THE EFFECTS OF TEMPERATURE AND DIETARY RESTRICTION ON AGING AND REPRODUCTIVE PATTERNS IN THE ROTIFER ASPLANCHNA BRIGHTWELLI, GOSSE

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ABSTRACT

THE EFFECTS OF TEMPERATURE AND DIETARY RESTRICTION ON AGING AND REPRODUCTIVE PATTERNS IN THE ROTIFER ASPLANCHNA BRIGHTWELLI, GOSSE

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In this study, the effects of different environmental temperatures and dietary restriction on the lifespan, reproductive cycle and fecundity of the rotifer Asplanchna brightwelli were examined. In the first set of experiments, it was observed that the mean lifespan and the times at which the reproductive cycle started and ended increased as environmental temperatures decreased. Dietary restriction by increasing the interval between feeding times from 12 to 36 hours also increased the mean lifespan and the length of the reproductive period in the rotifer. In further experiments examining these phenomena, it was observed that the rate of nuclear division in the gastric glands and vitellarium of the rotifer, as determined by daily nuclear counts, was retarded by dietary restriction. Although the growth rate of this eutelic organism during its lifespan was

not affected by dietary restriction, the size of the gastric glands was affected. The nuclear sizes of this organ were smaller in dietary-restricted rotifers but this difference was found not to be significant. Although the vitellarium was not affected by dietary restriction, it was observed that the nuclear sizes were significantly smaller in the dietary-restricted group. Spectrofluorometric measurements have shown that DNA content, as an index of DNA damage, throughout the lifespan of the rotifer is not affected by dietary restriction even though DNA concentration is constantly lower in underfed rotifers from day 2 to day 5 of life. Lactic dehydrogenase and malic dehydrogenase activities as indications of protein synthesis were determined through the release of fluorescent NAD. Results have shown that the level of activity was constantly lower during the first 3 days of life in rotifers subjected to dietary restriction but higher in this group during the reproductive cycle and the post-reproductive period. The results obtained in the various experiments are discussed in light of some current theories of aging.

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INTRODUCTION

Aging and senescence are biological processes that have been investigated very closely in recent years; the interest in this subject is due to the fact that the majority of organisms, invertebrates and vertebrates, go through the process of aging which in turn leads to the increased probability of death. Aging also involves a decrease in the ability of an organism to cope with its environment along with a decrease in vitality, "the ability to sustain life" (Lamb, 1977).

There is no single theory of aging that is generally accepted by researchers in the field. However, according to Comfort (1979), there are two general schools of thought that encompass the wide variety of aging theories.

Extrinsic or Random Theory

According to this view, aging results from the "wear and tear" of living and not from programmed development (Moment, 1978). For example, vital organs become damaged thus affecting important systems in the organism; these in turn cause accumulation of waste products (for example, the age-pigment lipofuscin) and lysosomes (Cristofalo & Stanulis, 1978). More importantly, there is thought to be an increase in

errors in DNA affecting repair and replication, along with errors in protein synthesis resulting in the production of faulty enzymes.

Intrinsic or Controlled Theory

Here aging is thought to be genetically programmed by an aging "clock" (Everitt, 1973; Lamb, 1977) or a "pacemaker" (Finch, 1977). The timepiece is thought to have the capacity of regulating the process of aging by controlling the rate of cell division in organs and tissues as well as other internal processes in the cell.

When considering the various theories that could be investigated regarding the different views on aging, the choice of organism is important for research in this field. Primarily, it is beneficial to work with a multi-cellular organism of constant genetic make-up and a short lifespan. With this in mind, the rotifer Asplanchna brightwelli, Gosse was selected for the present study.

These metazoans are ideal for aging studies since they meet the above criteria and are also easily cultured. Although they are small and thus easily stored, they can still be handled without much difficulty. They have few cell types, thereby permitting close observation of structural changes with aging. The lifespan of this rotifer is about

4 days and the short lifespan can be altered by modifying environmental conditions (Fanestil & Barrows, 1965). In the adult rotifer, growth occurs through cytoplasmic expansion rather than by cell division; there are only two organs in the rotifer that undergo cellular division and these are (1) a pair of gastric glands that act in digestion and (2) the vitellarium, a reproductive structure (Birky & Field, 1966). This eutelic characteristic is beneficial for aging studies since the same cells are observed throughout the lifespan of the rotifer.

Another characteristic of A. brightwelli which makes it an ideal specimen for aging studies concerns its mode of reproduction. The form of the rotifer used in this project is an amictic female which produces other amictic females through diploid parthenogenesis (Gilbert, 1963; Birky, 1967). Because of the dominant asexual form of reproduction, all the rotifers of the clones are genetically identical thus eliminating the influence of genetic variability on lifespan, although the possibility of mutations cannot be ignored. Under certain conditions, not used in this study, such as high environmental temperatures or the presence of algae or dietary tocopherol, mictic females are produced; the females produce haploid eggs which can develop into

males or into resting eggs if the females are fertilized by males (Birky & Gilbert, 1971).

In this study, temperature and diet are manipulated to see how the lifespan and reproduction of A. brightwelli are affected. Further experiments were conducted to see if certain factors in the rotifers are affected; these include body size and size of certain organs, nuclear division in the gastric glands and the vitellarium as well as DNA and enzyme content throughout the lifespan of the rotifers.

Various experiments in aging studies examine the effects of high and low temperatures on the lifespan of different organisms as well as the effects on their reproductive patterns. Experimental data involving changes in temperature and their effect in the rotifer Philodina acuticornis odiosa, Milne have shown that increased temperatures decreased life expectancy but did not affect fecundity in the rotifer (Meadow, 1967). Another experiment conducted by Fanestil and Barrows on the same rotifers also demonstrated that an increase in environmental temperature markedly decreased longevity but only very high temperatures affected fecundity (Fanestil & Barrows, 1965). Studies involving Drosophila have shown that fruit flies reared at high temperatures have a shorter lifespan than those reared

at low temperatures (Loeb & Northrop, 1917) and it was also shown that the lifespan of Daphnia varied inversely with environmental temperature (MacArthur & Bailie, 1929). The interpretation of such results concerning temperature and longevity will be discussed in more detail later on in this study.

Food availability and caloric intake have also been studied quite extensively by researchers in the field of aging. In a wide range of laboratory animals, it has been shown that dietary restriction, either by reducing caloric intake or by decreasing the times of feeding, significantly increased longevity. This was shown in invertebrates such as Tokophyra (MacKeen & Mitchell, 1975), Campanularia flexuosa (Brock, 1975), Daphnia (Ingel, Wood & Bantas, 1937), Drosophila (Loeb & Northrop, 1917) and in the guppy, Lebistes (Comfort, 1963).

It was also found that undernutrition lengthened the interval from birth to cessation of egg production in the rotifer Philodina (Barrows & Fanestil, 1965). Similar results showing the evidence of dietary restriction delaying maturity and the onset of reproductive period were obtained from experiments in rats (McCay, Crowell & Maynard, 1935; McCay, Maynard, Sperling & Barnes, 1939; McCay, Sperling & Barnes,

1943; Berg & Simms, 1960; Ross, 1959). In these experiments, the mean lifespan of the organisms was significantly increased through some form of dietary restriction. It was also observed that dietary restriction altered body size as well as the size of various organs in the majority of organisms mentioned above. Some proposed theories suggesting why dietary restriction has such effects on longevity and reproduction will also be discussed.

Since animals have a characteristic limited lifespan, it is conceivable that senescence and death are consequences of the limited lifespan of their cells. The idea of "programmed aging" of the nucleus is a concept that has raised numerous questions regarding cell death. There is a hypothesis stating that cell death occurs simply from "wear and tear" and the inability of cells to renew their vital resources (Sonneborne, 1978). Are cells therefore programmed to stop dividing after they have reached their characteristic number of divisions?

Limited capacity for cell division was shown in protozoan clones such as Paramecium; this "clonal senescence" was demonstrated by Maupas (1889) who presented evidence that the protozoans had a limited lifespan characterized by a decline in vigor and sharp decrease (even cessation) of cell division at the end

of the lifespan (Moment, 1978). The process was altered if sexual interchange occurred; that is, the exchange of nuclear material seemed to invigorate the cells and cell division occurred as previously until the limit was reached. The idea of senescence in clones sparked researchers into studying various other life forms in order to establish if cell division was also limited in certain organs and tissues.

Early experiments on cell cultures had reported that certain cells of vertebrate tissues could grow indefinitely in vitro (Carrel, 1912; Ebeling, 1913). Later studies however, have shown that cell cultures (such as fibroblasts and fetal tissue cells) proliferated for an extended period of time and then died (Swim & Parker, 1957; Hayflick & Moorhead, 1961). Hayflick (1965) presented the concept that limited lifespan of cultured cells was related to aging where "the declining vitality of cells was considered an expression of senescence at the cellular level" (Finch & Hayflick, 1977).

There are four major targets that play a significant role in molecular senescence: DNA, RNA, protein and lipid membranes (Brash & Hart, 1978). Obviously, DNA is considered the most important target because it is a large molecule, it has a unique copy of genetic instructions and it serves as the template

for all other targets. Any alterations in DNA can lead to changes in cell growth, division, mutation and even death.

Most extrinsic aging theories are based on the fact that aging is associated with some sort of DNA damage. There is Szilard's theory that mutations in DNA, caused by radiation result in improper functioning of cells (Moment, 1978) and Orgel's theory of "error catastrophe" which states that aging occurs through the production of faulty enzymes due to changes in the DNA-protein synthesizing machinery (Moment, 1978). Since Orgel's hypothesis also predicts that with increasing age there is an increase in the amount of defective protein (Lamb, 1977), alterations in enzyme levels or activities throughout the lifespan of the organism should occur. Gershon (1979) has shown that the accumulation of inactive enzyme molecules resulting from protein degradation is characteristic of senescence and that this contributes "to the declining ability of aging organisms to cope with environmental changes" (Gershon, 1979) and eventually, death. Changes in enzymatic activities also occur with age; some enzymes show decreased activity with age, some show increased activity while some show no change at all. These enzymatic activities differ in the tissues of various

species but even studies on the same organism are sometimes difficult to replicate. Because of these difficulties, studies of enzyme activity have not yet contributed much insight into metabolic activities during aging (Sanadi, 1977).

Some studies have shown that certain enzyme activities were altered as a result of dietary restriction in rats (Ross, 1959), in mice (Leto, Kokkonen & Barrows, 1971) and in rotifers (Fanestil & Barrows, 1965). The last experiment showed that enzymatic activities of malic dehydrogenase and lactic dehydrogenase in the rotifer Philodina increased in early life and decreased near the end of life. The pattern was slightly altered by different diets; in the group of rotifers fed less frequently which exhibited a longer lifespan, the changes in enzymatic activities occurred later in life (Fanestil & Barrows, 1965; Barrows, 1972). Further studies on the pattern of biochemical events in the cells and organs of organisms throughout their lifespan will undoubtedly shed more light on the mechanism of the aging process.

This project will therefore examine how aging is manifested in the rotifer A. brightwelli and how manipulations in its environment alter longevity and reproductive patterns. The experiments were designed

to test some of the aging theories described.

The first experiment involved varying the temperature at which rotifer cultures were reared. The reason for carrying out such an experiment in this rotifer was twofold: to study the effects of a certain range of temperatures on the lifespan and reproductive cycle of the rotifers and to find an optimal temperature in which to rear the cultures so that the greatest mean lifespan was achieved. This allowed the observation of some aging processes on a daily basis in the rotifer for the longest possible time.

The second manipulation involved limiting the amount of food available to the rotifers by increasing the time intervals between feedings and determining how this affected their longevity and reproductive patterns. The results of these two experiments were compared to similar studies and by taking into consideration some proposed aging theories, some suggestions were made concerning how the changes in the rotifer's environment affected its longevity.

Since many studies on dietary restriction have shown that organ and body size in the organisms are affected, the body size and sizes of various organs and their nuclei were measured throughout the lifespan of A. brightwelli to see if the type of dietary

restriction imposed affected such factors in this rotifer. Also since the gastric glands and the vitellarium are the only exception to the eutelic rule in this organism, it was determined whether the rate of nuclear division in these organs was affected by dietary restriction. The gastric glands lie on each side of the stomach in the rotifer and aid in digestion. The vitellarium is responsible for the production of yolk and for providing essential nutrients for the developing embryo.

The rate of nuclear division was monitored by simply counting the number of nuclei in these organs daily throughout the lifespan of the rotifer. The results will show if a certain pattern of nuclear division exists in these organs; the results will also show whether dietary restriction affected nuclear number in the two organs and if it could be correlated in any way to longevity and reproductive patterns displayed under the imposed regime. Another reason for this experiment was to see if these actively dividing glands went through a fixed number of divisions before death of the organism occurred thus testing the hypothesized "programmed aging" of certain cells and organs. Although rotifers are considered an example of aging through "wear and tear" on non-renewing cells because of their eutelic

condition and the short lifespan they display, the pattern of nuclear division in the gastric glands and the vitellarium may provide a clue as to how senescence is manifested in this organism.

Certain biochemical events were also monitored during the aging process of A. brightwelli. Although rotifers because of their small size were not the ideal organisms in which to study various aspects of DNA or protein damage, the DNA content throughout their lifespan was determined using spectrofluorometric methods. The quantitative analysis of DNA was carried out on a daily basis to see if differences occurred regarding DNA content throughout the lifespan of control rotifers and rotifers under dietary restriction.

As well as alterations in DNA which could accompany senescence and even death in organisms, alterations in proteins are thought to be closely linked with aging through the production of faulty enzymes or through alterations in enzyme activities. Using spectrofluorometric methods, it was also possible to determine if changes in the enzymatic activities of lactic dehydrogenase and malic dehydrogenase occurred throughout the lifespan of A. brightwelli and also if levels of activities were affected by dietary restriction.

The results of all the experiments described will be interpreted in light of some aging theories mentioned previously.

MATERIALS AND METHODS

Culture Methods

The rotifers used in this study were Asplanchna brightwelli Clone 4B61 originally obtained from Dr. John J. Gilbert, Dartmouth College, Hanover, New Hampshire.

The rotifers were fed paramecia cultured in a Cerophyll infusion medium prepared according to the method described by Bridger (1970). A 993-ml volume of distilled water containing 0.06g $CaCO_3$ and 7.0 mls of stock buffer solution (see Appendix I) was heated in a 4-liter Erlenmyer flask. When the solution was boiling, 0.75g of Cerophyll (obtained from Ward's Natural Science Establishment, Rochester, New York) was added and the whole suspension was boiled for 10 minutes. The solution was cooled down to room temperature in an ice bath and then filtered using Whatman #4 filter paper. The filtered solution was autoclaved for 20 minutes.

After the autoclaving, the solution was cooled down under running tap water to room temperature. The solution was then ready for inoculation with E. coli (Type K12, obtained from Dr. E. Newman, Concordia University). Using sterile technique, a loopful of E. coli was placed into 3.0 mls of distilled water, mixed, and this suspension was added to the solution.

The bacteria served as a food source for the paramecia. After the solution had been incubated at 37°C for 24 hrs, it was cooled down to room temperature and at this point the pH was checked; the ideal pH ranged between 6.5 - 7.0. The pH could be adjusted if need be, using 0.05N NaOH but this step was usually unnecessary.

A 50-ml culture of Paramecium caudatum (originally obtained from Boreal Laboratories Ltd., Mississauga, Ontario) was added to the solution, swirled and left at room temperature for 24 - 36 hrs. After this time, 150 mls of medium were placed into each of 4 250-ml Erlenmyer flasks and about 20 rotifers were placed in each flask. These were labelled "Stock Cultures". Every week, rotifers were removed and transferred to another flask containing new medium. About 100 mls of paramecia medium were kept aside to be used for new stock preparations which were made every 10 days.

Experimental Methods

For the first part of any experiment, rotifers were removed from the stock cultures and placed in a Petri dish for observation under a dissecting microscope; 24 adult rotifers were removed and placed individually in culture chambers. The removal and transfer of rotifers was done through mouth suction using a 50-µl micropipet fitted to a 55-cm rubber tubing with a mouthpiece.

This allowed individual rotifers to be picked up with very little of the surrounding medium. The experimental and/or culture chambers were Tissue Culture Clusters #3524 (obtained from Costar, Cambridge, Massachussets) made up of 24 wells or chambers measuring 16-mm in diameter with a capacity of 3.0 mls. A single rotifer was placed in each chamber along with 2.5 ml of paramecium medium.

The chambers were checked every 12 hrs for offspring. The newborn rotifers were separated from the parent and placed in new experimental chambers; only newborn rotifers were used for the experiments since their age could be estimated more accurately (0 - 12 hrs old). This procedure was carried out before the start of all the experiments conducted.

Temperature Experiments

Once the adult rotifers were isolated, the culture dishes were kept in the dark at the required temperature for the experiment (15°C, 17.5°C, 20°C, 23°C or 25°C) in an incubator. A 500-ml Erlenmyer flask containing paramecia medium was also kept at the appropriate temperature. When the offspring were born, they were transferred to new experimental chambers and these were filled with medium from the flask.

The readings were taken daily at approximately

9:30 AM and 9:30 PM; during the readings, the chambers were checked for offspring which were counted, removed and discarded when present. Also, 0.5 ml of medium was removed from the top layer of the chamber using a marked Pasteur pipet and replaced with an equal amount of fresh medium to assure a constant, fresh supply of food for the rotifer. It was important that the chambers were checked twice daily so that a close estimate of time of death of a rotifer could be made: the rotifer was considered dead when it was in a state of deterioration or when probing with a pipet did not induce any motion.

Dietary Restriction Experiments

In these experiments, all the experimental cultures were kept at 18°C since lower temperatures were found to be beneficial for the survival of the rotifers according to the previous experiment. Two other flasks were kept at this temperature: one contained paramecia medium and another flask contained medium without paramecia or E. coli labelled "Cerophyll Only".

Readings were taken every 12 hrs despite the various feeding intervals and replacement of 0.5 ml of medium was standard procedure. Since dietary restriction in these experiments involved decreasing the times of feeding, the flask containing Cerophyll solution was used as fresh medium replacement at the times when the rotifer was not

fed. The regular paramecia medium was used for the appropriate feeding times.

Six different groups of 24 rotifers were used for the experiment at feeding times of 12 hrs, 24 hrs, 36 hrs, 48 hrs, 60 hrs and 72 hrs. All isolated adult rotifers were fed twice daily and the newborn were initially fed paramecia and were transferred to the experimental chamber. The next feeding occurred after 12 to 72 hrs according to the group in which it was placed. Results were recorded as described previously.

Nuclear Counts/Size Measurements

The procedure for counting nuclei in the vitellarium (yolk gland) and the gastric glands in A. brightwelli was based on the method described by Birky and Field (1966) using A. sieboldi. The offspring from isolated rotifers were collected as described previously. The experimental rotifers were placed in control or experimental culture chambers and were fed every 12 or 36 hrs respectively.

For the nuclear counts, the rotifers were removed from the culture chambers on the appropriate day and time (Day 1 to Day 6) and were placed in Petri dishes containing distilled water to wash them of paramecia and medium. After a minute, they were transferred to another Petri dish containing 50% ethanol where they were fixed for 3 minutes. A few fixed rotifers were then placed on

a clean slide and after excess ethanol had been drained by tilting the slide, a coverslip was gently applied. This caused the rotifer to become squashed.

Through this method, the organs and their nuclei were clearly visible and could easily be counted using a Zeiss Ultraphot II Phase Contrast Microscope at a magnification of 200X. Only rotifers in which both the gastric glands and vitellarium were clearly seen were used so that precise counts could be obtained. Twenty rotifers from both control and experimental (dietary restricted) cultured were used for the nuclear counts from Day 1 to Day 6.

The same method was used for taking the various measurements in control and experimental rotifers. A 6X ocular micrometer was used instead of the regular (12X) eyepiece on the phase contrast microscope. Using the Ph 2/16X objective lens for a final magnification of 96X, the body size (length and width) of the rotifer and its vitellarium were measured. Using the Ph 2/40X objective (240X magnification), the size of the gastric glands (length and width) was measured. At this magnification it was possible to measure the diameter of nuclei of the gastric glands and of the vitellarium. Diagrammatic details of how these measurements were taken are given in Appendix III.

DNA Fluorescence

The method used for DNA fluorescence was the one described by LePecq and Paoletti (1967) where a dye, ethidium bromide, was used to bind nucleic acids. The binding caused fluorescence that could be measured using a spectrofluorimeter.

Preliminary tests were run to determine the sensitivity of the Aminco-Bowman spectrofluorimeter available for the assays. Also several trial runs were carried out to determine how many rotifers would be needed to observe changes in DNA levels using this particular method. The trial experiments provided the basis for future measurements.

Several culture dishes were placed in an incubator at 18°C containing isolated adult rotifers. The offspring were randomly placed in either control or experimental (dietary-restricted) groups and were fed every 12 or 36 hrs respectively. Since DNA content was to be measured for every day of life, the same procedure was used for rotifers 1 to 6 days old in each group.

Twenty rotifers were collected from both groups on the appropriate day and were placed in separate Petri dishes containing deionized-distilled water. The rotifers were transferred in two other changes of deionized-distilled water to remove all traces of

paramecia and medium. The rotifers were then pipetted into a calibrated test tube and deionized-distilled water water was added until a 1.0 ml volume was reached. The sample was carefully removed with a micropipet and placed in a sonication vessel. The rotifers were sonicated in an ice bath for 30 seconds using a Cell Membrane Disruptor/Sonic 300 working at 35% capacity.

Since DNA was the only nucleic acid to be measured, it was necessary to destroy RNA to prevent binding of the dye. Ribonuclease-A (from bovine pancreas, R-4875, obtained from Sigma Chemical Co., Saint Louis, Missouri) was added to the sonicate at a concentration of 20 µg/ml and left to act for 1 hour at room temperature. Also, a standard curve of DNA concentrations from 0.01 µg/ml to 5.0 µg/ml was prepared prior to each assay. The DNA used was the sodium salt (reagent grade) obtained from Eastman Kodak Co., Rochester, New York. Two main stock solutions of DNA were prepared in deionized--distilled water: Stock A was made up using 1.0 mg DNA/l to obtain standard DNA concentrations between 0.01 µg/ml and 0.8 µg/ml. Stock B containing 1.0 mg DNA/100 mls was used for standard DNA concentrations ranging from 1.0 µg/ml to 5.0 µg/ml.

Different aliquots from the stock solutions were put in each tube used for the construction of the

standard curve and the various amounts were made to equal 1.0 ml volumes by adding a calculated amount of buffer solution, 0.1 M Tris- HCl/NaCl, pH 7.5. To prepare the buffer 12.15g of THAM (Tris (hydroxymethyl) aminomethane) and 5.8g of NaCl (both obtained from Fisher Scientific Co., Fair Lawn, New Jersey) were added to a liter of deionized-distilled water. After thorough mixing using a magnetic stirrer, concentrated HCl was added until a pH of 7.5 was reached.

The ethidium bromide solution (2,7-diamino-10--ethyl-9-phenyl-phenanthridium bromide, obtained from Sigma Chemical Co., Saint Louis, Missouri) was prepared in the above buffer at a concentration of 7.5 µg/ml. Two mls of this solution were added to all the tubes prepared for the standard curve and those containing the sonicated rotifers. The reference blank contained 2.0 mls of this fluorescent dye and 1.0 ml of deionized--distilled water. After mixing, all tubes were read immediately on the spectrofluorimeter at $\lambda ex = 546$ mµ and $\lambda em = 590$ mµ.

Enzymatic Activities

The spectrofluorometric method used to assay enzyme activity of lactic dehydrogenase and malic dehydrogenase was based on the ones described by Lowry, Roberts and Kapphahn (1957) and Fanestil and Barrows

(1965). The activity of both enzymes was determined by the amount of NAD (β-nicotinamide adenine dinucleotide) formed which was the fluorescent component of the reaction.

