EFFECTS OF NUTRIENT ENRICHMENT AND A ROUGH FISH POPULATION (CARP) ON A GAME FISH POPULATION (SMALLMOUTH BASS)

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TERRY A. HAINES

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This is to certify that the

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Effects of Nutrient Enrichment and a Rough Fish Population (Carp) on a Game Fish Population (Smallmouth Bass).

presented by

Terry A. Haines

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ABSTRACT

EFFECTS OF NUTRIENT ENRICHMENT AND A ROUGH FISH POPULATION (CARP) ON A GAME FISH POPULATION (SMALLMOUTH BASS)

By

Terry A. Haines

This experiment was conducted to test the effects of nutrient enrichment and a rough fish population, individually and in combination, on a game fish population. The rough fish and game fish species used were carp (Cyprinus carpio L.) and smallmouth bass (Micropterus dolomieui Lacépède) respectively.

Using a series of artificial ponds, two fertility levels (low and high) were established. Low fertility ponds were left undisturbed, while inorganic nitrogen and phosphorus were added to the high fertility ponds at bi-weekly intervals. Replicates of three fish populations were established at each fertility level: game fish only, rough fish only, and game fish and rough fish in combination. The physical, chemical and biological conditions in the ponds were sampled regularly. The fish populations were censused after 15 months. Growth rate was used as an index of the status of the various fish populations.

RNA-DNA ratios also were measured to test the degree to which they reflect growth rate.

In low fertility ponds, bass and carp had similar growth rates and RNA levels. In high fertility ponds the growth rate and RNA levels of bass were significantly reduced ($p < 0.01$), while those of carp increased ($p < 0.01$).

The growth rate of each species was not significantly ($p = 0.5$) affected by species interactions at either fertility level. The RNA data showed no significant difference ($p = 0.4$) in the RNA level of bass in low fertility ponds when carp were present or absent. However, in high fertility ponds bass had higher RNA levels when carp were present ($p < 0.01$). Carp in low fertility ponds had higher RNA levels when bass were present ($p < 0.01$), but carp in high fertility ponds had lower RNA ratios when bass were present ($p = 0.01$).

The depression of bass growth rate and RNA concentrations in enriched ponds may have been a result of the increased durnal dissolved oxygen flux, decreased water clarity and increased ammonia concentrations in these ponds. Carp apparently were not affected by these factors.

The increase in RNA levels of bass in high fertility ponds when carp were present may be a result of bass feeding on carp eggs and fry, or to an increased availability of benthos resulting from the feeding activity of carp. Social facilitation in feeding response

may also be present. The reasons for the changes in carp RNA levels observed when bass were present are unknown.

The significant responses found in growth rate data also were present and significant in the RNA data; in several cases non-significant trends seen in growth rate data were present and significant in the RNA data. RNA concentration may be a more sensitive indicator of the condition of a fish than is growth rate.

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By

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INTRODUCTION

Much attention has been given to artificial eutrophication in recent years and an extensive literature is being developed (see National Academy of Sciences, 1969, for a recent review). It is now generally accepted that eutrophication is the result of excessive inorganic plant nutrients, especially phosphorus and nitrogen, entering surface waters from municipal and industrial wastes, and urban and rural runoff (Mackenthum, 1968; Ohle, 1953; Sawyer, 1954, 1965). The resultant increase in productivity and excessive plant growths are well documented (Hutchinson, 1969). The usual effect on fish is a decline in numbers of species and an increase in numbers of individuals (Gaufin and Tarzwell, 1952; Hasler, 1947; Thompson and Hunt, 1930). In lakes that stratify thermally, hypolimnetic oxygen depletion is commonly associated with eutrophication. This depletion has been blamed for the change in fish species by elimination of the habitat required by cold water species (Hasler, 1947; Larkin and Northcote, 1969). The same type of change also occurs in waters which do not stratify, and the species lost are

normally the desirable game fishes, while the rough fishes increase in population size (Katz and Gaufin, 1953; Tarzwell and Gaufin, 1953). Instead of hypolimnetic oxygen depletion, non-stratifying waters exhibit extreme diurnal variations in oxygen content because of plant activities (Mackenthum, Ingram and Porges, 1964; Schroepfer, 1942; Smith, 1934). It has been proposed that the low point in dissolved oxygen at night may limit fish distributions (Gaufin and Tarzwell, 1952, 1956).

