratus (Acanthocephala) (Eure and Esch, 1974).

Whether the cause of these differences is higher temperatures (Esch and Hazen, 1978), higher immediate nutrient levels, toxic substances such as mercury (Tilly, 1976, and unpublished data) or a synergism of these factors has not been conclusively established. None of the observed differences, including those in this study, have been dramatic enough to warrant remedial action by those in charge of reactor operations.

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APPENDIX A

ADDITIONAL LIMNOLOGICAL DATA

Introduction

The purpose of this section is to describe background physical, chemical, and biological data collected during the period of this study. Most of this data (with the exception of cladoceran abundances) were not collected as part of the overall design of this study and are not meant to support the data or conclusions already described. It is presented so that the limnologically oriented reader can have a general picture of Par Pond during the period of this study.

Physical-chemical parameters

Basic physical and chemical information on Stations MAS and CAS for the period of September 1974 to September 1976 was obtained from the Savannah River Laboratory. Below is a list of the types of information available. Methods are described in Standard Methods (1971).

1. Surface conductivity by a conductivity meter.
2. Light penetration at midday by secchi disk.
3. Surface dissolved oxygen at midday by Hydrolab surveyor.
4. Surface pH by a Hydrolab surveyor.
5. Surface Ca, Mg, K, Na, and Si ions by atomic absorption.
6. Surface Cl ions by the mercuric thiocyanate method.
7. Surface sulfates by the turbidimetric method.
8. Surface orthophosphates by the stannous chloride method.

9. Surface nitrates by the phenol-disulphuric acid method.
10. Turbidity with a Hach Turbidimeter.
11. Total surface alkalinity by titration.

Similar basic limnological information for Par Pond has been described by Grace (1977) for the periods of March through September of 1974 and 1975. Tilly (1975) has summarized the same data from Stations CAS collected from 1965 to 1973. In brief summary, according to Grace (1977) there were gradients of nutrients and light penetration down the middle arm of Par Pond (the effluent receiving arm) from the point of entry of the effluent. The direction of the gradients, however, depended upon the particular nutrient. For example, magnesium, sodium, calcium, and potassium ions were all found to be higher at unheated areas than at the discharge point. Nitrate levels, on the other hand, decreased at increasing distances from the discharge point, although the decrease was not linear. Oxygen levels were higher at the unheated than at the heated areas, but the differences could be completely explained by the different solubilities at different temperatures.

Tilly (1975) has reported that water quality at CAS parallels that of the Savannah River and that this was due to the additions of makeup water for the P reactor. Except for chlorides and bicarbonates, concentrations of major ions in Par Pond correlated significantly with those from the Savannah River as did the conductivities of these two systems. Additional evidence for the dependence of Par Pond on Savannah River water comes from comparison of the two systems with other SRP area Coastal Plain waters. For example, the relative ionic composition of Par Pond and the Savannah River is bicarbonate dominated while chloride domination is characteristic of most SRP area Coastal Plain water.

Table A-1 shows the monthly averages of the ten factors described for station CAS between September 1974 and August 1976 and Table A-2 for Station MAS. Table A-3 shows the results of a sign test to compare all measurements at Stations CAS and MAS for the same time period. The direction of differences are for the most part, the same as those described by Grace (1976) for March through September of 1974 and 1975. However, Grace reported no significant differences ($P<.05$) for chlorides during the growing seasons while this analysis shows a significant difference ($P<.05$) between the two stations for all the 1974 through 1976 data. Grace also reported significant differences for potassium in the 1975 growing season but not for the 1974 season. This analysis shows no overall significance for 1974 through 1976.

Phytoplankton community productivity

Primary productivity at Stations CAS and MAS was measured simultaneously once a month from September 1975 to August 1976 with the exception of June 1976 using the C14 method. At each station water samples were taken with a 5 liter Van Dorn bottle at one meter intervals down to 7 m. Between the surface and 3 meters, pairs of 135 ml Pyrex reagent bottles were placed at 25 cm intervals, at 50 cm intervals between 3 and 6 meters, and one pair placed at 7 meters. Each bottle was spiked with 100 ul of $NaH^{14}CO_3$ solution. Incubations were for three hour intervals always between 1100 and 1400 hours. The bottles were returned to the laboratory immediately, filtered through 0.45 u Millipore filters and the filters placed in scin-

Table A-1. Average monthly values for water quality parameters at Station CAS from September 1974 through August 1976. Number of measurements per month are in parentheses.

		Conductivity (umho)		Dissolved O_2 (ppm)		pH	
1974	September	52.7	(2)	5.96	(2)	7.50	(2)
	October	55.2	(2)	7.00	(2)	7.55	(2)
	November	52.2	(2)	8.12	(2)	7.70	(2)
	December	52.6	(3)	9.33	(3)	8.00	(3)
1975	January	51.5	(2)	9.26	(2)	7.53	(3)
	February	51.0	(2)	9.62	(2)	7.50	(4)
	March	52.0	(2)	9.46	(2)	7.27	(4)
	April	52.5	(1)	8.50	(1)	7.50	(4)
	May	50.6	(3)	8.46	(3)	7.56	(5)
	June	49.5	(1)	7.36	(1)	7.68	(5)
	July	49.2	(2)	7.26	(2)	7.67	(4)
	August	50.0	(1)	7.85	(1)	7.42	(4)
	September	53.7	(2)	7.50	(2)	7.15	(4)
	October	54.2	(2)	7.93	(2)	7.38	(5)
	November	43.6	(3)	6.90	(3)	7.16	(3)
	December	39.8	(2)	9.50	(2)	7.44	(5)
1976	January	--		--		7.37	(4)
	February	42.7	(2)	9.85	(2)	7.47	(4)
	March	44.4	(2)	9.05	(2)	7.46	(5)
	April	48.4	(2)	9.70	(2)	7.73	(3)
	May	47.5	(2)	8.95	(2)	7.90	(5)
	June	44.7	(2)	8.70	(2)	7.92	(4)
	July	53.9	(3)	8.96	(3)	7.88	(5)
	August	52.0	(2)	8.25	(2)	7.30	(4)

Table A-1 (cont'd)

Turbidity (jtu)		Secchi (m)		Alkalinity (mg/l)		Calcium (mg/l)		Chloride (mg/l)	
2.62	(2)	1.95	(2)	--		--		--	
1.50	(1)	2.07	(2)	--		--		--	
--		2.75	(2)	--		--		--	
--		2.53	(3)	--		--		--	
0.85	(2)	2.85	(2)	13.60	(1)	4.56	(1)	5.70	(1)
1.40	(2)	2.88	(2)	13.15	(2)	4.25	(2)	6.10	(2)
--		2.68	(2)	12.10	(2)	4.18	(2)	5.15	(2)
--		2.65	(2)	12.40	(2)	4.05	(2)	5.40	(2)
1.45	(2)	2.68	(2)	13.47	(3)	4.10	(3)	5.73	(3)
1.93	(3)	2.46	(3)	13.60	(2)	4.26	(2)	4.85	(2)
1.60	(2)	2.62	(2)	11.25	(2)	4.41	(1)	5.00	(2)
1.60	(2)	3.00	(2)	11.45	(2)	3.94	(2)	4.75	(2)
1.95	(2)	2.92	(2)	13.35	(2)	4.10	(2)	5.35	(2)
2.75	(2)	2.50	(2)	14.57	(3)	4.30	(3)	5.07	(3)
2.00	(2)	1.98	(2)	17.80	(1)	4.73	(1)	5.20	(1)
2.20	(3)	2.80	(3)	16.20	(2)	4.65	(2)	5.25	(2)
5.00	(2)	2.00	(2)	15.60	(2)	4.74	(2)	5.10	(2)
2.45	(2)	2.65	(2)	15.40	(2)	4.47	(2)	3.45	(2)
2.50	(1)	2.62	(2)	15.30	(3)	4.56	(3)	5.17	(3)
1.55	(2)	2.95	(2)	15.20	(1)	4.73	(1)	5.50	(1)
2.60	(2)	2.55	(2)	15.50	(3)	4.54	(3)	5.30	(3)
2.75	(2)	2.38	(2)	16.70	(2)	4.65	(2)	5.20	(2)
2.00	(1)	2.28	(3)	15.25	(2)	4.61	(2)	4.35	(2)
1.95	(2)	2.20	(2)	16.10	(2)	4.52	(2)	5.10	(2)

Table A-1 (cont'd)

Potassium (mg/l)		Magnesium (mg/l)		Sodium (mg/l)		Nitrate (ug/l)		Ortho-phosphate (ug/l)	
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--		--		--		--		--	
--		--		--		--		--	
--		--		--		--		--	
1.13	(1)	0.84	(1)	5.97	(1)	55.00	(1)	2.00	(1)
1.11	(2)	0.99	(2)	5.51	(2)	13.50	(2)	2.00	(2)
1.10	(2)	0.90	(2)	5.47	(2)	2.00	(2)	3.00	(2)
1.06	(2)	0.90	(2)	5.64	(2)	0.50	(2)	1.50	(2)
0.95	(3)	0.85	(3)	4.91	(3)	3.00	(3)	3.00	(3)
0.95	(2)	0.79	(2)	4.66	(2)	1.00	(2)	2.00	(2)
0.96	(1)	0.80	(1)	4.78	(1)	26.50	(2)	2.50	(2)
0.95	(2)	0.77	(2)	5.11	(2)	0.00	(2)	2.50	(2)
0.98	(2)	0.85	(2)	5.20	(2)	1.00	(2)	1.00	(2)
1.01	(3)	1.06	(3)	5.17	(3)	2.00	(1)	1.50	(2)
1.08	(1)	1.08	(1)	5.73	(1)	8.00	(1)	1.00	(1)
1.12	(2)	1.11	(2)	5.88	(2)	21.00	(2)	4.50	(2)
1.09	(2)	1.08	(2)	5.93	(2)	30.00	(2)	2.50	(2)
0.99	(2)	1.08	(2)	5.52	(2)	3.50	(2)	3.00	(2)
1.09	(3)	1.03	(3)	5.45	(3)	4.33	(3)	1.66	(3)
1.05	(1)	1.05	(1)	5.27	(1)	1.00	(1)	1.00	(1)
1.06	(3)	1.08	(3)	5.44	(3)	6.00	(3)	4.33	(3)
0.96	(2)	1.07	(2)	5.30	(2)	5.00	(2)	6.00	(2)
0.90	(2)	1.03	(2)	5.07	(2)	4.50	(2)	3.00	(2)
0.87	(2)	0.98	(2)	5.25	(2)	7.50	(2)	5.00	(2)

Table A-1 (cont'd)

Silicate (mg/l)		Sulphate (mg/l)	
--		--	
--		--	
--		--	
--		--	
3.49	(1)	3.40	(1)
2.42	(2)	5.00	(2)
2.07	(2)	5.50	(2)
3.48	(2)	4.35	(2)
2.83	(3)	4.60	(3)
1.96	(2)	4.10	(2)
2.16	(1)	3.75	(2)
2.65	(2)	3.50	(2)
2.83	(2)	3.55	(2)
3.08	(3)	2.00	(3)
2.77	(1)	1.80	(1)
3.11	(2)	1.35	(2)
3.48	(2)	1.45	(2)
3.22	(2)	2.10	(2)
2.81	(2)	3.97	(3)
1.92	(1)	3.40	(1)
1.92	(3)	3.20	(3)
2.44	(2)	4.55	(2)
2.56	(2)	2.80	(2)
2.00	(2)	4.00	(2)

Table A-2 - Average monthly values for water quality parameters at Station MAS from September 1974 through August 1976. Number of measurements per month are in parentheses.

		Surface Conductivity (umho)		Surface Dissolved O_2 (ppm)		pH	
1974	September	5.32	(2)	4.58	(2)	7.15	(2)
	October	5.75	(2)	6.20	(2)	7.50	(2)
	November	5.35	(2)	6.78	(2)	7.45	(4)
	December	5.25	(3)	8.69	(3)	7.60	(5)
1975	January	5.45	(2)	8.55	(2)	7.40	(4)
	February	5.40	(2)	8.67	(2)	7.37	(4)
	March	5.50	(1)	8.84	(2)	7.15	(4)
	April	4.70	(1)	8.26	(2)	7.57	(4)
	May	5.03	(3)	7.74	(2)	7.54	(5)
	June	5.42	(2)	7.02	(3)	7.66	(5)
	July	5.25	(1)	7.16	(2)	7.82	(4)
	August	5.27	(2)	7.19	(2)	7.12	(4)
	September	5.45	(2)	5.80	(2)	7.22	(4)
	October	4.24	(3)	6.88	(2)	7.16	(5)
	November	4.38	(2)	7.00	(2)	7.06	(3)
	December	4.31	(2)	8.76	(3)	7.48	(5)
1976	January	--		--		7.27	(4)
	February	4.54	(2)	9.20	(2)	7.47	(4)
	March	5.15	(2)	8.55	(2)	7.32	(5)
	April	5.15	(2)	8.40	(2)	7.56	(3)
	May	4.60	(2)	8.37	(2)	7.50	(5)
	June	4.86	(3)	8.02	(2)	7.82	(4)
	July	6.00	(2)	7.46	(3)	7.24	(5)
	August	5.60	(2)	7.25	(2)	6.87	(4)

Table A-2 (cont'd)

Turbidity (jtu)		Secchi (m)		Alkalinity (mg/l)		Calcium (mg/l)		Chloride (mg/l)	
2.15	(2)	1.95	(2)	--		--		--	
1.90	(1)	2.00	(2)	--		--		--	
--		2.00	(2)	14.51	(2)	4.26	(2)	5.25	(2)
--		2.83	(3)	15.10	(2)	4.50	(2)	3.45	(2)
1.15	(2)	2.93	(2)	14.45	(2)	4.32	(2)	5.40	(2)
3.80	(2)	2.27	(2)	13.60	(2)	3.82	(2)	4.65	(2)
--		2.30	(2)	12.15	(2)	2.49	(2)	5.40	(2)
--		2.48	(2)	12.20	(2)	3.53	(2)	5.10	(2)
3.35	(2)	2.00	(2)	13.50	(3)	3.66	(3)	5.17	(3)
3.00	(3)	2.05	(3)	13.35	(2)	3.93	(2)	4.40	(2)
2.60	(2)	2.00	(2)	11.95	(2)	4.42	(1)	4.20	(2)
2.35	(2)	2.50	(2)	10.80	(2)	4.11	(2)	5.35	(2)
2.45	(2)	2.38	(2)	13.20	(2)	3.13	(2)	3.70	(2)
1.90	(2)	2.28	(2)	14.27	(3)	3.97	(3)	4.20	(3)
3.20	(2)	2.08	(2)	20.00	(1)	4.32	(1)	5.00	(1)
2.26	(3)	2.78	(3)	15.55	(2)	4.52	(2)	5.05	(2)
4.50	(2)	2.00	(2)	15.50	(2)	4.36	(2)	4.50	(2)
2.70	(2)	2.55	(2)	14.70	(2)	4.15	(2)	3.60	(2)
1.60	(1)	2.43	(2)	15.23	(3)	4.21	(3)	4.50	(3)
1.65	(2)	2.93	(2)	14.70	(1)	6.94	(1)	6.00	(1)
2.45	(2)	2.63	(2)	15.37	(3)	3.86	(3)	4.97	(3)
3.05	(2)	2.25	(2)	17.00	(2)	6.78	(2)	5.30	(2)
2.30	(1)	2.16	(3)	16.15	(2)	5.28	(2)	5.30	(2)
2.35	(2)	2.00	(2)	15.40	(2)	3.75	(2)	4.70	(2)

Table A-2 (cont'd)

