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An Investigation of Nitrogen Utilization as a Factor in the Size Selective Feeding Habits of Lepomis Macrochirus

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AN INVESTIGATION OF NITROGEN
UTILIZATION AS A FACTOR IN THE SIZE
SELECTIVE FEEDING HABITS OF LEPOMIS MACROCHIRUS

by

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Stephen M. Hinckley

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WESTERN MICHIGAN UNIVERSITY, M.A., 1978

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INTRODUCTION

Many fish culturists recognize the importance of the relationship between food supply and fish production. However, much of the effort in investigating this relationship has been directed towards artificial food sources.

In the natural environment, planktonic organisms play an important role in the aquatic ecosystem. Planktonic crustacea, such as cladocerans and copepods, feed on phytoplankton and bacteria in aquatic systems. In turn, these entomostracans are consumed by fish which use the nutrients acquired from these organisms for maintenance, activity and growth. The zooplankton community contributes significantly to fish production and should, therefore, be given greater attention in fish production studies.

The consumption of zooplankton by fish is not a random process. Fish demonstrate definite preferences for specific sizes of zooplankton available to them in their natural habitats and in laboratory studies. (Lindstrom, 1955; Gerking, 1962; Brooks and Dodson, 1965; Grygierek et al., 1966; Reif and Tappa, 1966; Galbraith, 1967; Brooks, 1968; Hall et al., 1970; Wells, 1970; Hutchinson, 1971; Werner and Hall, 1974.)

The object of this study was to examine the size selective feeding habits of bluegill sunfish, (Lepomis macrochirus), and to determine if this selectivity is correlated with greater protein content in the prey selected and/or greater protein absorption by the fish.

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LITERATURE REVIEW

Planktonic organisms are an important source of food available to fish. During the first year of growth many fish are dependent upon zooplankton for nutrition. After the first year of growth, most fish have attained a size which allows them to consume larger organisms, Werner (1974) believes the graduation to larger food particles is primarily a result of increase in mouth gape.

Several investigations have indicated that fish will continue to select zooplankton as a food source after the first year of growth. Adult ciscoes, (Leucichthys artedi), and black crappies, (Pomoxis nigromaculatus), were found to consume primarily Daphnia pulex (Hall, 1964). Brooks and Dodson (1965) wrote that alewives, (Alosa pseudoharengus), and glut herring, (Alosa aestivalis), fed exclusively on the larger zooplankton species in Connecticut lakes. Galbraith (1967) presented evidence that both rainbow trout, (Salmo gairdneri), ranging in size from 20.1 cm to 43.7 cm and yellow perch, (Perca flavescens), with a size range of 7.1 cm to 24.9 cm, consumed cladocerans. Smelt, (Osmerus mordax), were reported by Reif and Tappa (1966) to select cladocera as food. Ball (1948) reported that adult bluegill sunfish exhibited a preference for insect larvae, however, a considerable number of zooplankton were also noted in the stomach contents of these bluegills. Adult bluegills of Wyland Lake, Indiana were determined to feed upon zooplankters also (Gerking, 1962). Moffett and Hunt (1943) concluded that bluegills of Cedar

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Lake, Michigan consumed primarily zooplankton during the winter months.

Although fish do exhibit tendencies towards selection of zooplankton for food, the more pronounced phenomena is their selection of the zooplankters with respect to size. There is no indication that fish indiscriminately select any plankter that is found in their habitat. Observations show that there is a marked preference towards those available planktonic organisms which are the largest (Gerking, 1962; Brooks and Dodson, 1965; Reif and Tappa, 1966; Galbraith, 1967; Hall, Cooper and Werner, 1970; Hutchinson, 1971).

Brooks and Dodson (1965) reported that after the introduction of glut herring into Crystal Lake, Connecticut there was a distinct change in the plankton population. This change was likely a result of predation by the glut herring population.

Prior to the introduction of herring, the zooplankton community of Crystal Lake was comprised primarily of Daphnia, Diaptomus and Mesocyclops. All of these genera being large forms of Entomostraca. Ten years following the introduction of herring, the plankton community was again sampled. The dominant zooplankton at this time was Bosmina longirostris, Cyclops bicuspidatus, and Tropocyclops prasinus. All of these are smaller than the previous dominant species, with the exception of C. bicuspidatus, which is similar in size to Diaptomus. The authors felt that this was the result of the tendency of C. bicuspidatus to concentrate in the benthic and littoral waters. The herring, being a pelagic feeder, would thus place less predation pressure on this particular species.

Similar results were obtained when another member of the herring family was examined. An alewife, (*Alosa pseudoharengus*) population was established in Black Pond, New York. Prior to the establishment of the alewife population the dominant Cladocera and copepods were; *Epischura lacustris*, *Diaptomus minutus*, *Mesocyclops edax*, *Daphnia catawba* and *Leptodora kindtii*. Following the introduction of the alewife population smaller forms, *B. longirostris* and *T. prasinus*, became the dominant species. Several large species of copepods, *Cyclops vernalis* and *Holopedium gibberum*, were also observed at this time. According to Hutchinson (1971), the selective predation by the alewives brought about the elimination of most of the large species of zooplankton. Those large zooplankters eliminated were replaced by smaller species and some larger species. These larger species were able to survive through their escape movements and habitat selection.

The examination of the size selective feeding habits of fish is facilitated by observing obligate planktivores such as alewives and glut herring. These are excellent subjects because of their selection of plankton as food, and their preference for larger zooplankton species. Other species of fish which are classified as piscivores or insectivores also show a distinct interest in plankton. These facultative planktivores also exhibit the capability to discriminate among sizes of zooplankton available to them in their foraging habits (Brooks, 1968; Morsell and Nordan, 1968).

Evidence of differential predation was noted in Harveys Lake, Pennsylvania after a population of smelt had been introduced (Reif and Tappa, 1966). Prior to the introduction of smelt the dominant

zooplankton species was D. pulex with a limited number of Leptodora also occurring. Six years following the planting of smelt the cladoceran population consisted of Daphnia dubia, which had not been observed in earlier samples, and D. pulex. At this time D. dubia was more numerous than D. pulex. Ten years following the introduction of smelt the only cladocerans found in plankton samples were D. dubia and Bosmina longirostris. This demonstrated that along with the change in the dominant species of cladoceran occurring in the lake there was a corresponding decrease in the size of the dominant cladoceran species.

Two lakes in Michigan, Sporley Lake and Stager Lake, were used by Galbraith (1967) to examine the impact that two facultative planktivores had upon a planktonic community. In the fall of 1955 Sporley Lake was treated with toxaphene to remove the fish fauna. At the same time the toxaphene treatment eliminated the zooplankton population of the lake. The year following the toxaphene treatment D. pulex established a population in this lake. Sampling of the population in subsequent years; 1957, 1958, 1959, revealed that the population of D. pulex ranged in size from .4 mm to 2.9 mm with the mean size being 1.5 mm.

In the fall of 1959 rainbow trout were introduced into Sporley Lake. The following year a population of fathead minnows, (Pimephales promelas), was detected. Smelt were illegally introduced into the lake in 1962.