Preliminary tests were carried out using standard amounts of β-NAD (stock no. 260-101, obtained from Sigma Chemical Co., Saint Louis, Missouri) to determine the sensitivity of the Aminco-Bowman spectrofluorimeter. Trial assays were first run to determine the number of rotifers that would be needed for the daily enzymatic assays.

The control group (rotifers fed every 12 hrs) and the experimental group (rotifers fed every 36 hrs) were kept at 18°C. The cultures were synchronized so that rotifers could be collected from day 1 to day 6 of life and assayed for enzyme activity. On the appropriate day, 3 rotifers from both control and experimental groups were removed and rinsed in deionized-distilled water. The rotifers were then rinsed in three changes of sonication medium which contained 0.005 M Tris-Cl, pH 8.2 (Tris (hydroxymethyl) aminomethane adjusted to the correct pH using concentrated HCl) and 0.7 M NaCl in 0.05% bovine plasma albumin (BPA, stock no. A-4628, 5% sterile filtered solution in 700 mg % NaCl, pH 7.0); all compounds were obtained from Sigma Chemical Co. The rinsing

procedure consisted of pipetting the rotifers into Petri dishes containing the solutions for a few minutes. After rinsing, the rotifers were placed into sonication vessels containing 1.0 ml of the above medium and were sonicated for 30 seconds.

The incubation medium for both dehydrogenases was made up of 0.1 M Tris, 0.05% BPA, 0.025 M nicotinamide and 0.001 M NADH (β-nicotinamide adenine dinucleotide, reduced form, stock nos. 340-102, 340-105) all obtained from Sigma Chemical Co. For the lactic dehydrogenase assay, 15 µls of the sonicate were placed with 15 µls of the incubation medium; for the substrate, 15 µls of 1×10^{-3} M sodium pyruvate, pH 7.4 (solution, 0.011 M in 0.1 M phosphate buffer, Sigma Chemical Co.) were added to the mixture and the tubes were incubated in a water bath at 37.5°C for 90 minutes. For the malic dehydrogenase assay, the procedure was the same except that the substrate used here was 7.5×10^{-4} M neutralized oxalacetate, pH 8.8; this reagent was in cis-enol form 90 - 95% (obtained from Sigma Chemical Co.) and the solution was titrated to pH 8.8 using 0.1 N NaOH. This reaction mixture was incubated in a water bath at 37.5°C for 45 minutes.

The standard curve for NAD ranged from 0.05 - 3.0 µg β-NAD/ml and was prepared by using different

aliquots from 2 stock solutions: Stock A make up of 1.0 mg β-NAD/100 mls of sonciation medium and Stock B containing 1.0 mg β-NAD/10 mls of sonication medium.

Following incubation, the tubes were placed in an ice bath and 20 µls of 0.45 N HCl were added to all the tubes in order to remove residual NADH. Also, 10 µls of 6 N NaOH were added to the mixtures and the tubes were heated in a water bath at 60°C for 10 minutes to develop fluorescence. After this time period, 2.0 mls of deionized-distilled water were added and the tubes were read on the spectrofluorimeter at λex = 360 mµ and λem = 460 mµ.

Statistical Analysis

A one-way analysis of variance (ANOVA) followed by a post hoc Tukey test were employed to determine any difference between the treatments for the measures of lifespan and fecundity for both the temperature and dietary restriction studies (Bruning & Kintz, 1977). Life tables were constructed for both these studies according to Lamb (1977) and these are included in Appendix II.

For nuclear counts study, t-tests were conducted for the gastric glands and the vitellarium nuclei between control and experimental groups for every day of life (Sokal & Rohlf, 1969). For the various measurements

taken of both groups of rotifers during their lifespan, a comparison of slopes for the regression lines as described by Sokal and Rohlf (1969) was performed. To determine whether the range of nuclear sizes in the vitellarium on each day of life between control and experimental rotifers differed, a paired t-test was performed between the smallest and the largest nuclei observed daily of both groups throughout the lifespan.

A paired t-test was performed to find out if the DNA content was different between control and experimental rotifers throughout their lifespan.

A paired t-test was also performed to determine if the enzymatic activities of lactic dehydrogenase and malic dehydrogenase were different between control ahd experimental rotifers throughout a 6-day lifespan.

RESULTS

In this section, the effects of temperature on lifespan will be presented first, followed by data on the effects of dietary restriction on lifespan. The effects of the two environmental manipulations of lifespan, fecundity and reproductive profile of A. brightwelli will be analyzed. To follow up on the the observations obtained from the studies on dietary restriction, results of nuclear counts will be presented as well as results of organ- and nuclear-size measurements made on both groups of rotifers. Following this, the results of DNA and enzyme assays will be shown for both control and dietary-restricted rotifers throughout the lifespan.

Temperature

Longevity. The results show that longevity in the rotifer was influenced by temperature; the results are given in Table 1a. A one-way analysis of variance showed there were significant differences among the mean lifespan of A. brightwelli reared at various temperatures ($F(4,115) = 17.874$, $p<.001$). The results indicated that increasing enviromental temperatures decreased the lifespans of rotifers. The optimal temperature in which to rear the cultures was at 17.5°C since in this group,

Table 1a

Mean lifespan observed and life expectancy as derived from life tables (see Appendix II) for Asplanchna brightwelli at various environmental temperatures. (N = 24)

Temperature (^{o}C)	Mean Lifespan, $\bar{x}$ (days) ± S.E.	Life Expectancy, $e\dot{x}$ (days)
15	5.62 ± .33	4.91
17.5	5.91 ± .41	5.66
20	3.93 ± .29	3.29
23	3.35 ± .20	3.12
25	3.04 ± .25	2.33

Table 1b

Longevity - Table of significant differences within the treatment groups according to a post hoc Tukey test.

Temperature (°C)	15	17.5	20	23	25
15	-	ns	**	**	**
17.5		-	**	**	**
20			-	ns	ns
23				-	ns
25					-

ns = not significant

* = $p<.05$

** = $p<.01$

the greatest mean lifespan was obtained, $\bar{x}$ = 5.91 ± .41 days. A post hoc Tukey test revealed that this mean was not significantly different from the mean lifespan of rotifers reared at 15°C where $\bar{x}$ = 5.62 ± .33 days (q = .9333, $p>.05$). The mean lifespans of A. brightwelli reared at 15°C and 17.5°C were significantly longer than the mean lifespan of rotifers reared at 20°C where $\bar{x}$ = 3.93 ± .29 days (q = 5.41; 6.34, $p<.01$), at 23°C where $\bar{x}$ = 3.35 ± .20 days (q = 7.28; 8.21, $p<.01$) and at 25°C where $\bar{x}$ = 3.04 ± .25 days (q = 8.28; 9.22, $p<.01$). The mean lifespan of rotifers reared at 20°C was not significantly different from the mean lifespans of rotifers kept at 23°C (q = 1.869, $p>.05$) and at 25°C (q = 2.873, $p>.05$). Table 1b summarizes the statistical analysis among all the groups.

These results are graphically shown in Figures 1 and 2. Figure 1 shows the survival curves of groups of A. brightwelli reared at the 5 different temperatures. The survival curve of rotifers reared at 17.5°C, lies farthest to the right showing that at this temperature, the number of rotifers still alive at increasing age was greater than at any other temperature. At higher temperatures, the survival curves of the groups lie toward the left part of the graph showing that increasing environmental temperatures were

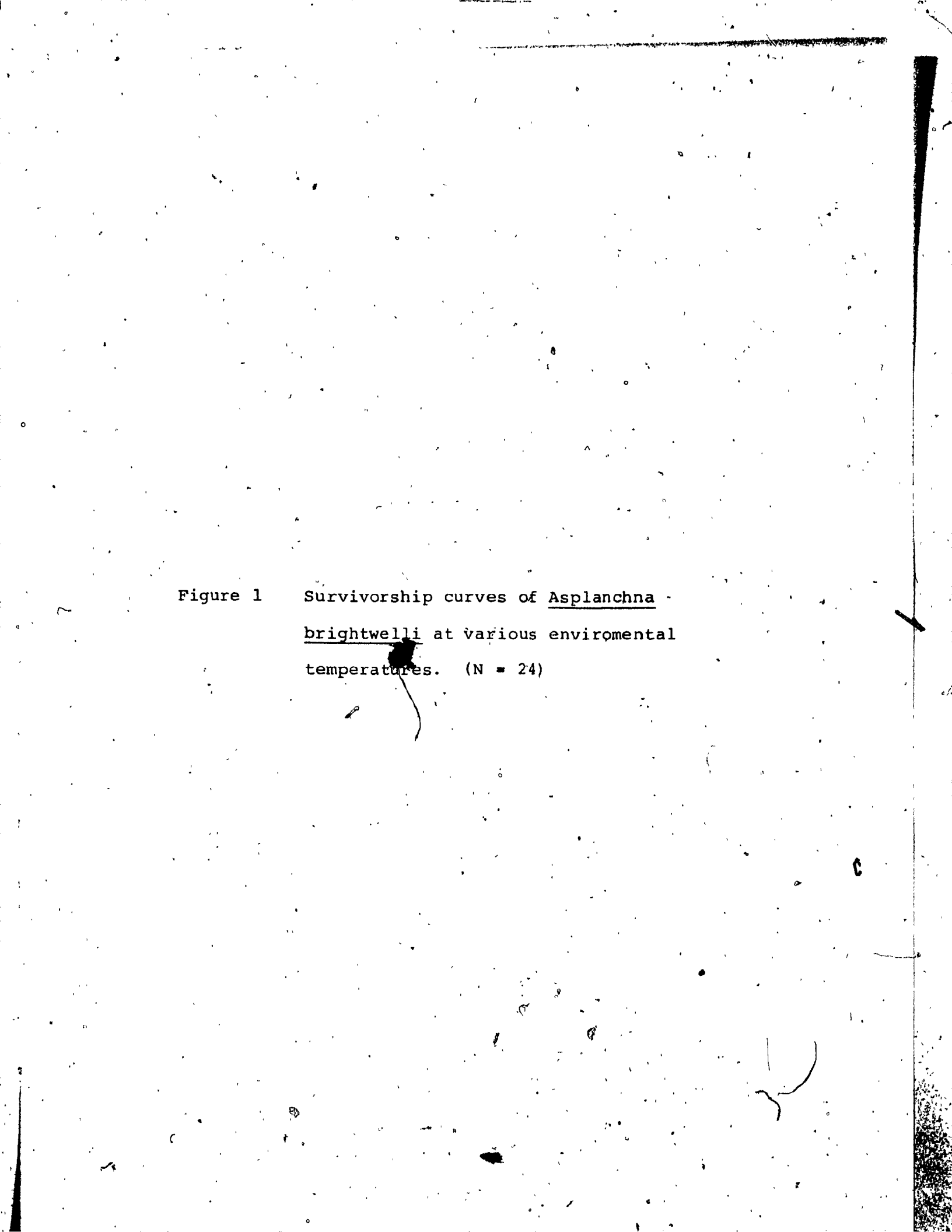
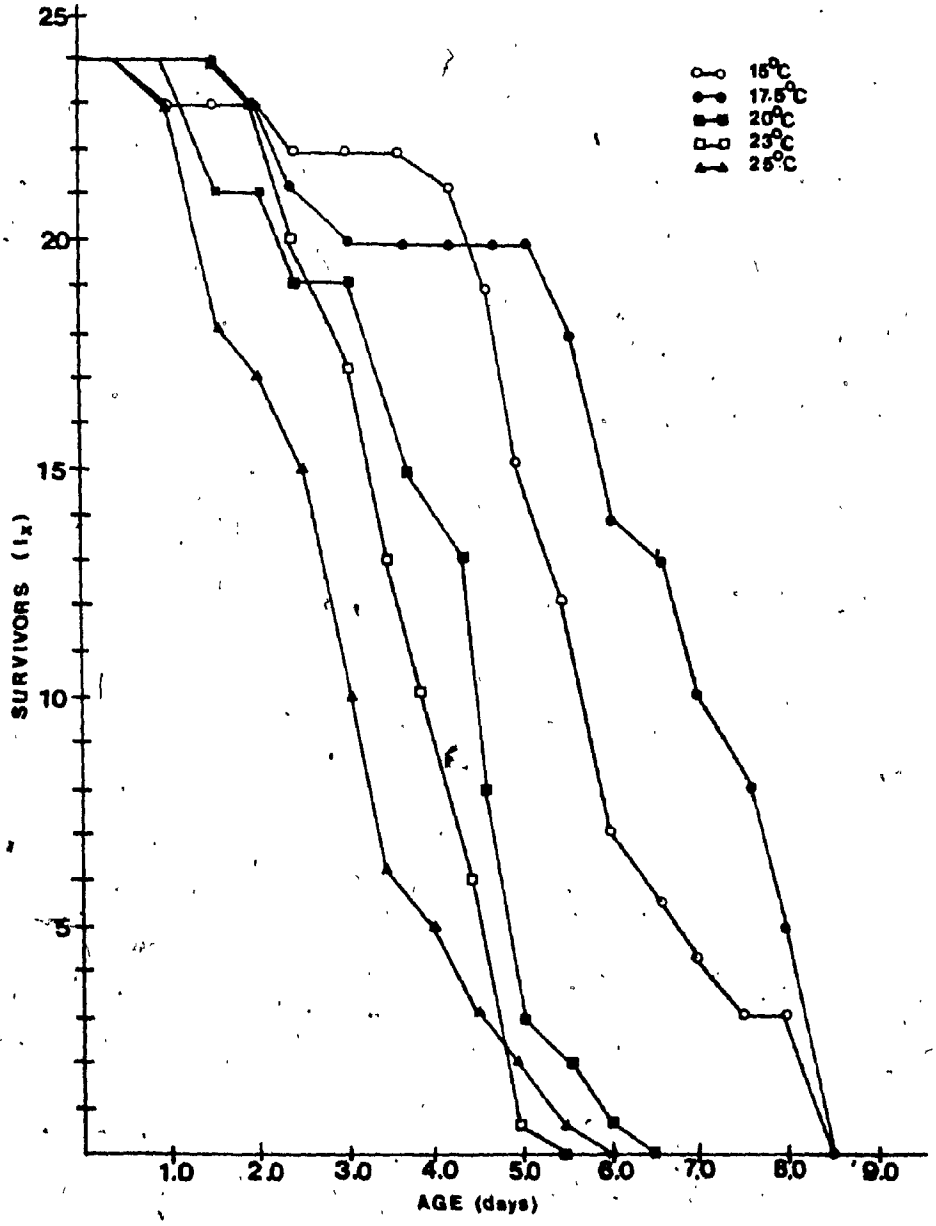


Figure 1 Survivorship curves of Asplanchna brightwelli at various enviromental temperatures. (N = 24)


25
20
15
10
5
SURVIVORS (l_x)
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
AGE (days)
15°C
17.5°C
20°C
23°C
26°C

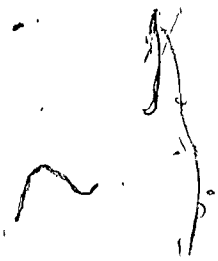
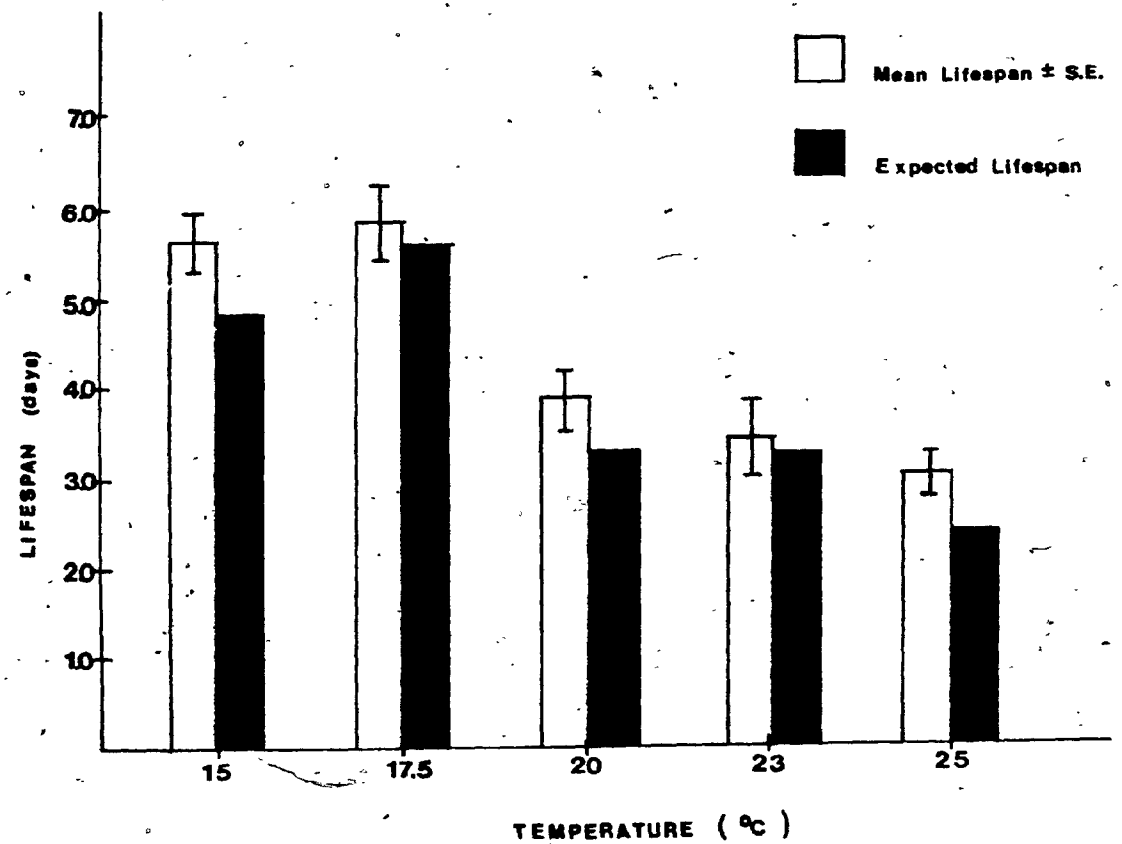


Figure 2 Histogram of mean and expected lifespan of Asplanchna brightwelli at various environmental temperatures. (Expected values are derived from Life Tables in Appendix II). (N = 24)


Mean Lifespan ± S.E.
Expected Lifespan
LIFESPAN (days)
70
60
50
40
30
20
10
15
17.5
20
23
25
TEMPERATURE (°C)

detrimental to the survival of A. brightwelli. Figure 2 is a histogram which represents mean and expected lifespan of rotifers at the different temperatures; mean lifespan was derived by taking the average of total lifespan of all rotifers in the experiment. Expected lifespan was derived from life tables (refer to Appendix II).

Fecundity. Table 2a shows the different reproductive patterns exhibited by A. brightwelli when kept at the different temperatures. A one-way analysis of variance showed that there were significant differences in the times at which these rotifers entered the reproductive cycle at various temperatures ($F(4,115) = 14.350$, $p<.001$). The beginning of the reproductive cycle was the time when rotifers started producing offspring. At the lowest temperature, 15°C, the rotifers started reproducing after an average of 3.83 ± .31 days which was significantly later, according to a post hoc Tukey test, than the average start of reproduction in rotifers kept at 17.5°C where $\bar{x}$ = 2.20 ± .19 days ($q = 6.84$, $p<.01$). Similarly, rotifers reared at 15°C entered the reproductive cycle significantly later than rotifers kept at 20°C where the start occurred after an average of 2.00 ± .25 days ($q = 7.68$, $p<.01$), in rotifers at 23°C where the

Table 2a

Fecundity - Reproductive profile of Asplanchna brightwelli reared at various temperatures. (N = 24) Diagram shows a visual representation of these results.

Temp. (^{o}C)	Ave. START of *R.P. (days) ± S.E.	Ave. END of R.P. (days) ± S.E.	Ave. LENGTH of R.P. (days) ± S.E.	Peak day of R.P.	Ave. number of offspring/ rotifer
15	3.83 ± .31	4.85 ± .43	1.02 ± .28	4.5	2
17.5	2.20 ± .19	5.39 ± .47	3.19 ± .34	4.0	7
20	2.00 ± .25	3.37 ± .42	1.38 ± .23	3.5	4
23	1.81 ± .10	2.93 ± .19	1.15 ± .12	2.0	4
25	1.54 ± .26	2.37 ± .40	.83 ± .19	2.5	2

*R.P. = Reproductive Period

Table 2a (Cont'd)

Temperature (°C)

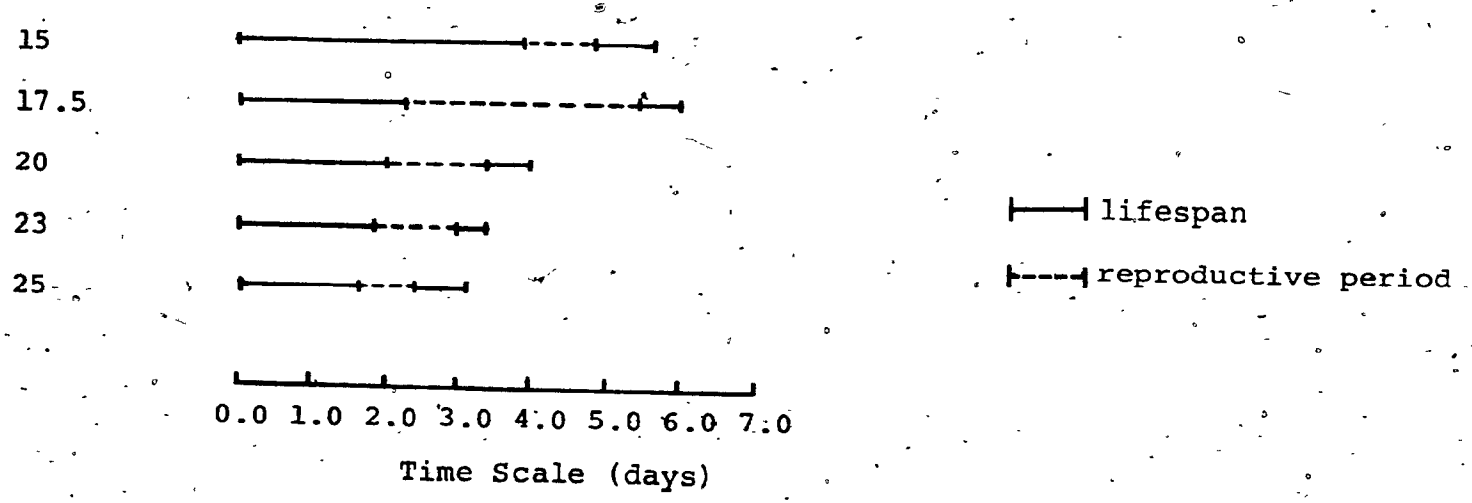


Table 2b

Reproductive Cycle - Table of significant differences within the treatment groups according to a post hoc Tukey test.