Potassium (mg/l)		Magnesium (mg/l)		Sodium (mg/l)		Nitrate (ug/l)		Orthophosphate (ug/l)	
--		--		--		--		--	
--		--		--		--		--	
3.29	(2)	1.05	(2)	6.18	(2)	2.00	(2)	2.00	(2)
1.05	(2)	0.99	(2)	5.64	(2)	46.00	(2)	3.00	(2)
1.15	(2)	0.86	(2)	5.78	(2)	43.50	(2)	2.00	(2)
1.03	(2)	0.84	(2)	4.62	(2)	20.00	(2)	1.00	(2)
1.09	(2)	0.85	(2)	5.34	(2)	9.50	(2)	2.00	(2)
1.03	(2)	0.79	(2)	5.00	(2)	2.50	(2)	3.00	(2)
0.85	(3)	0.77	(3)	4.73	(3)	3.00	(3)	2.33	(3)
0.90	(2)	0.73	(2)	4.33	(2)	11.50	(2)	1.50	(2)
0.97	(1)	0.82	(1)	4.87	(1)	48.00	(2)	5.50	(2)
0.95	(2)	0.77	(2)	5.24	(2)	25.00	(2)	2.00	(2)
0.87	(2)	0.61	(2)	4.01	(2)	10.00	(2)	3.50	(2)
0.92	(3)	0.96	(3)	4.67	(3)	16.33	(3)	2.33	(3)
1.05	(1)	1.01	(1)	5.18	(1)	11.00	(1)	1.00	(1)
1.05	(2)	0.98	(2)	5.33	(2)	21.50	(2)	2.00	(2)
0.98	(2)	0.91	(2)	5.07	(2)	33.00	(2)	4.50	(2)
0.95	(2)	0.99	(2)	5.09	(2)	5.00	(2)	4.50	(2)
1.05	(3)	0.97	(3)	5.05	(3)	11.67	(3)	3.66	(3)
1.06	(1)	1.05	(1)	5.66	(1)	1.00	(1)	3.00	(1)
1.03	(3)	0.91	(3)	4.68	(3)	21.33	(3)	7.33	(3)
1.37	(2)	0.99	(2)	5.45	(2)	8.50	(2)	2.50	(2)
0.93	(2)	1.00	(2)	5.10	(2)	11.50	(2)	2.00	(2)
0.79	(2)	0.76	(2)	4.41	(2)	17.00	(2)	5.00	(2)

Table A-2 (cont'd)

Silicate (mg/l)		Sulphate (mg/l)	
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--		--	
.92	(2)	3.65	(2)
.92	(2)	5.10	(2)
2.23	(2)	5.20	(2)
2.15	(2)	4.25	(2)
2.26	(2)	5.75	(2)
3.21	(2)	3.15	(2)
3.00	(3)	4.10	(3)
1.83	(2)	4.55	(2)
2.20	(1)	3.35	(2)
2.46	(2)	3.40	(2)
1.73	(2)	3.30	(2)
3.24	(3)	2.10	(3)
3.04	(1)	3.10	(1)
3.09	(2)	1.05	(2)
2.92	(2)	1.25	(2)
3.30	(2)	2.05	(2)
2.76	(2)	4.03	(3)
2.62	(1)	3.80	(1)
1.85	(3)	2.73	(3)
3.05	(2)	3.95	(2)
2.83	(2)	3.55	(2)
2.00	(2)	4.00	(2)

Table A-3. Results of sign tests on water quality parameters of Stations CAS and MAS from September 1974 through August 1976.

Factor	Probability	Station with positive difference
Conductivity	.1188	n.d.[1]
Dissolved oxygen	.0000	CAS
pH	.0026	MAS
Turbidity	.0588	n.d.
Calcium	.0002	CAS
Chloride	.0286	CAS
Potassium	.1010	n.d.
Magnesium	.0002	CAS
Sodium	.0002	CAS
Nitrate	.0001	MAS
Phosphate	.1646	n.d.
Silicon	.7238	n.d.
Sulfate	.7490	n.d.
Total alkalinity	.6242	n.d.
Seechi disk	.0026	CAS

[1]n.d. - no significant difference between stations at alpha level of 0.05.

tillation fluid. Counting was done by the Savannah River Laboratory.

Alkalinity and pH measurements for the determination of total available carbon were made immediately after the bottles were placed in the water. Light penetration and temperature measurements were made immediately before removing the bottles from the water. Days with extreme cloud cover were avoided, when possible, for this study.

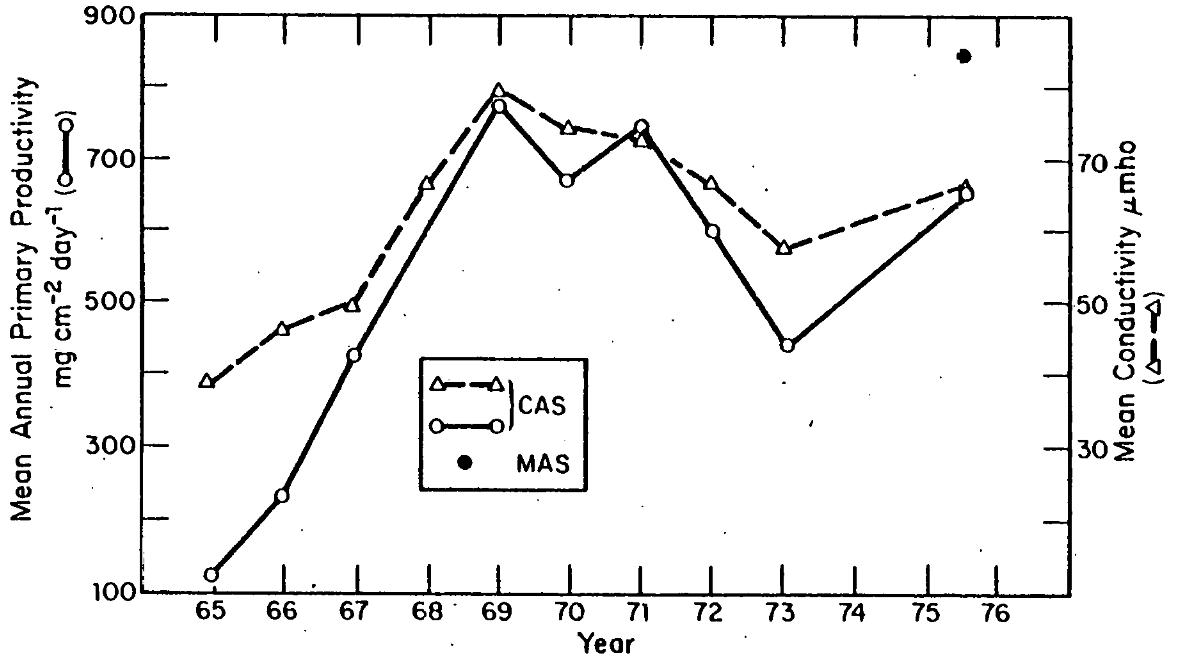
The results were plotted on graph paper, curves eye-fitted, and integrals determined by planimetry. The curves of even numbered and odd numbered bottles were considered to be replicates and three times the mean values of the integrals were used as the best estimates of primary productivity for the day. A more detailed description of the method and experimental verification of it can be found in Marshall and Tilly (1971) and Tilly (1973).

Table A-4 presents the average productivity levels and associated light, average water column conductivity, and temperature measurements for the period studied. Integral productivity and the maximum productivity observed were significantly different ($P<.05$, sign test) between Stations CAS and MAS with the average levels higher at MAS. Average water column conductivity was significantly different ($P<.05$, sign test) with the average lower at MAS than CAS. The temperature, surface light, and depth of maximum productivity (P-max) were not significantly different ($P>.05$, sign test) between the two stations. Figure A-1 shows this data in relation to that reported by Tilly (1975) for Station CAS. Productivity during 1975-76 appears to have returned to the 1972 level, reversing the downward trend observed from 1971 to 1973. Tilly (1975) had also reported a significant correlation between mean water column conductivity and primary pro-

Table A-4. Averages of primary productivity determinations made at Stations CAS and MAS once a month from September to August 1975-1976.

	CAS	MAS
Average maximum productivity ($mgC/m^3/3$ hr)	72.0	110.0
Average depth of maximum productivity (cm)	155	116
Average temperature at depth of maximum productivity (°C)	22.8	25.2
Average surface light at depth of maximum productivity (microeinsteins)	200	190
Average integral productivity ($mgC/m^2/day$)	654	867
Average water column conductivity (umho)	67.1	64.5

Figure A-1 - Mean annual primary productivity and conductivity at Station CAS (January-December 1965-1973, September to August 1975-1976) and Station MAS (September to August 1975-1976).



Mean Annual Primary Productivity
mg cm⁻² day⁻¹
Mean Conductivity μmho
900
700
500
300
100
70
50
30
65
66
67
68
69
70
71
72
73
74
75
76
Year
CAS
MAS

ductivity for Station CAS. This correlation is further supported by the 1975-76 results (Figure A-1) for Station CAS, but Station MAS had a significantly higher productivity than would have been predicted by the relationship of conductivity and productivity at CAS.

Limnological description: January to August 1976

Table A-5 summarizes the means and ranges of measurements associated with the routine limnological characterization from January to August 1976. The 4 meter depth was chosen for comparison of dissolved oxygen, pH, and conductivity because it lies above the regular thermocline in the summer at both stations and below the secondary thermocline due to the hyperthermal effluent at Station MAS. Dissolved oxygen and pH were significantly higher at this depth at Station CAS according to a sign test. There were no significant differences in Secchi desk transparency and conductivity. Integral productivity, maximum productivity at a depth, and depth of maximum productivity were also significantly higher at Station MAS according to the Wilcoxon-Matched Pairs Signed Rank Test.

Generally, during the winter months light penetration and temperature did not differ below 4 meters, and pH was similar throughout the water column. Only dissolved oxygen was consistently higher at each depth at Station CAS. In the summer at Station MAS light penetration was generally lower throughout the water column as was dissolved oxygen and pH. Temperature was again the same below 4 meters.

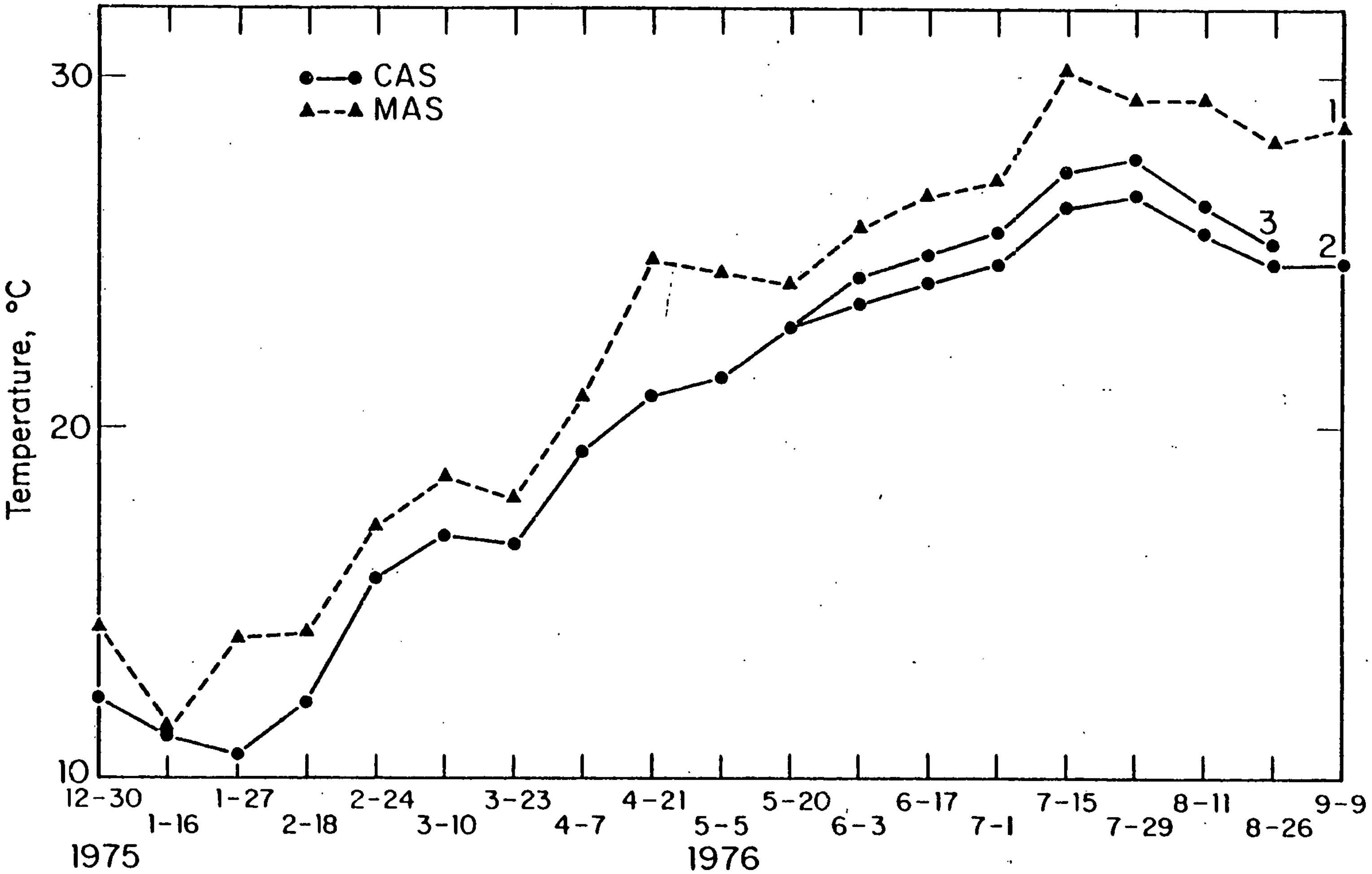
Figure A-2 shows the average water column temperatures in winter and summer at Stations CAS and MAS. The average difference between

Table A-5. Mean and range of monthly routine limnological measurements made at Stations CAS and MAS from January 1 to August 31, 1976.

	Jan. - Aug. 1976 (none in June)	
	CAS	MAS
Integral productivity* mgC/m²/3 hr	345.9 138.4 - 396.0	356.8 274.5 - 453.8
P max* mgC/m²/3 hr	77.5 36.0 - 107.7	121.0 94.2 - 176.4
Depth of P max* cm	196 87 - 225	146 75 - 200
Secchi disk m	2.44 1.75- 3.0	2.36 1.75- 3.0
Conductivity (4 M) umhos/cm²	478 422 - 555	471 420 - 550
pH (4 M)* (pH units)	6.3 5.0 - 8.35	6.0 4.6 - 7.8
DO (4 M)* ppm	8.3 6.4 - 9.8	6.5 4.1 - 9.3

* differed significantly ($P < .05$) according to sign test.

Figure A-2 - Average water column temperatures at Station MAS (0 to 7 meters) from December 1975 to September 1976 (1), Station CAS (0 to 12 meters) from December 1975 to September 1976 (2), and Station CAS (0 to 10 meters) from June 1976 through August 1976 (3).


CAS
MAS
Temperature, °C
30
20
10
1
2
3
12-30
1-16
1-27
2-18
2-24
3-10
3-23
4-7
4-21
5-5
5-20
6-3
6-17
7-1
7-15
7-29
8-11
8-26
9-9
1975
1976

The stations was 1.7°C in the winter and 2.1°C in the summer (using the differences between Station MAS means and CAS means for 0 to 12 meters depth for the summer calculations.

APPENDIX B

VERTICAL MIGRATION

Introduction

Gehrs (1974) conducted a laboratory study on the vertical movement of Diaptomous sanguineus (Copepoda) and Daphnia parvula (Cladocera) in response to heated water using 90 CM tall plexiglass tubes. All animals moved deeper when the temperature of the water at the level in which they resided was increased 2°C. Gehrs, based on these data and a review of the literature, concluded that thermal discontinuity can block vertical movement either by a large thermal gradient over a short vertical range, or by temperature that is above the range acceptable to the zooplankton.

Vertical migration sampling

Sampling for vertical migration was conducted from an anchored houseboat by lowering a 30-li Schindler trap (Schindler, 1969) with a winch and davit equipped with a meter wheel. Two samples were taken at each of eight depths beginning at the surface and ending at 7 m. Each time the Schindler trap was lowered so that its top was at the specified depth. Because the trap was 0.6 m deep, 60% of the entire 7-m water column was sampled by a set of samples.