The primary species of fish in Stager Lake prior to the experiment were yellow perch, bluegills, smallmouth bass, (Micropterus

dolomieui), common suckers, (Catostomus commersoni) and pumpkinseeds, (Lepomis gibbosus). In the fall of 1958 rainbow trout were added to the species of fish found in Stager Lake.

To examine the foraging habits of the rainbow trout and yellow perch Daphnia from net and stomach samples were examined for species and size. The size of the Daphnia, in combined samples from both lakes, was .4 mm to 2.9 mm. Examination of the stomach samples disclosed that yellow perch and rainbow trout usually chose Daphnia over 1.3 mm in size, with 96 percent of the Daphnia consumed by the trout and 82 percent consumed by the perch being larger than 1.3 mm. This was strong evidence supporting the size selective nature of the feeding habits of these fish.

Comparing the size range of the Daphnia available to the trout and the perch in Galbraith's study to the size of those daphnids actually consumed indicated that the trout and the perch used some manner of discrimination among sizes in the daphnid population. One popular theory was that gill-raker spacings would give an indication to the size of the planktonic organism which would be filtered out of the water column and consumed by the fish. Galbraith measured gill-raker spacings in both yellow perch and rainbow trout. He found that a large number of the gill-raker spacings were of a size that should have allowed for filtering daphnids smaller than those found in the stomach samples. Galbraith further stated that he had observed schools of trout feeding on Daphnia which were concentrated along the shore of Johnson Lake, Michigan. Closer examination of the concentration of Daphnia revealed that the larger Daphnia were floating

along the surface of the water and the smaller individuals were below
the surface. On examining the stomach contents of these trout it was
established that the trout were only feeding on the larger organisms.
Thus there is some indication that visual discrimination plays an
important role in size selective predation. The importance of visual
discrimination is also emphasized in Werner and Hall's work (1974).

Evidence has been presented by previous workers that bluegill
sunfish selectively feed upon planktonic crustacea. Of the Entomo-
straca selected, the major organisms consumed were Cladocera and cope-
pods (Ewers and Boesel, 1935; Leonard, 1939; Moffett and Hunt, 1943;
Ball, 1948; Gerking, 1962; Turner, 1955).

The size selective feeding by Wyland Lake bluegills was investi-
gated by Gerking (1962). He compared the species and size of the
Entomostraca found in the stomach with those in the lake. His results
revealed that bluegills select Cladocera and copepods on the basis of
size. Those Daphnia taken in the plankton samples varied in size
from .3 mm to 1.8 mm, with the mode being .7 mm to .8 mm. The size
of the Daphnia in the stomach samples ranged from .5 mm to 1.8 mm
with the mode of 1.11 mm to 1.12 mm. Size distribution for the
copepods varied in the same manner. Gerking also pointed out that
the small cladoceran, Bosmina, was prevalent in the plankton samples,
but, none were found in stomach samples.

The impact of bluegills on the size and community structure of
Crustacea was not fully examined until Hall, Cooper and Werner (1970)
presented a study examining production and structure in aquatic
communities. In their study bluegills varying in age from fry to

adults were introduced into experimental ponds with previously established aquatic communities and varying nutrient levels. These ponds were manipulated by the investigators. From net plankton samples taken prior to fish stocking, it was determined that the dominant zooplankton in each pond was <u>Ceriodaphnia</u>. Examination of the net plankton samples following the introduction of bluegills revealed that in those ponds not stocked with bluegills, <u>Ceriodaphnia</u> comprised an average of 53 percent of the total biomass. In ponds containing bluegill populations the <u>Ceriodaphnia</u> comprised an average of three percent of the total biomass. Thus there is good indication that the bluegill sunfish uses some form of discrimination when selecting its prey.

There is a great deal of literature discussing the feeding habits of fish. Most of this work is directed towards the identification of organisms which are consumed by fish. Recent literature has taken previous work and advanced it one step further. Workers are now, not only concerned with those organisms consumed by fish, but are also interested in determining the impact that fish have upon the aquatic community. Research has been conducted on the aquatic communities of lakes and ponds which indicates that many fish are size selective when consuming prey.

It is, therefore, important to determine if fish obtain any nutritional advantage through the selection of specific sized zooplankton as food. Much of the work to date concerning nutritional value of fish foods has been conducted on artificial foods employed

by fish culturists to increase fish production. Of importance in the aquatic ecosystem is the use of natural food organisms by fish for maintenance and growth. The use of natural food organisms by fish for maintenance and growth has been examined by several workers (Birkett, 1969; Gerking, 1952, 1954, 1955, 1971; Pandian, 1967; Ricker, 1949; Savitz, 1969, 1971).

The simplest type of experiment to be performed along these lines is one where the weight of the food consumed by the fish is compared to the weight gain and increase in length of the fish. This is basically what Ricker (1949) did when examining the utilization of food by bluegills for growth. Initially the experimental fish were weighed and their length recorded. The fish were then fed weighed aliquots of earthworms. During the course of the experiment the fish were weighed and their length measured twice a week. At the end of the experiment the increase in growth of the fish was correlated with the amount of food consumed.

Experiments, such as the one mentioned above, provide data on the actual physical growth acquired by fish while feeding on specific food. However, it does not provide relative efficiency data as to what part of the food is actually absorbed by the fish and used for growth or metabolic waste.

A classic experiment devised to examine food utilization by fish was the nitrogen balance sheet experiment described by Karzinkin and Krivobok (1964). The nitrogen balance method has been widely used by Soviet fishery scientists whose works have been reviewed by

Winberg (1956). Other workers have used the nitrogen balance experiment in determining the protein requirements and deposition in fish (Birkett, 1969; Gerking, 1952, 1954, 1955, 1962, 1971; Pandian, 1967; Savits, 1969, 1971).

Nitrogen balance sheet experiments are important for the examination of protein requirements and utilization in an organism. When fish are the experimental animals being investigated, the amount of nitrogen taken in with the food, as well as the amount of nitrogen excreted in the feces, the urine and through the gills is measured. The amount of nitrogen deposited in the body resulting from growth is determined by examining the difference between the average nitrogen content in the body of the fish at the initiation of the experiment and at the completion of the experiment. Comparing these values allows for the determination of the amount of nitrogen that is digested, absorbed, excreted and converted into body tissues as growth. This can be accomplished by applying the above values to the equation proposed by Warren and Davis (1967): $C = F + U + \Delta B + R$, where C equals the energy value of the consumed food, F equals the energy value of the feces, U represents the energy value of the materials excreted in the urine or through the gills and skin, ΔB is the total change in the energy value of the materials of the body (growth), and R is the total energy of metabolism. This equation is applicable to nitrogen balance studies if all terms are expressed in nitrogen concentrations and if R is redefined as the nitrogenous products excreted as the result of metabolic processes and respiration. R would then be incorporated into the term U. Leaving the

following equation: $C = F + U + \Delta B$, with C equaling the amount of
nitrogen in consumed food, F equal to the nitrogen in feces, U being
the amount of nitrogen lost in the form of urea via the kidney and
ammonia by the gills and ΔB representing the total change in nitrogen
content of the body (growth). Birkett (1969) defined a similar
equation for nitrogen balance studies which includes a value for the
amount of nitrogen which is absorbed:

> Total N consumed - fecal N = N absorbed = N retained + N
> excreted.