START of reproductive cycle	T 15°C	T 17.5°C	T 20°C	T 23°C	T 25°C
T 15°C	-	**	**	**	**
T 17.5°C		-	ns	ns	ns
T 20°C			-	ns	ns
T 23°C				-	ns
T 25°C					-

END of reproductive cycle	T 15°C	T 17.5°C	T 20°C	T 23°C	T 25°C
T 15°C	-	ns	ns	*	**
T 17.5°C		-	**	**	**
T 20°C			-	ns	ns
T 23°C				-	ns
T 25°C					-

Table 2b (Cont'd)

LENGTH of reproductive cycle	T 15°C	T 17.5°C	T 20°C	T 23°C	T 25°C
T 15°C	-	**	ns	ns	ns
T 17.5°C		-	**	**	**
T 20°C			-	ns	ns
T 23°C				-	ns
T 25°C					-

Number of offspring/rotifer	T 15°C	T 17.5°C	T 20°C	T 23°C	T 25°C
T 15°C	-	**	ns	ns	ns
T 17.5°C		-	*	*	**
T 20°C			-	ns	*
T 23°C				-	*
T 25°C					-

ns = not significant * = $p<.05$ ** = $p<.01$

average start was after 1.81 ± .10 days ($q = 8.47$, $p<.01$) and in rotifers kept at 25°C where the average start of reproduction was even earlier at 1.54 ± .26 days old ($q = 9.61$, $p<.01$). In comparing rotifers kept at 17.5°C, 20°C, 23°C and 25°C, the average start of reproduction was not significantly different among these groups.

A one-way analysis of variance also revealed that the time at which the rotifers stopped reproducing differed significantly at various temperatures ($F(4,115) = 56.422$, $p<.001$). At 15°C, the rotifers ended their reproductive phase after an average of 4.85 ± .43 days of life; according to a post hoc Tukey test, this was not significantly different from the end of reproduction in rotifers reared at 17.5°C where the average end of the reproductive period was after 5.39 ± .47 days ($q = 1.335$, $p>.05$). At 15°C, the end of the reproductive period did not occur significantly later than in rotifers kept at 20°C where $\bar{x} = 3.37 \pm .42$ days ($q = 3.66$, $p>.05$) but it was significantly later than in rotifers kept at 23°C where $\bar{x} = 2.93 \pm .19$ days ($q = 4.74$, $p<.05$) and at 25°C where $\bar{x} = 2.37 \pm .40$ days ($q = 6.13$, $p<.01$). Rotifers reared at 17.5°C stopped producing offspring after an average of 5.39 ± .47 days of life and this was found to be significantly later

than in rotifers kept at 20°C ($q = 4.99$, $p<.01$), at 23°C ($q = 6.08$, $p<.01$) and at 25°C ($q = 7.46$, $p<.01$). There were no significant differences in the average end of reproductive phases among rotifers kept at 20°C, 23°C and 25°C. The statistical differences for these and other parameters concerning the reproductive cycle are summarized in Table 2b. The pattern has shown that as lifespan was increased by lower temperatures, the time at which the rotifers stopped reproducing was also delayed.

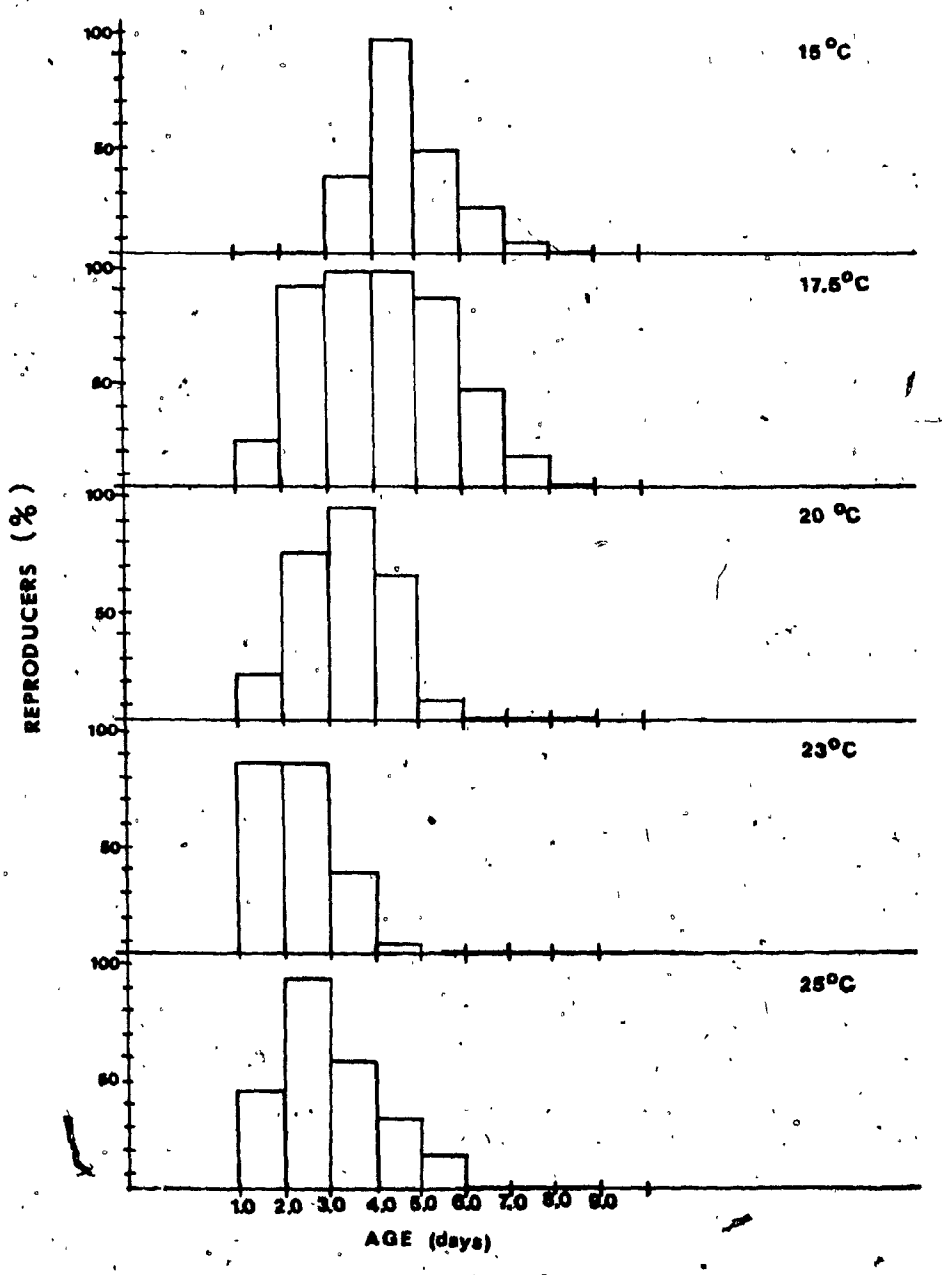
A one-way analysis of variance also showed differences in the average lengths of the reproductive period among the rotifers reared at various temperatures ($F(4,115) = 13.930$, $p<.001$). At the optimal temperature of 17.5°C, the average length of the reproductive period was 3.18 ± .34 days; according to the post hoc Tukey test this was significantly longer than in rotifers kept at 15°C where reproduction only lasted 1.02 ± .28 days ($q = 8.42$, $p<.01$). Similarly, rotifers at 17.5°C had significantly longer reproductive cycles than rotifers kept at 20°C where $\bar{x} = 1.37 \pm .34$ days ($q = 7.06$, $p<.01$), those kept at 23°C where $\bar{x} = 1.14 \pm .12$ days ($q = 7.95$, $p<.01$) and rotifers kept at 25°C where $\bar{x} = 0.83 \pm .19$ days ($q = 9.16$, $p<.01$). The average length of the reproductive periods in rotifers reared at 20°C, 23°C

and 25°C were not significantly different from each other.

These results are represented in Figure 3; the histogram represents the percentage of rotifers, using sample sizes of 24, which were producing offspring on each day of life. The pattern indicated that the longer the lifespan, the longer was the reproductive period. At low temperatures, 15°C in this case, the cycle started late in life and ended later, and the total length of the period was very short (about one day). During this period, an average of only 2 offspring per rotifer was produced. At 17.5°C, the average length of the reproductive period was longer, corresponding to an increase in longevity. In this group, the average number of offspring per rotifer was 7. At increasingly higher temperatures, the length of the reproductive cycle became progressively shorter and this could be correlated directly to the decrease in the number of offspring per rotifer. At 20°C, each rotifer produced an average of 4 offspring and at 23°C and 25°C, an average of 2 offspring was produced per rotifer.

Figure 3 shows not only the start, the end and the length of the reproductive cycle but also the peak days of reproduction for A. brightwelli at various environmental temperatures. The peak days occurred

Figure 3 Percentage of Asplanchna brightwelli in reproductive cycle at various days during lifespan exposed to different environmental temperatures. (N = 24)


15 °C
17.5°C
20 °C
23°C
25°C
100
50
REPRODUCERS (%)
1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0
AGE (days)

progressively earlier as temperatures increased. The peak day of reproduction in rotifers reared at 15°C was at 4.5 days while the peak occurred at 4.0 days of age in rotifers kept at 17.5°C. At 20°C, the peak day occurred at 3.5 days of life while at 23°C and 25°C, rotifers reached their peak at 2.5 days of age.

The results of this experiment have shown that longevity, fecundity and reproductive patterns of A. brightwelli were significantly affected by changes in environmental temperatures.

Dietary Restriction

Longevity. Food availability was the next environmental variable that was manipulated to evaluate its effect on the lifespan of A. brightwelli. A one-way analysis of variance showed that dietary restriction conducted in the manner described previously significantly altered the mean lifespan of rotifers ($F(5,138) = 6.294$, $p<.001$). These results are shown in Table 3a.

The feeding intervals tested were spaced at 12 hrs, 24 hrs, 36 hrs, 48 hrs, 60 hrs and 72 hrs. Rotifers fed every 12 hrs had a mean lifespan of 5.44 ± .28 days while those fed every 24 hrs lived an average of 5.91 ± .21 days and rotifers fed every 36

Table 3a

Mean lifespan observed and life expectancy as derived from life tables (see Appendix II) for Asplanchna brightwelli on various restricted feeding intervals. (N = 24, T = 18°C)

Feeding Interval (hrs)	Mean Lifespan, $\bar{x}$ (days) ± S.E.	Life Expectancy, ex (days)
12	5.44 ± .28	5.66
24	5.91 ± .21	5.66
36	6.08 ± .34	5.77
48	4.41 ± .36	3.73
60	4.41 ± .32	4.14
72	4.35 ± .35	4.02

Table 3b

Longevity - Table of significant differences within the treatment groups according to a post hoc Tukey test.

Feeding Interval (hrs)	12	24	36	48	60	72
12	-	ns	ns	ns	ns	ns
24		-	ns	**	**	**
36			-	**	**	**
48				-	ns	ns
60					-	ns
72						-

ns = not significant

* = $p<.05$

** = $p<.01$

hrs had an even greater mean lifespan of 6.08 ± .34 days. A post hoc Tukey test revealed, however, that these results were not significantly different from one another.

When dietary restriction was more severe, the results were detrimental to the longevity of A. brightwelli. Rotifers fed at 48 hrs and 60 hrs intervals had a mean lifespan of 4.41 ± .36 days and 4.41 ± .32 days respectively; those fed at 72 hrs intervals had a mean lifespan that was slightly shorter at 4.35 ± .35 days. Post hoc analysis showed that these means were not significantly different from one another. However, rotifers fed every 48 hrs and every 60 hrs had a significantly shorter mean lifespan than those fed every 24 hrs ($q = 4.87$, $p<.01$) and rotifers fed every 36 hrs ($q = 5.42$, $p<.01$). Similarly, rotifers fed every 72 hrs had a significantly shorter mean lifespan than rotifers fed every 24 hrs ($q = 5.08$, $p<.01$) and every 36 hrs ($q = 5.62$, $p<.01$). The statistical results are summarized in Table 3b.

Figure 4 represents the survival curves for A. brightwelli under the various restricted diets. There is a clear separation of the survival curves of the control group (12 hrs feeding interval), the group fed every 24 hrs, the group fed every 36 hrs, and the

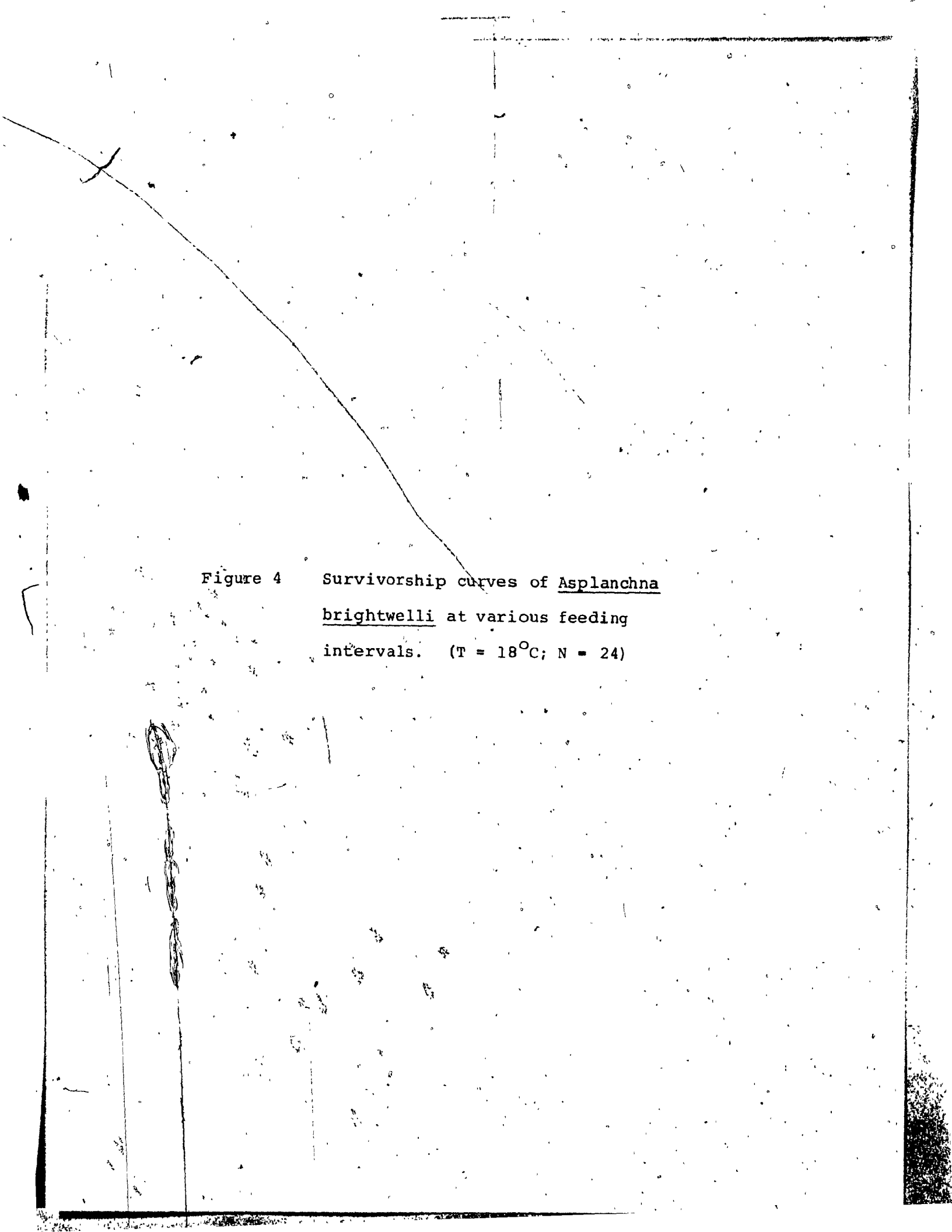


Figure 4 Survivorship curves of Asplanchna brightwelli at various feeding intervals. (T = 18°C; N = 24)

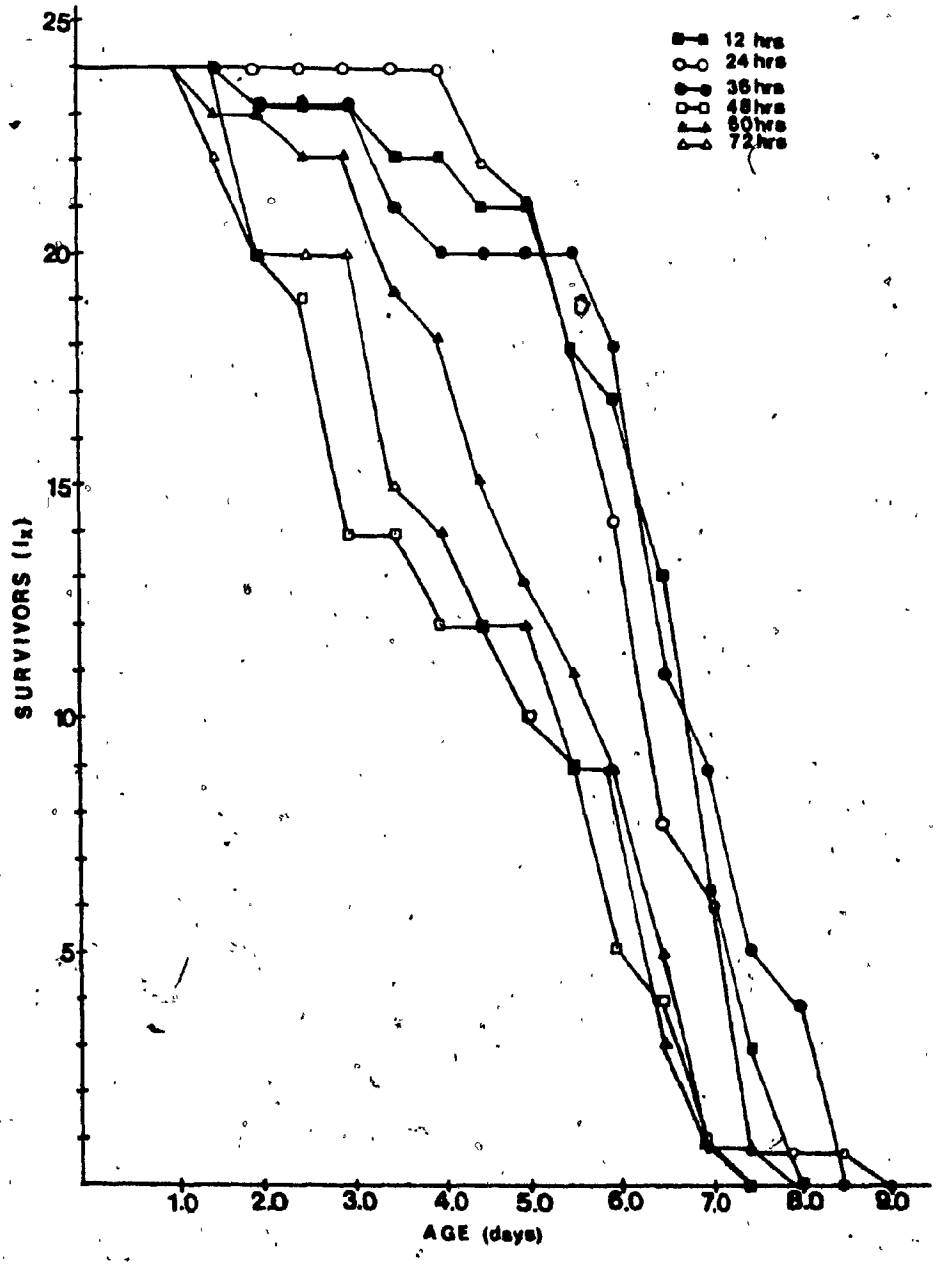
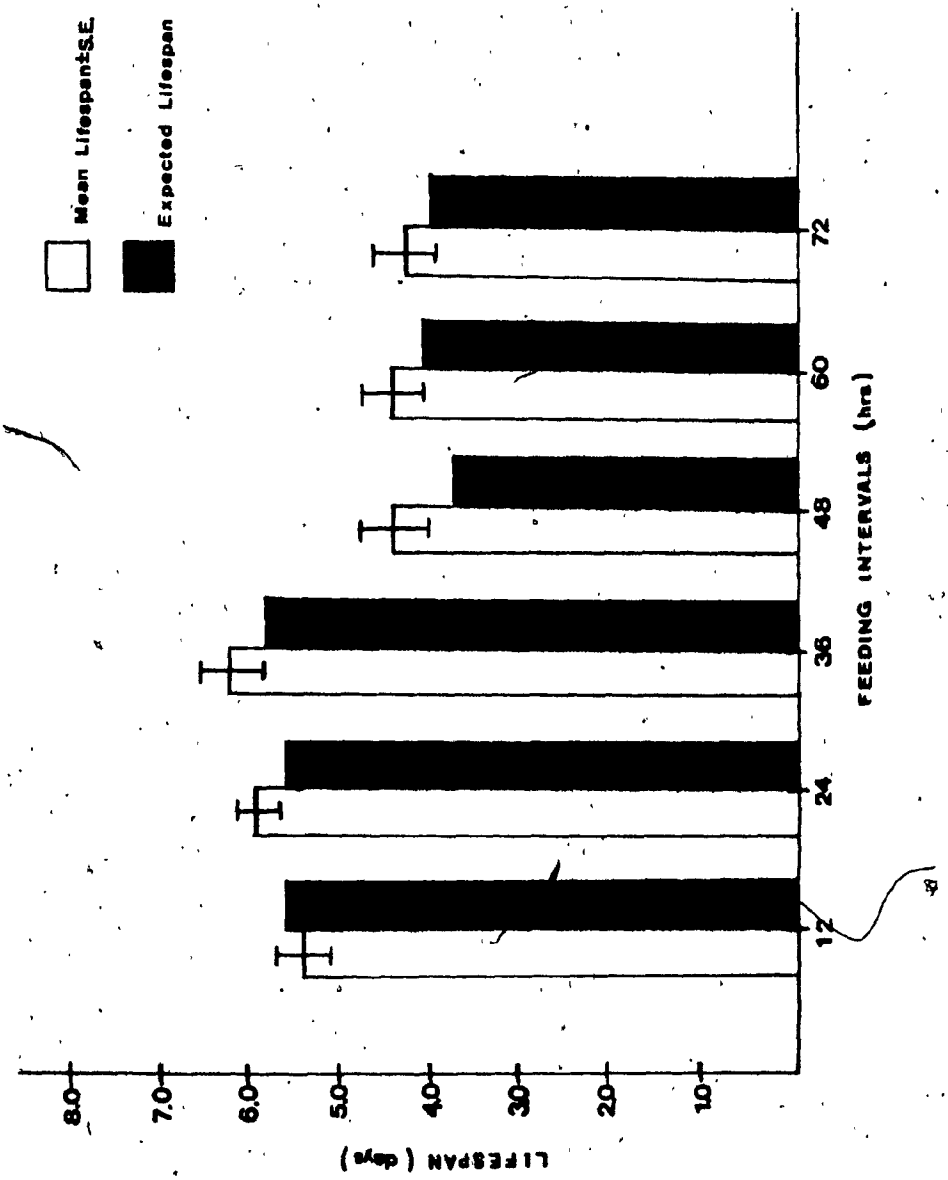

12 hrs
24 hrs
36 hrs
48 hrs
60 hrs
72 hrs
SURVIVORS (l_x)
25
20
15
10
5
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
AGE (days)

Figure 5 Histogram of mean and expected lifespan of Asplanchna brightwelli at various feeding intervals. (Expected values are derived from Life Tables in Appendix II). ($T = 18^{\circ}C$; $N = 24$)


Mean Lifespan±S.E.
Expected Lifespan
LIFESPAN (days)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
12
24
36
48
60
72
FEEDING INTERVALS (hrs)

more severely restricted groups fed every 48, 60 and 72 hrs. The first three groups lie on the right part of the graph and rotifers fed every 36 hrs show the best group survival. The other three groups all lie on the left side of the graph, showing how severe dietary restriction shortened lifespan in these rotifers.

The histogram in Figure 5 clearly shows the pattern obtained in the mean and expected lifespans of rotifers fed at the various intervals: an increase in the mean lifespan with moderate dietary restriction followed by a decrease in rotifers under severe dietary restriction. The mean and expected lifespans were derived as described previously. The reason the expected lifespan of rotifers fed every 48 hrs was shorter than the expected lifespan of those fed every 60 hrs was probably a result of the high number of rotifers in the former group which died during the first 3 days of life. The reason this occurred is unkown.