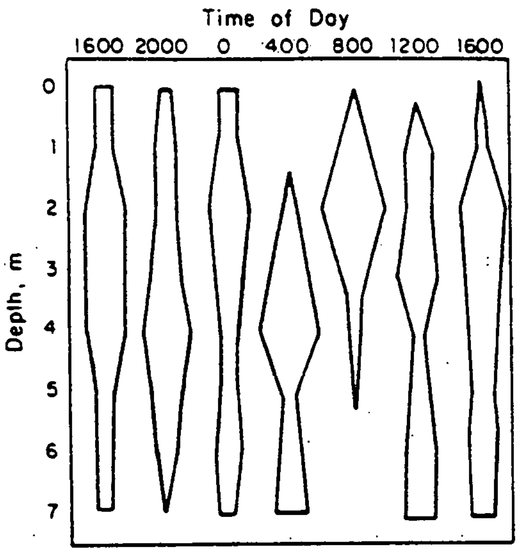
In the winter of 1975, samples were taken every four hours from 4 PM on December 2 through 4 PM on December 3 (seven sampling periods) at the thermally-elevated Middle Arm Station (MAS) having a depth of 8.0 m (See figure 2 of thesis). In the summer of 1976, samples were taken at MAS and a station of equal depth in the ambient temperature South Arm (SAS). Samples were taken every four hours at MAS beginning at 1 PM on August 5 through 9 AM on August 7 (12 samples), and at SAS from August 9 to August 11 (12 samples). Temperatures were recorded at the beginning of each sampling period for every sampling depth using a Hydrolab temperature probe.

Results

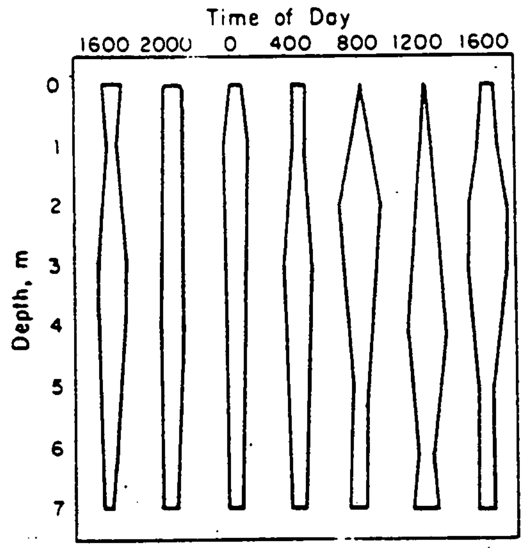
All samples were counted in their entirety for three species of Cladocera, Bosmina longirostris, Ceriodaphnia lacustris, and Diaphanosoma brachyurum. Because this study was focused on the distributional pattern of a species population in relation to depth (and temperature) in the water column, percentages were calculated for each species by dividing the numbers of individuals of a given species per sample by the total number of that species sampled in the entire water column during each sampling interval.

The distribution of each species at each depth over the seven sampling intervals in December 1975 is graphically illustrated in Figure B1). Analysis of variance (ANOVA) (using an arc-sine transformation on the percentage values) showed that distributions of means at each depth over time were significantly different ($P<.05$) for Bosmina longirostris and Ceriodaphnia lacustris, but not for Diaphonosoma brachyurum. Figure B2 illustrates the same data for these species for the August 1976 samples. Unfortunately, samples taken during 4:45 PM on August 10 at SAS were lost. To maintain a balanced design, samples from 4 PM on August 9 were therefore not included in the ANOVA. During August all there species had significantly different depth distributions ($P<.05$) over time at each station. Taking a group of six samples (five, in the case of SAS)

Figure B-1 - Depth distribution of *Bosmina longirostris*, *Ceriodaphnia lacustris*, and *Diaphanosoma brachyurum* at the Middle Arm Station of Par Pond in December 1975. Total sample sizes were 372 animals for Bosmina, 4544 animals for *Ceriodaphnia*, 1187 animals for *Diaphanosoma*.

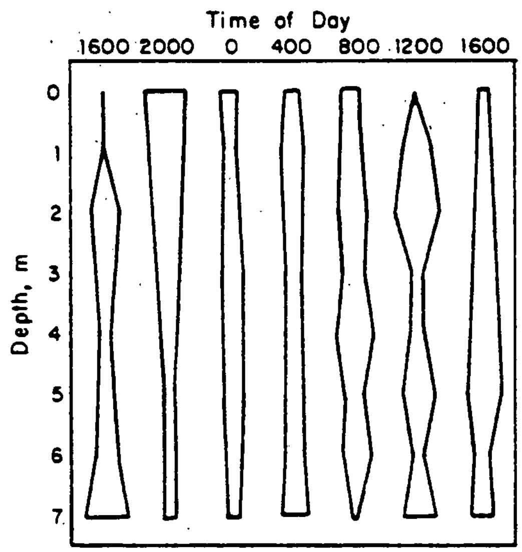


Bosmina longirostris



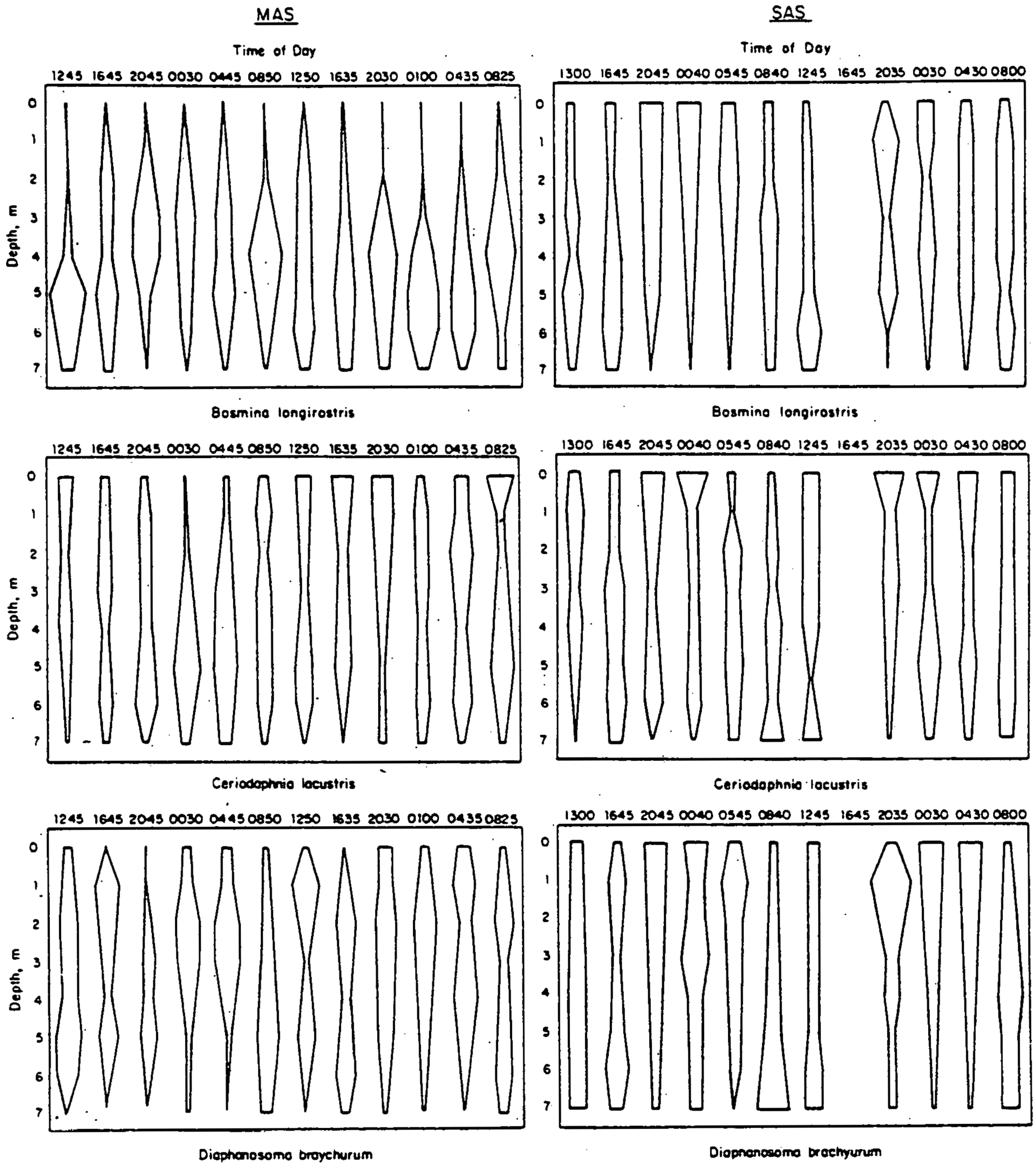
Ceriodaphnia lacustris

MAS



Diaphanosoma brachyurum

Figure B-2 - Depth distribution of *Bosmina longirostris*, *Ceriodaphnia lacustris*, and *Diaphanosoma brachyurum* at the Middle Arm (MAS) and South Arm (SAS) stations of Par Pond during August 1976. Total sample sizes at MAS: for *Bosmina*, 44,637 animals, for *Ceriodaphnia*, 8,723 animals, for *Diaphanosoma*, 3,730 animals. At SAS, sample size was 7,280 for *Bosmina*, 922 for *Ceriodaphnia*, and 2,093 for *Diaphanosoma*.


MAS
SAS
Time of Day
1245 1645 2045 0030 0445 0850 1250 1635 2030 0100 0435 0825
1300 1645 2045 0040 0545 0840 1245 1645 2035 0030 0430 0800
Depth, m
0
1
2
3
4
5
6
7
Bosmina longirostris
Ceriodaphnia lacustris
Diaphanosoma braychurum
Diapnanosoma brachyurum

to represent a day, the ANOVA showed that the pattern of distributions changed significantly from day-to-day for Ceriodaphnia lacustris at both stations, for Bosmina longirostris at both stations, for Bosmina longirostris at MAS, and for Diaphanosoma brachyurum at SAS ($P<.05$).

Although the formation and dispersal of clumps of animals at different times were observed for each species (Figures B1 and B2) and the changing distributions were significantly different, there was no easily discernible pattern of change over a 24-hour period. Only Bosmina longirostris in winter (Figure B1) showed a definite movement out of the surface in the early morning hours. Unfortunately, because of the lack of replication in that sampling interval, no statement of statistical confidence can be made for that observation.

The distribution of Bosmina at MAS in the summer samples (Figure B2) is interesting because of the temperature-depth distribution there. Thermal effluent at MAS forms a heated stratum of approximately 3 m thickness (Table B1). At and below 3 meters, temperatures at SAS are almost identical to those at MAS. If a species were to stay below 3 m in the Middle Arm, its thermal history might be nearly identical to another population of the same species in the South Arm. To compare distributions in the upper 3 m of the water column for each species at the two stations, I calculated percent total numbers of each species population present at each sampling period at each station in the upper 3 meters. These comparisons were made for 23 independent observations in August and seven in December. For all but one comparison, mean percentages were strongly similar during both August and December sampling among all species at both stations. Only relative abundance of Bosmina longirostris at MAS in August was

Table B-1. Mean ± S.E. of temperatures (°C) at middle arm (MAS) and south arm (SAS) in summer 1976 and MAS in winter 1975.

	Winter	Summer			
		Day 1		Day 2	
Depth	MAS	MAS	SAS	MAS	SAS
	Dec. 3-4	Aug. 5-6	Aug. 9-10	Aug. 6-7	Aug. 10-11
Surface	20.00 ± 0.14	32.9 ± 0.8	29.8 ± 0.7	34.3 ± 1.1	29.8 ± 0.4
1	19.9 ± 0.21	32.6 ± 0.7	29.5 ± 0.2	34.2 ± 1.1	29.6 ± 0.3
2	17.4 ± 0.42	30.8 ± 1.2	28.8 ± 0.2	31.3 ± 1.5	29.2 ± 0.2
3	16.6 ± 0.1	28.6 ± 0.2	28.5 ± 0.2	29.2 ± 0.4	29.0 ± 0.2
4	16.5 ± 0.1	28.2 ± 0.2	28.1 ± 0.04	28.4 ± 0.2	28.4 ± 0.2
5	16.4 ± 0.08	28.0 ± 0.1	28.0 ± 0.04	28.1 ± 0.1	27.9 ± 0.3
6	16.3 ± 0.08	27.3 ± 0.2	27.0 ± 0.6	27.4 ± 0.3	27.1 ± 0.2
7	16.2 ± 0.12	25.1 ± 0.2	24.5 ± 1.0	24.8 ± 0.8	25.6 ± 0.4
$WC\overline{X}$[1]	17.4	29.2	28.0	29.7	28.3

[1]$WC\overline{X}$ = water column average (mean of means)

significantly lower ($P<.05$) in the upper 3 m of the water column (Figure B3).

After describing both the vertical distribution of each species and the temperature profile in the water column, I estimated the thermal history of each species for the time period. This was done using the formula

$$\text{mean temperature for population per day} = \frac{\sum_{i=1}^{N} \text{percentage at depth}_i \times \text{temperature at depth}_i}{\sum_{i=1}^{N} \text{percentage at depth}_i}$$

where N is the number of samples taken.

Using daily data, separate calculations can be used to estimate variance of the mean temperatures for the populations (Figure B4). Because distributions over time were not significantly different for different days for Bosmina at SAS and Diaphanosoma at MAS, variability could not be estimated. Therefore, both values appear separately in Figure B4. For Bosmina at SAS, both estimates are higher than the mean value at MAS, but one lies within one standard error of the mean at MAS. The mean temperature for Ceriodaphnia is higher at MAS, but again the standard error estimates overlap for the two stations. Both estimates for Diaphanosoma at MAS are higher than two standard errors around the mean at SAS.

In comparison to the mean water column temperature at each station, calculated population microhabitat temperature means at MAS were higher, with the exception of Ceriodaphnia and Bosmina. For Ceriodaphnia and Diaphanosoma at both stations and Bosmina at

Figure B-3 - The average percent (± s.e.) of *Bosmina longirostris* (B), *Ceriodaphnia lacustris* (C), and *Diaphanosoma brachyurum* (D) occurring in the upper 3.0 m at the Middle Arm (MAS) and South Arm (SAS) stations sampled during two days in August 1976 (S) and one day in December 1975 (W).