This formula can be condensed and rearranged to:

> Total N consumed = fecal N + N excreted + N retained

which is the same as the above modification of Warren and Davis.

Therefore, it is possible to determine the amount of nitrogen
which is retained for short term growth by knowing the amount of
nitrogen in the food consumed, the amount of nitrogen lost in the
feces and that excreted via the kidney and gills.

MATERIALS AND METHODS

This study included two separate experiments. 1) an investigation of the size selective feeding habits of bluegills and 2) the utilization of protein nitrogen from natural foods by bluegills. The organisms presented to the bluegills in each experiment were cladocerans. Four species of Cladocera were used in the study. The selection of these crustaceans was dependent upon their size. Table 1 lists the species and size range of the Cladocera used.

Table 1
Species and Size Range
of Cladocera Used

Species	Size Range (Edmondson, 1966)
Daphnia magna	2.0mm - 5.0mm
Daphnia pulex	1.3mm - 2.2mm
Ceriodaphnia reticulata	.6mm - 1.4mm
Chydorus sphaericus	.3mm - .5mm

All of the Crustacea, with the exception of D. magna, were initially obtained from either Wolf Lake, Van Buren County, Michigan or Little Asylum Lake, Kalamazoo County, Michigan. D. magna was originally obtained from American Science and Engineering Corporation of Boston, Massachusetts. The initial samples of Cladocera obtained were cultured under laboratory conditions. Cultures of D. magna and D. pulex were maintained in 38 liter and 57 liter aquaria.

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C. reticulata and C. sphaericus were cultured in four liter glass jars or 16 liter plastic containers. All cultures were placed in indirect sunlight and were maintained on a natural light cycle throughout the duration of the experiment. The media on which the zooplankton were maintained was a mixed culture of Chlamydomonas sp. and Chlorella sp..

To maintain a constant supply of algae it was necessary to culture the algae in the laboratory. During the summer of 1975 two methods were employed, Pringsheim's Biphasic Soil-water Medium, and Bold's Basal Medium and Modifications (Bold, 1967). Both methods produced enough algae to maintain cultures of the cladocerans in quantities sufficient to run the size selective feeding experiment. However, neither method produced enough algae to stimulate the large growths of Cladocera needed in the nitrogen utilization phase of this study. Both media also became contaminated with molds that interfered with algal growth, therefore, it was necessary to find another means of culturing the algae in sufficient quantities to yield greater growth in the zooplankton cultures.

It was discovered that if a quantity of the mixed algae was introduced into an aquarium which contained a population of fish that the nutrients in the waste products of the fish were sufficient to produce an algal bloom within 10 to 14 days. Abundant algal growth was obtained using a total of 30 centrachids and Notropis sp., none longer than six centimeters, in a 189 liter aquarium with aerated, aged tap water. The fish were fed maintenance rations of Tetra-Min Flakes three times a day. After a period of two weeks four liters of the mixed algal culture were introduced into the aquarium. Within

two weeks following the introduction of the algae, dense growths of algae were obtained. It was found that if 57 liters were removed from the algal culture weekly and replaced with 57 liters of aged tap water the algal culture would continue to grow in profuse quantities.

The best results for growth and population numbers of D. magna and D. pulex were achieved when the plankton were placed in containers containing algae taken directly from the algal culturing vessels. To keep the algae concentrated in the D. magna and D. pulex culture containers eight liters of water were siphoned from the cladoceran aquaria every other day and replaced with eight liters of algae taken from the algal culturing containers.

Ceriodaphnia and Chydorus were more difficult to culture. These two cladocerans had to be cultured in containers having a capacity of no more than 16 liters. Neither of these organisms tolerated introduction into undiluted algae taken directly from the algal culturing vessels. It was, therefore, necessary to keep the algae diluted. This was accomplished by introducing both Ceriodaphnia and Chydorus into containers containing one liter of undiluted algae for every four liters of aged tap water. Every third day five liters of water were removed from the Ceriodaphnia and Chydorus culturing containers and replaced with an equal amount of the above mentioned diluted algae solution. The Ceriodaphnia and Chydorus cultures did not respond as favorably as the D. magna and D. pulex cultures, and as a result were not cultured in sufficient numbers to use throughout the entire study. However, the culturing methods used did provide

adequate numbers of these two cladocerans for use in the size selective feeding experiment.

The standard length of the bluegills used in this study was 40 mm to 50 mm. The standard length was considered as the distance from the most anterior part of the head to the end of the vertebral column. The length of the fish was determined at the time of collection. The length of the fish used in the size selective feeding experiment was also determined at the time of the experiment.

The fish used in the size selective feeding experiment were obtained from Dr. Earl Werner of the W. K. Kellogg Biological Station, Hickory Corners, Michigan. These fish were seined from research pond number two and immediately transferred to the Warm Water Development Building at Wolf Lake State Fish Hatchery. The bluegills were maintained in a fiber glass trough in aerated well water at $14^{\circ}C$ to $17^{\circ}C$. The exchange rate of the water was three liters per minute.

These bluegills fed daily on plankton obtained from Wolf Lake, which consisted primarily of D. pulex and copepods. The zooplankton were collected by net and concentrated in a 20 liter bucket until the bottom of the bucket could no longer be seen. The plankton were then introduced into the fish trough. The collection of the zooplankton and the feeding process was conducted in the evening prior to sunset. It was observed that a few zooplankton were still present 10 to 15 hours later and that the fish were foraging on the remaining plankters. The feeding process was discontinued 24 hours prior to each size selective feeding experiment.

The aquaria used in the size selective feeding experiment were 40 liters in capacity. Two bluegills were introduced into each aquarium containing 40 liters of aerated well water. The fish were allowed to acclimate to the experimental containers for four hours prior to beginning the experiment.

To ensure that the zooplankton presented to the fish were of the appropriate size, each species was filtered through "Nitex" monofilament nylon screening having a specific mesh opening. The size of the mesh was selected so as to prevent any overlapping among the sizes of the species presented to the fish. The size of the mesh used to filter D. magna was 2.38 mm, that for D. pulex was 1.32 mm, for C. reticulata .63 mm and for C. sphaericus .31 mm.

The filtering procedures involved siphoning zooplankton from the appropriate culture and passing the water and plankton through the specific sized "Nitex" screen. Organisms that were too small passed through the screen and were collected in another container. Plankters of the correct size did not pass through the screen and were concentrated on the top of the "Nitex" screening. When it appeared that there were sufficient numbers, the screen was quickly immersed in a large beaker of water and gently agitated to ensure that all the plankters were removed from the screen. After this process the zooplankton were counted and pipetted into another container from which they were introduced into the aquarium containing the experimental fish.

Twenty zooplankton, of each species, per fish were introduced

into the experimental aquarium. For each aquarium, all four cladoceran

species were mixed in one container prior to the beginning of the

experiment. The contents of this container were poured into the

middle of the aquarium along the long axis, to avoid concentrating

the zooplankton in one central area.