Fecundity. Table 4a shows the reproductive patterns of A. brightwelli when subjected to the different intensities of dietary restriction. There were no significant differences in the times at which the various groups entered the reproductive phase according to a one-way analysis of variance ($F(5,138) = .8916$, $p>.05$). In the control group (12 hrs

Table 4a

Fecunidty - Reproductive profile of Asplanchna brightwelli under various restricted diets. (N = 24, T = 18°C) Diagram shows a visual representation of these results.

Feeding Interval (hrs)	Ave. START of *R.P. (days) ± S.E.	Ave. END of R.P. (days) ± S.E.	Ave. LENGTH of R.P. (days) ± S.E.	Peak day of R.P.	Ave. number of offspring/ rotifer
12	2.81 ± .14	5.29 ± .30	2.48 ± .21	3.5	7
24	2.95 ± .09	5.64 ± .21	2.69 ± .21	3.5	5
36	2.87 ± .20	5.64 ± .42	2.78 ± .28	2.5	6
48	2.29 ± .33	3.52 ± .55	1.27 ± .26	3.5	2
60	2.60 ± .33	3.60 ± .46	1.06 ± .21	4.0	2
72	2.47 ± .37	3.45 ± .54	.98 ± .23	4.0	2

*R.P. = Reproductive Period

Table 4a (Cont'd)

Feeding Interval (hrs)

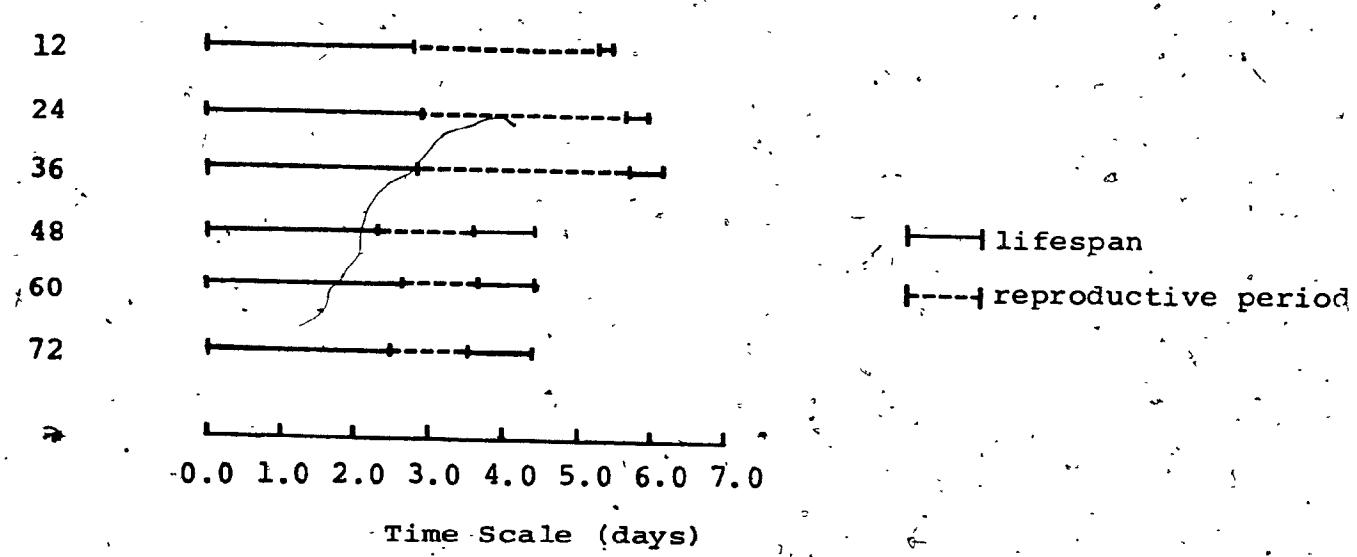


Table 4b

Reproductive Cycle - Table of significant differences within the treatments groups according to a post hoc Tukey test.

START of reproductive cycle

No significant differences $F(5,138) = .8916$, $p>.05$

END of reproductive cycle	I 12	I 24	I 36	I 48	I 60	I 72
*I 12	-	ns	ns	ns	ns	*
I 24		-	ns	*	*	**
I 36			-	*	*	**
I 48				-	ns	ns
I 60					-	ns
I 72						-

*I = feeding interval

Table 4b (Cont'd)

LENGTH of reproductive cycle	I 12	I 24	I 36	I 48	I 60	I 72
I 12	-	ns	ns	**	**	**
I 24		-	ns	**	**	**
I 36			-	**	**	**
I 48				-	ns	ns
I 60					-	ns
I 72						-

Number of offspring/rotifer	I 12	I 24	I 36	I 48	I 60	I 72
I 12	-	*	ns	**	**	**
I 24		-	ns	**	**	**
I 36			-	**	**	**
I 48				-	ns	ns
I 60					-	ns
I 72						-

ns = not significant * = p<.05 ** = p<.01

feeding interval), the average start of reproduction was after 2.81 ± .14 days of life, in the rotifer fed every 24 hrs it was after an average of 2.95 ± .09 days and in rotifers fed every 36 hrs, the average start of reproduction was after 2.87 ± .20 days; in the more severely restricted groups, rotifers fed every 48 hrs started to reproduce after 2.29 ± .33 days, those fed every 60 hrs started after 2.60 ± .33 days and rotifers fed every 72 hrs started to produce offspring after 2.47 ± .37 days old.

Significant differences were found in the average times at which these rotifers stopped producing offspring according to a one-way analysis of variance ($F(5,138) = 6.25$, $p<.001$). In the control group (12 hrs feeding interval), the average end of reproduction occurred after 5.29 ± .30 days while in rotifers fed every 24 hrs and every 36 hrs, the end of the reproductive cycle occurred after 5.64 ± .21 days and 5.64 ± .42 days respectively. These means were not significantly different from each other according to post hoc analysis. The average end of reproduction in rotifers fed every 48 hrs was after 3.52 ± .55 days which was significantly earlier than in rotifers fed every 24 and 36 hrs ($q = 4.81$, $p<.05$). Rotifers fed at 60 hrs intervals ended reproduction after an average of 3.60 ± .46 days which was also

significantly earlier than in those fed at 24 and 36 hr intervals ($q = 4.58$, $p<.05$). Rotifers fed every 72 hrs stopped producing offspring after 3.45 ± .54 days which was significantly earlier than in control rotifers ($q = 4.18$, $p<.05$) and in rotifers fed at 24 and 36 hr intervals ($q = 4.97$, $p<.01$). In the three severely restricted groups, the average end of the reproductive cycles were not significantly different.

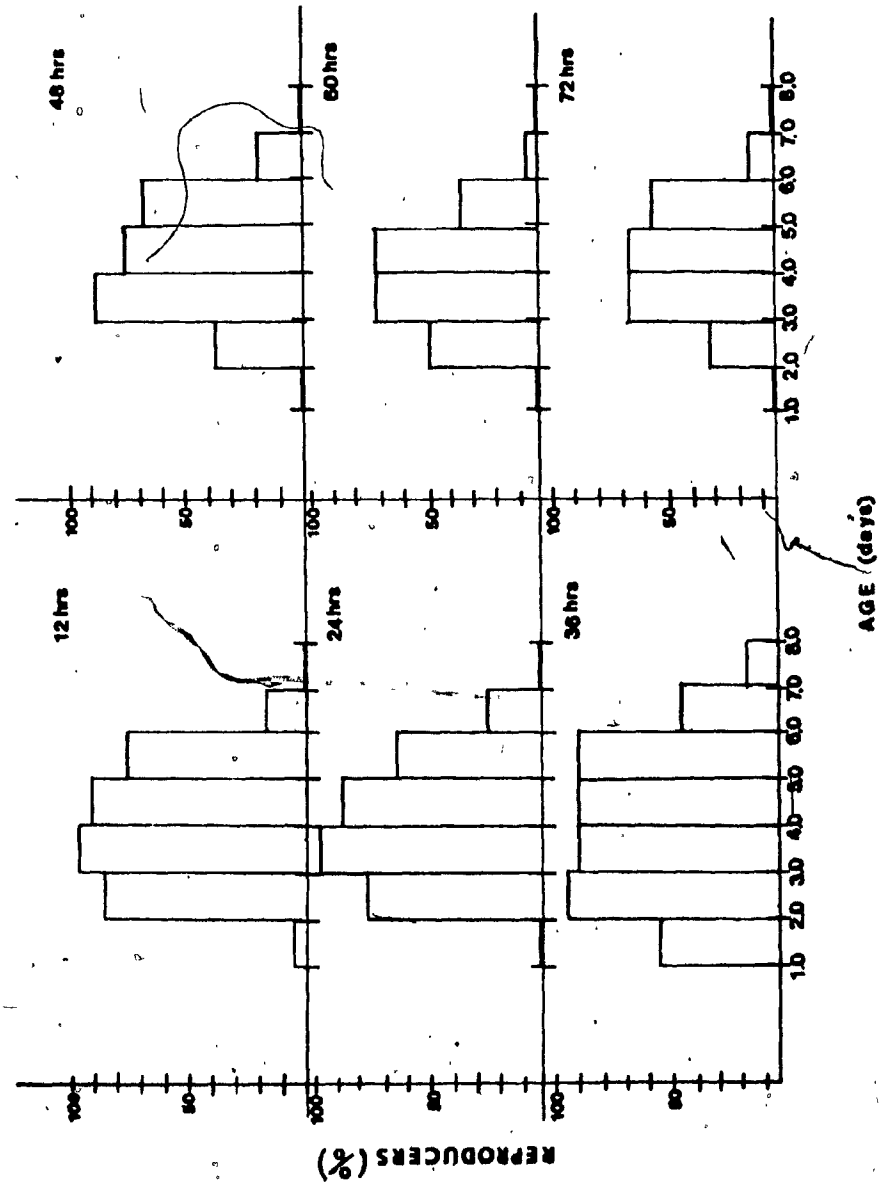
The length of reproductive periods was also significantly different among the six groups of rotifers as indicated by a one-way analysis of variance. In rotifers where diet was not severely restricted (12, 24 and 36 hrs feeding intervals) the average length of the reproductive periods increased as the mean lifespan increased through dietary restriction. Rotifers fed every 12 hrs had a mean reproductive cycle of 2.47 ± .20 days, those fed every 24 hrs had a mean reproductive cycle lasting 2.60 ± .21 days and in those fed every 36 hrs, the reproductive cycle lasted slightly longer, an average of 2.77 ± .28 days. These means were not significantly different from one another as revealed by post hoc analysis. Rotifers that were severely restricted had increasingly shorter reproductive cycles correlating with the shorter lifespans. Rotifers fed every 48, 60 and 72 hrs had an average reproductive

period lasting 1.27 ± .26 days, 1.06 ± .24 days and 0.98 ± .23 days respectively. These means were not statistically different from one another but all 3 means were significantly shorter than the average reproductive periods of rotifers fed at 12, 24 and 36 hrs intervals.

As can be expected, the number of offspring produced by A. brightwelli under various dietary restrictions was affected; the average number of offspring produced per rotifer in the control (12-hr feeding) group was 7, exactly the same number as obtained in the previous experiment when the groups were kept at 17.5°C, while rotifers in the 24-hr feeding group produced an average of 5 offspring; rotifers in the 36-hr feeding interval produced an average of 6 offspring. In all other groups, the average number of offspring produced per rotifer was 2. The statistical results for all the parameters of the reproductive cycle are summarized in Table 4b.

A histogram given in Figure 6 describes the reproductive patterns obtained in the 6 different groups of rotifers. The peak days of reproduction in all groups occurred after 3.5 or 4.0 days; in rotifers fed every 36 hrs, the peak day was reached earlier, after 2.5 days, but until day 6, this peak was maintained. Therefore, according to these results,

Figure 6 Percentage of Asplanchna brightwelli in reproductive cycle at various days during lifespan under different feeding intervals. ($T = 18^{o}C$; $N = 24$)


48 hrs
60 hrs
72 hrs
12 hrs
24 hrs
36 hrs
AGE (days)
REPRODUCERS (%)

rotifers fed every 36 hrs showed the longest reproductive cycle and during this time a high average number of offspring was produced by each rotifer.

Nuclear Counts. The results for the nuclear counts in the vitellarium and gastric glands of A. brightwelli throughout a 6-day lifespan are given in Table 5. Two groups of 20 rotifers were used to obtain the daily averages in the two groups. The control groups were fed twice daily (approximately every 12 hrs) and the experimental groups were fed every 36 hrs. It has been shown in the previous experiment that A. brightwelli fed at 36-hr intervals had a greater mean lifespan that those fed at 12 hrs intervals.

Photomicrographs #1 and #5 show the morphological characteristics of the organs studied, the vitellarium and the gastric glands. Fixation of the rotifer in 50% ethanol for a few minutes caused the nuclei to show up very clearly for counting. Photomicrographs #2 and #3 show a close-up of the nuclei of the vitellarium which are polyploid (Jones & Gilbert, 1977). Photomicrograph #5 shows a squash preparation of a fixed rotifer when the body contents were extruded; this photomicrograph shows the everted stomach with the two gastric glands on each side. Photomicrograph #6

Table 5

Average number of nuclei in the vitellarium and gastric glands of control and experimental (dietary-restricted) Asplanchna brightwelli on each day of life. (N = 20)

(Note that there are 2 gastric glands/rotifer and that the average is given.)

	VITELLARIUM Average number of nuclei ± S.E.		GASTRIC GLANDS Average number of nuclei ± S.E.	
Age Interval (days)	Control	Experimental	Control	Experimental
0 - 1	20.5 ± .57	19.6 ± .62	5.93 ± .15	5.50 ± .14
1 - 2	28.4 ± .63	26.4 ± .60*	6.70 ± .22	6.20 ± .18*
2 - 3	29.9 ± .70	27.2 ± .78*	9.80 ± .19	9.40 ± .25
3 - 4	28.6 ± .77	29.8 ± .79	10.70 ± .33	9.50 ± .28**
4 - 5	30.9 ± .98	31.3 ± .65	10.20 ± .27	9.40 ± .29**
5 - 6	24.8 ± .57	30.3 ± .90*	9.80 ± .28	10.30 ± .25
6 - 7	-	(26.5 ±1.15)[1]	-	-

[1] = (N = 6)

Significant differences between control and experimental groups according to t-test:

* = $p<.05$

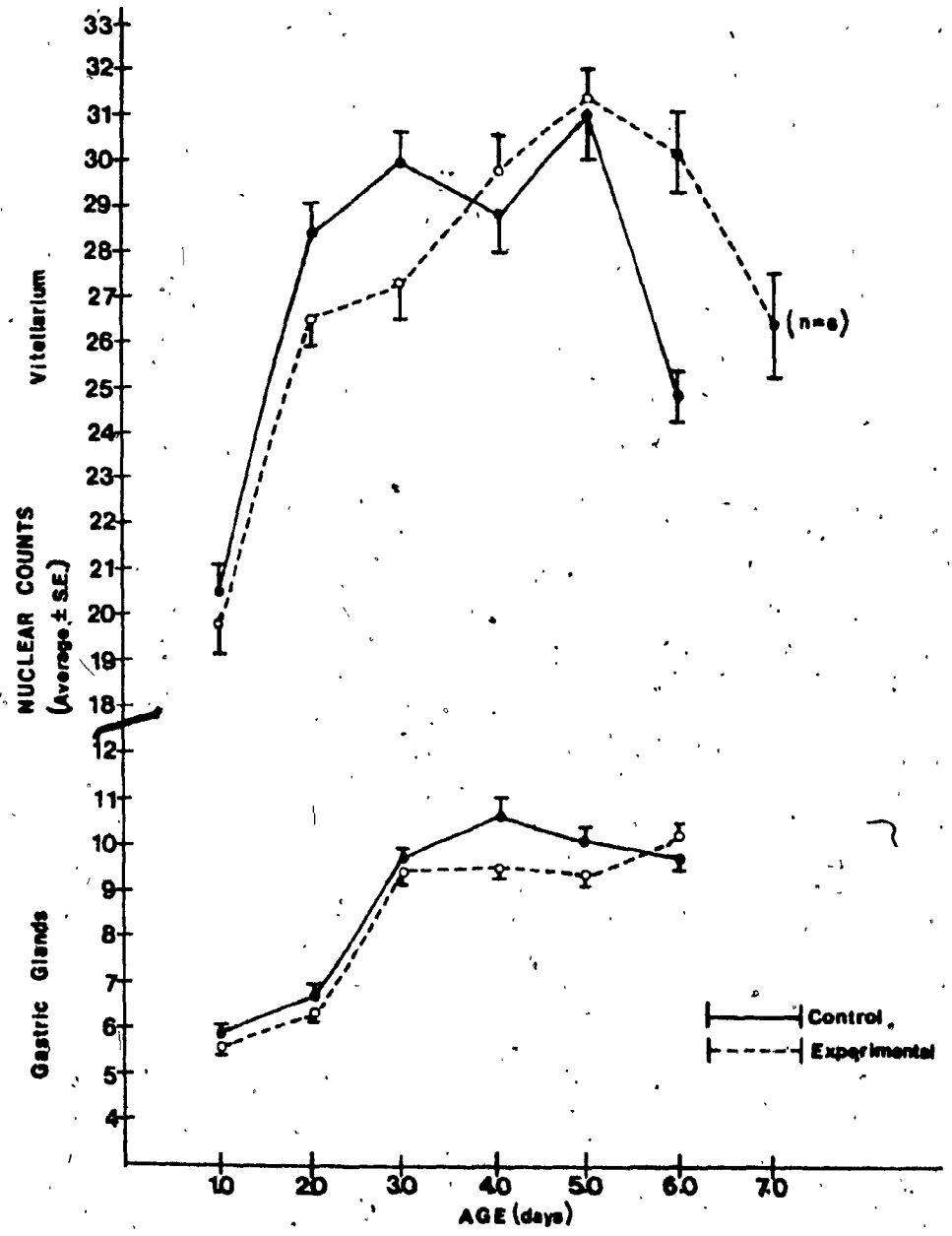
** = $p<.01$

is a close-up of a gastric gland showing the small, dark nuclei present. These and other photomicrographs are given in Appendix IV.

In the control rotifers, the average number of nuclei in the vitellarium on day 1 was 20.5 ± .57 and the average steadily increased until a peak was reached of 30.9 ± .98 nuclei by day 5. On day 6, the average nuclear number in the vitellarium decreased to 24.8 ± .57. In the experimental rotifers, the average number of nuclei in the vitellarium was 19.6 ± .62 on day 1, increasing at a slower rate than in the control group until a peak of 31.3 ± .65 nuclei was reached on day 5. After this time, the decrease in the average nuclear number was not as sharp as seen in the control rotifers; on day 6, experimental rotifers had an average of 30.3 ± .90 nuclei in the vitellarium. A smaller sample of rotifers was collected to see if this average nuclear number kept on decreasing after this day. Nuclear counts taken of 6 experimental rotifers on day 7 have shown the average number of nuclei in the vitellarium was 26.5 ± 1.15.

Figure 7 graphically represents these results. On day 1, the average number of nuclei in the vitellarium of control and experimental rotifers were not significantly different ($t = 1.32$, $p > .05$). On day 2,

Figure 7 Number of nuclei in the vitellarium and gastric glands of Control (12 hr feeding intervals) and Experimental (36 hr feeding intervals) Asplanchna brightwelli during 6 days of life. (T = 18°C; N = 24)


NUCLEAR COUNTS
(Average ± S.E.)
Vitellarium
Gastric Glands
(n=6)
Control
Experimental
AGE (days)
10
20
30
40
50
60
70

the average nuclear number in the vitellarium of control rotifers was 28.4 ± .63 which was significantly greater than the average found in the vitellarium of experimental rotifers, 26.4 ± .60 nuclei ($t = 2.40$, $p<.05$). On day 3, control rotifers again had a significantly greater number of nuclei in their vitellarium, 29.9 ± .70, as compared to experimental rotifers which had 27.2 ± .78 nuclei present in the vitellarium ($t = 2.49$, $p<.05$). Although the average number in the vitellarium of experimental rotifers was increasing at a significantly slower rate as compared to controls, by the time days 4 and 5 of life were reached, the average number of nuclei in the vitellarium was not significantly different. On day 4, the control rotifers had an average of 28.6 ± 1.60 nuclei in their vitellarium whereas experimental rotifers had an average of 29.8 ± 1.63 nuclei ($t = 1.23$, $p>.05$). On day 5, control rotifers had an average of 30.9 ± .98 nuclei in the vitellarium and experimental rotifers had a mean of 31.3 ± .65 nuclei ($t = .467$, $p>.05$). On day 6, the average number of nuclei in the vitellarium of control rotifers decreased rapidly to 24.8 ± .57 nuclei while the mean in experimental rotifers remained significantly higher at 30.3 ± .90 nuclei ($t = 5.98$, $p<.001$). After this day, the nuclear number in experimental rotifers

decreased to a low of 26.5 ± 1.15 nuclei.

Table 5 also shows the results obtained for nuclear counts in the gastric glands of A. brightwelli throughout a 6-day lifespan. Since 2 gastric glands are present in each rotifer, the average of the two is given in this table. A t-test was conducted to see if there were any significant differences between the number of nuclei in the two gastric glands within the same organism. Using the results of a sample of 20 control and experimental rotifers, no significant differences were observed in the nuclear counts of the gastric glands in the same rotifers.

On day 1, there was an average of 5.93 ± .15 nuclei in the gastric glands of control rotifers which was not significantly different from the mean of 5.50 ± .14 nuclei found in the gastric glands of experimental rotifers ($t = 1.95$, $p>.05$). On day 2, although the average number of nuclei increased in the gastric glands of both control and experimental rotifers, the average nuclear number in control rotifers was 6.70 ± .22 nuclei which was significantly greater than the average of 6.20 ± .18 nuclei in the experimental group ($t = 2.60$, $p<.05$). On day 3, a further increase in nuclear number occurred in the gastric glands; in the control group there was an average of 9.82 ± .19 nuclei present in the gastric glands and this was not found to be significantly

greater than the average of 9.40 ± .25 nuclei found in the gastric glands of experimental rotifers ($t = 1.06$, $p > .05$).

On days 4 and 5 however, the average number of nuclei in the gastric glands of control rotifers increased to 10.7 ± .33 nuclei and then decreased slightly to 10.2 ± .27 nuclei on day 5. In the experimental group, there was an average of 9.50 ± .28 nuclei in the gastric glands on day 4 and a mean of 9.40 ± .29 nuclei on day 5. On these two days, the means between control and experimental groups were significantly different ($t = 2.80, 2.47$, $p < .05$). On day 6, the nuclear number in the gastric glands of experimental rotifers reached a peak of 10.3 ± .25 nuclei while the average found in the control group declined to 9.80 ± .28 nuclei; these two means were not significantly different from one another ($t = 1.63$, $p > .05$).

Size measurements. The measurements of body size and various organs were taken as described in Appendix III.