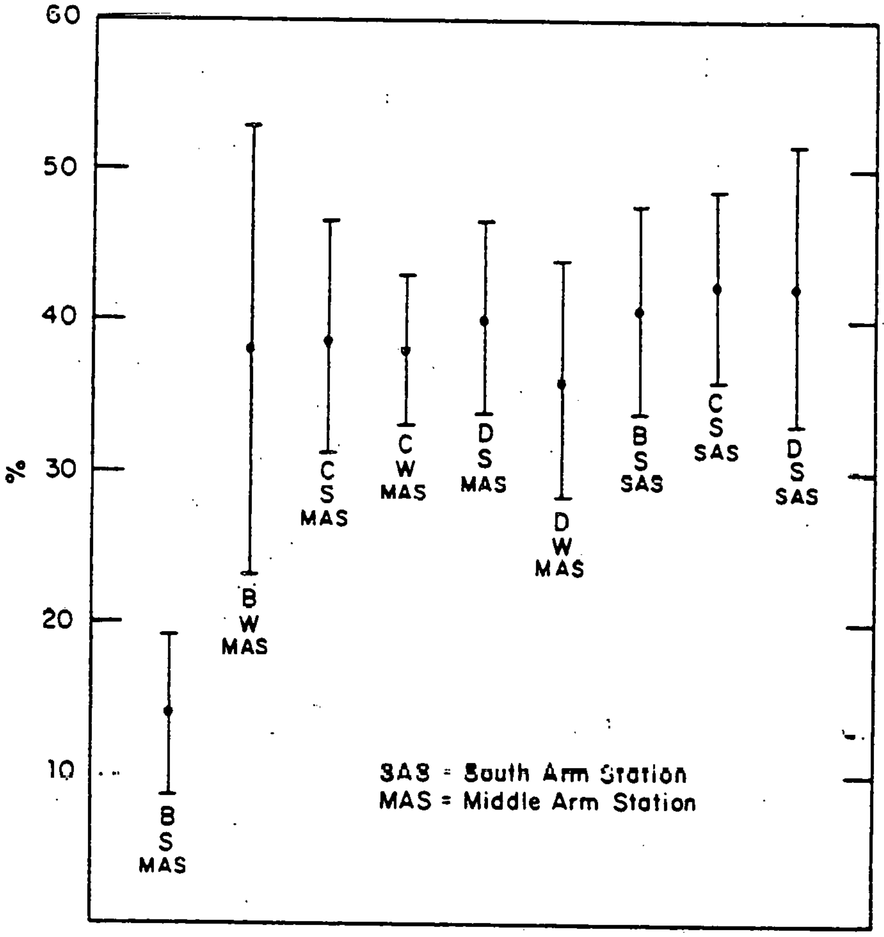
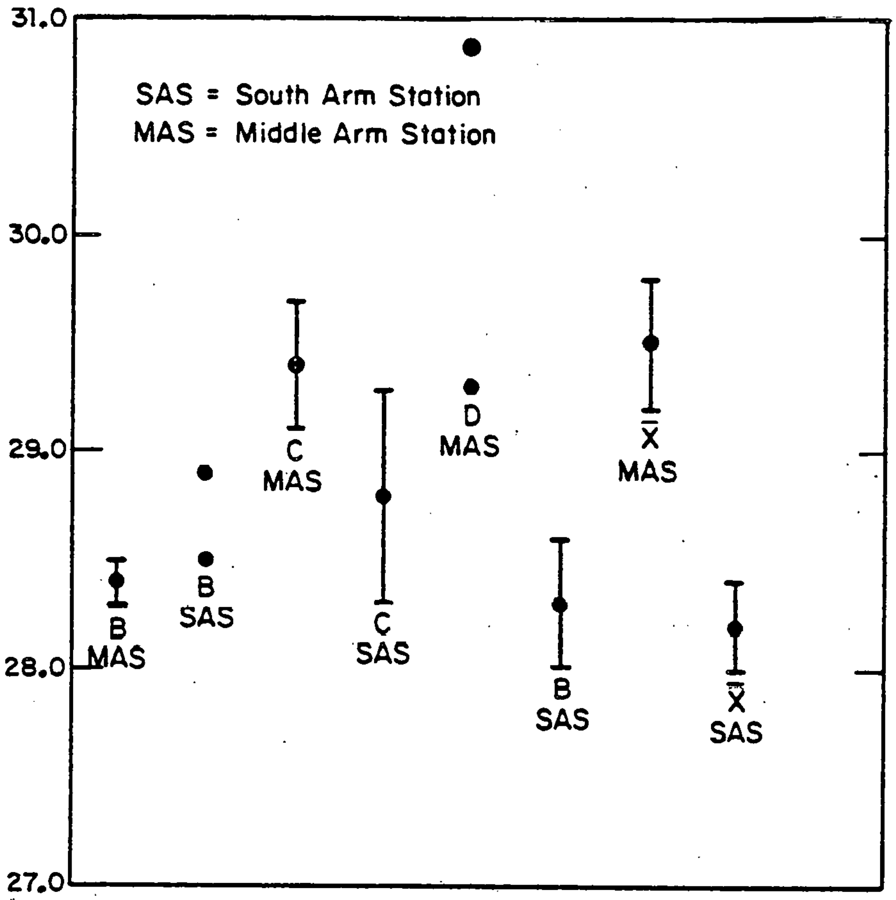

60
50
40
% 30
20
10
B S MAS
B W MAS
C S MAS
C W MAS
D S MAS
D W MAS
B S SAS
C S SAS
D S SAS
SAS = South Arm Station
MAS = Middle Arm Station

Figure B-4 - Mean temperature ($\pm$ s.e.) of populations of *Bosmina longirostris* (B), *Ceriodaphnia lacustris* (C), and *Diaphanosoma brachyurum* (D) at the Middle Arm (MAS) and South Arm (SAS) stations and average water column temperatures ($\overline{X}$) at the two stations in August 1976.


31.0
30.0
29.0
28.0
27.0
SAS = South Arm Station
MAS = Middle Arm Station
B MAS
B SAS
C MAS
C SAS
D MAS
B SAS
$\bar{X}$ MAS
$\bar{X}$ SAS

SAS, the standard errors (or at least one of the single estimates) overlap with two standard errors of the water column averages. For Bosmina at MAS, however, the population average was two standard errors below the average water column temperature.

Discussion

The lack of a strikingly discernible pattern to the vertical migration of the three species is not surprising (Figures B1 and B2). Hutchinson (1967) reported that the most dramatic vertical migrations for zooplankton occur in deep oligotrophic lakes with high transparency. Par Pond, with an average yearly phytoplankton productivity of approximately 450 mg C/M2/day, an average Secchi disk transparency of 2.5 m, and an average depth of 6.2 m (Tilly, 1975), meets neither of these qualifications.

As was discussed in the introduction of this appendix, Gehrs (1974) suggested that heated effluents could restrict movement of zooplankton in two ways. Our observations, especially for Bosmina, tend to support the hypothesis that vertical movement can be impeded by a temperature range above that acceptable to the zooplankton. Our observations do not support the hypothesis that a large thermal gradient over a short vertical range will block vertical movement. In December samples, all species had approximately equal percentages of their population occurring within the plume; i.e. the top 3 meters (Figure B1), when a steep thermal gradient of 1 to 2 °C per meter (Table B1) was present. In August, only Bosmina had a significantly lower percentage of its total numbers occurring within the effluent plume (Figure B3). Temperatures were near what the thermal tolerance data from Pond C suggests is the upper lethal limit (36°C) for *Bosmina*, (See Appendix D). Thus, although a few *Bosmina* were found in the plume, the population in this two day period in August generally avoided it. However, the study was too short in duration to support this observation as a general rule. For two of the three species there were no statistically significant differences between calculated population temperature means and water column temperature means. The use of average water column temperatures for the determination of Cladocera birth rate cannot be rejected by this study. This assumes, however, that all egg bearing females wander vertically throughout the entire range of the water column and that the fraction of time spent by an individual is shown by the fraction of the population in that stratum. If the animals remain in a separate strata, use of average water column temperature will automatically be inappropriate because of the non-linear relationship between temperature and birth rate (Prepas and Riger, 1978). This assumption was not tested by this study.

APPENDIX C

SYNCHRONOUS EGG DEVELOPMENT

Edmondson (1960) noted that a stable egg age distribution was important to the accuracy of his model. A simple measure of the age structure of a cladoceran egg population is the ratio of females bearing eyed embroyos to females bearing all other stages (referred to hereinafter as the eyed embryo ratio). To check the assumption of a stable egg age distribution the eyed embryo ratio was calculated from data on fecundity described in the materials and methods section.

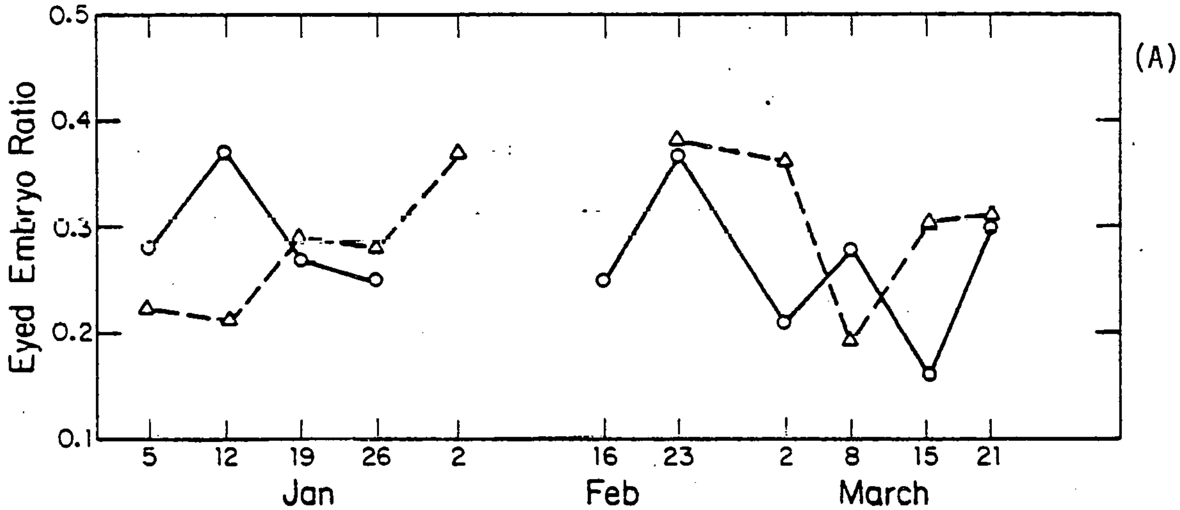
During the winter of 1976 (Figure C-1A) there was no significant pattern to the eyed embryo ratio over time at either station according to a runs test. There was also almost no correlation (Spearman's rank correlation coefficient = .10, $P > .05$) of the eyed embryo ratio between the stations. In contrast, and what is quite perplexing, the summer data (Figure C-1B) showed a significant increase in the eyed embryo ratio at both stations between the periods of June 1 to July 10 and July 10 to Augusta 31 ($P \leq .05$, Mann-Whitney Test). The correlation of the eyed embryo ratios between the two stations was also relatively high and statistically significant (rho = +.70, $P < .05$).

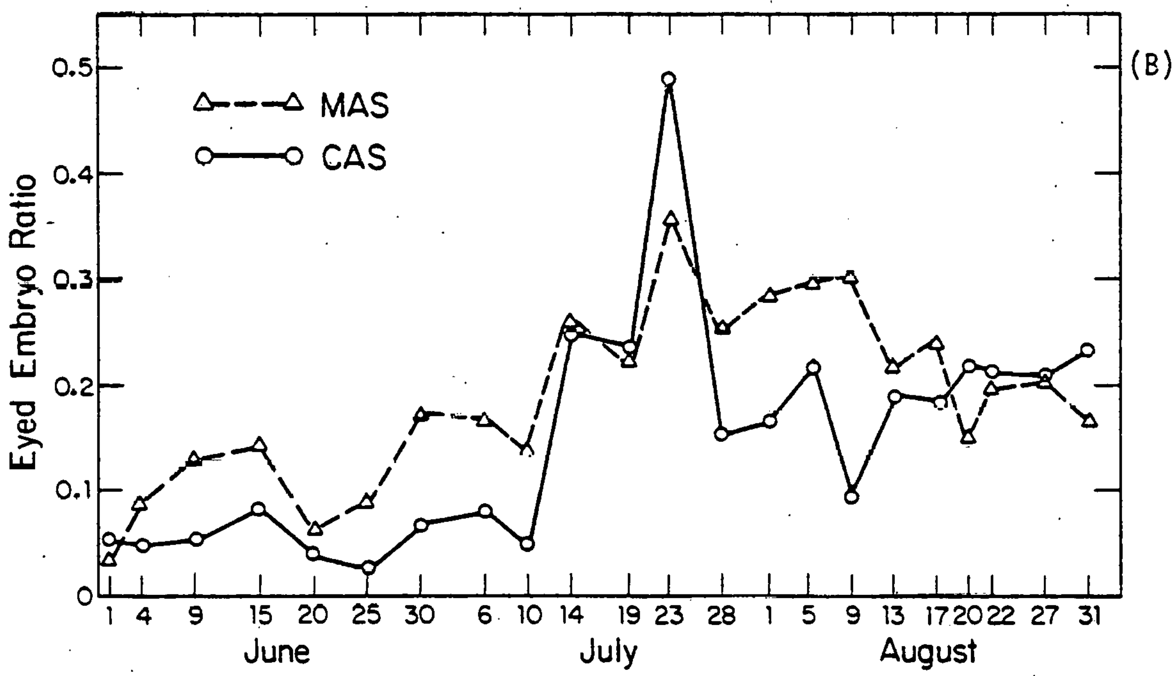
One speculation is that the Q10's for instar duration and egg development were dissimilar, causing the eggs to become mature and remain in the brood pouch for a number of hours before hatching. This would increase the likelihood of observing the eyed embryo stage. Bottrell (1975), however, found no difference in egg development time and instar duration at 15°C and 20°C in eight

Figure C-1 - Ratio of females bearing eyed-embryos to females bearing all other stages (eyed-embryo ratio).

A. Winter, 1976

B. Summer, 1976


(A)
Eyed Embryo Ratio
0.5
0.4
0.3
0.2
0.1
5
12
19
26
2
16
23
2
8
15
21
Jan
Feb
March


(B)
MAS
CAS
Eyed Embryo Ratio
0.5
0.4
0.3
0.2
0.1
0
1
4
9
15
20
25
30
6
10
14
19
23
28
1
5
9
13
17
20
22
27
31
June
July
August

species of Cladocera from the Thames River, England. Hall (1964) reported the same results at various temperatures and food levels for Daphnia galeata mendotae. A shorter instar duration in the summer, which would decrease the likelihood of observing the last stage of egg development, could explain the lower eyed embryo in June (Figure C-1B) as compared with the winter values (Figure C-1A).

The variation in the eyed embryo ratio from sampling date to sampling date, therefore suggested that the egg age distribution was not always stable.

A non-stable egg age distribution has been previously reported for the cladoceran, Daphnia magna, in a shallow pond by Green (1956). He speculated that his observations could have been caused by synchronization of egg laying or by extreme patchiness of females carrying specific egg stages that biased his samples.

The possibility of cyclic egg production, suggested by Green, was examined for rotifers using a graphical model by Ruttner - Kolisko (1975). She concluded that fluctuating temperatures could induce not only a rhythmic egg deposition in individual animals but also a synchronic egg laying of entire populations. The extent of the synchronization of the population would depend upon the life schedule and the type of thermal oscillation.

Gophen (1978) has reported synchronization of egg laying in a limnetic planktonic species, the copepod, Mesocyclops leuckarti in Lake Kenneret, Israel. In two 24-hour sampling programs (one in March and one in June) he found maximum egg and minimum naupli counts to occur between 1400 and 2200 hours. He attributed the synchronization to regular pattern of diurnal migration which exposed the adults to a temperature range of 25 to 28°C in the epilimnion during most of the day and early night, and a lower temperature range in the hypolimnion, 20 to 24°C, during the remainder of the night. This would result in the cyclic thermal history for the population required by the Ruttner-Kolisko hypothesis.

A set of samples which could be used to examine the change in the eyed embryo ratio intensively over a short period of time and possible reveal synchronous egg production was available in the vertical migration samples. From these samples (described in the Appendix B, Vertical Migration) a single sample for each sampling interval was made by compositing one sample from each pair for each depth. The females in these composites were tabulated into the categories, females with eggs-embryos, females with eyed embryos, females without eggs. From station MAS, the first two females encountered in the sample were tabulated. From station SAS, the entire sample was tabulated because of the small number of animals in each sample. Each samples, however, did contain at least 100 animals. From these counts the eyed embryo ratio and the percentage of gravid females was determined for each four hour period.

The results from the 48 hour sampling periods during August are shown in Table C1. At both stations there was an apparent increase in the ratios between the hours of 5 AM and 9 AM on both days. Table C1 also shows the changes in percentage gravid over the same intervals. At station MAS the lowest percent gravid was observed at 8 AM(on both days when the eyed embryo ratios were highest). At station SAS, the lowest percent gravid occurred at SAS on both days, but the value at SAS on the first day tied the value observed at 8 PM on that day.

Table C-1. Eyed embryo ratio (ratio) and percentage of gravid females (% gravid) in diurnal samples taken at Stations MAS and SAS in August 1976.