The fish were allowed to forage for 15 minutes. At the end

of this time the fish were removed and sacrificed by cutting the

spinal cord just posterior of the head. The stomach was removed and

an incision made along the greater curvature. The contents of the

stomach were preserved in a five percent formalin solution. The

esophagus and mouth were examined for the presence of any zooplankton

that may have been regurgitated. Zooplankton present in these areas

were added to the preserved stomach contents.

Examination of the stomach contents was performed with a com-

pound microscope. Identification of those species present was

accomplished with the aid of Fresh-water Biology (Edmondson, 1966).

Because of the short time span that the organisms were in the fish

stomach, most of the species could be identified by size and carapace

shape. Those plankton which were crushed or broken during ingestion

or removal of the stomach contents were identified by examination of

the anal spines or combs. Only those larger species used, D. magna

and D. pulex, were damaged and as a result were identified by this

latter method. The smaller species, C. reticulata and C. sphaericus,

were not observed to be damaged and were, therefore, easily identified.

The bluegills used in the nitrogen utilization phase of this study were obtained from Three Lakes, Kalamazoo County, Michigan. Only enough fish were obtained at the time of each collection to run one experiment. Each experiment consisted of running one group of controls and one or two groups of experimental fish. Each group was composed of 17 to 22 fish.

The fish were transported back to the laboratory, divided into two or three equal groups and placed in two or three 40 liter aquaria containing 20 liters of aerated tap water which had been aged for a minimum of 24 hours. The temperature of the water varied from 18°C to 21°C. Every second day the water in the aquaria was siphoned out and replaced with aged tap water to prevent fouling. Each time the water was changed the amount replaced was decreased by 4.6 liters. After the final changing there were only six liters of water in each aquarium. This was done to accustom the fish to the low water levels used in the experiments. The fish were acclimated to laboratory conditions for seven days. During this time they were fed daily on zooplankton obtained from Wolf Lake. A period of fasting was initiated 24 hours prior to the beginning of each experiment.

On the day that a nitrogen utilization experiment began, the bluegills were transferred from the holding aquaria to two or three clean 40 liter aquaria containing six liters of aerated, aged tap water. Because of this transfer the fish became excitable and it was necessary to allow them to stabilize for a period of two hours before introducing the zooplankton. The sides of each aquarium were

covered with paper to prevent each group from observing another group

and to reduce the probability of startling the fish when approached

quickly.

The zooplankton used in the nitrogen experiment were either $\underline{D}$.

$\underline{magna}$ or $\underline{D}$. $\underline{pulex}$. Neither $\underline{C}$. $\underline{reticulata}$ nor $\underline{C}$. $\underline{sphaericus}$ could

be cultured in quantities large enough for this experiment. Both of

the plankters used were collected from the laboratory cultures as

described previously. Three, or four, two gram samples of cladocerans

were obtained for each nitrogen utilization experiment. Two of the

samples were used to determine the nitrogen content of the plankton.

The remaining samples were fed to the experimental fish. The

cladocerans were presented to the fish by submerging the "Nitex"

screen in the experimental tank and agitating gently to remove all of

the plankters from the screen. The fish immediately began to forage

following introduction of the zooplankton into the aquaria. Periodic

examination of the aquaria revealed that the cladocerans introduced

into the aquaria were consumed within 45 minutes. The fish remained

in the aquaria for 24 hours to ensure that all food consumed had

passed through the digestive system. Windell (1966) reported that

natural food organisms were completely digested by bluegills in 18

hours.

No plankton were presented to the control fish. However, they

remained in the aquarium for the same period of time as the experi-

mental fish.

After the 24 hour time period the experimental fish were removed

from the aquaria and sacrificed. An incision was made in the

abdominal cavity on the ventral side. The anterior end of the small intestine was ligated at the pyloric valve and the posterior end of the large intestine was cut at the urogenital sinus. The intestine was then cut just anterior to the pyloric valve and removed. Removal of the contents of the intestine was accomplished by carefully sliding a finger or surgical probe along the intestine forcing the contents out the posterior end of the intestine. Intestinal contents for each group of fish were collected on separate pieces of Munktell's number 1F, 5.5 cm, filter paper. These samples were examined for cladoceran remains to ensure that digestion was complete.

A 20 ml sample of water was removed from each aquarium and analyzed for the amount of nitrogen excreted from the kidneys and gills of the fish. Fecal matter was collected from the aquaria by siphoning the water through Munktell's number 1F, 11 cm, filter paper and a Buchner funnel. After filtration the filter paper was examined for the presence of scales dislodged from the fish during the capturing process. These were removed to prevent any erroneous readings in the nitrogen content of the feces. It was observed that mucous was also present on the bottom of the aquaria along with the fecal matter. This was either mucous from the lining of the intestinal tract which was excreted or mucous secreted through the skin of the bluegills. The mucous was analyzed along with the fecal matter for nitrogen content for it was impossible to isolate it from the feces.

Examination of the feces revealed that much of it appeared to be small pieces of chitin from the zooplankton exoskeletons.

The determination of nitrogen in the samples taken was accomplished using direct nesslerization (Koch and McMeekin, 1924 and Wicks, 1941). The samples were digested in the following manner:

1. Weighed samples were placed in 30 ml micro Kjeldahl flasks.

2. Two milliliters of concentrated sulfuric acid (H_2SO_4) and two glass boiling beads were added.

3. The flasks were placed on a Rotary Kjeldahl Digestion Apparatus (American Instrument Company) and heated until white fumes from the sulfuric acid filled the flask (approximately 30 minutes).

4. Once the white fumes formed, the flasks were removed from the heat source and allowed to cool.

5. After cooling, three drops of concentrated hydrogen peroxide (30% H_2O_2) were added.

6. The contents were heated for 15 minutes, removed from the heat source and allowed to cool.

7. Three more drops of 30 percent peroxide were added to each flask and the contents heated again for 15 minutes (usually following the second addition of peroxide the contents of the digestion flask became colorless during the heating process. When this was observed the flask was left on the heat source for an additional 15 minutes to drive off the excess peroxide).

8. If the digestion solution did not become colorless, the peroxide process was repeated.

9. Once the solution in the digestion flasks became colorless
   they were allowed to cool.

10. Ten milliliters of ammonia free water were then added to
    each flask followed by the addition of 10 ml of six normal
    sodium hydroxide (6N,NaOH).

11. The contents of the digestion flasks were then diluted to
    250 ml with ammonia free water.

The heat used in the digestion procedures was very high and
vigorous boiling of the solutions occurred. When the digestion
process was performed on the water samples, the heat was initially
lowered until boiling occurred to avoid bumping.

The weight of the samples of feces and cladocerans varied with
each experiment. This was because different numbers of fish were
used for each experiment and different weights of zooplankton were
fed to the fish in each experiment. The feces were collected on
Munktell's 1F filter paper necessitating the determination of the
amount of nitrogen contained in the filter paper.