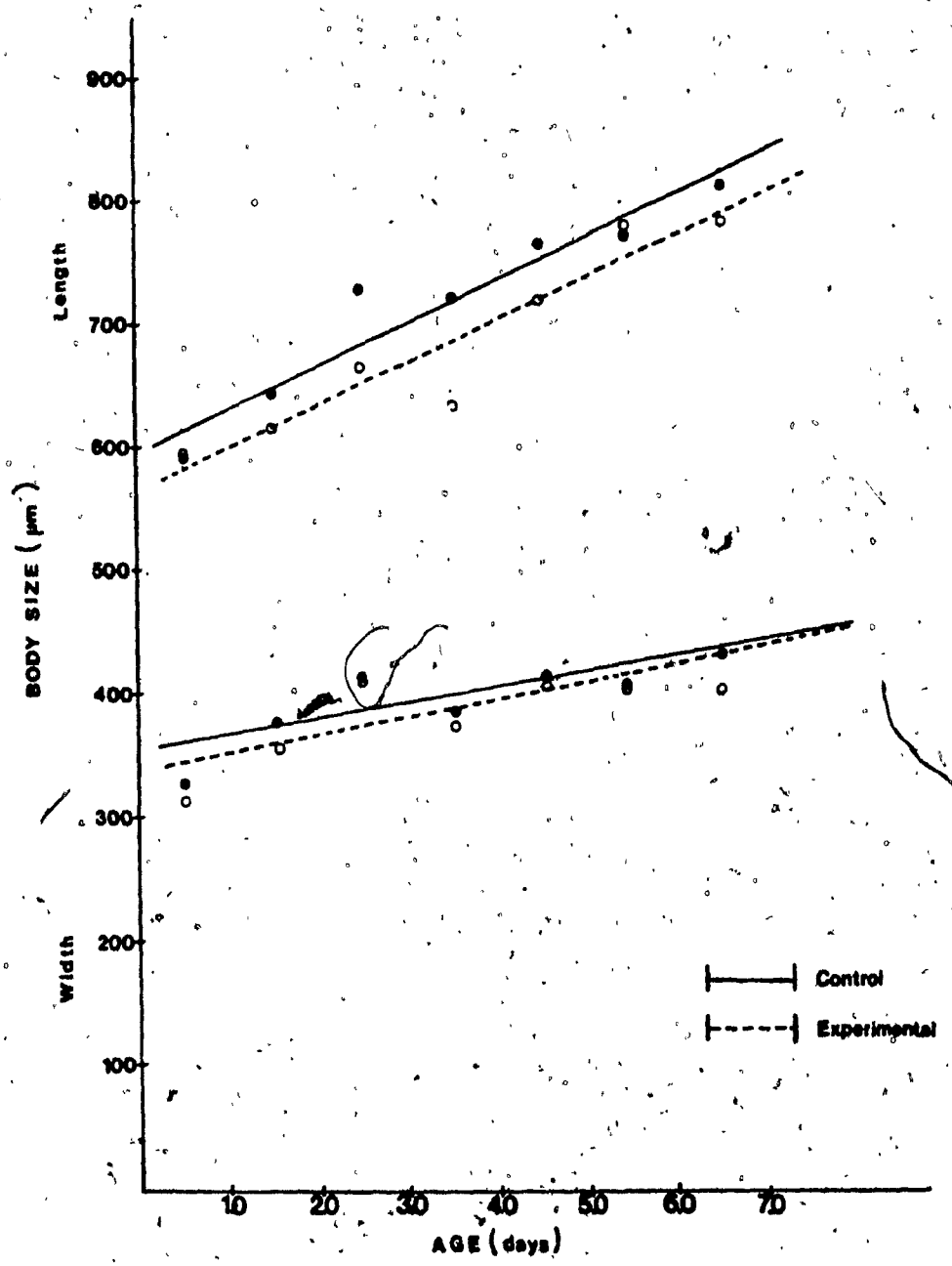
Table 6 shows the growth in body size of A. brightwelli during a 6-day lifespan under control (fed twice daily) and experimental (fed every 36 hrs) conditions. A sample size of 5 rotifers was used

Table 6

Body measurements of control and experimental (dietary-restricted) *Asplanchna brightwelli* at various ages. (N = 5)

Age (days)	Control or Experimental	Average Body Size ± S.E. LENGTH (µm)	WIDTH (µm)
0 - 1	C	591.33 ± 11.3	326.00 ± 8.12
	E	597.00 ± 4.4	311.00 ± 5.26
1 - 2	C	641.60 ± 10.2	377.60 ± 13.20
	E	616.00 ± 22.8	368.80 ± 20.10
2 - 3	C	728.00 ± 24.5	414.40 ± 7.33
	E	667.20 ± 18.0	412.80 ± 11.50
3 - 4	C	723.20 ± 16.1	387.20 ± 9.70
	E	636.80 ± 11.5	374.40 ± 12.20
4 - 5	C	768.00 ± 11.0	417.60 ± 7.60
	E	720.00 ± 13.7	408.00 ± 13.40
5 - 6	C	771.20 ± 9.0	409.60 ± 9.60
	E	780.80 ± 9.0	406.40 ± 6.90
6 - 7	C	813.30 ± 7.1	432.00 ± 4.62
	E	786.70 ± 11.7	405.30 ± 9.62

Figure 8 Body size of Asplanchna brightwelli throughout lifespan in Control and Experimental (dietary-restricted) groups. (N = 5)


900
800
700
600
500
400
300
200
100
Length
Width
BODY SIZE (μm)
10
20
30
40
50
60
70
AGE (days)
Control
Experimental

for the daily measurements in both groups. By doing a regression analysis using the body size measurements in both groups, Figure 8 was obtained. As can be seen, there was a slight increase in body width and a gradual increase in body length with age in both groups of rotifers. Although the dietary-restricted rotifers showed a smaller increase in both length and width, this was not significantly different from the control rotifers according to the test for comparison of slopes.

Table 7 shows the results obtained when measurements of the gastric glands were taken throughout the lifespan of control and dietary-restricted rotifers. A regression analysis was obtained for the length and width of these glands in both groups of rotifers for 6 days of life. A test for the comparison of slopes revealed that although the width of the gastric gland was not significantly different between the control and the experimental groups, the length of the gastric gland was different. In control rotifers, the length of the gastric gland remained more or less the same throughout the lifespan but in experimental rotifers, this length increased gradually with age until it reached the size mesaured in the control group.

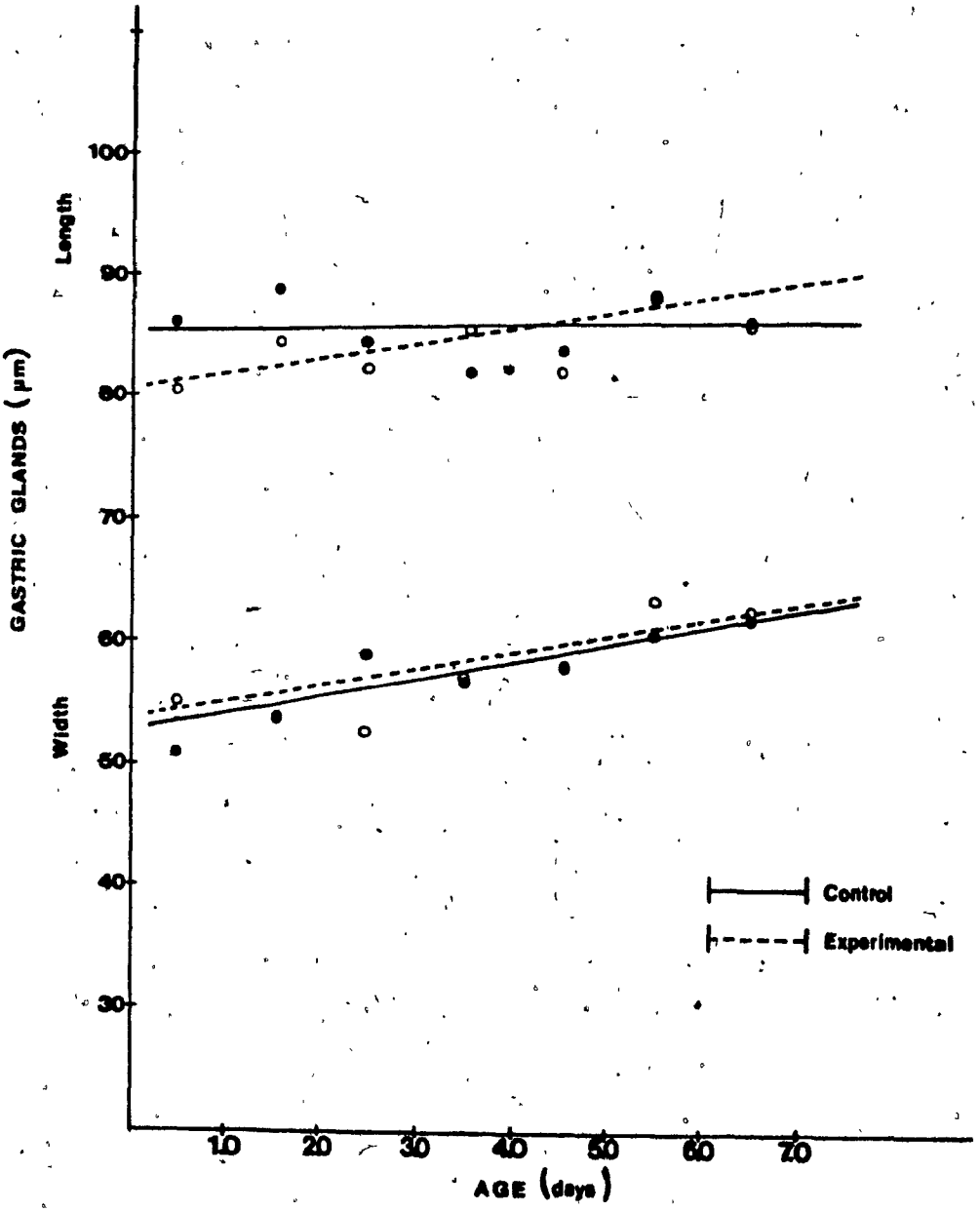
Table 7 also shows the results of measurements taken on the nuclei in the gastric glands in control

Table 7

Measurements of gastric glands and nuclei of gastric glands in control and experimental (dietary-restricted) Asplanchna brightwelli at various ages. (N = 5)

Age (days)	Control or Experimental	GASTRIC GLANDS (µm) Ave. Length ± S.E.	GASTRIC GLANDS (µm) Ave. Width ± S.E.	Average size of nuclei of GASTRIC GLANDS (µm) ± S.E.
0 - 1	C	86.0 ± 5.50	50.8 ± 1.90	7.80 ± .48
	E	80.7 ± 2.80	54.9 ± 1.90	7.50 ± .53
1 - 2	C	88.8 ± 4.80	54.0 ± 2.10	7.60 ± .40
	E	84.6 ± 4.80	53.8 ± 8.10	7.00 ± .40
2 - 3	C	84.8 ± 3.80	58.8 ± 1.80	7.20 ± .46
	E	82.5 ± 1.20	52.3 ± 3.80	7.60 ± .61
3 - 4	C	81.6 ± 5.30	56.8 ± 4.60	8.00
	E	85.6 ± 4.80	57.2 ± 2.60	7.60 ± .40
4 - 5	C	84.0 ± 2.40	58.0 ± 2.10	7.20 ± .46
	E	82.2 ± 5.20	58.2 ± 2.90	7.00 ± .40
5 - 6	C	88.3 ± 6.20	60.8 ± 2.90	7.70 ± .32
	E	88.6 ± 3.40	64.6 ± 2.90	7.00 ± .40
6 - 7	C	86.4 ± 1.80	61.9 ± 2.10	8.00
	E	86.0 ± 3.70	62.9 ± 2.80	8.00

Figure 9 Gastric gland measurements in Control and Experimental (dietary-restricted) Asplanchna brightwelli throughout lifespan. (N = 5)


GASTRIC GLANDS (µm)
Length
Width
100
90
80
70
60
50
40
30
10
20
30
40
50
60
70
AGE (days)
Control
Experimental

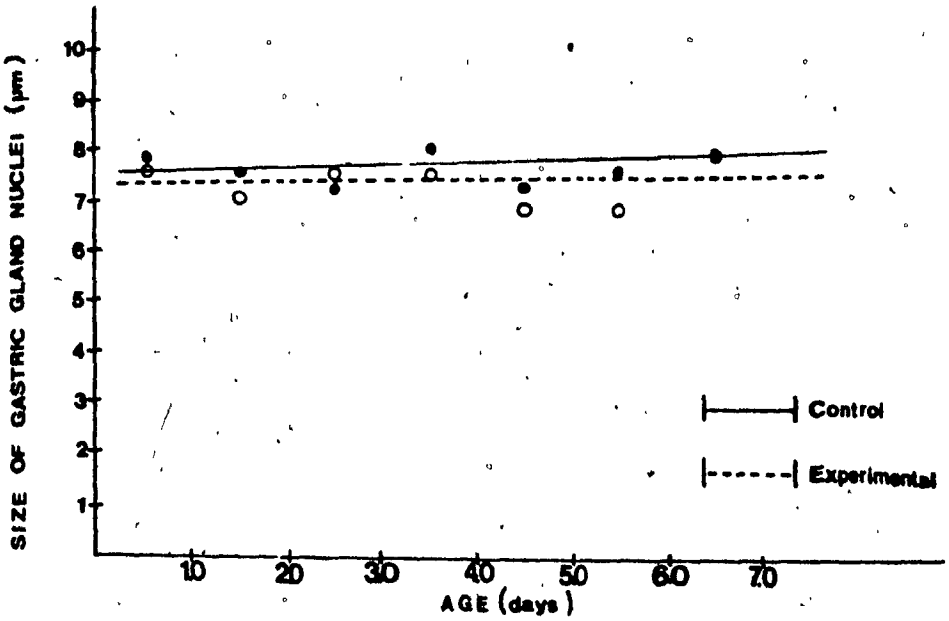


Figure 10 Size of nuclei in gastric glands of Control and Experimental (dietary-restricted) Asplanchna brightwelli throughout lifespan. (N = 5)

and experimental rotifers. Figure 10 shows a regression analysis of these nuclear measurements throughout the lifespan. A test for the comparison of slopes has shown that the nuclei of the gastric glands in experimental rotifers were not significantly smaller than the nuclei present in the gastric glands of controls during the lifespan.

The measurements of the vitellarium and its nuclei for both control and experimental (dietary-restricted) rotifers are given in Table 8. The vitellaria were only measured when at least one side-arm was fully extended to permit as close an estimate possible of the actual size of that part of the organ. Figure 11 shows that the size of the vitellarium in experimental rotifers was constantly smaller than in control rotifers throughout the lifespan. A comparison of slopes test revealed that this difference was not significant.

Since the nuclei in the vitellarium vary a great deal in size due to mitotic activity, a range of nuclear sizes was recorded in an attempt to find a more accurate representation than simply taking the average size. To do this, the smallest and largest observed nuclei in the vitellarium were measured for every day of life for both control and experimental rotifers.

Figure 12 shows the results obtained in both

Table 8

Measurements of one side-arm of the vitellarium and nuclear sizes in this organ in control and experimental (dietary-restricted) Asplanchna brightwelli at various ages. (N = 5)

Age (days)	Experimental	VITELLARIUM (½) (µm) Ave. size ± S.E.	NUCLEAR SIZES (µm) Average ± S.E. Smallest	Largest
0 - 1	C	204.00 ± 13.10	10.40± .69	16.53±1.20
	E	264.00 ± 13.90	10.80±1.00	18.06± .94
1 - 2	C	237.60 ± 12.80	16.00±1.00	24.64± .64
	E	224.00 ± 13.40	12.80±1.40	23.04±1.10
2 - 3	C	282.00 ± 6.80	14.40± .92	24.40± .77
	E	235.20 ± 12.00	11.52± .78	21.12± .93
3 - 4	C	240.00 ± 10.40	12.80	24.64± .39
	E	200.80 ± 8.10	9.60	22.08±1.30
4 - 5	C	230.40 ± 7.80	13.44± .64	24.64± .39
	E	220.80 ± 9.70	10.88± .78	22.08±1.30
5 - 6	C	220.80 ± 9.30	14.72± .78	24.96± .39
	E	233.60 ± 14.10	13.44±1.10	24.32± .32
6 - 7	C	253.30 ± 16.20	16.00	29.86±1.10
	E	274.70 ± 18.70	14.93±1.10	25.06±5.30

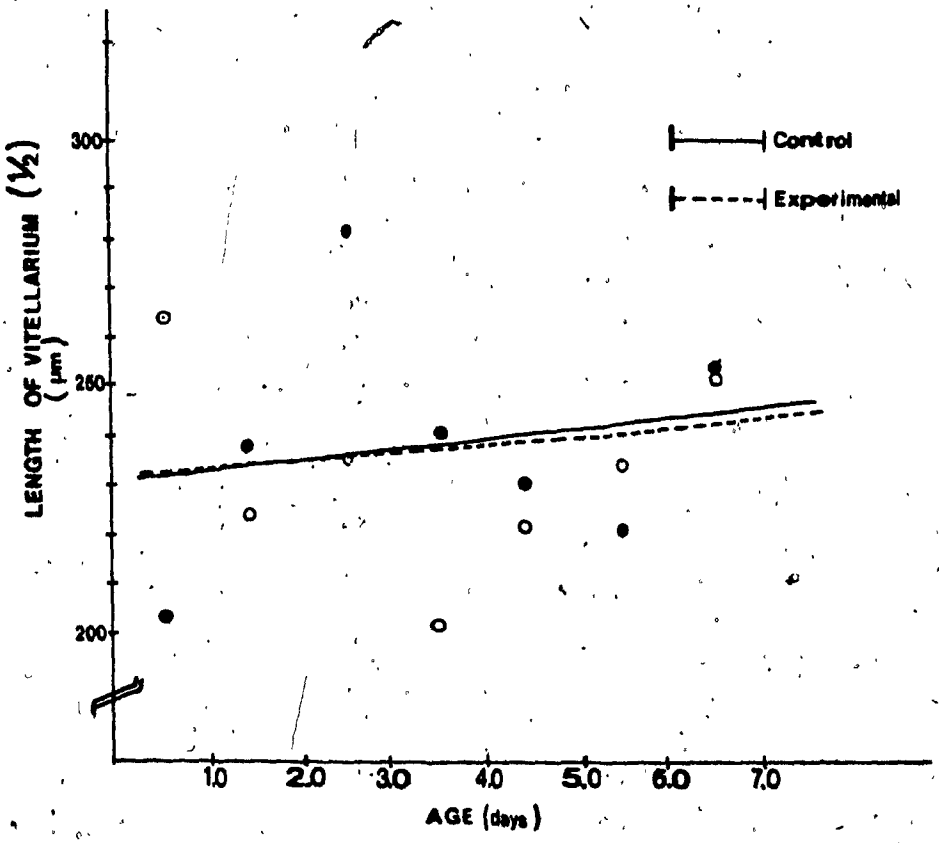


Figure 11 Length of one side-arm of vitellarium in Control and Experimental (dietary-restricted) Asplanchna brightwelli throughout lifespan. (N = 5).

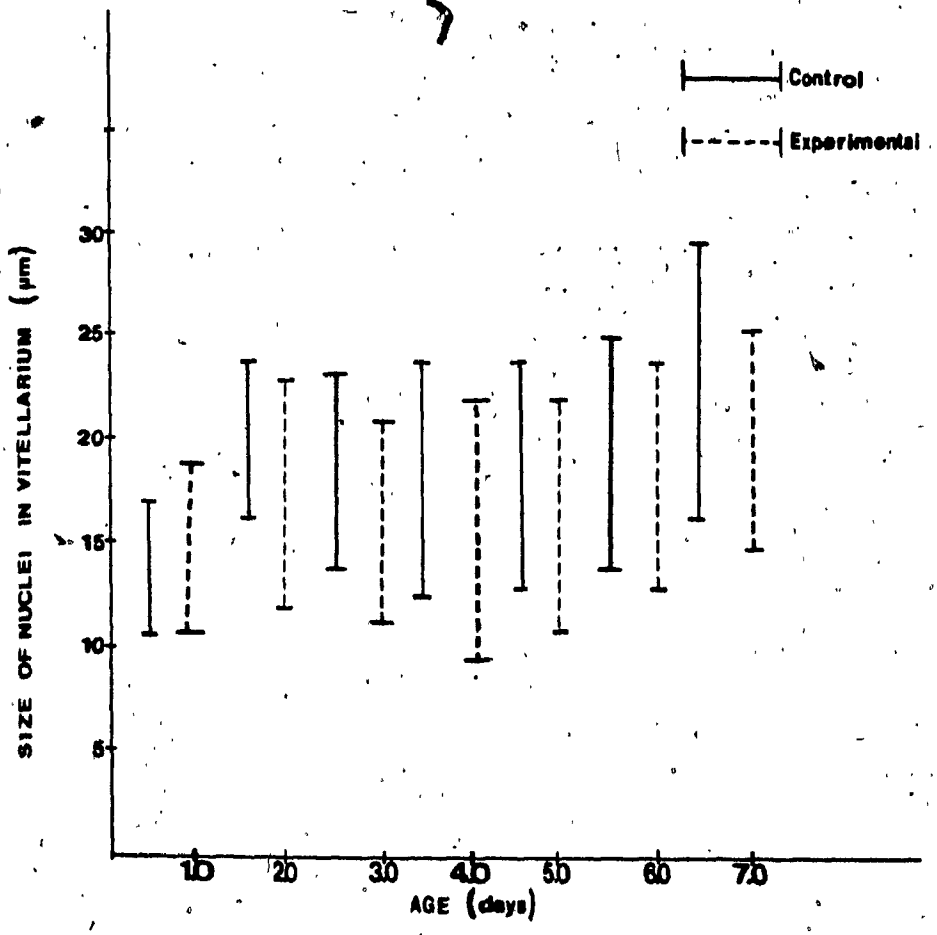


Figure 12 Range of nuclear sizes (smallest and largest) observed in the vitellarium of Control and Experimental (dietary-restricted) Asplanchna brightwelli throughout lifespan. (N = 5)

groups. On day 1, the nuclei found in both groups were relatively small, ranging from 10 - 17 µm in the control group and 11 - 18 µm in the experimental group. From days 2 to 6, although the largest observed nuclei were constantly between 21 and 24 µm in both groups, there was a greater variety of sizes in the smallest observed nuclei, that is, from 9 to 15 µm. By the last day of life measured, the nuclei in the vitellarium of the control group were mostly large, ranging from 16 - 30 µm while those in the experimental group of rotifers ranged from 15 - 25 µm.

In control rotifers, the nuclear size of the vitellarium increased with age especially from day 4 to day 7 and this pattern was also seen in experimental rotifers but to lesser extent. The smallest and largest vitellarial nuclei measured for each day of life were constantly of a smaller size in experimental rotifers. This difference was shown to be significant only when the smallest nuclei were compared in the two groups ($t = 3.86$, $p<.01$) but not when the largest nuclei were compared ($t = 1.39$, $p>.05$).

Photomicrographs #3 and #4 (see Appendix IV) show the morphological differences in the vitellarium and its nuclei in young and old rotifers. Photomicrograph #3 shows the polyploid nuclei of various sizes seen in

a young vitellarium; in photomicrograph #4, the vitellarium has a large amount of fat deposit and the nuclei are very large. Also, the nucleoli are broken up, looking very different from the dark, compact forms seen in the vitellarium of younger rotifers.

DNA Content. The DNA content for groups of 20 control and experimental (dietary-restricted) rotifers during each day of life was measured spectrofluorometrically and the results for these daily assays are given in Table 9.

DNA concentration was similar in both groups on the first and last day (day 6) of life measured. The variation in DNA content on days 2 to 5 was attributed to changes due to reproduction and the presence of offspring in various developmental stages within the adult rotifers. Figure 13 shows a graphic representation of these results. The increase followed by a decrease in DNA content could be correlated with reproductive activity. Although dietary-restricted rotifers produced offspring more actively on the last three days of life, this was not indicated in the results. The difference may have been too small for detection using the technique described.