	MAS 8/5 - 8/7			SAS 8/9 - 8/11	
Time	Ratio	% Gravid	Time	Ratio	% Gravid
1245	.26	51	1300	.23	41
1645	.18	50	1645	.17	52
2045	.10	48	2045	.11	38
0030	.12	50	0040	.11	53
0445	.52	47	0545	.71	38
0850	.41	44	0840	1.00	49
1250	.29	47	1245	.12	43
1635	.30	54	1645[1]	---	--
2030	.17	60	2035	.48	46
0100	.26	56	0030	.17	47
0435	.70	48	0430	.77	41
0825	.44	42	0800	.77	53

[1] 1645 hour samples for station SAS were lost during original migration study.

The lack of replication of samples in the 48 hour sampling periods in August at SAS and MAS and the small number of individuals observed at SAS (<200 per sample) makes a statement of statistical confidence impossible. Nevertheless, the highest ratios were observed between 4:00 and 9:00 AM at both stations on both days (Table C1). At MAS, the lowest percentage gravid was also observed the same time on both days (8-9 AM), also during the period of highest ratios, while the period from 4:00 to 6:00 AM had the lowest percentage gravid on both days (also during the period of highest ratios). However, two other times during the two day period had the same percentage gravid values. The percentage gravid values were more sensitive to counting errors at SAS than at MAS because of the smaller sampling sizes (see Materials and Methods). The evidence, weak as it is, suggests that synchronous egg production was occurring at both stations.

APPENDIX D

FIELD DETERMINATION OF UPPER THERMAL TOLERANCE

Introduction

Bunting (1974) has reviewed most of the literature on temperature effects on Cladoceran life cycles that has immediate relevance to power plant operation. Goss and Bunting (1976) list all the studies of thermal tolerance on zooplankton. Brown and Crozier (1927-28) found that *Daphnia pulex* would grow and reproduce at 30°C but died at 32°C and *Moina macropa* would live and reproduce at 39°C but died at 40°C. Graphs were presented which allow the prediction of the percent mortality of these two species based on time of exposure to various temperatures. Brown (1929) reared 19 identifiable species of Cladocerans at room temperature and subjected them to instantaneous immersion for one minute at high temperatures in an effort to determine upper tolerance limits. The following text table shows the results of these experiments.

Species	Lethal Temperature (°C)
Daphnia longispina	42
Daphnia magna	41
Daphnia pulex	44
Latonopsis occidentalis	46
Macrothrix rosea	50
Moina macrocopa	48
Moina rectirostris	47
Psuedosida bidentata	48
Scapholebris mucronata	43

Species	Lethal Temperature (°C)
Sida crystallina	40
Simnocephalis exspinosus	43
Simnocephalis vetulus	43
Ceriodaphnia latidaudata	43

Goss and Bunting (1976) report on a variety of thermal tolerance tests on *Daphnia magna* and *Daphnia pulex*. The two species were able to withstand instantaneous changes in temperature of 20° to 25°C but time-to-mortality at 35°C was 1/2 hour for *D. magna*. Based on their entire set of experiments they speculated that both species would be eliminated from natural waters at temperatures greater than 30°C.

Methods and Materials

When the P reactor is not operating for approximately one week, all species of limnetic Cladocera found in Par Pond are transported through the reactor and the canal system into Pond C. When the reactor comes back into operation, hot water enters as a sheet across the surface of the pond. The effluent water heats the entire water column of Pond C from above, sometimes in 3 to 4 days. The hot water eventually enters Par Pond, forming a surface plume (Lewis, 1974). The arm of Par Pond receiving the thermal effluent may take one week to reach maximum temperature. Cooling canal temperatures are monitored from a road approximately 4 km upstream from Pond C. This distance allows sampling in Pond C to begin as soon as the reactor is operating, but before hyperthermal effluent reaches the Pond C sampling station. Because it is separated from the body of Par Pond by a dam and culvert it serves as a large water bath analagous to the standard laboratory beaker. The thermally affected middle arm of Par Pond simulates more closely the thermal situation created by a power generating plant, which can allow us to test the values of our critical thermal maxima data for the prediction of consequences of thermal waste water additions. Samples from the ambient portion of the reservoir thus can serve as a 'control' for our observations. They can insure that species did not undergo changes in population levels or did not disappear as a result of naturally occurring biological events during the course of studies.

On March 15-18, 1976, Cladocera were sampled in the cooling canal with a #10, 13-cm-diameter plankton net allowed to drift out with the current. On each of these four sampling days, four vertical tows were taken at the Pond C station (depth, 7 m) with a 1/2-m-diameter, #10 net. During July 27-29, 1976, samples were made every 12 hours (beginning at noon on the 27th) using a 13-cm-diameter, #10 net to take a series of vertical tows from depths of 1 to 7 m at 1-meter intervals. This procedure allowed the observation of the vertical depth distribution of Cladocera in the water column. This sampling method was continued during September 20-28, but only once a day, around noon.

During the entire course of this study vertical tows were taken at least weekly in the ambient portion of Par Pond (Station CAS, Fig. 1). Concurrent to the March 1976 sampling of Pond C, four vertical tows were taken at a station in the middle arm of Par Pond (Station MAS), the arm which directly receives thermal effluent, and at CAS. Samples were taken with a 1/2-m-diameter, #10 net on three dates: March 15, 21, and 28. By March 28, temperatures at the middle arm station had stabilized.

Entire samples from Pond C in July and September 1976 were counted. The samples taken from Par Pond and Pond C in March 1976 were subsampled using a 2-ml Hensen-Stempel pipette. One subsample was counted from each sample. Relative abundance of cladoceran species in a sample was determined by frequency counting each species in the first 200 Cladocera encountered in subsamples. All counts represent non-parametric estimates of abundance. Temperature measurements were made with a thermister probe.

Results

March 15 - 28

All limnetic cladoceran species present at Station CAS were found in the cooling canal on March 15, 1976, when the reactor was not operating. By March 17, canal temperatures had risen 6°C, and based on a subjective examination of a net haul taken across the canal, the number of animals alive seemed reduced. On March 18, the canal temperature had risen 23°C (to 44°C) at the monitoring station, and no living plankton were found.

In Pond C samples taken on March 15, all the Station CAS species were present in similar relative abundances (Table D-1). By March 18, the upper two meters had been heated 6.5°C (Figure D-1). A trend of decrease in abundance of Bosmina longirostris and Ceriodaphnia lacustris began on this date so that by March 21 both species were rare in the tows (Figure D-2). In contrast, Chydrous sphaericus standing stocks appeared to increase during the sampling period, an observation for which there is no explanation. The increase in total cladocerans from the 15th to the 17th can be attributed to immigration

Figure D-1 - Vertical temperature profiles of the Pond C Station taken at 1500 EST from March 17 to March 21, 1976.

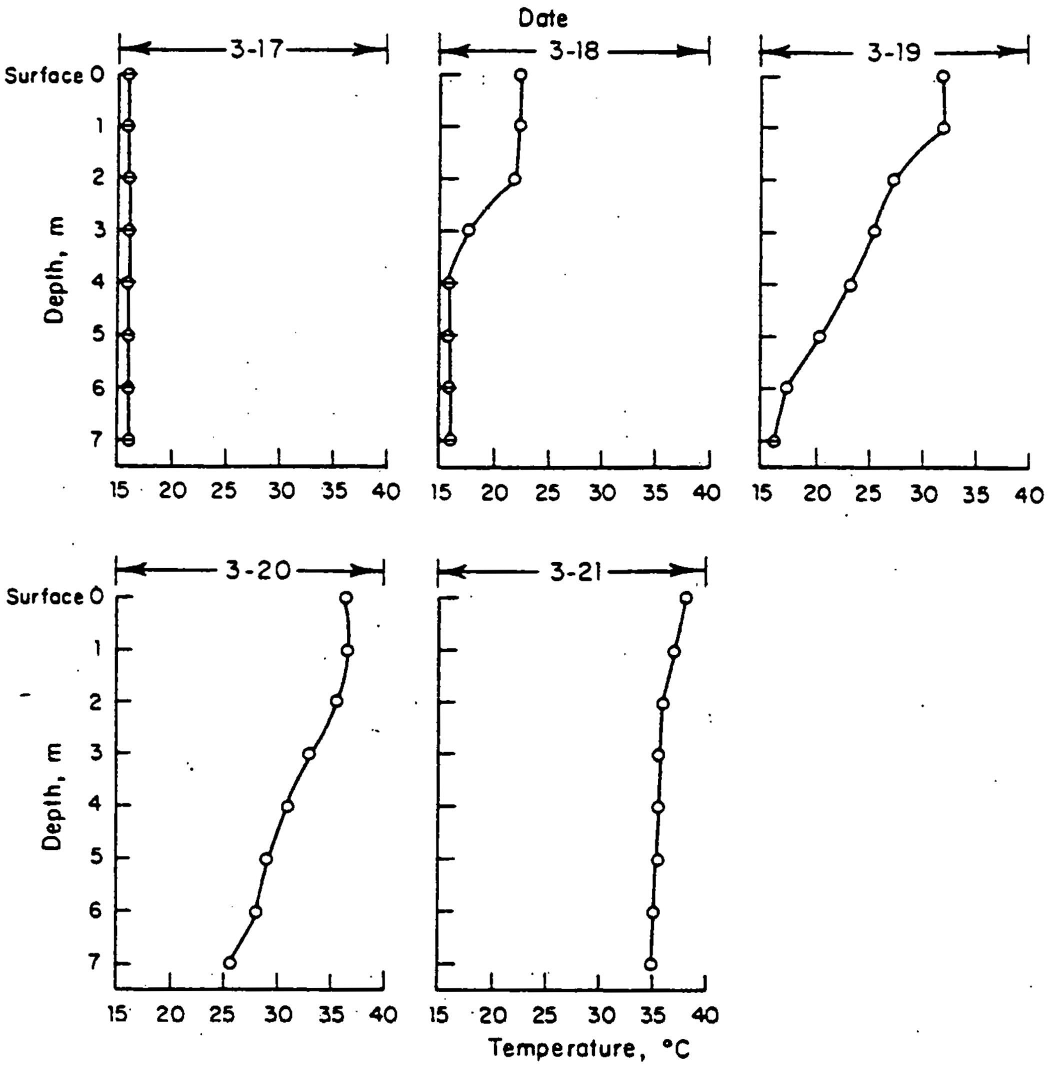

Date
3-17
3-18
3-19
3-20
3-21
Surface 0
1
2
3
4
5
6
7
Depth, m
15
20
25
30
35
40
Temperature, °C

Figure D-2 - Numbers of individuals of Bosmina longirostris, Ceriodaphnia lacustris, Chydorus sphaericus and total Cladocera in a 2 ml subsample from each of four plankton tows taken on March 15, and daily from March 17 to March 21, 1976, at Pond C Station.

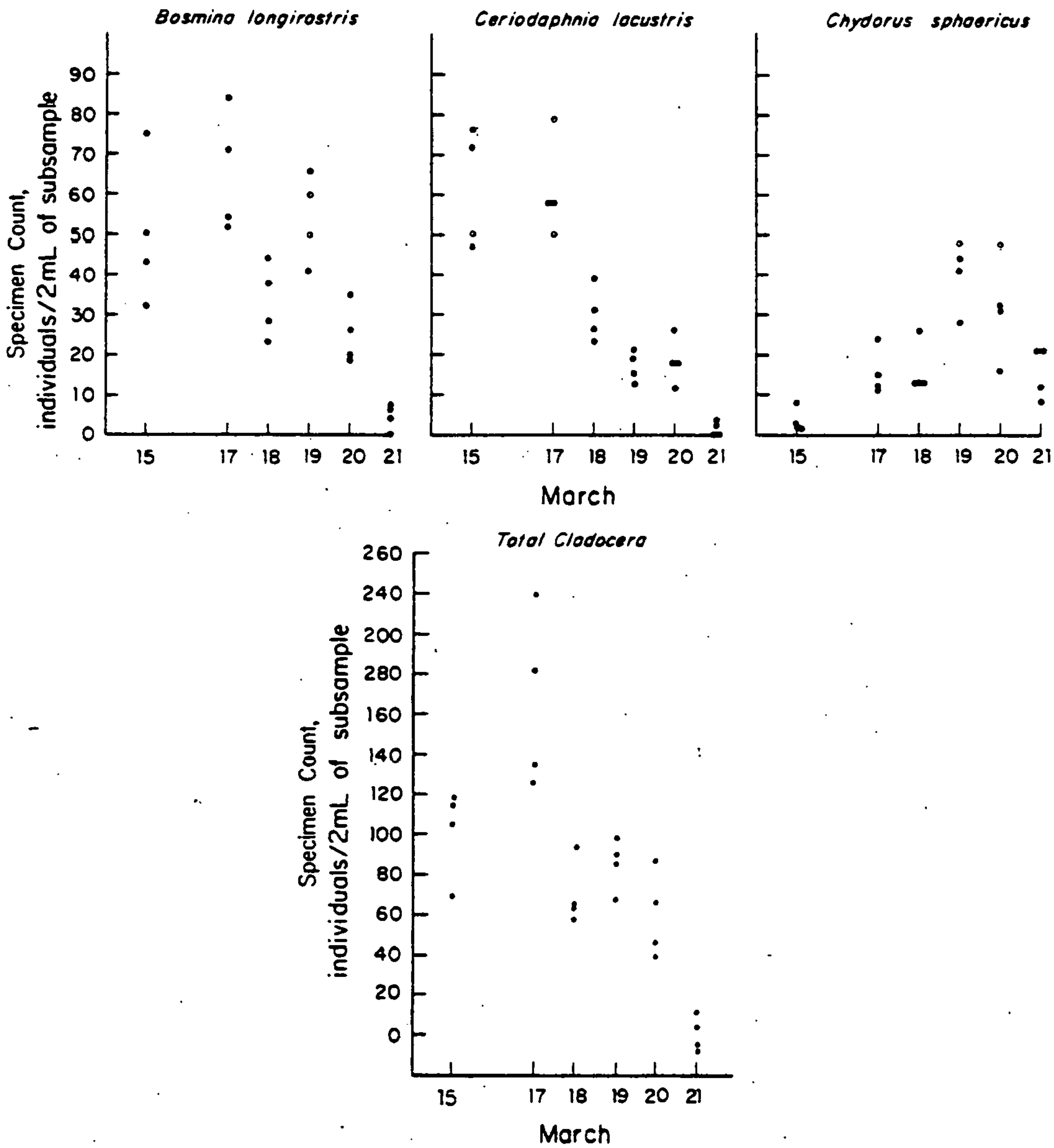

Bosmina longirostris
Ceriodaphnia lacustris
Chydorus sphaericus
Specimen Count,
individuals/2mL of subsample
90
80
70
60
50
40
30
20
10
0
15
17
18
19
20
21
March
Total Cladocera
260
240
200
280
160
140
120
100
80
60
40
20
0
March

Table D-1. Range of percentage relative abundance of Cladocera species in four replicate plankton tows taken at Station CAS in Par Pond and Station Pond C on March 15, 1976.

Species	Range of Percent at Station CD	Range of Percent at Pond C Station
Bosmina longirostris	41 - 33.5	40.5 - 27.0
Ceriodaphnia lacustris	61.5 - 46.5	68.5 - 52
Other (*Chydorus sphaericus*, *Diaphanosoma brachyurum*, *Daphnia parvula*, *Eubosmina tubicen*, *Holopedium amazonicum*)	12.5 - 6.0	8.5 - 4.0

Table D-2. Number of each cladoceran species counted from two vertical tows taken at Station Pond C at noon on July 28, midnight July 28, and noon July 29, 1976.