Twenty-five samples of Munktell's filter paper were analyzed for
nitrogen content and an average value was obtained. Using this
value and the dry weight of the filter paper used for each nitrogen
determination it was possible to subtract the amount of nitrogen in
the filter paper from that obtained for the total sample.

The Nesslers reagent used in the nitrogen determinations was
prepared by dissolving 51 grams of potassium iodide, analytical
reagent quality (KI, AR) in 200 ml of ammonia free water. Sixteen
and two-tenths grams of red mercuric oxide powder (HgO, AR), in small

portions, was added to the KI solution. This solution was stirred on

a hot plate until all of the HgO was dissolved. After allowing the

solution to cool, 160 ml of 50 percent NaOH was added and the solution

was made to one liter with ammonia free water (ibid.).

Ammonium sulfate $(NH_4)_2SO_4$, which had been dried in a dessicator,

was used to prepare the solution used as the nitrogen standard. This

was made by dissolving 4.719 grams of $(NH_4)_2SO_4$ in ammonia free water

and bringing the volume to one liter. One half milliliter of con-

centrated H_2SO_4 was added as a preservative before the one liter mark

was reached. One milliliter of this stock solution was diluted to

100 ml to give a working standard of 10 micrograms per milliliter.

The process of direct nesslerization consisted of transferring

one milliliter aliquots of the digested samples into test tubes.

Ammonia free water was then added to bring the volume to six milliliters.

Three milliliters of Nesslers reagent was added to this and the con-

tents mixed thoroughly. For the blank, one milliliter of ammonia

free water was used. The concentration of the standards was dependent

upon which sample was being examined. That is, when fecal samples

were being examined the standards were 5, 10 and 20 micrograms per

milliliter. However, when examining the water samples the standards

were .2, .5 and one microgram per milliliter. All samples and

standards were run in duplicate. The optical density of each sample

was measured in a Beckman Spectrophotometer (model H420-H15) at a

wavelength of 415 millimicrons. All measurements were made between

10 and 30 minutes after mixing.

In evaluating the concentration of nitrogen in the zooplankton it is important to consider the amount of chitin in their exoskeletons. Chitin is composed of 6.9 percent nitrogen (Richards, 1951). Therefore, when examining the amount of nitrogen digested and retained by the bluegill, the amount of chitin consumed with the food must be determined. This was accomplished by using approximately 1.5 grams wet weight of the cladocerans being investigated. All chitin assays were run with triplicate samples. The cladoceran samples were dried at $60^{\circ}C$ for 24 hours. The dried samples were then completely covered by a five percent acetic acid solution. The samples remained in this solution until bubbles ceased forming on the exoskeletons. Following this the samples were washed four times in distilled water and four times in 80 percent ethyl alcohol. This was followed by oven drying for 24 hours at $60^{\circ}C$. The dried, decalcified exoskeletons were then extracted in 2N NaOH for three to four hours at $110^{\circ}C$. The remaining residues were washed two times in 10 percent hydrochloric acid (HCl), four times in distilled water and four times in 80 percent ethyl alcohol followed by drying at $60^{\circ}C$ for 24 hours. The remaining colorless residue, which was chitin (Welinder, 1974), was then weighed. With this value it was possible to determine what percent of the dried zooplankton was chitin and how much nitrogen was tied up in the chitin. Because chitin is indigestible to fish, the value obtained for the amount of nitrogen present was subtracted from the total nitrogen content of the cladocerans and feces.

An experiment was conducted to examine the size selective nature of the feeding habits of bluegill sunfish ranging in size from 40 mm to 50 mm in standard length. In this experiment 30 fish were used in total. Each test in this experiment was conducted with one pair of bluegills of approximately the same size. For each test the fish were presented four different sizes of cladocerans from which to select. Twenty cladocerans of each size class per fish were introduced into the experimental tanks. The results of this experiment are summarized in Table 2. The fish demonstrated the greatest preference for D. magna, consuming an average of 16.40 D. magna per fish. D. pulex were preferred second to D. magna with an average of 13.37 consumed per fish. Next in descending order of preference was C. reticulata with an average of .63 per fish and least important was C. sphaericus with an average of .13. The results were statistically examined using a two group t-test to compare the mean values obtained. There was no significant difference (P > .05) between the mean values for D. magna and D. pulex. However, there were significant differences (P < .05) among all remaining paired combinations. Statistical results are listed in Table 3. The results indicate that the larger cladocerans, D. magna and D. pulex, were selected in preference to the smaller cladocerans, C. reticulata and C. sphaericus.

Table 2
Results of the Size Selective Feeding Experiment.
The Average Number of Cladocerans Consumed per Fish.

Cladoceran	Number of Cladoceran Consumed	Standard Deviation	Number of Fish
Daphnia magna	16.40	7.03	30
Daphnia pulex	13.37	6.46	30
Ceriodaphnia reticulata	.63	1.16	30
Chydorus sphaericus	.13	.35	30

Table 3
Statistical Comparison of the Mean Number
of Cladocerans Consumed per Fish in the
Size Selective Feeding Experiment.

t-values With Associated Probabilities

Cladoceran	D. magna	D. pulex	C. reticulata
D. pulex	1.741 $P = .087$		
C. reticulata	12.12* $P < .05$	10.63* $P < .05$	
C. sphaericus	12.66* $P < .05$	11.21* $P < .05$	2.26* $P = .027$

*These combinations are significantly different at $\propto$ equal to .05.

The remaining portions of this study were concerned with the quality of protein which was retained by the fish after consuming a

known weight of a specific cladoceran. This was accomplished by examining nitrogen concentrations. The initial tests in this phase of the experiment were aimed at determining the amount of nitrogen in each species of cladoceran.

Cladocerans are branchiopod crustaceans having an exoskeleton composed of chitin. As mentioned previously in this paper, chitin is not digested by fish. Chitin, therefore, passes through the digestive system in an unchanged state (Gerking, 1955). Richards (1951) revealed that 6.9 percent of chitin is composed of nitrogen. This means that the nitrogen present in the chitin is not available for growth or metabolic processes, therefore, it was necessary to assay the amount of chitin in each species of cladoceran used and from that value calculate the amount of nitrogen present in the chitin. The concentration of nitrogen in the chitin was then sub-tracted from the total nitrogen content of the zooplankton and the total nitrogen content of the solid excreta.

The chitin analysis revealed that 5.69 percent of the dry weight of D. magna was composed of chitin. For D. pulex chitin was 5.58 percent of the dry weight and for C. reticulata chitin composed 5.81 percent of the dry weight. The chitin analysis was not performed on C. sphaericus for insufficient numbers of this cladoceran were cultured. The results of the chitin assays were compared statistically with t-tests which showed no significant difference (P > .05) among any of the values (Table 5).

In analyzing the cladocerans for their nitrogen concentration it was not possible to examine C. sphaericus. Again this was the result

of the inability to culture sufficient numbers of this organism.

However, D. magna, D. pulex and C. reticulata were analyzed for their

nitrogen content. D. pulex had the largest value which was an average

of 82.87 mg of nitrogen per gram of the dried organism. C. reticulata

had an average value of 81.66 mg of nitrogen per gram of dry weight,

and D. magna had an average of 80.51 mg of nitrogen per gram of dry

weight. There was no significant difference noted among these values

$(P > .05)$ when examined with a t-test (Table 6).