The results did show that the DNA content was constantly lower in experimental rotifers from day 2

Table 9

DNA content in control and experimental (dietary-restricted) Asplanchna brightwelli for each day of life according to spectrofluorometric results. (20 rotifers/group)

Age (days)	DNA (µg/ml) Control	Experimental
1.0	0.2610	0.2610
2.0	1.2720	0.8446
3.0	1.4857	1.0580
4.0	0.9810	0.4231
5.0	0.4231	0.3302
6.0	0.2714	0.2714

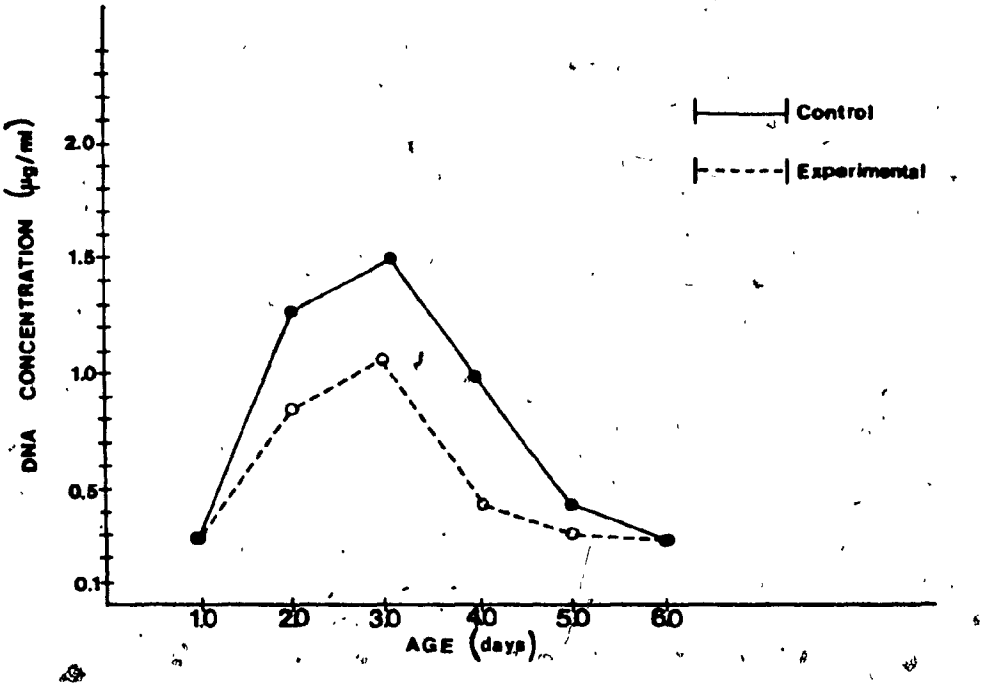


Figure 13 : DNA concentration during each day of lifespan in Control and Experimental (dietary-restricted) Asplanchna brightwelli. (T = 18°C; 20 rotifers/group)

to day 5 as compared to control rotifers but the same pattern was obtained in the two curves; the difference may have been related to the smaller number of offspring produced by the experimental rotifers. A paired t-test proved that these results were not significantly different from each other ($t = 2.47$, $p > .05$).

Enzymatic Activities. Lactic dehydrogenase and malic dehydrogenase activities were determined by the amount of NAD released, the fluorescent component of the reactions. The amount of NAD formed when the sonicate of 3 rotifers was incubated with β-NADH and sodium pyruvate indicated lactic dehydrogenase activity; the malic dehydrogenase activity was also determined by the amount of NAD released after the sonicates were incubated with β-NADH and cis-oxalacetate. The results of the daily enzymatic activities for both enzymes in control (fed every 12 hrs) and experimental (fed every 36 hrs) A. brightwelli throughout a 6-day lifespans are given in Table 10 and Table 11.

Figure 14 shows that in control rotifers, the amount of lactic dehydrogenase activity increased slowly during the first 3 days of life. This was followed by a sharp increase from day 3 to day 4. After this time, a slight increase in lactic dehydrogenase activity

Table 10

NAD release as an indication of lactic dehydrogenase activity for control and experimental (dietary-restricted) Asplanchna brightwelli throughout lifespan (T = 18°C; 3 rotifers/group).

Age (days)	Fluorescent NAD released (µg/ml)	
	Control	Experimental
1.0	0.3331	0.3076
2.0	0.4097	0.2565
3.0	0.4352	0.4097
4.0	2.0949	2.7076
5.0	2.5544	2.8608
6.0	2.5544	3.0906

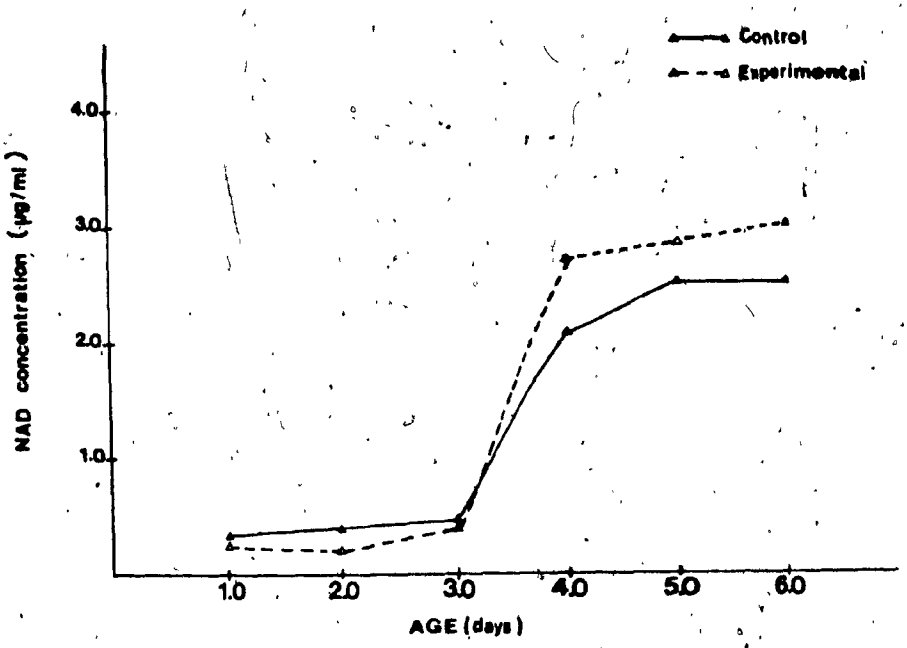


Figure 14 Lactic dehydrogenase activity as determined by NAD release for control and experimental (dietary-restricted) Asplanchna brightwelli throughout lifespan. (T = 18°C; 3 rotifers/group)

Table 11

NAD release as an indication of malic dehydrogenase activity for control and experimental (dietary-restricted) Asplanchna brightwelli throughout lifespan (T = 18°C; 3 rotifers/group).

	Fluorescent NAD released (µg/ml)	
Age (days)	Control	Experimental
1.0	0.5884	0.0460
2.0	0.4608	0.3331
3.0	0.5629	0.5118
4.0	2.7079	2.9374
5.0	3.2488	3.3204
6.0	3.3204	3.5502

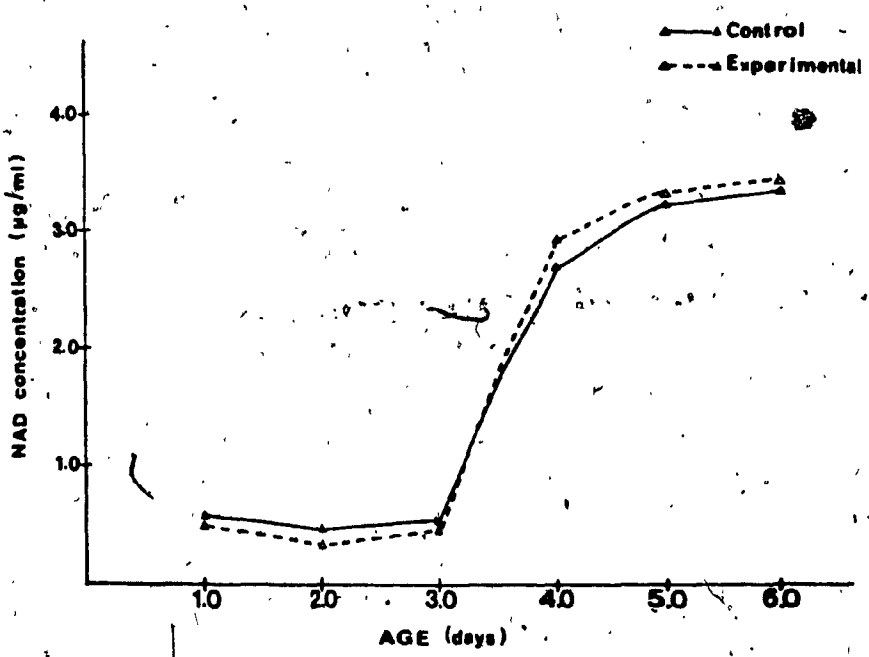


Figure 15 Malic dehydrogenase as determined by NAD release for control and experimental (dietary-restricted) Asplanchna brightwelli throughout lifespan. (T = 18°C; 3 rotifers/group)

occurred from day 4 to day 5 and this level of activity remained unchanged on day 6. In the experimental group, the lactic dehydrogenase activity decreased a little from day 1 to day 2 but then increased from day 2 to day 3. For the first 3 days of life, the level of enzymatic activity was constantly lower in the experimental group as compared to the control group. In this group there was also a sharp increase in lactic dehydrogenase activity from day 3 to day 4, a much greater increase than in the control group and the activity kept on increasing on day 5 and day 6 of life. For the last 3 days of life measured, the level of lactic dehydrogenase activity was higher in the experimental group of rotifers.

Figure 15 shows the malic dehydrogenase activity throughout the lifespan of control and experimental rotifers. In the control group, the malic dehydrogenase activity decreased slightly from day 1 to day 2 but then increased from day 2 to day 3. Again there was a sharp increase in enzymatic activity from day 3 to day 4 and the level of malic dehydrogenase activity gradually increased on days 5 and 6 of life. In the experimental group, the same pattern was obtained except that in the first 3 days of life, the level of malic dehydrogenase activity was lower than in the controls.

However, during the last 3 days of life, the level of enzymatic activity of this enzyme was higher in the experimental group.

A paired t-test performed on both sets of results revealed that there were no significant differences in enzymatic activities between control and experimental groups throughout the 6-day lifespan ($t = 1.58, .553$, $p > .05$).

DISCUSSION

Temperature

The results have shown that manipulating certain environmental factors such as temperature and diet markedly affected lifespan and reproductive patterns in the rotifer A. brightwelli.

The studies describing the effects of temperature on lifespans of various organisms are numerous. One of the first theories concerning temperature on "rate of living" was proposed by Pearl (1928) which stated that the "length of life was somehow related to the rates at which chemical reactions occurred within the body" (Van Heukelem, 1978). It is important to have a clear idea of the "rate of living" theory of aging since many of the results presented here can be interpreted in relation to this theory. The theory implies that the length of life of an organism can be correlated with the amount of energy expenditure throughout its lifespan. It was observed that in the majority of organisms reared at high temperatures, the metabolic rates were higher, the rate of development was increased, the onset of sexual maturity occurred earlier and senescence and death occurred earlier than in organisms reared at lower temperatures. These observations confirmed

an earlier hypothesis by Rubner (1908) who proposed that length of life was inversely proportional to the rate of metabolism (Strehler, 1977).

These results were similar in Drosophila (Loeb & Northrop, 1917; Miguel, Lundgeren, Bensch & Atlan, 1976), mosquitoes (Briegel & Kaiser, 1973), nematodes (Byerly, Cassada & Russell, 1976), rotifers (Meadow & Barrows, 1969) and various fish and insects (Van Heukelem, 1973). In other words, the "rate of living" theory proposed that when two groups of organisms of the same chronological age were reared at two different temperatures, the group reared at the lower temperature was physiologically younger.

Maynard Smith (1959) proposed the concept of "vitality" to explain results he obtained with fruit fly experiments. Two groups of Drosophila were used. One was kept at 20°C throughout their lifespan and another kept at 30°C for part of their lifespan and then switched to 20°C. The lifespans of these two groups were not significantly different. Since these results were inconsistent with the proposed "rate of living" theory, Maynard Smith presented the concept that lifespan was divided into two phases: 1) an aging phase and 2) a dying phase. The first phase would not be affected by temperature but a decline in vitality occurred during

this phase. As vitality decreased, the rate of dying increased and this phase was affected by temperature - the higher the temperature, the faster the dying rate (Maynard Smith, 1959).

Various experiments using different organisms have shown that both theories could be used to explain lifespan results: therefore these two theories are not mutually exclusive.

Lamb (1977) proposed that high temperatures increased the probability of death by increasing metabolic rates and thus raising the level of metabolic errors. Whatever the reason, it seemed that most invertebrates and "cold-blooded" animals displayed a longer lifespan at the lower end of the temperature scale.

Fanestil and Barrows (1965) found that Philodina acuticornis had a mean lifespan of 33.6 ± .86 days when reared at 25°C but at 31°C and 35°C, the mean lifespans were shortened to 21.9 ± 1.07 days and 18.1 ± 1.20 days respectively. In 1967, Meadow found that average lifespan of Philodina acuticornis odisoa, Milne at 27°C was 16.3 ± .16 days and at 24°C it was 22.2 ± .30 days.

In the present experiment using the rotifer A. brightwelli, the results shown in Table 1a revealed

that at 17.5°C, rotifers displayed the longest mean lifespan. At increasingly higher temperatures of 20°C, 23°C and 25°C, the mean lifespan decreased. At a temperature of 15°C, the mean lifespan was slightly shorter but this was not significantly different from the mean lifespan obtained at 17.5°C. The results of temperature of lifespan were consistent with results obtained in other rotifer studies.

In the present experiment, it was also found that when groups of A. brightwelli were reared at decreasing temperatures, the time at which these rotifers started to produce offspring occurred later (see Table 2a). the end of reproduction also occurred earlier in these rotifers as the temperature increased. Except for rotifers kept at 15°C, the length of the reproductive cycle decreased as the temperature increased; in A. brightwelli kept at 15°C, the length of the reproductive period averaged only a day; this was due to the fact that the start of the reproductive period occurred so late in life. For this reason also, an average of only 2 offspring per rotifer was produced. It was also found that as temperature increased, the average number of offspring produced per rotifer decreased, this being a direct function of the shortened reproductive phase with increasing

temperatures.

In the experiment by Fanestil and Barrows (1965), the effects of temperature on fecundity was also studied. Here it was found that the total number of eggs produced in Philodina kept at 25°C and 31°C was not significantly different (41 and 39 eggs respectively) but the total found in rotifers kept at 35°C was significantly reduced (29 eggs). The authors have also shown that in these rotifers, the time at which reproduction started and ended was not different. Meadow (1967) also reported that in Philodina reared at 27°C and 24°C, the total number of eggs laid per animal was similar (52.5 and 52.9 eggs respectively). Although the study reported that egg laying began and ended earlier in rotifers kept at 27°C as compared to those kept at 24°C, it did not mention whether this difference was significant.

The results obtained in this first series of experiments using A. brightwelli have shown that various environmental temperatures affected both lifespan and fecundity. The reproductive period appeared to be proportional to the average lifespan; that is, the longer the mean lifespan, the longer was the reproductive phase. Fecundity was also dependent on lifespan, since the average number of offspring produced per rotifer increased as the reproductive cycle was lengthened.

One interesting aspect of this study was that the time at which reproduction started and ended occurred earlier as temperatures increased. The results seemed to indicate that metabolism was increased at higher temperatures causing the rotifers to mature and senesce faster as environmental temperatures increased. Thus, the results of the present experiment were in agreement with the "rate of living" theory. Since no other changes were studied, no conclusions can be made at this point concerning other mechanisms such as DNA deficiency or enzymatic activities or the accumulation of waste products in the cells of the organism.

This experiment yielded interesting observations: (1) there was a definite extension of time from birth to sexual maturity and to senescence as environmental temperature decreased and this was reflected by changes in fecundity; the delay in the onset of sexual maturity was an observation from which stemmed from the "rate of living" theory. Correlated with this, the period of senescence was delayed and so, death occurred later in A. brightwelli reared at lower temperatures; (2) a temperature optimum did exist for the rotifers in which the greatest mean lifespan, the longest reproductive period and maximum fecundity were achieved. From these results it was decided that cultures should be kept at

approximately 18°C for future experiments.

Dietary Restriction

In both invertebrates and vertebrates dietary manipulations have been considered to be important factors in manipulating lifespan. Although most of the work concerning dietary restriction involved mammalian systems, quite a few studies were also carried out on invertebrates and some of these will be briefly described.

In an experiment using the ciliate Tokophyra, it was shown that if these organisms were fed twice daily instead of 7 to 8 times daily, the mean lifespan increased from 9.62 days to 22.8 days. In a group fed ad libitum, the mean lifespan observed was only 4.48 days (MacKeen & Mitchell, 1975). Similarly, when another invertebrate Campanularia flexuosa was fed Artemia every third day instead of every day, the mean lifespan was significantly increased (Barrows & Kokkonen, 1978).

In the rotifer Philodina, Fanestil and Barrows (1965) have also shown that limiting the times of feedings as well as the type of diet lengthened lifespan. When rotifers were fed algae and pond water daily, the mean lifespan obtained was 34.0 ± 1.1 days; when no algae were included in the daily feedings, the mean lifespan was increased to 45.3 ± 1.7 days and if this diet was given on alternate days, the mean lifespan

was further increased to 54.7 ± 1.3 days (Fanestil & Barrows, 1965).

Early experiments by Minot (1908; 1913) suggested that factors which retarded growth and development or that reduced metabolism tended to postpone senescence (Comfort, 1977). The age at which dietary restriction was imposed on an organism was also thought to be important since some researchers proposed that the period of senescence occurred only after cessation of growth (McCay et al., 1935, 1939). From studies on rats, it was thought that growth retardation due to dietary restriction was associated with a longer lifespan. Recent studies have shown that dietary restriction in the adult still resulted in the prolongation of life, thus disproving the former ideas on diet and senescence. It was also reported that in mammals, nutritional deprivation retarded sexual maturity (Osborne & Mendel, 1915; McCay et al., 1943). In 1948, Lansing correlated the cessation of growth in rotifers with the appearance of an unidentified cytoplasmic factor, a manifestation of the aging process.

In invertebrates, very little information was found regarding delayed maturation and dietary restriction. In the Fanestil and Barrows (1965) study on aging in the rotifer Philodina, it was reported that in the two

groups of rotifers where diet was limited, the lifespan increased along with the interval between the start and the end of egg production. In the control group (algae and pond water daily) the duration of this interval was 8 days, while in the second and third regimes (pond water daily and every second day) the interval lasted 21 and 29 days respectively.

There are numerous theories explaining how dietary restriction has such a pronounced effect on lifespan in the majority of organisms. Moment (1978) proposed that the answer may involve the reduction in the use of the genetic code thus decreasing the wear and tear on DNA (Moment, 1978). This concept was agreed upon by other authors, namely Barrows and Kokkonen (1978), who stated that dietary restriction increased lifespan by decreasing protein synthesis; in this manner, the use of the genetic code was decreased thereby "minimizing genetic imperfections as they may occur in later life" (Barrows & Kokkonen, 1978).

Lamb (1977) proposed that dietary restriction slowed down the "biological clock" that governs the rate of aging. The reasoning here was that if the growth period were prolonged by undernutrition, the period of senescence must also be extended. In the mammalian systems, it was suggested that dietary restriction

also delayed the onset of certain "aging diseases" such as leukemia and kidney failure and that the incidence of tumors was reduced in underfed animals (Berg & Simms, 1962; Bras & Ross, 1964; Saxton et al., 1944; Ross & Bras, 1965). There was also a study showing that mice on dietary restriction had a lower body temperature but higher oxygen consumption (Leto, Kokkonen & Barrows, 1976); although the decreased body temperature could be explained in terms of decreased metabolic rates, there was no apparent reason why organisms with a longer lifespan should display higher oxygen consumption.

The indication that dietary restriction increased lifespan again seemed to point toward the "rate of living" theory. Through dietary restriction, growth was delayed; this delay was correlated with a delay in senescence as well as in certain diseases attributed to aging. The "biological clock" theory was an extension of the "rate of living" theory since it also proposed a slowing down of events. The above theory can be used to explain the results obtained in the second experiment using A. brightwelli.

When manipulating diets of various invertebrates, it was difficult to monitor the diets so as to

limit caloric intake; an easier method therefore involved decreasing the amount of food available to the organism. Lengthening the feeding intervals was the method employed in this experiment using A. brightwelli.

Under normal experimental conditions, the rotifer cultures were monitored and fed twice daily (approximately every 12 hrs), therefore the control group in this study was defined as the one being fed at these times. The experimental groups were fed every 24 to 72 hrs; all groups were kept at a temperature of 18°C. The results showed that underfeeding these rotifers by extending the feeding interval up to 36 hrs lengthened mean lifespan (see Table 3a).

In the present experiment it was also found that the times when these rotifers started to produce offspring were the same in all 6 groups (after about 2.5 days). In the groups that were fed every 24 and 36 hours, the mean length of the reproductive period showed a slight increase as compared to the controls. After this point, the more severely restricted groups showed a decrease in the duration of the reproductive phase as lifespan decreased (see Table 4a). Again, it could be concluded that the length of the reproductive period was proportional to the lifespan, that is, the extension of reproduction

was dependent upon increased longevity.

This study has also shown that the average number of offspring per rotifer was high when the reproductive period was extended but in the more severely restricted groups displaying the shorter lifespans and reproductive periods, the average was only 2 offspring per rotifer. Although rotifers fed every 12 hours (control group) had a slightly shorter reproductive period than rotifers fed at 24 and 36 hours intervals, this was not found to be significant according to the statistics performed on these 3 groups; however, the highest number of offspring per rotifer was attained in this group. The reason for this is unknown.

In the present experiment, although the onset of sexual maturity was not delayed through moderate dietary restriction, the period of reproduction was lengthened and senescence was delayed. Since rotifers are eutelic, it cannot be assumed that a "biological clock" based on the number of cell divisions is regulating the rate of the aging process in a non-dividing system; certain events may be going on at a slower rate, though, and some of these were investigated.

Although most of the theories mentioned above have been derived from studying mammalian systems, some variables can be studied using invertebrates. Since

moderate dietary restriction did show promising results in A. brightwelli, this phenomenon was studied in greater detail to see what other processes were affected in the rotifer during dietary restriction, and to see if the results agree in some way with the "rate of living" theory of aging.

Nuclear Number

The first aspect that was studied concerned the pattern of nuclear division in the two organs that are exceptions to the eutelic condition of the rotifer, the vitellarium and the pair of gastric glands. With the "rate of living" theory in mind, if the rates of cellular events are delayed by dietary restriction, this delay may be expressed by a change in pattern in the dividing organs of the rotifer.

A study examining the nuclear number in the vitellaria and gastric glands of Asplanchna sieboldi was carried out by Birky and Field (1966) to see (1) if rotifers of the same clone showed a characteristic number of nuclei in these organs and (2) if different diets affected the nuclear number. From this study, it was observed that there existed intraclonal variation regarding nuclear number in the vitellarium and gastric glands and that nuclear number fluctuated from day to day. Of prime importance though, it was found that in three

parallel cultures of A. sieboldi, significant differences occurred depending on the source of food given. The authors concluded that environmental factors such as diet could affect nuclear number and this effect was detected even after two generations (Birky & Field, 1966).

In the present experiment, although the food source was the same (Paramecium caudatum), the times at which the rotifers were fed differed. The first group (control) of A. brightwelli were fed every 12 hours while the second group (experimental) were fed every 36 hours; in both groups, the numbers of nuclei in the pair of gastric glands and in the vitellarium were counted for all 6 days of life.

The results suggested that there was a certain pattern of nuclear division in the gastric glands and vitellaria of A. brightwelli; also, the pattern was slightly different in rotifers fed every 36 hours as compared to those fed every 12 hours (see Table 5).