Depth m	Temp. °C	*Bosmina longirostris*	*Ceriodaphnia lacustris*	*Diaphanosoma brachyurum*	*Moina micrura*
		Noon, July 28			
1	41.2	0-0	0-0	0-0	0-0
2	41.0	0-0	0-0	0-0	0-0
3	37.8	0-0	0-0	0-0	0-0
4	36.5	0-0	14-16	0-0	5-2
5	34.6	1-0	15-23	0-1	3-4
6	32.6	58-64	22-13	5-3	6-2
7	32.5	79-139	12-11	7-11	4-10
		Midnight, July 28			
1	43.6	0-0	0-0	0-0	0-0
2	41.8	0-0	0-0	0-0	0-0
3	40.3	0-0	0-0	0-0	0-0
4	38.9	0-0	8-9	0-0	0-1
5	37.9	0-0	10-5	0-0	4-2
6	36.9	8-3	22-11	4-0	4-2
7	34.6	4-11	9-14	5-15	1-3
8	33.2	35-98	9-14	13-35	1-3
		Noon, July 29			
1	43.9	0-0	0-0	0-0	0-0
2	43.9	0-0	0-0	0-0	0-0
3	43.1	0-0	0-0	0-0	0-0
4	42.0	0-0	0-0	0-0	0-0
5	40.8	0-0	0-0	0-0	0-0
6	40.4	0-0	0-0	0-0	0-1
7	39.3	0-1	1-1	0-1	1-2
8	38.2	2-0	2-0	0-1	3-1
9	36.8	0-2	3-3	0-1	2-3

through the cooling canal system from Par Pond.

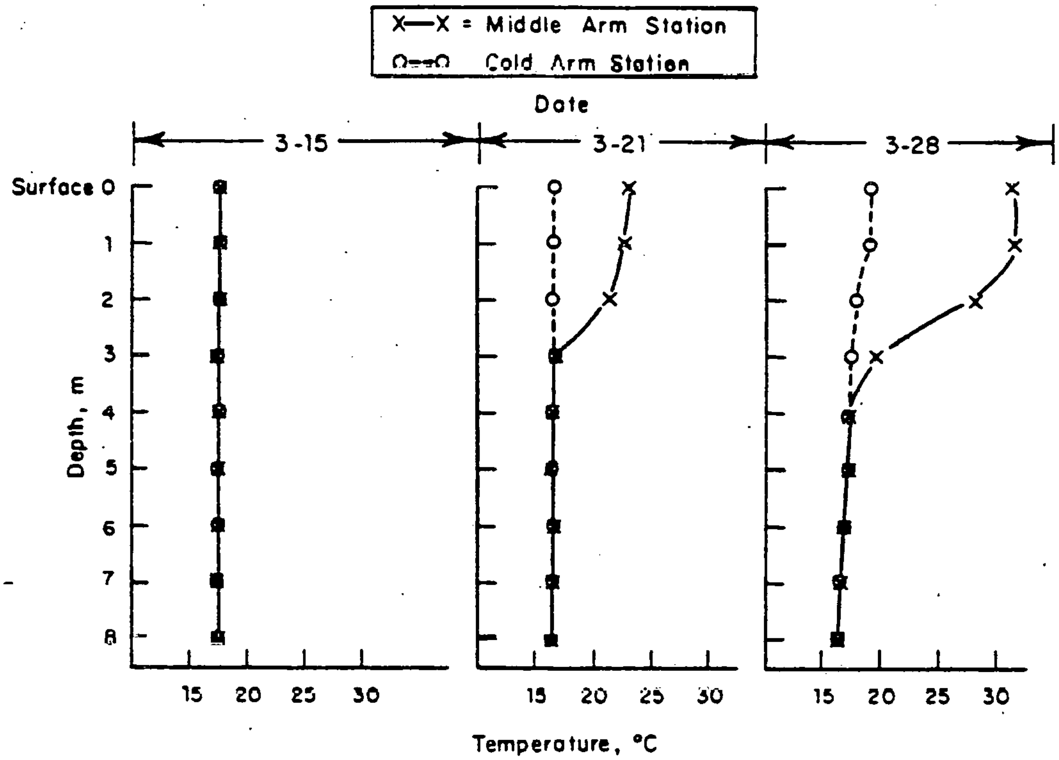
The species rare on March 15 (designated "other" on Table D-1) were still rare (less than 10% of the total Cladocera) on March 21. The temperature of the entire 8 m water column was above 35°C by this date. All depths had undergone a change in temperature of at least 7.8°C over a 24-hour period, and all but the 4 m and 5 m isobaths had undergone a temperature change of at least 9.3°C in 24 hours (Figure D-1).

Temperatures in the middle arm, Station MAS, of Par Pond did not reach a maximum until March 28 (Figure D-3). By this date at Station MAS observed changes were a significant drop in standing crop of Bosmina and Ceriodaphnia and a significant increase of Daphnia (Figure D-4, Mann Whitney U Test; $p<0.05$, Siegel, 1956). Also during this period, standing crops of Chydorus, Diaphanosoma, and Holopedium did not change relative to March 15. Station CAS remained at the same level of standing crop throughout the sampling period. Only Ceriodaphnia decreased in abundance.

July 26 - 29

On July 26, the water temperature was almost uniformly 32°C to the bottom of Pond C (Figure D-5). On July 26 and 27, tows taken in the upper 5 m of our Pond C station contained approximately 70% Bosmina longirostris, 16% Ceriodaphnia lacustris, 12% Diaphanosoma brachyurum, and 2% Moina micrura. Water temperatures on July 27 ranged from 35.5 to 32°C between the surface and the 8 m isobaths. By noon on July 28 (Table D-2), Bosmina was still the most abundant (approximately 40% of the total Cladocera captured). Ceriodaphnia and Moina still had similar standing stocks at all depths sampled below 4 m. At noon, July 29, Bosmina, Diaphanosoma, and now Ceriodaphnia were all reduced in number

Figure D-3 - Temperature profiles at Station CAS (dotted line) and Station MAS (solid line) in Par Pond on March 15, 21, and 28, 1976.



X—X = Middle Arm Station
O--O Cold Arm Station
Date
3-15
3-21
3-28
Surface 0
1
2
3
4
5
6
7
8
Depth, m
15 20 25 30
15 20 25 30
15 20 25 30
Temperature, °C

Figure D-4 - The number of the three dominant species in a 1 ml subsample from each of four samples taken at Station CAS and Station MAS on March 15, 21, and 28, 1976.

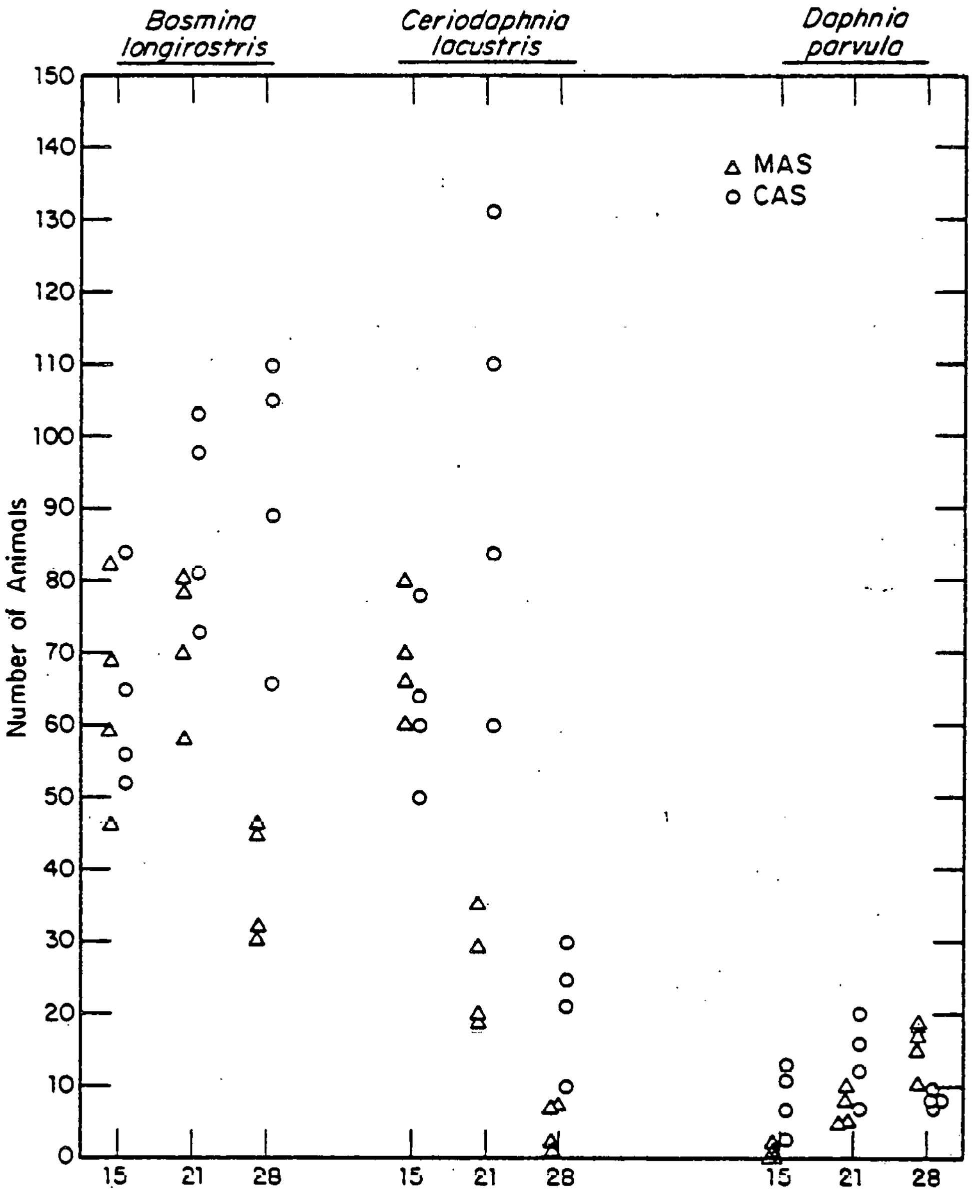
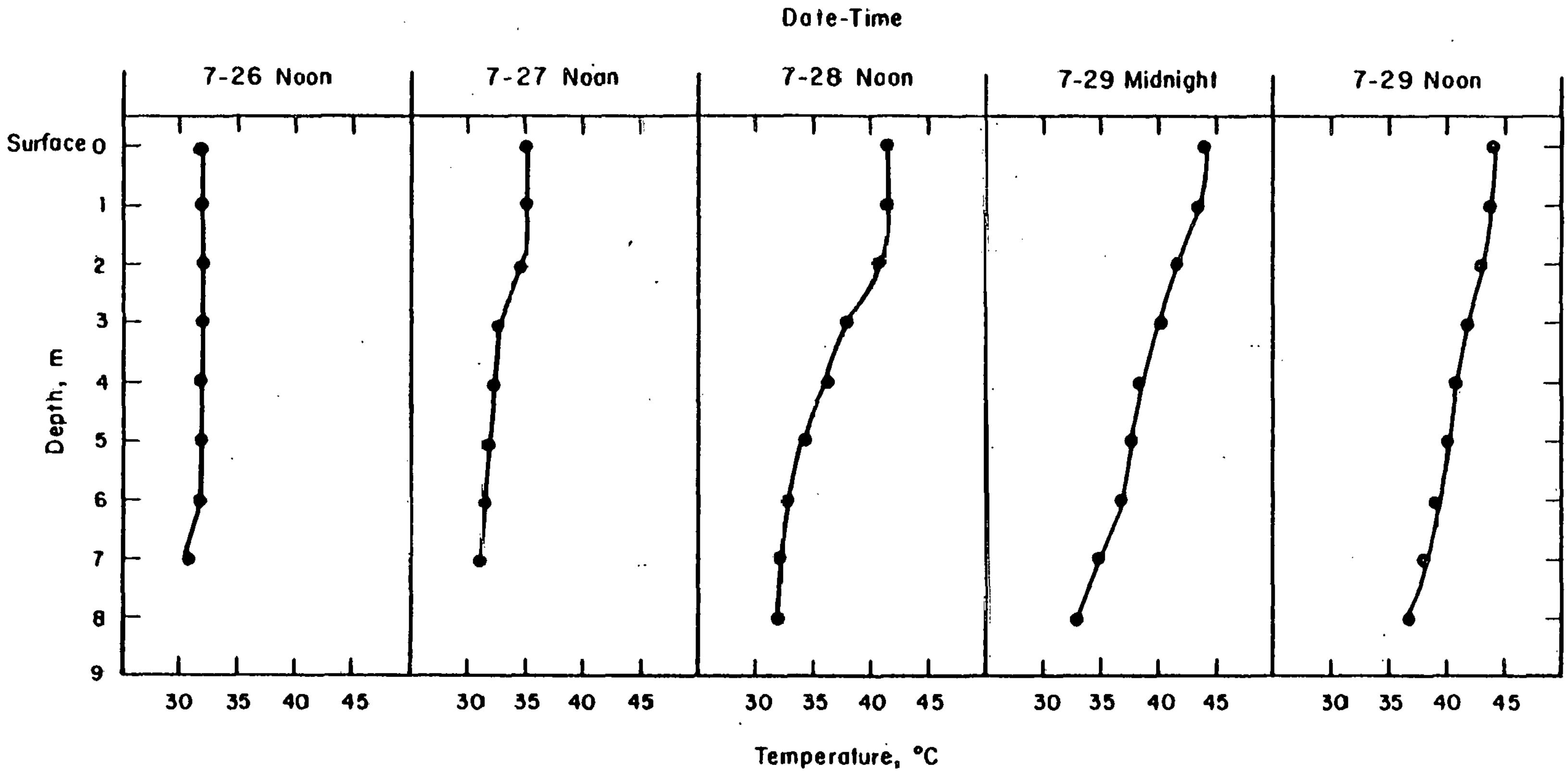

Bosmina longirostris
Ceriodaphnia lacustris
Daphnia parvula
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Number of Animals
15
21
28
15
21
28
15
21
28
MAS
CAS

Figure D-5 - Temperature profiles at Pond C Station from July 26 to July 29, 1976.


Date-Time
7-26 Noon
7-27 Noon
7-28 Noon
7-29 Midnight
7-29 Noon
Surface 0
1
2
3
4
5
6
7
8
9
Depth, m
30 35 40 45
Temperature, °C

and were rare throughout the water column. Only Moina continued to maintain a constant, but low, standing stock. Except in one instance, when all but the last meter of the water column was close to or above 38°C (noon, July 29), Bosmina and Diaphanosoma were never found in that part of the water column. These Cladocera were found in greatest abundance in that part of the water column below 34°C. In contrast, Moina and Ceriodaphnia were found in higher temperatures (greater than 38°C but less than 41°C). Ceriodaphnia was in greatest abundance below the 37°C isotherm, but Moina was always rare.

All species with the exception of Moina micrura, were present at stations CAS and MAS during the entire month of July and of August.

September 20 - 27

Pond C temperatures during the September observation period rose slowly and did not exceed 37°C even at the surface (Figure D-6). Although there appeared to be large fluctuations in standing crops at our station on Pond C during the sampling period, the abundances of Ceriodaphnia lacustris, Diaphanosoma brachyurum, and Moina micrura in our tows on September 27 were not different from those in tows taken on September 20 (Figure D-7). The large increase in Moina standing stocks on the 21st and 22nd are quite puzzling. Swarming of Moina affinis has been reported by Ratzlaff (1974), but we have no data to support or reject this possibility. Bosmina was not found in our tows on September 26 and 27. On September 28, we took four 7 m vertical tows with a 1/2 m diameter, #10 net and confirmed the absence of Bosmina. Bosmina disappeared from the upper 2 m of the water column by September 24 and from the entire water column by September 26 (Figure D-6). The upper 2 m had heated to more than 35°C on September 24; the entire water column exceeded that

Figure D-6 - Temperature profiles at the Pond C Station from September 20 to 27, 1976.