Table 4
Results of Nitrogen Determinations
Performed on Cladocerans

Cladoceran	Percent Chitin	Total Nitrogen in Chitin per g Dry Weight of Organism	Total Nitrogen per g Dry Weight of Organism	Total Nitrogen in Cladoceran Minus Nitrogen in Chitin
D. magna	5.69	3.93 mg	84.44 mg	80.41 mg
D. pulex	5.58	3.85 mg	86.72 mg	82.87 mg
C. reticulata	5.81	4.01 mg	85.67 mg	81.66 mg

Table 5
Statistical Comparison of the Percent Chitin
Present in the Three Cladoceran Species Analyzed

Cladocerans Compared	t-value	Probability	Degrees of Freedom
D. magna vs D. pulex	.778	.480	4
D. magna vs C. reticulata	.996	.375	4
C. reticulata vs D. pulex	1.800	.146	4

Table 6
Statistical Comparison of the Total Nitrogen
Present in the Cladoceran Species Analyzed

Cladoceran Compared	t-value	Probability	Degrees of Freedom
D. magna vs D. pulex	.777	.460	8
D. magna vs C. reticulata	.618	.556	7
C. reticulata vs D. pulex	.374	.719	7

In order to determine the amount of nitrogen retained by the fish it was necessary to examine the solid excreta for total nitrogen content. This was facilitated by siphoning the water and the solid excreta from the experimental tanks and passing this through a Buchner funnel which was fitted with a piece of Munktell's number 1F filter paper which had been dried and weighed. It was necessary to analyze the filter paper, that the feces had been collected on, for nitrogen content as it was impossible to remove all of the dried excreta from it.

Twenty-five samples of Munktell's filter paper were analyzed for total nitrogen content. The results of this analysis showed that there were .181 mg of nitrogen per gram of dried filter paper. Using this value and the dry weight of the filter paper used in each experiment it was possible to calculate the amount of nitrogen in each piece of filter paper used. The appropriate value was subtracted from the results of the analysis of the total nitrogen present in the fecal matter. All results for total nitrogen found in the solid excreta in the remaining sections of this paper have this calculation taken into account.

Following the filtration step, the solid excreta and the filter paper it was collected on were dried and analyzed for total nitrogen content. The results of these analyses are presented in Table 7.

A t-test was used to compare the results of the analysis of the solid excreta. Comparison of the combined control groups of fish to the combined experimental groups of fish revealed that there was a significant difference between the two means ($P < .05$). No significant

difference was noted between the mean values of nitrogen in the feces of the fish fed $\underline{D}$. pulex and that in the feces of the fish fed $\underline{D}$. magna (P > .05) (Table 8).

Table 7
Results of the Analysis of the Feces
for Total Nitrogen Concentration

Group of Fish	Mean Value of Nitrogen in Feces (mg)	Number of Fish	Standard Diviation	Number of Analysis Performed
Combined Controls	1.066	209	.447	10
Combined Experimentals	1.569	300	.629	14
Experimentals Fed $\underline{D}$. pulex	1.261	147	.245	7
Experimentals Fed $\underline{D}$. magna	1.876	153	.760	7

Table 8
Statistical Analysis of the Results of the
Total Nitrogen Determination on the Feces

Groups Compared	t-value	Probability	Degrees of Freedom
Combined Controls vs. Combined Experimentals	2.16*	.042*	22
Experimentals Fed $\underline{D}$. pulex vs. Experimentals Fed $\underline{D}$. magna	2.03	.081	7

*Combinations significantly different at α equal to .05.

A good portion of the nitrogenous wastes of fish are lost through means other than in the form of solid excreta. These means include the secretion of nitrogenous wastes through the gills and the elimination of nitrogenous material in liquid form through the excretory system by the kidneys (Fromm, 1963; Smith, 1929; Wood, 1958). These forms of nitrogenous wastes are very soluble in water, therefore, to detect the amount of nitrogenous wastes lost in these ways it was necessary to analyze the water in which the fish were maintained during the experiment.

Table 9 summarizes the results of the analysis of the water for total nitrogen content. The water for the combined control groups had an average value of 2.43 mg of nitrogen. In comparison, the water in which the combined experimental groups were maintained had an average nitrogen concentration of 2.71 mg. These values were compared statistically using a t-test which revealed that there was no significant difference ($P > .05$) between them (Table 10). The water which contained the D. pulex fed fish had an average nitrogen concentration of 2.40 mg. The water which contained the bluegills fed D. magna had an average value of 3.02 mg of nitrogen. There was no statistical difference observed between these values either ($P > .05$) (Table 10).

Table 9
Results of the Analysis for Nitrogen in the Water
in which the Fish were Maintained During
Experimental Procedures.

Group Examined	Average Nitrogen Concentration in Total Water Volume (mg)	Standard Diviation	Number of Samples
Combined Controls	2.43	1.49	10
Combined Experimentals	2.71	1.40	14
Experimentals Fed D. pulex	2.40	1.39	7
Experimentals Fed D. magna	3.02	1.45	7

Table 10
Statistical Analysis of the Results of the Analysis
for Nitrogen in the Water in Which the Fish
were Maintained During Experimental Procedures

Groups Compared	t-value	Probability	Degrees of Freedom
Combined Controls vs. Combined Experimentals	.476	.639	22
Experimentals Fed D. pulex vs. Experimentals Fed D. magna	.826	.425	12

To determine the quantity of nitrogen retained by the bluegills,
the amount of nitrogen excreted by the control fish was subtracted
from the nitrogen excreted by the experimental fish. The value
obtained was then subtracted from the total nitrogen consumed to

determine how much nitrogen was retained by the fish. Table 11

presents the data and the results of this calculation. The percent

nitrogen retained by the bluegills fed D. pulex was statistically

compared to the percent nitrogen retained by the fish fed D. magna

using a t-test. There was no significant difference observed between

these two values (P > .05) (Table 12).

Table 11

Mean Values of Nitrogen Involved in Nitrogen Retention Equation

Experimental Group	Total Nitrogen Consumed (mg)	Total Nitrogen in Feces (mg)	Total Nitrogen in Water (mg)	Total Nitrogen Excreted by Experimentals (mg)	Total Nitrogen Excreted by Controls (mg)	Total Nitrogen Retained (mg)	Percent Nitrogen Retained
Fish Fed D. pulex	11.435	1.261	2.397	3.658	2.986	10.763	93.86
Fish Fed D. magna	13.624	1.876	3.023	4.899	4.002	12.727	93.30

Table 12
t-value and Associated Probability for
Comparison of Percent Nitrogen Retained
by Fish Fed D. pulex vs. Fish Fed D. magna

Groups Compared	t-value	Probability	Degrees of Freedom
Fish Fed D. pulex vs. Fish Fed D. magna	.4318	.674	12

DISCUSSION

The results of the phase of this investigation concerned with the size selective feeding habits of the bluegill sunfish demonstrated that these fish preferred cladocerans which were 1.3 mm and larger, up to 4 mm in size. This size category included the two largest cladocerans used in this investigation. The selection of the largest cladocerans corresponded with the work of Brooks and Dodson (1965) who explained the impact of the introduction of glut herring upon the zooplankton community structure of a lake. They observed that following the introduction of the herring the larger species of zooplankton, which had been most abundant, were no longer predominant in numbers. The dominant species now were the smaller zooplankters. The same type of differential predation was observed by Reif and Tappa (1966) with smelt. Prior to the introduction of smelt into a lake the dominant plankton species was _D. pulex_. Following the introduction of the smelt it was observed that the dominance was shared by _D. dubia_ and _B. longirostris_. This demonstrated again that there was a change in the population from a dominant large zooplankter to a smaller species of zooplankton when subject to predation by a species of fish.