In the gastric glands of the controls, the number of nuclei present increased from the first to the fourth day of life followed by a decrease on day 5 and on day 6. In the experimental group, the nuclear number in the gastric glands also increased from day 1 to day 4 but on all these days, the nuclear number was always lower than found in the control group. The nuclear

number decreased slightly on day 5 (the same pattern as in the former group) but on day 6 it increased, finally reaching the peak obtained much earlier in life in the control group. Although the pattern of nuclear division found in the gastric glands on each day of life was similar in both groups, the time at which the same events occurred in the experimental group was slower as compared to the controls.

The same kind of situation occurred when observing the nuclear number in the vitellarium. In the control group the nuclear number increased from day 1 to day 5 and after this time the nuclear number decreased on day 6. In the experimental group, the nuclear number in the vitellarium also increased from day 1 to day 5 but for the first three days of life, the nuclear number was always lower than in the control group. On days 4 and 5, the values were statistically the same in both groups but on day 6, the nuclear number in the vitellarium in the experimental group decreased very slightly. A smaller sample showed that a further decrease was observed by day 7 in the experimental group.

In the vitellarium, the nuclear division pattern was similar to that seen in the gastric glands. The nuclear number increased until a peak was reached and then decreased late in life. In the experimental

rotifers, the same events occurred but in this group, the events occurred later than in the controls. Figure 7 is a graphic representation of the division events in both organs of the control and experimental rotifers. The delay of events in the experimental group is shown as a shift to the right on the graph, that is, the "experimental" curve lies to the right of the "control" curve.

The results of this experiment yielded two interesting observations. First, the patterns of nuclear divisions obtained indicated that in the vitellarium and the gastric glands, the nuclear number reached a maximum value and then the number of nuclei decreased after this peak. Mammalian cells in culture have been shown to undergo a finite number of population doublings before death (Hayflick & Moorhead, 1961; Hayflick, 1965); manifestation of senescence at the cellular level was therefore defined as the time at which cells lost this proliferative capacity. Although cellular division in invertebrate organs has never been studied in a comparative manner, the results of this experiment seemed to indicate that nuclei in the only two dividing cell lines of the rotifer reached a certain peak and then decreased before death.

Although it would be difficult to analogize the

results of this experiment with those from cell cultures, the underlying concept is interesting. Hayflick suggested that the decline in cell proliferation may be due to depletion of essential nutrients of the cell (Lamb, 1977); it is possible that nuclear division stopped in the vitellarium and gastric glands of the rotifer due to the depletion of necessary metabolites or the accumulation of waste products in these glands. Thus a decrease in nuclear number may be a manifestation of senescence in this rotifer.

Secondly, the results have shown that dietary restriction did affect the nuclear number in the gastric glands and vitellarium of the rotifer. In the longer-lived rotifers (those fed every 36 hrs), the nuclear pattern observed was similar to the shorter-lived rotifers except that the timing of events was slower. Many gerontologists have hypothesized that aging was genetically programmed through a "biological clock" so that longer-lived organisms exhibited an aging program that was slowed down (Lamb, 1977). Again, since rotifers are eutelic organisms, the results of this experiment cannot be explained in terms of this theory; the delay in events observed could be related to the "rate of living" theory which, as mentioned before, stated that the rate of occurrence of cellular

events or chemical reactions within the organism influenced its longevity. The experiment suggested that the pattern of nuclear division in the vitellarium and gastric glands of A. brightwelli was slowed down through dietary restriction; since this manipulation increased the mean lifespan of the organism, it could be assumed that the slower rate of nuclear division in these organs was a factor in extending the lifespan.

Measurements

The effect of dietary restriction was further studied to see if it produced any significant changes in the growth of the rotifer throughout its lifespan or if it altered the size of the gastric glands, the vitellarium and their nuclei. The body lengths and widths of control rotifers and dietary-restricted rotifers (experimental) were measured throughout a 6-day lifespan. The results showed that statistically, there were no alterations in body size as a result of dietary restriction even though the experimental rotifers were slightly smaller than the controls (see Figure 8). Since growth in the rotifer after embryological development occurs through cytoplasmic expansion and not by cellular division (Gilbert, 1974), these results were not surprising.

Measurements of the gastric glands taken in both groups throughout the lifespan showed that significant differences occurred. The length and width were measured as precisely as possible after a squash preparation and in both control and experimental groups, the widths of the gastric glands were similar throughout the lifespan (see Table 7). When the length of the gastric glands was measured however, it was found that these were statistically different in the two groups. The length of the gastric glands in the control groups remained fairly constant throughout the lifespan of the rotifer but in the experimental rotifers there was a greater increase in the length of the gastric glands from day 1 to day 6. Graphically, the slopes of the two groups are obviously different. During the last days of life in the experimental rotifers, the size of the gastric glands reached the average size maintained throughout the lifespan of control rotifers. Figure 9 shows this clearly, the regression line representing the length of the gastric glands of experimental rotifers is increasing while the regression line depicting the length of the gastric glands in control rotifers lies flat.

Although the size of the nuclei of the gastric glands was smaller throughout the lifespan of the

experimental rotifers, this difference was not statistically significant. The gastric glands act in digestion of food so it may be possible that dietary restriction by limiting food intake affected the size of these glands. Possibly, the development of the gastric glands in the dietary-restricted rotifers was slower. If nuclear division in the gland were an indication of its physiology, this could explain why the nuclear number in these glands was consistently lower in the experimental rotifers according to the previous experiment. No statistical differences were found in nuclear number on the last day of life between the two groups and similarly, the lengths of the gastric glands reached the same size at the end of life.

The various sizes of the nuclei in the gastric glands were probably indicative of the stage of division observed at the time, that is, the smallest nuclei were the ones that had just divided while the larger ones were those in resting phase or preparing to divide. The reason the nuclei in the gastric glands of experimental rotifers were consistently smaller than those in control rotifers may be related to the size of the gastric gland itself. The increase in size of the gastric glands throughout the lifespan of experimental rotifers while this organ remained the same size in the

control group could mean that the nuclei in the gastric glands of the group under dietary restriction may have been dividing for a longer period of time. This would not necessarily be seen in terms of a greater nuclear number present on each day of life but rather as a gradual increase in the number of nuclei present in the glands of experimental rotifers after the time nuclear division stopped in control rotifers.

It was therefore assumed that the lower nuclear number constantly found in the gastric glands of experimental rotifers throughout their lifespan was reflected by the size of the gastric gland itself. When nuclear number equalled that found in the gastric glands of control rotifers, the lengths of the gastric glands reached a similar size. During experimental observation, it was noticed that young rotifers (about 1 day old) gorged themselves with paramecia prior to entering the reproductive phase. The number of paramecia could actually be seen through the phase contrast microscope as bright, yellow bodies within the stomach. It was speculated that this high feeding rate may be some kind of preparatory behavior coinciding with the bearing of embryos to provide an adequate supply of nutrients for the embryos. The increased food intake surely must affect the digestive capacities of the

gastric glands, thus mitotic activity is high. If the available food is limited, the nuclear number and size of the gastric glands may be reduced. These interpretations are based on the effects noted concerning the results observed of dietary restriction in A. brightwelli since these types of observations have never been reported before.

The vitellarium and its nuclei in control and experimental rotifers were measured following squash preparations. The size of this organ was difficult to measure since it is a U-shaped organ and the extended arms often bent or folded during a squash preparation. For this reason, only vitellaria that had a side-arm fully extended were measured but still a high variability in the measurements occurred. A regression analysis and comparison of slopes for the measurements of the vitellaria in control and experimental rotifers throughout lifespan have shown that no significant differences occurred between the two groups.

Figure 11 shows that the size of this organ was slightly shorter through dietary restriction but again since this was a difficult organ to measure, no definite conclusions could be drawn from the experiment. The results did, on the other hand, follow the pattern

obtained in the study of the size of gastric glands in control and experimental rotifers.

What was interesting to observe in this organ however were the different sizes of nuclei present. The variety was so great that a range of nuclear sizes for each day of life was presented in Figure 12 instead of the average nuclear size observed. Again, the smaller nuclei were thought to be ones having just completed division while the larger ones were preparing to divide or simply in resting stage. The vitellarium or yolk-gland is responsible for providing nutrients to the developing embryo; the nuclear counts study showed that mitotic activity in this gland was high during the reproductive phase.

Although the results were difficult to interpret because there were no definite pattern seen in the groups concerning nuclear sizes, the following observations could be made. (1) The smallest observed nuclei in the vitellarium of experimental rotifers was constantly smaller than in control rotifers. This was also true for the largest sizes observed except for day 1. (2) From day 4 to day 7, both groups have shown that mostly large nuclei were present in the vitellarium. This may have been an indication of decline in mitosis towards the end of the reproductive cycle. The reason

the largest nuclei in the vitellarium of experimental rotifers were so much smaller on day 7 as compared to those in control rotifers may have been because the length of the reproductive period was slightly longer in dietary-restricted rotifers therefore all the nuclei may not have been in resting stage. Since no significant changes were found between the two groups concerning the time at which reproduction started or ended, the statistical differences found in these results between the two groups could not be accounted for.

Relating these findings to the previous experiment, it could be seen that as nuclear sizes increased in the vitellarium, the nuclear number decreased. In other words, the same amount of space in the yolk-gland was occupied by fewer but larger nuclei as age increased. During the active reproductive phase, many small nuclei were seen as a result of greater mitotic activity and nuclear number was also high according to the results of the previous experiment.

Visually, it was also observed that the nuclei were fragmented in older rotifers and not of distinct polyploid appearance (see photomicrographs #2 - #4 for comparison in Appendix IV). Also, the vitellarium itself seemed to contain many lipid globules, another

characteristic of an organ in a degenerating state.

To summarize, the results of these measurements have shown that dietary restriction did not affect body size in A. brightwelli but the size of the gastric glands was affected. Nuclear size was not significantly affected in the dietary-restricted group. Also, there were no significant differences in the length of the vitellaria throughout the lifespan of the two groups but significantly smaller nuclei were found in the vitellarium of the experimental group. Some correlations were drawn concerning the various nuclear sizes in the vitellarium and nuclear number throughout lifespan.

The results seem to fit the "rate of living" theory. The gastric glands of rotifers under dietary restriction took a longer time to attain the size of those in controls; the smaller nuclear sizes in these glands may suggest that the rate of development was a little slower in rotifers under dietary restriction. The vitellarium was slightly shorter throughout the lifespan of experimental rotifers. The significant delay in the growth of the gastric glands may be a condition that occurred along with the extension of life through dietary restriction.

Since significant differences occurred between the nuclear sizes in the vitellarium of the two groups of

rotifers, the next question asked concerned the actual amount of DNA in these rotifers; that is, if differences occurred regarding nuclear number and sizes of gastric glands and vitellarium between control rotifers and those on dietary restriction, did DNA content also vary between the two groups? Also, DNA content should reveal whether DNA changes occurred with aging and if the results differed between dietary-restricted rotifers and controls.

DNA Content

Most experiments conducted that looked at DNA content during aging dealt with mammalian systems and cell cultures. For example, it was observed that old human diploid cells were larger and had increased nuclear size as compared to a young population but DNA content was approximately the same (Cristofalo & Stanulis, 1978; Enesco, 1967). In the Leto et al. (1976) study on dietary restriction and mice, it was shown that DNA concentrations were not markedly affected with age in either control or restricted mice except for kidney and liver; in these organs DNA content was higher in restricted mice (Leto et al., 1976).

DNA content was measured spectrofluorometrically. The results given in Table 9 and Figure 13 show that although the experimental group of rotifers had a

consistently lower DNA concentration from day 2 to day 5 of their lifespan, this was not significantly different from the control group of rotifers. On day 1 and day 6, the DNA content was identical in both groups; the increase in DNA content in both groups on day 2 and day 3 could be attributed to reproductive activity. The presence of offspring within the adult rotifer contributed to the DNA content that was measured. Near the end of the reproductive cycle, days 4 and 5, the DNA content decreased in the two groups.

The slightly higher DNA content in the control group of rotifers could only be attributed to a slightly greater number of offspring produced by this group as compared to the experimental rotifers. In the first experiment carried out, it was observed that although the reproductive cycle was slightly shorter in rotifers fed every 12 hrs as compared to the group fed every 36 hrs, a higher number of offspring occurred in the former group. This may account for the increase in DNA content in this group from day 2 to day 5. When the DNA concentration on day 1 and day 6 was compared, the results were identical in both groups; on these days, the probability of the presence of offspring was lower than on any other day thus a more precise estimate of

DNA content was obtained.

Dietary restriction studies have shown no change in DNA content between control and dietary-restricted organisms. If DNA repair were deficient in aging cells and if aging is retarded through dietary restriction, the level of DNA should be higher in longer-lived animals.

The results of the present experiment have revealed that the DNA content remained the same throughout the lifespan of this rotifer; there was no indication of DNA changes or deficiency since the DNA content did not decrease from the first to the last day of life measured. Since the same results occurred in control and experimental rotifers, dietary restriction has not been shown to affect DNA levels during aging based on the method of DNA detection used.

Other cellular conditions that are thought to change with age are RNA content, protein content, cellular lipids and presence of the age pigment, lipofuscin. The next biochemical study therefore involved looking at changes in enzymatic activities with age in control and dietary-restricted rotifers.

Enzyme Activity

Since no correlations could be made concerning the "rate of living" theory and DNA content throughout the lifespan of A. brightwelli, a study on enzyme activity

throughout lifespan was conducted to see (1) if enzyme levels changed during the 6-day lifespan and (2) if changes in enzyme levels differed between control and experimental (dietary-restricted) rotifers. The "rate of living" theory would predict that if protein damage occurred during aging, enzyme levels should be affected and the rate at which these events occurred would be slower in the longer-lived rotifer under dietary restriction.

Fanestil and Barrows (1965) have shown that levels of lactic dehydrogenase and malic dehydrogenase activity were markedly affected in underfed Philodina as compared to controls. This rotifer had a much longer lifespan than A. brightwelli; Philodina fed algae and pond water daily lived for about 34 days while those fed pond water on alternate days lived an average of 55 days. The activities of lactic dehydrogenase and malic dehydrogenase were assayed every 5 days thoughout the lifespans and the pattern obtained showed that enzymatic activities increased during early life becoming relatively stable during adulthood and then decreased during senescence. The results have shown that the occurrence of these events was delayed in the longer-lived (dietary-restricted) Philodina. Therefore, the pattern of enzymatic activities was similar in

control and underfed rotifers but the timing of these biochemical events was different; in the longer-lived Philodina, the level of lactic dehydrogenase and malic dehydrogenase activities increased much more slowly in early life and decreased more slowly during senescence.

The pattern of enzymatic activity of lactic dehydrogenase throughout a 6-day lifespan of A. brightwelli is represented in Figure 14 and is similar in control (fed every 12 hrs) and experimental (fed every 36 hrs) rotifers. The initial increase during the first three days of life could partially be attributed to an increase in body mass (Fanestil et al., 1965) but it must also reflect changes in the physiological processes such as the bearing of offspring. The results indicated that there was lower enzymatic activity during the first 3 days of life in the experimental rotifers as compared to the control rotifers. By day 4, both groups of rotifers were actively producing offspring which may have caused the amount of fluorescent NAD to increase sharply from day 3 to day 4.

In a previous experiment, it was observed that the peak of reproductive activity occurred after 3.5 days in rotifers fed every 12 hrs and kept at 18°C;

although rotifers reared at this temperature and fed every 36 hrs displayed a peak of reproductive activity a little earlier, this high rate of fecundity was maintained until day 6 of life. Therefore the results showing a sharp rise in enzymatic activity between day 3 and day 4 could be attributed to the presence of offspring and other cellular events occurring within the organism during the reproductive cycle.

During the last three days of life, the level of lactic dehydrogenase activity was higher in the experimental group of rotifers as compared to the control group. In the control group, there was a slight increase in enzymatic activity from day 4 to day 5 but then the level of lactic dehydrogenase activity remained the same on days 5 and 6 of life. In experimental rotifers, lactic dehydrogenase activity increased from day 4 to day 6 of life. The slightly longer reproductive period obtained with dietary-restricted rotifers may explain the continued increase in enzymatic activity on the last 2 days of life measured while controls levelled off.

Malic dehydrogenase activity throughout the lifespan of both groups of A. brightwelli is represented in Figure 15. The reason for the small decrease in malic dehydrogenase activity on day 2 is unknown. Once again, the level of malic dehydrogenase

activity was lower for the first 3 days of life in experimental rotifers as compared to controls. The sharp increase in malic dehydrogenase activity occurred from day 3 to day 4 of life for the same reasons mentioned before concerning lactic dehydrogenase activity. The increase in malic dehydrogenase activity in experimental rotifers during the last 3 days of life was again higher than in controls, the only reason for this being the longer reproductive period brought about by dietary restriction in the rotifer.

The reasons dietary restriction increased lifespan in A. brightwelli could not be fully explained in light of the results obtained in this experiment. The "rate of living" theory predicted that chemical processes in a longer-lived organism occurred at a slower rate than in shorter-lived organisms. The only evidence that dietary restriction may have slowed down biochemical processes in these rotifers was related to the levels of lactic dehydrogenase and malic dehydrogenase which were constantly lower during the first 3 days of life in experimental rotifers as compared to control rotifers. This seemed to indicate that increase in the lifespan of A. brightwelli by lengthening the feeding interval occurred during the pre-reproductive period of the lifespan. The age at which dietary restriction was

imposed on these rotifers must therefore have been a primary factor in increasing its lifespan. Since the mean lifespan of this rotifer was so short, it was assumed dietary restriction must be started very early in life to have a beneficial effect on the lifespan in the rotifers. This, of course, could only be verified by imposing the restricted diet at various times during its lifespan and determining the effects on longevity.

The times at which dietary restriction is imposed upon an organism have been a controversial subject since some studies have shown that only dietary restriction imposed early in life affected the lifespan of animals (McCay et al., 1935; Barrows, 1972) while some studies have shown that the lifespan of organisms could be increased even when dietary restriction was imposed later in life (Fanestil et al., 1965). Orgel's "error catastrophe" theory suggests that aging occurs from the high production of deleterious enzymes later in life (Gershon, 1979); it has been suggested that dietary restriction may retard the appearance of these faulty enzymes in later life by reducing protein synthesis and delaying genetic informational transfer in early life (Barrows et al., 1978). This would therefore retard senescence by delaying the accumulation of deleterious enzymes.

Although there was no evidence of this in this study, lower levels of enzymes were present in the experimental group of rotifers during the first 3 days of life. Even though the differences were not statistically significant, this observation is consistent with lower levels of metabolic activity which would be expected in the rotifers on dietary restriction according to the "rate of living" theory.

As noted throughout the discussion, this theory has been most useful of the many theories of aging in the interpretation of the results.

SUMMARY

Asplanchna brightwelli has proved a useful organism for the study of some aging processes. The various experiments conducted show that manipulation of certain environmental factors - temperature and food availability - affect lifespan and reproductive patterns in this rotifer.

The life cycle of this rotifer is divided into 3 phases: 1) birth and the pre-reproductive cycle, 2) the reproductive cycle and 3) the post-reproductive cycle shortly followed by death. The greatest mean lifespan achieved at an optimum of 17.5°C was a factor of the increase in the first 2 phases of the life cycle. The first 2 phases were progressively shorter as temperatures increased. Dietary restriction at 18°C, i.e., 36-hour feeding intervals, produces an increase in mean lifespan and occurs mainly by a lengthening of phase 2 (the reproductive cycle), more severe restriction did not promote a longer lifespan.

Daily nuclear counts within the vitellarium and gastric glands show that cellular division increases during the pre-reproductive period, reaches a peak during the reproductive cycle and decreases prior to death. In dietary-restricted rotifers, the timing of

the events is delayed compared with controls. Size measurements taken throughout the lifespan of this eutelic organism show that growth in body size is unaffected by dietary restriction but the size of the gastric glands was affected. The vitellarium length was not significantly shorter but its nuclei were significantly smaller in experimental rotifers; this may reflect a longer period of cell division in this group. Correlating the size data with the nuclear counts indicates that cellular events and growth of these organs are delayed through dietary restriction. The patterns of delay support the theory of aging by "rate-of-living" which predicts that processes in longer-lived organisms occur at a slower rate than in shorter-lived organisms.

DNA content throughout the lifespan of A. brightwelli is not significantly different between dietary-restricted and control organisms. Lactic dehydrogenase activity in control rotifers increases gradually during the pre-reproductive phase, rises sharply during the reproductive cycle and levels off before death. In dietary-restricted rotifers, the level of enzymatic activity is constantly lower during the pre-reproductive phase but higher during and after the reproductive cycle; compared with controls, there is no

levelling off of activity prior to death. Malic dehydrogenase activity for the first 3 days of life is lower in dietary-restricted rotifers than in controls and for the last 3 days of life, experimental levels exceed control levels.

Although the activity of neither enzyme throughout the lifespan of A. brightwelli is significantly different between the controls and dietary-restricted rotifers the lower level of activity during the pre-reproductive phase of experimental rotifers may account for the longer lifespan attained in this group. Such results indicating a lower level of enzyme activity during this period may be one of the ways dietary restriction increases lifespan in this rotifer.

The delay of senescence in A. brightwelli through a decrease in environmental temperature and a decrease in food intake must occur through the lengthening of at least one phase of the life cycle. Low temperatures used in this study lead to an extension of the pre-reproductive and reproductive phases. Dietary restriction produces an extension of the reproductive phase. As well, there is a delay in growth and cellular division in the vitellarium and gastric glands, and in possible enzyme synthesis during the pre-reproductive phase. The delay of these processes may explain how

limiting food intake could extend the lifespan in the rotifer.

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APPENDIX I

Stock Buffer Solution

Used for culturing Paramecia/rotifers.

COMPONENT	MOLECULAR WEIGHT	AMOUNT USED
H_2O d	-	500.0 mls
NaH_2PO_4	137.99	69.0 g
NaOH	40.0	10.0 g

Use 7.0 mls for every liter of Medium prepared.

APPENDIX II

Life Tables

These Life Tables for temperature and dietary restriction experiments are constructed as described by Lamb (1977).