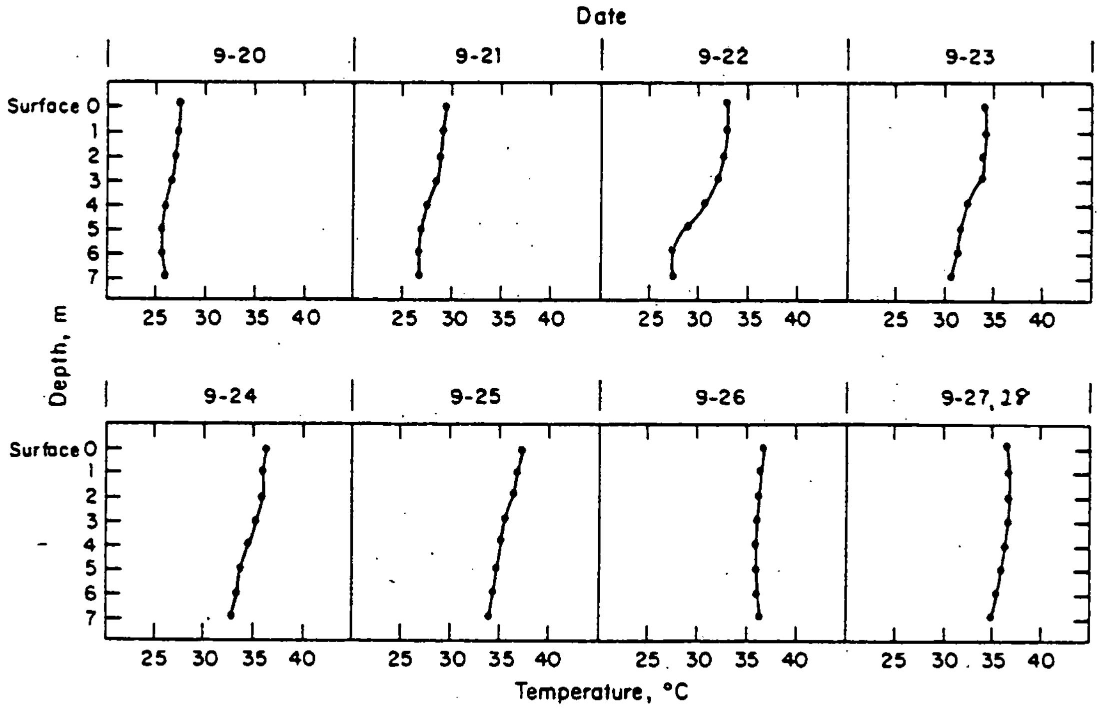
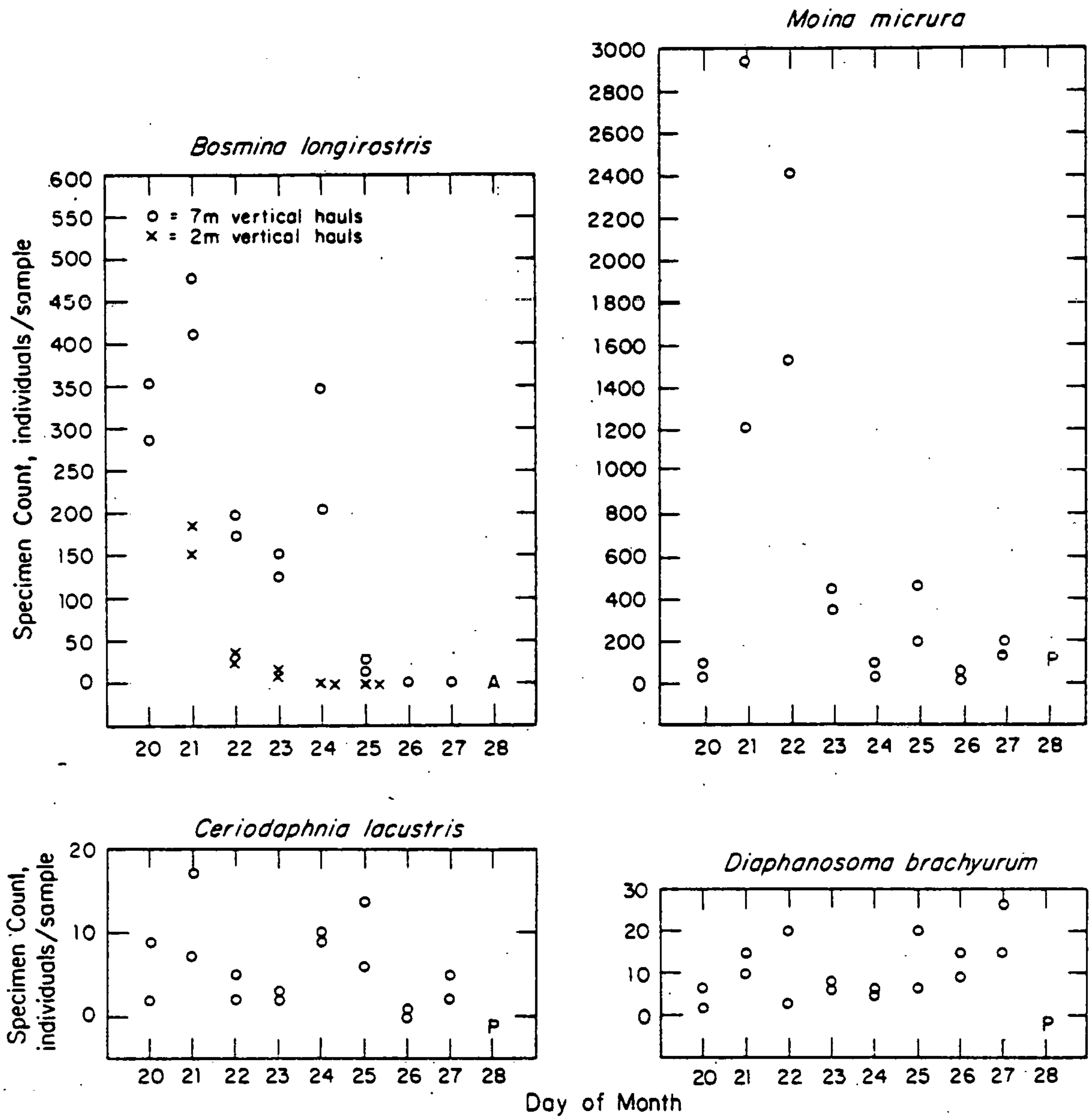

Date
9-20
9-21
9-22
9-23
Surface 0
1
2
3
4
5
6
7
25 30 35 40
Depth, m
9-24
9-25
9-26
9-27, 28
Temperature, °C

Figure D-7 - Number of each cladoceran species counted from vertical tows taken at Pond C Station from September 20 to 28, 1976. (P = present; A = absent in tows which were not quantified.)


Bosmina longirostris
o = 7m vertical hauls
x = 2m vertical hauls
Specimen Count, individuals/sample
Moina micrura
Ceriodaphnia lacustris
Specimen Count, individuals/sample
Diaphanosoma brachyurum
Day of Month

temperature by September 26.

Discussion

There are three general thermal situations created by power plants utilizing cooling lakes: the temperatures in the heat exchangers, the temperatures in the cooling canals, and the temperatures in the lake itself. The experiments intended to measure thermal tolerance can be applied to the latter two situations. The data discussed in the introduction text table apply to the former situation where residence time is very short.

Cooling Canals

All species we observed can withstand temperatures up to 36°C for at least 24 hours. We would, therefore, expect these species to be able to tolerate the thermal stress of passage through a cooling canal below this temperature.

Cooling Lakes

Heated effluents may affect the water-column temperatures of cooling lakes to different extents. The water of a mixing zone or the littoral areas of the lakes will be heated completely by the effluent, while the deeper, non-mixing areas (> 4 m) may be heated only in part. In the latter case, the effluent is called a surface plume. Our data from Pond C can be used to predict the consequences of heated effluent on the species composition of the littoral zones or mixing areas, but are probably not useful for predicting the consequences of surface plumes.

Of the species we observed more than once, Bosmina longirostris appears to have the lowest thermal tolerance. Each time (March, July, and September) this species became rare or disappeared when the water column became heated to more than 36°C. In July (Table D2) as the temperature at a particular depth in the water column went above 36°C, it became rare (< 8 individuals per tow). In September, when the upper two meters of the water column was at 36°C, Bosmina was absent from the tows taken from two meters to the surface. It did not disappear from the entire water column until two days later when the bottom waters had reached 36°C (Figures D6 and D7). In March (Figure D4), July (Figure 6B, Results, pg. 71) and September (unpublished data from the Savannah River Laboratory routine surveys) it was present at Stations CAS and MAS and did not undergo diamatic (> 50%) changes in population levels during the period of these observations in Pond C. Results from March (Figure D2) suggest that Ceriodaphnia lacustris is as sensitive to temperature near or above 36°C as Bosmina, but the results from July (Table D2) and September (Figure D7) show that it can tolerate temperatures greater than 36°C at least for a few days. Diaphanosoma brachyurum was only present during two of the three sampling periods and also appears to tolerate temperatures greater than 36°C. July results suggest that both species cannot withstand temperatures greater than 40°C. However, a lack of control observations from Par Pond for Diaphanosoma and only March Par Pond observations for Ceriodaphnia makes these conclusions very tentative.

Moina micrura, however, is able to survive exposure to temperatures up to 40°C for at least 24 hours (July). Chydrous sphaericus can tolerate temperatures greater than 36°C for several days (March). July observations suggest that all species are able to maintain a position in the water column below lethal temperatures, but conclusive evidence demands that we observe them reappearing higher in the water column as it cools, a situation which is impossible due to the way the reactor operations proceed.

An alternative hypothesis to these conclusions is that the cladocerans simply flow through the drainpipe from Pond C into Par Pond. (See Figure 2, Materials and Methods). The sampling station in Pond C is approximately 60 m from the outlet pipe which lies in the bottom of the pond in 9 m of water. This pipe delivers 681.4 cubic meters of water per minute to Par Pond. If we approximate the cross-sectional area of Pond C at our sampling station as the distance across the pond perpendicular to the dam (282 m) times the mean depth of the Pond (3 m) and assume uniform flow, the estimated velocity at this sampling station would be 15 mm/sec. This is well below 30 mm/sec., the velocity at which zooplankton are moved as though they were inert suspended particles (Einsele, 1960; as cited by Whitehouse, 1971). The turnover rate of Pond C is conservatively estimated to be on the order of three days. If the disappearance of cladocerans were due to this factor alone, assuming now that the cladocerans are not able to maintain their position in the water column, we would expect to see a uniform removal of total numbers and species. Only the total Cladocera for the September 1976 sampling period has the appearance of a simple dispersion event; but, even in this period, only Bosmina disappeared completely. If Bosmina were the only species unable to maintain its position in the water column, we would expect it to disappear at the same rate in each sampling period. However, in March it was present four days after the hot water began to enter Pond C; in July, it disappeared completely in four days; and in September, it took six days to disappear. Each point of disappearance coincided with the water column being heated to above 36°C. We, therefore, believe this alternative hypothesis should be rejected.

Vigerstad and Tilly (1977) had concluded from a comparison of littoral zone populations in the middle arm with data from an ambient station in the summer of 1974 that _Bosmina_ standing crops were (at least indirectly) reduced by hyperthermal temperatures, while _Ceriodaphnia_ and _Diaphanosoma_ crops were favored by the higher effluent temperatures. The average maximum temperature of the littoral zone area in the middle arm was 37.9°C at that time, the average minimum, 30.9°C. The Pond C results support the hypothesis that the reduced standing crops of _Bosmina_ are directly a result of the hyperthermal temperatures. The results for comparison of _Ceriodaphnia_ and _Diaphanosoma_ standing crops between stations differing in water temperature suggest that the temperatures in the littoral zone are probably below physiologically stressful levels for those species, a result which is also supported by our Pond C observations.

Our samples from the middle arm station in March (Figure D4) demonstrate the difficulty of using thermal tolerance data along to predict the short-term consequences of surface plume additions in the limnetic zone. For example, the Pond C results from March suggest that little or no effect from the surface plume additions to Par Pond should be observable because all species in Pond C were able to withstand temperature increases of 7°C per 24 hours and tolerate temperatures up to 36°C for several days, and Par Pond thermal conditions were less extreme. On the other hand, results from Pond C might be used as a relative index of susceptibility (reasoning from the observation that both species decreased in abundance with increasing temperature in Pond C (Figure D2)). We might predict that Bosmina and Ceriodaphnia would both be affected to some extent by the heated waters, but that no reduction in numbers should be observed for the other species. This latter pair of predictions seems to describe more closely the series of events for the different species. The different species may actually be more sensitive to changes in reactor operations than the observed values for our Pond C results indicate. Based upon our CAS samples, however, we would have expected a decline in Ceriodaphnia by March 28 even without the addition of hyperthermal effluents.

Certainly, our Pond C data are not useful to predict long-term consequences of existing thermal conditions at the central middle arm station (Station MAS) during reactor operations. Temperatures within the surface plume are well below observed tolerance limits. Below the 3-m isobath, water temperatures at Stations CAS and MAS are identical. Cladocera could select normal lake temperatures by migration.

APPENDIX E

PAR POND CIRCULATION PATTERNS AND PLANKTON SAMPLES[1]

T. J. Vigerstad and D. L. Kiser

Introduction and summary

Circulatory patterns in a cooling reservoir in the vicinity of a hot water discharge can influence the temperatures experienced by biota in the reservoir. Sampling of Cladocera and subsequent dye studies in the Hot Arm of Par Pond (a cooling reservoir for nuclear reactor water on the Savannah River Plant) indicate that reactor effluents can be expected to transport biota from the limnetic portion of the lake in the vicinity of the effluent discharge to areas further down the effluent-receiving-arm or to the littoral zone. This transport can confound sampling programs and complicate attempts to describe the thermal history of organisms.

Plankton sampling

In the summer of 1975, plankton samples were taken by 12 vertical tows at a locality in the littoral zone of Par Pond (Station MAS-1,Figure E1). The tows were made with a 13-cm diameter, No. 10 plankton net. Each sample was counted in its entirety for *Bosmina longirostris*. During the sampling period, the nuclear reactor discharging heat water to the cooling reservoir stopped and restarted operations twice. The plot of standing stock of *Bosmina* (Figure E2)

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[1]This report appeared in Savannah River Laboratory Environmental Transport and Effects Research, *Annual Report - 1977*, Savannah River Laboratory, Aiken, SC, pgs. 93-96. However, due to a printing error Figures B1 and B2 were omitted. Because this report is important to understanding this thesis and to understanding reports from Par Pond research it is reprinted in this appendix.

Figure E-1 - Map of Par Pond system showing sampling location.