The above studies described observations made on plankton communities. However, there was no conclusive evidence that the fish were actually selecting these larger zooplankton species. To approach this question Galbraith (1967) examined the stomach contents of yellow perch and rainbow trout. The results of his investigation of the

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stomach samples disclosed that both rainbow trout and yellow perch

consumed Daphnia which were larger than 1.3 mm. This agreed with the

laboratory results reported in this paper. The size of the zooplankton

available to both the trout and the perch ranged from .4 mm to 2.9 mm,

similar to the size range used in the study reported in this paper.

Work has also been conducted on bluegills with respect to the

size of Entomostraca selected in their natural environment. Hall,

Cooper and Werner (1970) performed experiments using ponds which were

either stocked or not stocked with bluegills. In both types of ponds

the largest cladoceran species was Ceriodaphnia. In those ponds which

did not contain bluegills Ceriodaphnia comprised 53 percent of the

total biomass. In the ponds having populations of bluegills the

Ceriodaphnia composed three percent of the total biomass. This

evidence suggests that the bluegills selected and consumed the

largest zooplankton species available to them.

Additional evidence concerning the size selective nature of the

bluegills feeding habits in its natural habitat has been reported by

Gerking (1962). Gerking's study examined the sizes of Entomostraca

available to the bluegills and the size of Entomostraca found in the

stomachs of the bluegills. The size distribution of the Daphnia

found in the stomachs was .5 mm to 1.8 mm with the mode being 1.11 mm

to 1.12 mm. Daphnia in the plankton samples ranged from .3 mm to

1.8 mm with the mode at .7 mm to .8 mm. In comparing the modes of

each sample it was noted that the bluegills were selecting the larger

of the Daphnia community. As with the work of Hall, Cooper and

Werner (1970), the largest zooplankter available was smaller than the

largest cladoceran used in this study. However, the size of the Daphnia selected most often approximated the 1.3 mm threshold observed in this study.

Another factor examined was the availability of the different sizes of Daphnia. The frequency distribution of Daphnia sizes available to the bluegills, in Gerking's investigation, approximated a normal distribution ranging from .3 mm to 1.8 mm. In the study reported in this paper the four different size classes of cladocerans were presented to the fish in equal numbers. This was done to prevent any interference in the examination of the problem of size selectivity by having more of one size class or less of one size class than another. Werner and Hall (1974) proved that a change can occur in the size selectivity pattern of the bluegill as a result of varying the number of prey in a size class.

Many of the studies which have been conducted on the feeding patterns of fish have been done in the natural habitat of the fish. That is, the fish have been removed from the lake and their stomach contents examined and compared to the zooplankton community present in the lake. Problems can arise with this type of study. One problem is the strata that some of the plankton occupy may not conform to the strata that the specific fish being examined patrols in search of food. Thus, the plankton may be of the appropriate size, however, because these plankton are not encountered in the foraging pattern, they are not consumed. This was a problem encountered in the work of Hutchinson (1971). Another problem to consider is that not all zooplankton have the same escape movement patterns, therefore, some

of the plankton available to the fish may not be able to escape as readily as others. This can be demonstrated by comparing the escape movements of copepods to cladocerans. Many copepods are able to escape predation by quickly darting away from approaching objects. Cladocerans, while they do move away from approaching objects, are not as fast and, therefore, are easier prey.

Both of the above problems were eliminated in this study by conducting the feeding experiment under laboratory conditions. The use of the aquaria eliminated the problem of spatial distribution. Along with this, cladoceran species were selected which appeared to have the same movement patterns as determined by the investigator. Observation of the size selective feeding experiments in progress revealed that the fish patrolled the aquaria at mid depths and made darting movements either up or down or to the side to capture prey. There was no indication of any of the size classes of prey congregating by size class.

Body morphology is another factor which could confuse the question of size selective feeding. The cladoceran species used were selected with this factor in mind. However, there were slight differences in the morphologies of the bodies of the cladocerans used. This factor was not considered to be a major problem in this study.

The selective predation exerted on a zooplankton community by fish has several effects. The main one previously pointed out is the decline in numbers of the larger zooplankton species. This can result in the complete disappearance of the larger zooplankton or they may respond by maturing at a smaller size and thus maintain a

population which is smaller in size and less dense with respect to
population numbers. As there is a decrease in the numbers of the
larger zooplankton, there is a concomittant shift in the dominance to
the smaller zooplankton. These changes result in an alteration of the
whole aquatic community structure. Those foods which are consumed by
the larger zooplankton are now free to increase in numbers.
Accompanying the shift of the dominance to the smaller zooplankton
species is a decrease in the foods which are consumed by the smaller
zooplankton. The change in size of the dominant zooplankton to a
smaller size can also have an adverse affect upon the growth of the
fish present in the system. There have been studies performed which
report that differences in growth rates of fish can be associated with
food size (Parker and Larkin, 1959; Paloheimo and Dickie, 1966).

The correlation between the size of the food organisms selected
by the fish and the increase in growth rate brings out an interesting
point. Is there more protein available to the fish, per gram, in the
larger organisms than in the smaller organisms, or do the fish retain
more protein from the larger zooplankton than they do from the small
zooplankton thus allowing greater growth rates?

Protein is used as an indication of growth, for the common
property of growth is the synthesis of new protoplasm and protein is
the least variable and most abundant constituent of protoplasm
(Gerking, 1954). The investigation of protein utilization in fish is
achieved by examining the total nitrogen injested and excreted by
the fish. Other methods have been used to examine the growth of
fishes, however, some of these are questionable. Ricker (1946) used

the wet-weight of fish as an indication of growth. The disadvantage with this approach is that changes in the water and fat content of the fish, resulting from differing nutrition conditions, influence the wet weight. The use of dry weight has its limitations also. Gerking (1954) found that the fat content of fish removed from their natural habitat varies from eight percent to 33 percent of the dry weight.