Explanation of Terms

x	Age interval (in days)
lx	Number of animals alive during age interval, x
dx	Number of animals dying during each age interval, x $dx = lx - l(x + 1)$
qx	Age-specific Death Rate, i.e. probability of death at any day $qx = dx/lx$
Lx	Age-structure, i.e. Average number of animals alive between 2 intervals $Lx = lx + l(x + 1)/2$
Tx	Total number of animal-age-units left by animals at beginning of each interval $Tx = Lx + L(x + 1) + \ldots + Lw$
ex	Further expectation of life at each interval $ex = Tx \cdot a/lx$ Where a = size of age interval

APPENDIX II (CONT'D)

Temp. = 15°C (N = 24)

x	lx	dx	qx	lx	Tx	ex
0.0 - 0.5	24	1	0.041	23.5	236.0	4.91
0.5 - 1.0	23	0	0.000	23.0	212.5	4.61
1.0 - 1.5	23	0	0.000	23.0	189.5	4.11
1.5 - 2.0	23	1	0.043	22.5	166.5	3.61
2.0 - 2.5	22	0	0.000	22.0	144.0	3.27
2.5 - 3.0	22	0	0.000	22.0	122.0	2.77
3.0 - 3.5	22	1	0.045	21.5	100.0	2.27
3.5 - 4.0	21	2	0.095	20.0	78.5	1.86
4.0 - 4.5	19	4	0.210	17.0	58.5	1.53
4.5 - 5.0	15	3	0.200	13.5	41.5	1.38
5.0 - 5.5	12	5	0.416	9.5	28.0	1.16
5.5 - 6.0	7	1	0.142	6.5	18.5	1.32
6.0 - 6.5	6	2	0.333	5.0	12.0	1.00
6.5 - 7.0	4	1	0.250	3.5	7.0	.875
7.0 - 7.5	3	0	0.000	2.0	3.5	.583
7.5 - 8.0	3	3	1.000	1.5	1.5	.250
8.0 - 8.5	0	0	-	-	-	-
8.5 - 9.0	0	0	-	-	-	-

APPENDIX II (CONT'D)

Temp. = 17.5°C (N = 24)

x	lx	dx	qx	Lx	Tx	ex
0.0 - 0.5	24	0	0.000	24	272.0	5.66
0.5 - 1.0	24	0	0.000	24	248.0	5.16
1.0 - 1.5	24	1	0.041	23.5	224.0	4.66
1.5 - 2.0	23	2	0.086	22	200.5	4.35
2.0 - 2.5	21	1	0.047	20.5	178.5	4.25
2.5 - 3.0	20	0	0.000	20	158.0	3.95
3.0 - 3.5	20	0	0.000	20	138.0	3.45
3.5 - 4.0	20	0	0.000	20	118.0	2.95
4.0 - 4.5	20	0	0.000	20	98.0	2.43
4.5 - 5.0	20	2	0:100	19	78.0	1.95
5.0 - 5.5	18	4	0.222	16	59.0	1.63
5.5 - 6.0	14	1	0.071	13.5	43.0	1.53
6.0 - 6.5	13	3	0.230	11.5	29.5	1.13
6.5 - 7.0	10	2	0.200	9	18.0	.90
7.0 - 7.5	8	3	0.375	6.5	9.0	.56
7.5 - 8.0	5	5	1.000	2.5	2.5	.50
8.0 - 8.5	0	0	-	-	-	-
8.5 - 9.0	0	0	-	-	-	

APPENDIX II (CONT'D)

Temp. = 20°C (N = 24)

x	lx	dx	qx	Lx	Tx	ex
0.0 - 0.5	24	0	0.000	24	158.0	3.29
0.5 - 1.0	24	3	0.125	22.5	134.0	2.79
1.0 - 1.5	21	0	0.000	21	111.5	2.65
1.5 - 2.0	21	2	0.095	20	90.5	2.15
2.0 - 2.5	19	0	0.000	19	70.5	1.85
2.5 - 3.0	19	4	0.210	17	51.5	1.35
3.0 - 3.5	15	2	0.133	14	34.5	1.15
3.5 - 4.0	14	5	0.384	10.5	20.5	.79
4.0 - 4.5	8	5	0.625	5.5	10.0	.63
4.5 - 5.0	3	1	0.333	2.5	4.5	.75
5.0 - 5.5	2	1	0.500	1.5	2.0	.50
5.5 - 6.0	1	1	1.000	.5	.5	.25
6.0 - 6.5	0	-	-	-	-	-
6.5 - 7.0	0	-	-	-	-	-
7.0 - 7.5	0	-	-	-	-	-
7.5 - 8.0	0	-	-	-	-	-
8.0 - 8.5	0	-	-	-	-	-
8.5 - 9.0	0	-	-	-	-	-

APPENDIX II (CONT'D)

Temp. = 23°C (N = 24)

x	lx	dx	qx	Lx	Tx	ex
0.0 - 0.5	24	0	0.000	24	150.0	3.12
0.5 - 1.0	24	0	0.000	24	126.0	2.62
1.0 - 1.5	24	1	0.041	23.5	102.0	2.12
1.5 - 2.0	23	3	0.130	21.5	78.5	1.70
2.0 - 2.5	20	3	0.150	18.5	57.0	1.42
2.5 - 3.0	17	4	0.235	15	38.5	1.13
3.0 - 3.5	13	3	0.230	11.5	23.5	.90
3.5 - 4.0	10	4	0.400	8	12.0	.60
4.0 - 4.5	6	5	0.833	3.5	4.0	.33
4.5 - 5.0	1	1	1.000	.5	.5	.25
5.0 - 5.5	0	-	-	-	-	-
5.5 - 6.0	0	-	-	-	-	-
6.0 - 6.5	0	-	-	-	-	-
6.5 - 7.0	0	-	-	-	-	-
7.0 - 7.5	0	-	-	-	-	-
7.5 - 8.0	0	-	-	-	-	-
8.0 - 8.5	0	-	-	-	-	-
8.5 - 9.0	0	-	-	-	-	-

APPENDIX II (CONT'D)

Temp. = 25°C (N = 24)

x	lx	dx	qx	Lx	Tx	ex
0.0 - 0.5	24	1	0.041	23.5	112.0	2.33
0.5 - 1.0	23	5	0.217	20.5	88.5	1.92
1.0 - 1.5	18	1	0.055	17.5	68.0	1.88
1.5 - 2.0	17	2	0.117	16.0	50.5	1.48
2.0 - 2.5	15	5	0.333	12.5	34.5	1.15
2.5 - 3.0	10	4	0.400	8.0	22.0	1.10
3.0 - 3.5	6	1	0.160	5.5	14.0	1.16
3.5 - 4.0	5	2	0.400	4.0	8.5	.85
4.0 - 4.5	3	1	0.333	2.5	4.5	.75
4.5 - 5.0	2	1	0.500	1.5	2.0	.50
5.0 - 5.5	1	1	1.000	.5	.5	.25
5.5 - 6.0	0	-	-	-	-	-
6.0 - 6.5	0	-	-	-	-	-
6.5 - 7.0	0	-	-	-	-	-
7.0 - 7.5	0	-	-	-	-	-
7.5 - 8.0	0	-	-	-	-	-
8.0 - 8.5	0	-	-	-	-	-
8.5 - 9.0	0	-	-	-	-	-

APPENDIX II (CONT'D)

Dietary Restriction = 12 hrs (Control) (T = 18°C; N = 24)

x	lx	dx	qx	Lx	Tx	ex
0.0 - 0.5	24	0	0.000	24	272.0	5.66
0.5 - 1.0	24	0	0.000	24	248.0	5.16
1.0 - 1.5	24	1	0.041	23.5	224.0	4.66
1.5 - 2.0	23	0	0.000	23	200.5	4.79
2.0 - 2.5	23	0	0.000	23	177.5	3.85
2.5 - 3.0	23	1	0.043	22.5	154.5	3.35
3.0 - 3.5	22	0	0.000	22	132.0	3.00
3.5 - 4.0	22	1	0.045	21.5	110.0	2.50
4.0 - 4.5	21	0	0.000	21	88.5	2.10
4.5 - 5.0	21	3	0.142	19.5	67.5	1.60
5.0 - 5.5	18	1	0.055	17.5	48.0	1.33
5.5 - 6.0	17	4	0.235	15	30.5	.89
6.0 - 6.5	13	7	0.538	9.5	15.5	.59
6.5 - 7.0	6	3	0.500	4.5	6.0	.50
7.0 - 7.5	3	3	1.000	1.5	1.5	.25
7.5 - 8.0	0	0	-	-	-	-
8.0 - 8.5	0	0	-	-	-	-
8.5 - 9.0	0	0	-	-	-	-

APPENDIX II (CONT'D)

Dietary Restriction = 24 hrs (T = 18°C; N = 24)

x	lx	dx	qx	Lx	Tx	ex
0.0 - 0.5	24	0	0.000	24	272.0	5.66
0.5 - 1.0	24	0	0.000	24	248.0	5.16
1.0 - 1.5	24	0	0.000	24	224.0	4.66
1.5 - 2.0	24	0	0.000	24	200.0	4.16
2.0 - 2.5	24	0	0.000	24	176.0	3.66
2.5 - 3.0	24	0	0.000	24	152.0	3.16
3.0 - 3.5	24	0	0.000	24	128.0	2.66
3.5 - 4.0	24	2	0.083	23	104.0	2.16
4.0 - 4.5	22	1	0.045	21.5	81.0	1.84
4.5 - 5.0	21	3	0.142	19.5	59.5	1.41
5.0 - 5.5	18	4	0.222	16.0	40.0	1.11
5.5 - 6.0	14	5	0.357	11.0	24.0	.85
6.0 - 6.5	8	2	0.250	7.0	13.0	.81
6.5 - 7.0	6	5	0.833	3.5	6.0	.50
7.0 - 7.5	1	0	0.000	1.0	2.5	1.25
7.5 - 8.0	1	0	0.000	1.0	1.5	.75
8.0 - 8.5	1	1	1.000	.5	.5	.25
8.5 - 9.0	0	0	-	-	-	-

APPENDIX II (CONT'D)

Dietary Restriction = 36 hrs (T = 18°C; N = 24)

x	lx	dx	qx	Lx	Tx	ex
0.0 - 0.5	24	0	0.000	24	277.0	5.77
0.5 - 1.0	24	0	0.000	24	253.0	5.27
1.0 - 1.5	24	1	0.041	23.5	229.0	4.77
1.5 - 2.0	23	0	0.000	23	205.5	4.46
2.0 - 2.5	23	0	0.000	23	182.5	3.96
2.5 - 3.0	23	2	0.086	22	159.5	3.46
3.0 - 3.5	21	1	0.047	20.5	137.5	3.27
3.5 - 4.0	20	0	0.000	20	117.0	2.92
4.0 - 4.5	20	0	0.000	20	97.0	2.42
4.5 - 5.0	20	0	0.000	20	77.0	1.92
5.0 - 5.5	20	2	0.100	19	57.0	1.42
5.5 - 6.0	18	7	0.388	14.5	38.0	1.05
6.0 - 6.5	11	2	0.181	10.0	23.5	1.06
6.5 - 7.0	9	4	0.444	7.0	13.5	.75
7.0 - 7.5	5	1	0.200	4.5	6.5	.65
7.5 - 8.0	4	4	1.000	2.0	2.0	.25
8.0 - 8.5	0	0	-	-	-	-
8.5 - 9.0	0	0	-	-	-	-

APPENDIX II (CONT'D)

Dietary Restriction = 48 hrs (T = 18°C; N = 24)

x	lx	dx	qx	Lx	Tx	ex
0.0 - 0.5	24	0	0.000	24	179.5	3.73
0.5 - 1.0	24	0	0.000	24	155.5	3.23
1.0 - 1.5	24	4	0.166	22	131.5	2.73
1.5 - 2.0	20	1	0.050	19.5	109.5	2.73
2.0 - 2.5	19	5	0.263	16.5	90.0	2.36
2.5 - 3.0	14	0	0.000	14	73.5	2.62
3.0 - 3.5	14	2	0.142	13	59.5	2.12
3.5 - 4.0	12	0	0.000	12	46.5	1.93
4.0 - 4.5	12	2	0.166	11	34.5	1.43
4.5 - 5.0	10	1	0.100	9.5	23.5	1.17
5.0 - 5.5	9	4	0.444	6.5	14.0	.77
5.5 - 6.0	5	1	0.200	4.5	7.5	.75
6.0 - 6.5	4	3	0.750	2.5	3.0	.37
6.5 - 7.0	1	1	1.000	.5	.5	.25
7.0 - 7.5	0	-	-	-	-	-
7.5 - 8.0	0	-	-	-	-	-
8.0 - 8.5	0	-	-	-	-	-
8.5 - 9.0	0	-	-	-	-	-

APPENDIX II (CONT'D)

Dietary Restriction = 60 hrs (T = 18°C; N = 24)

x	lx	dx	qx	Lx	Tx	ex
0.0 - 0.5	24	0	0.000	24	199.0	4.14
0.5 - 1.0	24	1	0.041	23.5	175.0	3.64
1.0 - 1.5	23	0	0.000	23	151.5	3.29
1.5 - 2.0	23	1	0.043	22.5	128.5	2.79
2.0 - 2.5	22	3	0.136	20.5	100.0	2.40
2.5 - 3.0	19	1	0.052	18.5	85.5	2.25
3.0 - 3.5	18	3	0.166	16.5	67.0	1.86
3.5 - 4.0	15	2	0.133	14	50.5	1.68
4.0 - 4.5	13	2	0.153	12	36.5	1.40
4.5 - 5.0	11	2	0.181	10	24.5	1.11
5.0 - 5.5	9	4	0.444	7	14.5	.80
5.5 - 6.0	5	2	0.460	4	7.5	.75
6.0 - 6.5	3	2	0.666	2	3.5	.58
6.5 - 7.0	1	0	0.000	1	1.5	.75
7.0 - 7.5	1	1	1.000	.5	.5	.25
7.5 - 8.0	0	-	-	-	-	-
8.0 - 8.5	0	-	-	-	-	-
8.5 - 9.0	0	-	-	-	-	-

APPENDIX II (CONT'D)

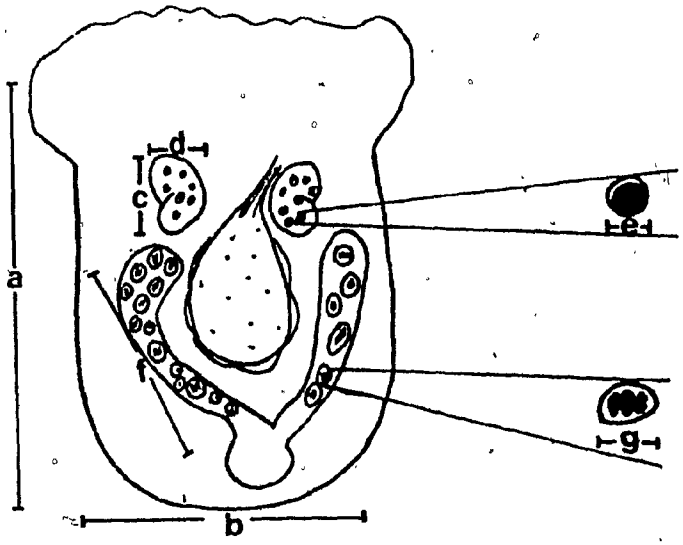
Dietary Restriction = 72 hrs ($T = 18^{\circ}C$; N = 24)

x	lx	dx	qx	Lx	Tx	ex
0.0 - 0.5	24	0	0.000	24	193.0	4.02
0.5 - 1.0	24	2	0.083	23	169.0	3.52
1.0 - 1.5	22	2	0.090	21	146.0	3.31
1.5 - 2.0	20	0	0.000	20	125.0	3.12
2.0 - 2.5	20	0	0.000	20	105.0	2.62
2.5 - 3.0	20	5	0.250	17.5	85.0	2.12
3.0 - 3.5	15	1	0.066	14.5	67.5	2.25
3.5 - 4.0	14	2	0.142	13	53.0	1.89
4.0 - 4.5	12	0	0.000	12	40.0	1.66
4.5 - 5.0	12	3	0.250	10.5	28.0	1.16
5.0 - 5.5	9	0	0.000	9.0	17.5	.97
5.5 - 6.0	9	6	0.666	6.0	8.5	.47
6.0 - 6.5	3	2	0.666	2.0	2.5	.41
6.5 - 7.0	1	1	1.000	.5	.5	.25
7.0 - 7.5	0	0	-	-	-	-
7.5 - 8.0	0	0	-	-	-	-
8.0 - 8.5	0	0	-	-	-	-
8.5 - 9.0	0	0	-	-	-	-

APPENDIX III

Size Measurements

This diagram shows how the various measurements were taken in the rotifer Asplanchna brightwelli.



a, b: length and width of body (at 96X).
c, d: length and width of gastric glands (at 96X)
e: diameter of nuclei of gastric glands (at 240X)
f: length of one side-arm (or ½) of vitellarium (at 240X)
g: diameter of several nuclei of vitellarium (at 240X)

Callibration of Ocular Micrometer (6X)

Power of Objective	Ocular Micrometer Units	Stage Micrometer mm	Stage Micrometer µm
6.3X	1	.200	200.0
ph2/16X	1	.080	80.0
ph2/40X	1	.032	32.0
ph2/100X (oil)	1	.013	13.3

APPENDIX IV

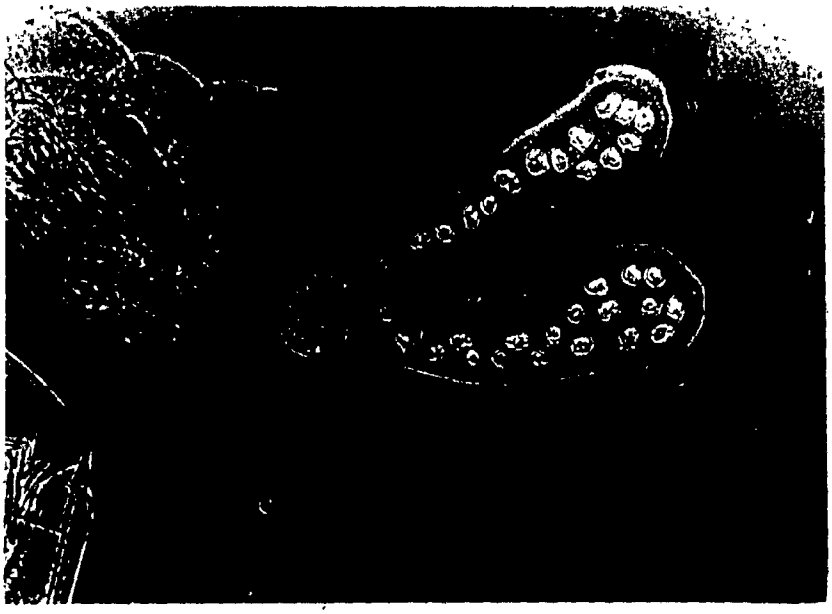
Photomicrographs taken with a Zeiss-Ultraphot II Phase Contrast Microscope. Kodak Panatomic-X (ASA 32) was used.

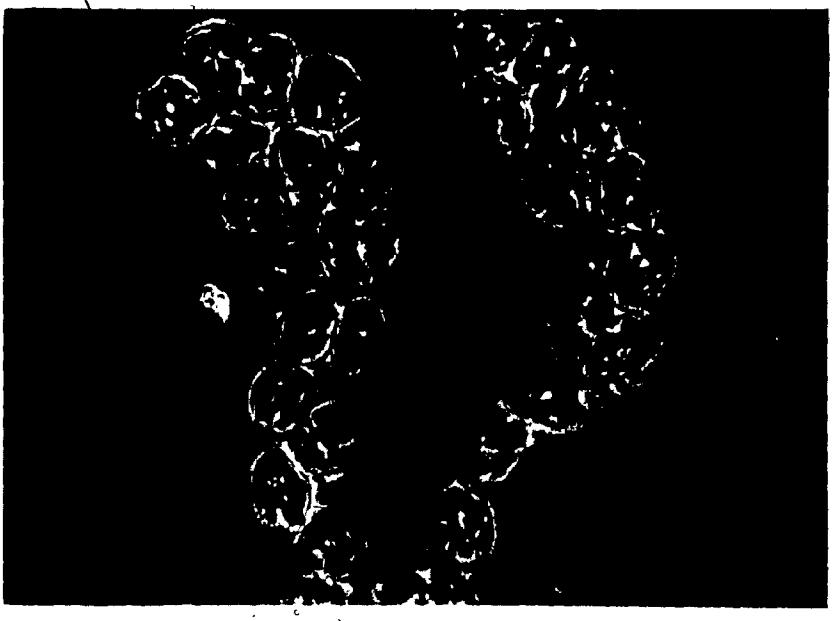
#1

Vitellarium of a 2-3 day old rotifer with about 30 nuclei present. Embryo develops in the ovary located at the junction of the 2 arms of the vitellarium. (Magnification = 160X)

#2

Vitellarium of 4-5 day old rotifer. Polyploid nuclei are big and can easily be counted at this and lower magnifications. (Magnification = 400X)



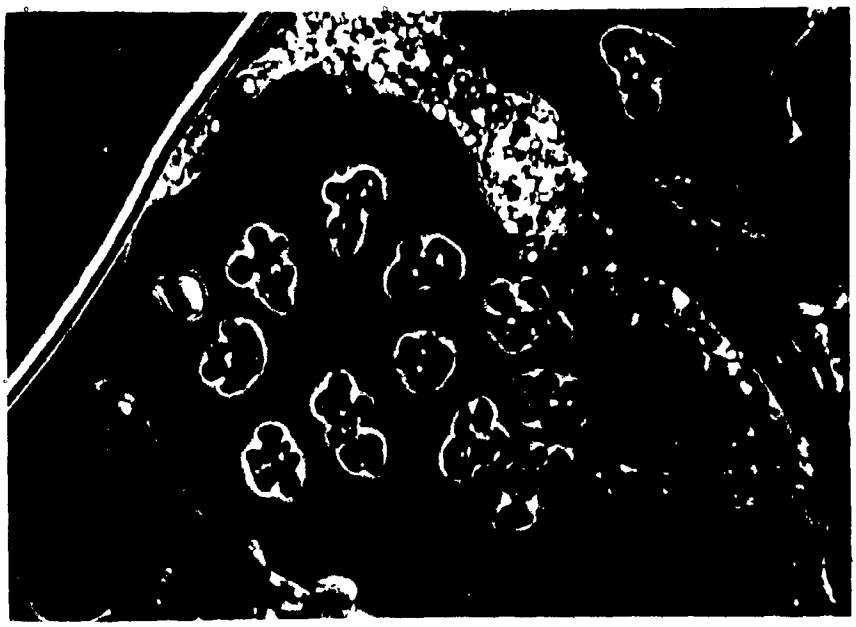


#3

Section of vitellarium of young rotifer with polyploid nuclei. Note absence of fat in the organ as compared to other photomicrograph below. (Magnification = 400X)

#4

Vitellaria of 6-7 day old rotifers. Note fat bodies in the vitellarium and large, fragmented nuclei. (Magnification = 400X)

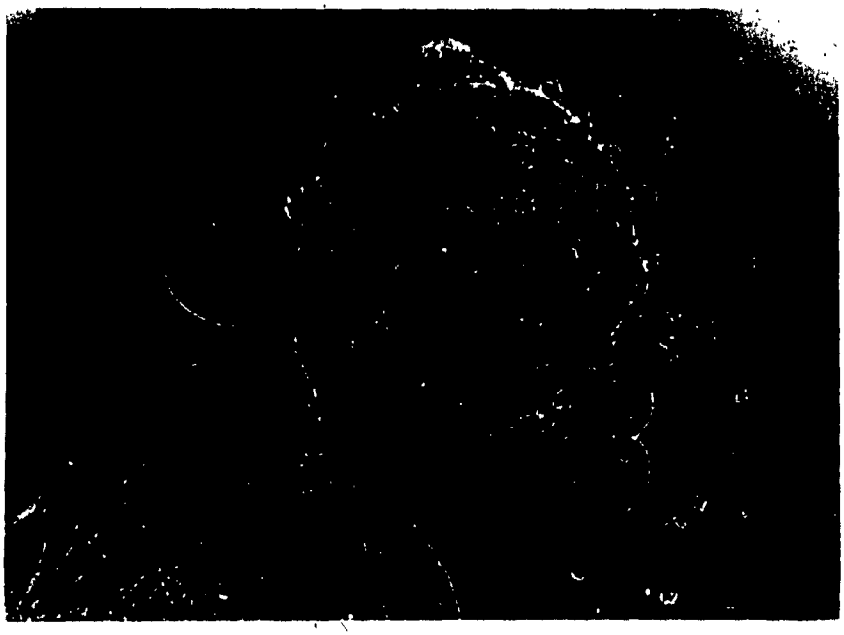


#5

Everted stomach with 2 gastric glands of 3-4 day old rotifer. About 10 small, dark nuclei are present in each gastric gland. (Magnification = 160X)

#6

Magnified gastric gland showing the small, round nuclei in a 3-4 day old rotifer. (Magnification = 400X)



#7

Sclerotized jaws (mastax) of Asplanchna brightwelli showing a) the ramus, b) the median fulcrum and c) a pair of manubrium. These parts are collectively called the trophi. (Magnification = 400X)

#8

Body of rotifer showing a) cuticular cells, b) nerve cells, c) muscle cells and d) sensory cells. (Magnification = 400X)