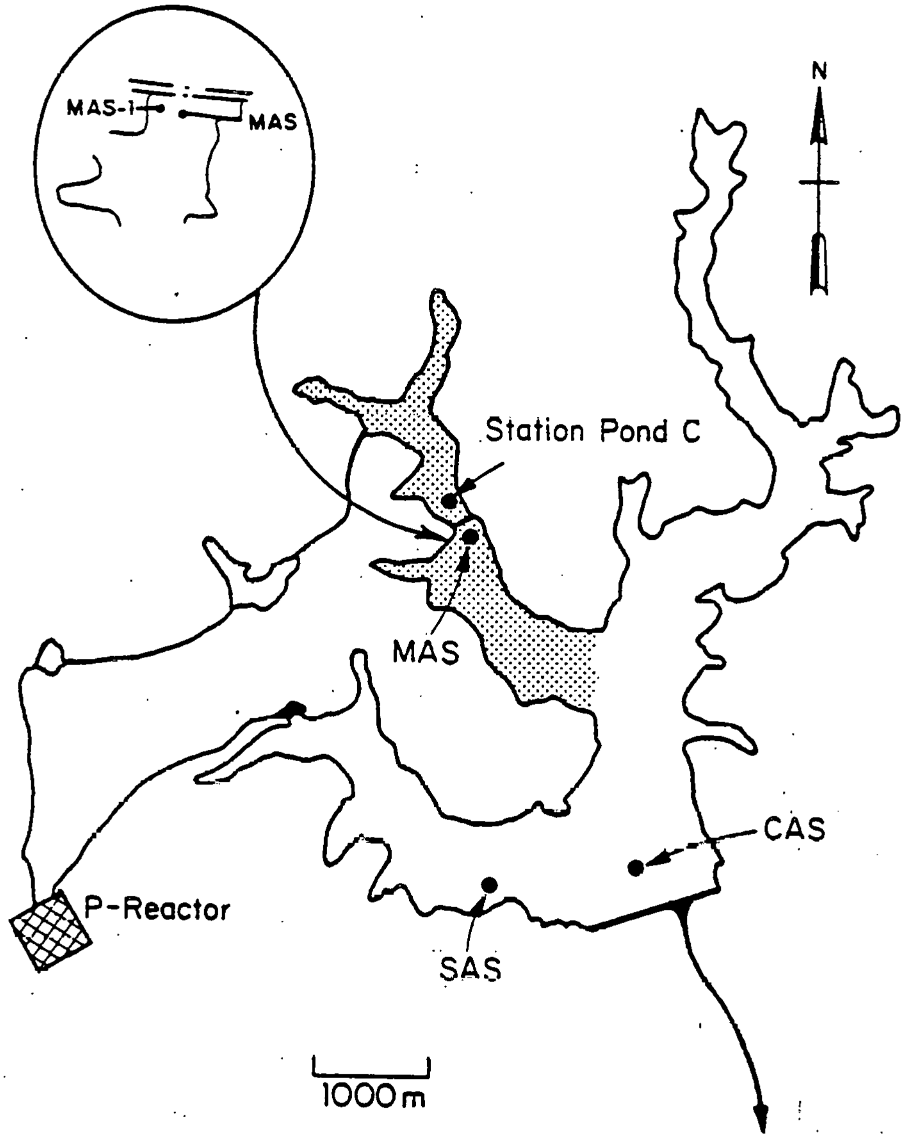
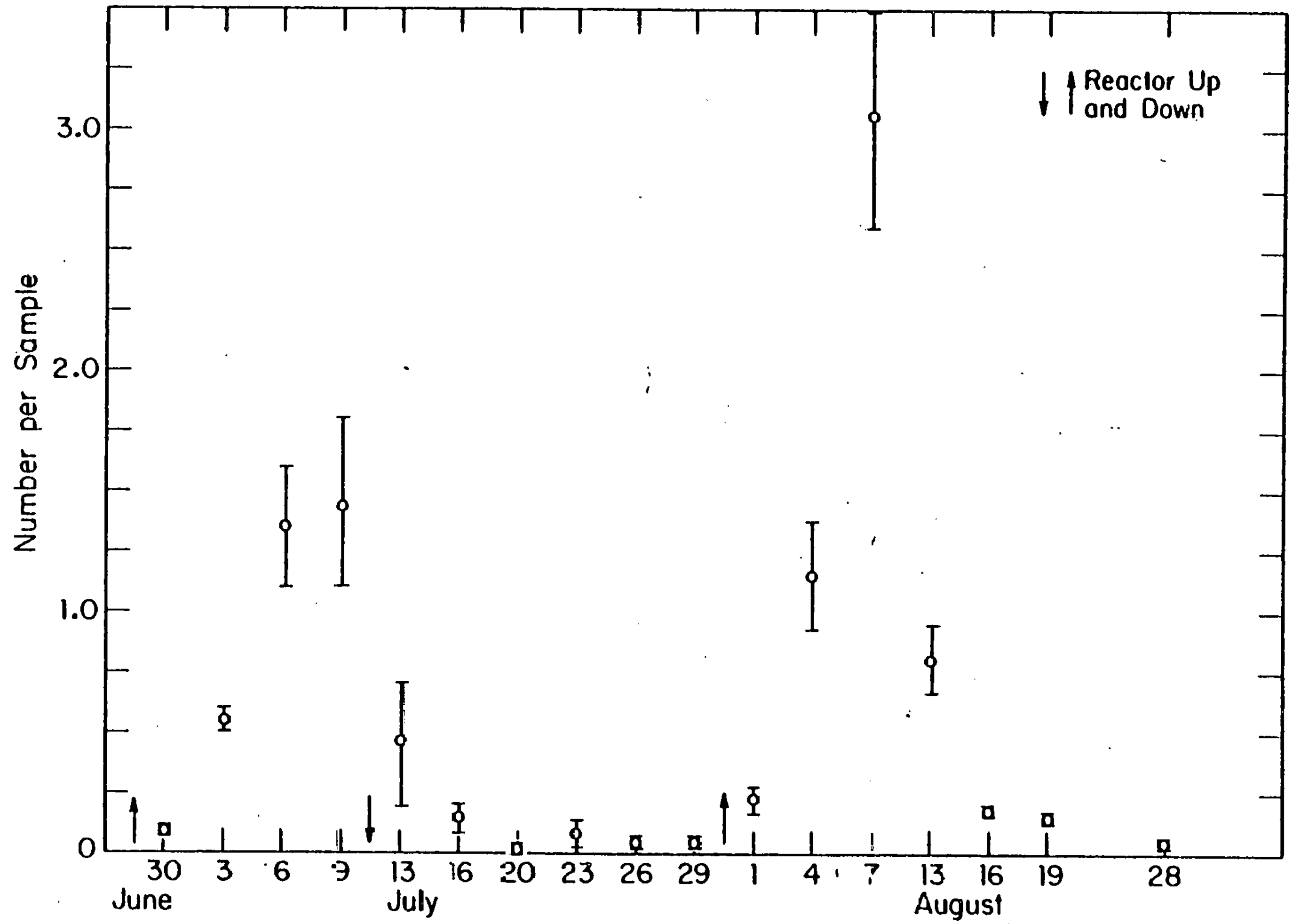

MAS-1
MAS
N
Station Pond C
MAS
P-Reactor
CAS
SAS
1000m

Figure E-2 - Mean and standard deviation of standing stock estimates of Bosmina longirostris in the summer of 1975, Station MAS-1. Arrows indicate up (starting) and down (stopping) of P Reactor.



Reactor Up
and Down
3.0
2.0
1.0
0
Number per Sample
30
3
6
9
13
16
20
23
26
29
1
4
7
13
16
19
28
June
July
August

suggested the stock was influenced in some way by the operation of the reactor.

To determine if Station MAS-1 could become exhausted of its population of Bosmina by sampling, 300 consecutive tows were made on August 19, 1975. The 300th consecutive tow contained as many Bosmina as in each of the first two tows.

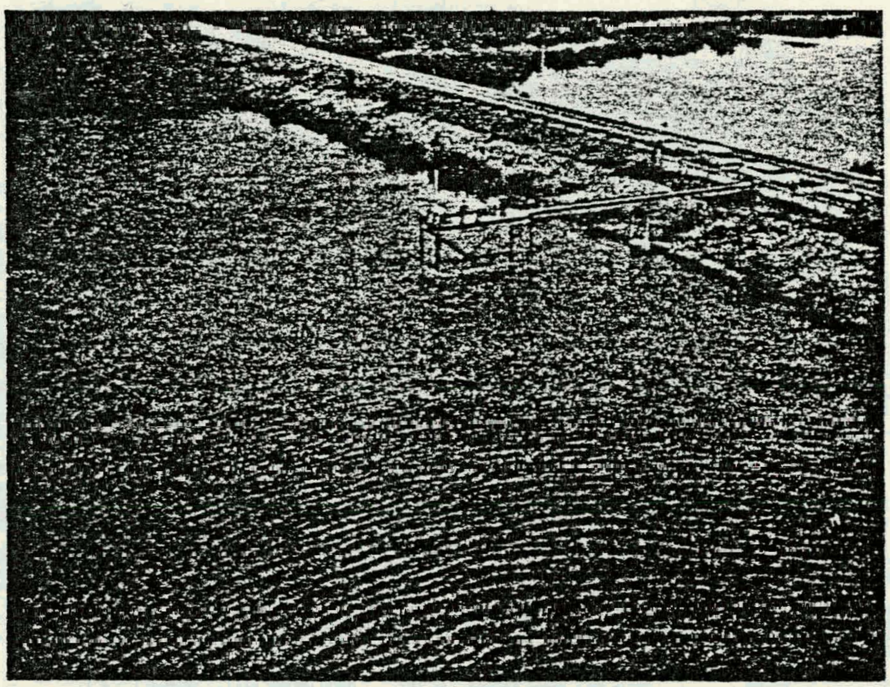
To examine the possibility of horizontal displacement of Bosmina at Station MAS-1, two 0.5-m diameter, #10 plankton nets were placed with their openings facing the dam in 0.5 and 1.25 m of water for three 1/2-hour periods on August 30, 1975. The surface flow was 14 ft/min as measured with a current meter at the station. The nets placed horizontally captured as many animals in 1/2 hour as were captured when taking 12 vertical tows with the 13-cm net on that date. The data suggests that Station MAS-1 is receiving a large input of Bosmina from "upstream."

Dye study

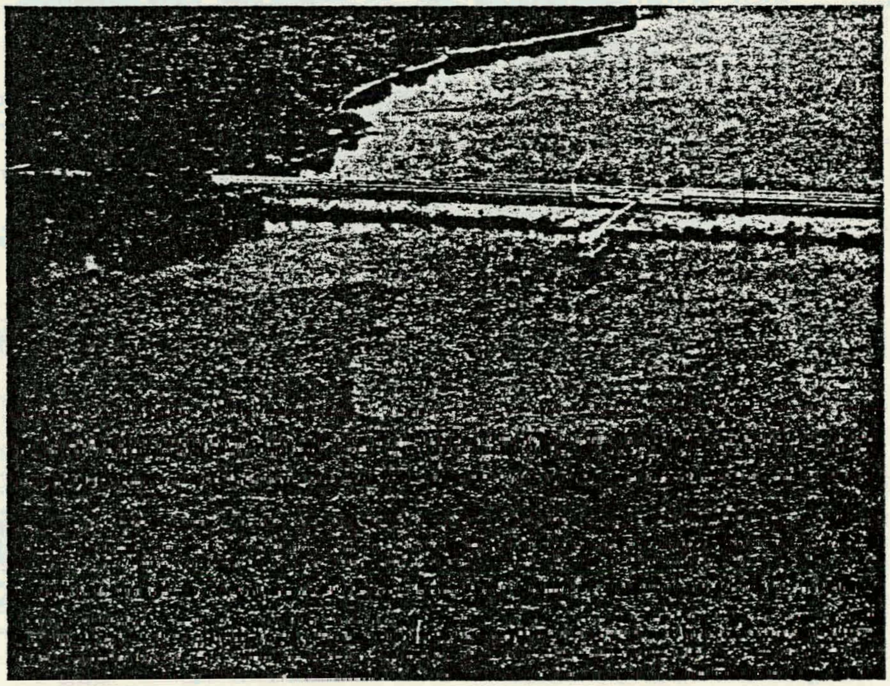
Circulatory patterns in the Hot Arm were examined there times in June 1977 by releasing Rhodamine WT dye as an instantaneous injection into the Hot Arm via the "Bubble-up" (the structure through which the effluent enters Par Pond from Pond C, Figure E1). Water samples were taken at different stations to determine longitudinal, lateral, and depth patterns. Aerial photographs were made of the last release.

All three runs revealed the dye first splits into two major plumes, each near the two shores and longitudinal with the reservoir. These plumes then converge into one below Beyers Bay Inlet (Figures E1 and E3). A "tear drop" effect of the water is created by a

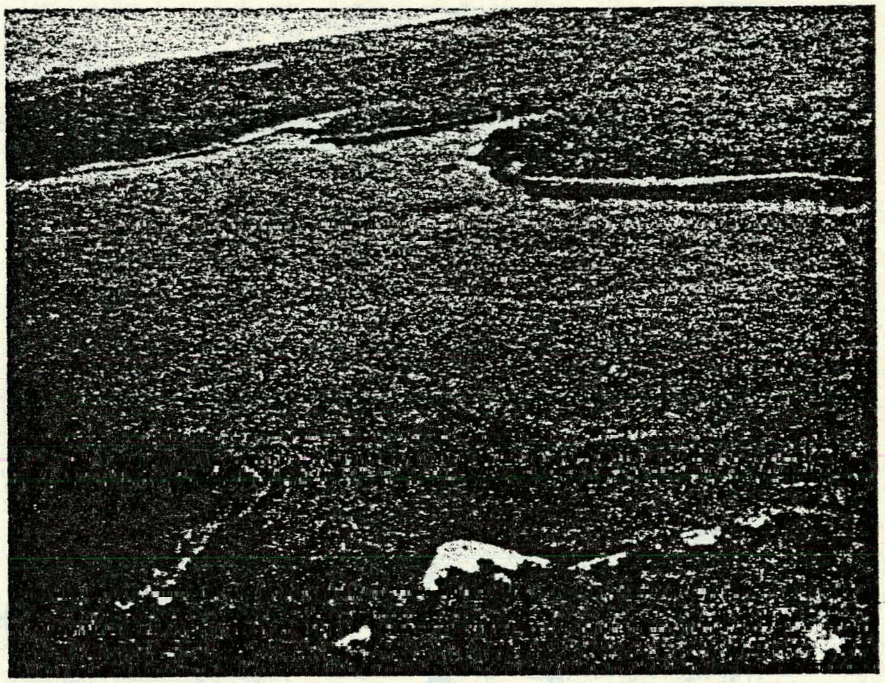
Figure E-3 - Dye flow pattern in the hyperthermal effluent receiving arm of Par Pond.



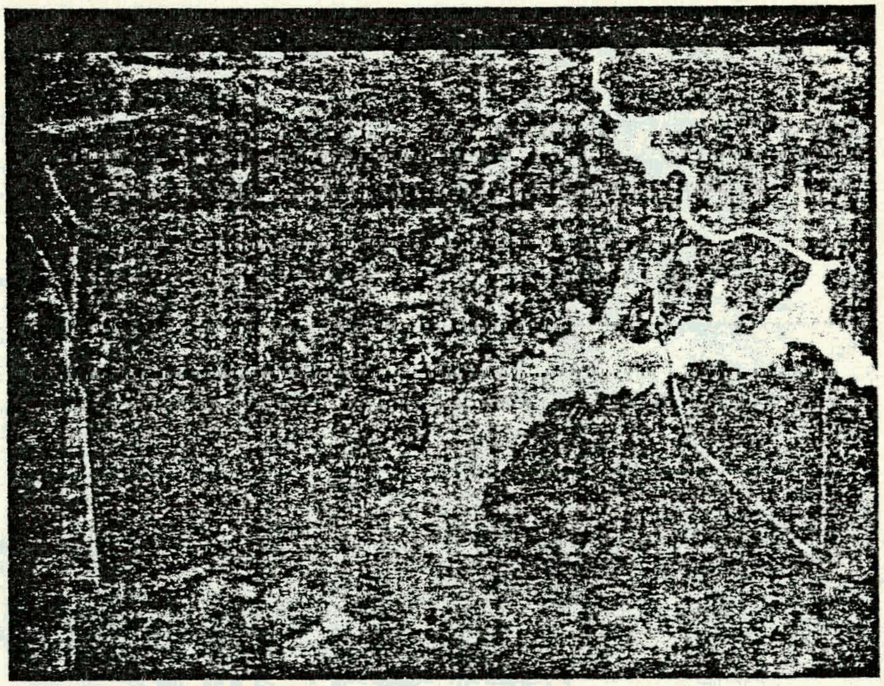
a. Initial Spread



b. Beginning of Lateral Spread



c. Developed "Tear-Drop" Pattern



d. Infrared Imagery of "Tear-Drop" Pattern minimum of dye in the center of the Hot Arm between the Bubble-up and Beyers Bay Inlet. This tear drop had been seen on infrared photographs (photographs which can detect small differences in temperature within an area) made at the Hot Arm in 1976. Depth sampling on the first and third runs revealed that the dye was confined to less than two meters of the surface. These observations indicate that the dye transport could be modeled by a two-dimension (planar) model.

Discussion

Our sampling experience with Bosmina longirostris in the littoral zone and the subsequent dye studies indicate that reactor operations do produce complicated flow patterns in Par Pond. A Bosmina in the upper 2 m of the water column near the discharge, if it acts similar to a dye particle, may be transported from the limnetic zone into the littoral zone and then out again into the limnetic zone. Hence, the thermal history of the population further down the Hot Arm than Beyers Bay Inlet may be unknowable, and sampling for standing crop in the littoral zone near the discharge is confounded.