Examination of the protein efficiency of members of the Centrarchidae family was performed by Gerking (1952). Gerking examined protein utilization of the longear sunfish, L. megalotis, and the green sunfish, L. cyanellus, by studying the efficiency of protein absorption and protein retained by the fish. The absorption efficiency of protein was examined by first determining the amount of protein nitrogen absorbed. Protein nitrogen was determined by subtracting the nitrogen present in the chitin of the food organisms from the total nitrogen content of the organism. The amount of protein nitrogen absorbed was determined by subtracting the protein nitrogen in the feces from the protein nitrogen consumed. Once the protein nitrogen absorbed was determined the efficiency of protein absorption, in percent, was calculated using the following equation:

$$\text{Efficiency of protein absorption in percent} = \frac{\text{Protein N absorbed}}{\text{Protein N consumed}} \times 100$$

The results of these calculations for Gerkings experiments were; 97.4 percent for the longear sunfish and 95.7 percent for the green sunfish. The same calculation was performed using the appropraite values listed in Table 11. The results revealed that those bluegills which consumed D. pulex had an average value of 93.86 percent for the

efficiency of protein absorption and those bluegills fed D. magna had
an average value of 93.30 percent. These values are lower than those
determined by Gerking. An explanation for this difference could lie
in the difference in the experimental designs used. The fish that
Gerking examined remained in an aquarium for a period of 10 days prior
to the removal of the feces. This 10 day period would allow the
excretion rate of the fish to stabilize after the initial handling.
In the experiment reported in this paper the fish were introduced
into the experimental aquaria and allowed to acclimate for a period
of four hours prior to beginning the experiment. It is possible that
the handling of the fish stressed them enough to interfere with their
metabolic processes resulting in an increase in nitrogen excretion.
Savits (1973) examined the effect of handling stress on starved blue-
gill sunfish. His results indicated that handling had no effect on
the excretion rate of starved fish. However, he did not examine the
effect on feeding fish.

Another method used by Gerking in examining growth efficiencies
was protein nitrogen retention. This was accomplished by taking
control fish at the beginning of the experiment, sacrificing them and
determining the dry weight and total nitrogen content of the fish.
The experimental fish were maintained for a period of 50 days on a
specific diet. At the end of that time period the fish were sacrificed
and analyzed for dry weight and total nitrogen. The total nitrogen
of the control fish was then subtracted from the total nitrogen of
the experimental fish and the resulting value was determined to be
the protein nitrogen retained by the fish for growth. The main

difference between Gerking's experiment and the one reported in this paper is in the analysis of the water for nitrogenous products lost through the gills and the kidneys of the fish. This value allows one to calculate the amount of nitrogen which is retained with each meal. The type of experiment conducted in this report could be administered over a longer time period. In the process some of the experimental error, which resulted from the variability noted between each different group of fish, may be removed. This merits further investigation.

Gerking's methods, mentioned above, have also been applied to the investigation of the nitrogen balance in Megalops cyprinoides and Ophiocephalus striatus by Pandian (1967). The values he obtained for the efficiency of protein absorption were 97.2 percent for M. cyprinoides and 97.1 percent for O. striatus. These values again are higher than the efficiency of protein absorption of the bluegill sunfish examined in this study. The explanation of this difference partially lies in the design of the experiment.

The experiment reported in this paper was conducted in the same manner as Gerking (1954, 1955), Savits (1971, b), Pandian (1967) and Birkett (1969), with the exception that the amount of nitrogen retained by the fish after one meal was determined without analyzing the body for total nitrogen content. This can be accomplished by applying the formula of Warren and Davis (1967) to the nitrogen determinations performed in this experiment. As discussed earlier, the formula of Warren and Davis was condensed to; $C = F + U + \Delta B$, with C representing the amount of protein nitrogen present in the food, F being the amount of protein nitrogen present in the feces, U equaling

the amount of nitrogen lost in the form of urea via the kidney and ammonia via the gills, and $\triangle B$ describing the total change in the nitrogen of the body which is the nitrogen retained for growth. By rearranging the formula to; $\triangle B = C - F - U$, the amount of nitrogen retained by the bluegills examined in this experiment can be determined.

Fish have a protein reservoir composed of proteins stored in organs and plasma proteins. According to Brody (1964) the protein reserve can be used to partially maintain the balance between protein anabolism and protein catabolism for an undetermined period of time. The bluegills in this study were starved for 24 hours prior to the initiation of the experiment. This period of time was not long enough to deplete the protein reserve. Therefore, control fish were run to indicate the amount of nitrogen which would be excreted by the fish due to metabolism, activity and the protein reserve. The control fish were given no food during the course of the experiment. The amount of nitrogen that they excreted into the water was then sub-tracted from the total protein nitrogen consumed along with the values of the nitrogen determinations for the experimental fish. The equation is as follows, with U_C representing the amount of nitrogen lost in the urea via the kidneys and ammonia via the gills of the control fish. U_E represents the same for the experimental fish.

$$\triangle B = C - F - U_E - U_C$$

Using the value obtained for $\triangle B$, dividing that by C and multiplying the results by 100 calculates the percent protein nitrogen retained by

the fish;

$$\frac{\triangle B}{C} \times 100 = \text{Percent protein nitrogen retained}$$

Gerking (1955) determined the utilization of protein for growth in the following manner;

$$\frac{\text{Utilization of protein}}{\text{for growth in percent}} = \frac{\text{Protein N used for growth}}{\text{Protein N absorbed}} \times 100$$

Protein nitrogen used for growth is the same as $\triangle$ B. The protein nitrogen absorbed was determined by the following formula: Protein N consumed – Protein N in feces = Protein N absorbed. Using the appropriate values in Table 11, it was determined that those fish fed D. pulex used 52.08 percent for growth and the bluegills fed D. magna used 47.65 percent. These values exhibit a close relationship to the work done by Gerking. The smallest longear sunfish used by Gerking weighed 9.1 grams and utilized 33 percent of the protein for growth and the smallest green sunfish, which weighed 7.1 grams, utilized 38 percent for growth. Gerking's results, for his complete experiment, demonstrated that with increasing weight of the fish the ability of the fish to utilize nitrogen for growth decreases. The bluegills used in the study reported in this paper had an approximate average weight of 4.5 grams. These fish were smaller than the smallest fish used by Gerking and the corresponding increase in the percent utilization is likely a result of this.

The purpose of this study was to investigate the size selective feeding habits of bluegill sunfish. If the fish were selecting their food on the basis of size it was important to determine if there was any nutritional benefit in this selection with respect to protein availability and/or retention.

Only three species of the cladocerans could be analyzed for nitrogen content. There was no significant difference noted in the nitrogen content of these cladocerans. In examining the question whether or not the bluegills retain more protein from one cladoceran species than another, it was possible to examine only two of the cladoceran species. These two cladocerans were the largest used in this experiment and they were the ones selected by the fish in the size selective feeding phase of the experiment. There was no significant difference observed between the values for protein nitrogen by fish fed D. pulex or D. magna.

Variability was observed in many of the observations obtained when determining the amount of nitrogen found in the feces of the fish and the nitrogen excreted in the water. Much of this variability could be the result of using a new group of fish with each experiment. Although all of the fish were obtained from the same lake, there must be natural variations in the population. These variations could have been eliminated if one group of fish had been used in a similar experiment conducted over a period of 20 to 30 days. This way handling would have been kept to a minimum. At the same time the procedures used by Gerking (1955) could have been performed and the results of both procedures compared more exactly.

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