Rough fish have been accused of interfering with game fish in a variety of ways, including competition for food, roiling of the water and spawning interference (Aldrich, 1943; Bennett, 1943; Krumholz, 1956). Rough fish also are associated with waters of naturally high nutrient levels (Moyle, 1956; Moyle, Kuehn and Burrows, 1950). There is thus some question concerning the reason for the observed species change in fish populations in eutrophic waters. The decline of game fish may be due to physical changes in the environment, to interference from rough fish which are better adapted to enriched conditions, or to some combination of these factors. This experiment was designed to test the effects of nutrient enrichment and a rough fish population, individually and in combination, on a game fish population.

MATERIALS AND METHODS

Experimental Design

A series of 12 small artificial ponds was used in this experiment. Two fertility levels were established by leaving half of the ponds undisturbed (low fertility) and enriching the others by the regular addition of nitrogen and phosphorus (high fertility). Within each fertility level replicate fish populations of each of three types were established: game fish only, game fish and rough fish in combination, and rough fish only. This gave eight experimental conditions:

1. Game fish only in low fertility ponds.
2. Game fish with rough fish present in low fertility ponds.
3. Game fish only in high fertility ponds.
4. Game fish with rough fish present in high fertility ponds.
5. Rough fish only in low fertility ponds.
6. Rough fish with game fish present in low fertility ponds.
7. Rough fish only in high fertility ponds.
8. Rough fish with game fish present in high fertility ponds.

The fish populations were exposed to these conditions for 15 months and were censused at the end of this time. The growth rate of each species under each of the experimental conditions was used as an index of the

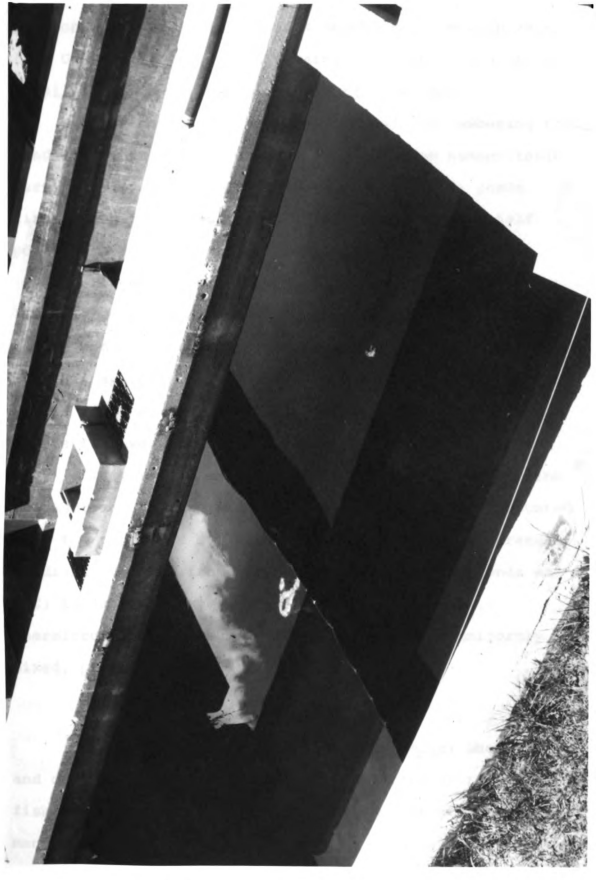
suitability of that condition for that species. Growth rate has been reported to be the major mechanism by which fish adjust to environmental conditions (Allen, 1951; Backiel and Le Cren, 1967; Ricker, 1958). A biochemical indicator of growth also was used secondarily in an attempt to determine the extent to which it agrees with growth rate in semi-natural conditions. Since growth in fish is due primarily to protein synthesis, the amount of ribonucleic acid (RNA), the protein organizer, should be a reflection of the growth rate of that fish (Leslie, 1955).

Description of the Experimental Ponds

The ponds used in the study were former waste water treatment ponds located at the corner of Kalamazoo and Harrison Streets, East Lansing, Michigan. Six identical ponds (7.2 x 7.2 x 1.8 m deep), arranged in two rows of three ponds each, were available (Figure 1). To obtain a sufficient number of ponds for replication of experimental conditions, the ponds were divided in half by the use of black polyethylene film reinforced with nylon net (Figure 2). Prior to use in this study these ponds twice had been allowed to stand full of water for several months, then were drained and cleaned. To prevent nutrients bound in the bottom of the pond from entering the water and thus raising the fertility level of the low fertility ponds, the bottom of each pond was covered with a layer of polyethylene film and filled to a depth of 0.3 m with peastone gravel.

Figure 1. The experimental ponds.

Figure 2. An experimental pond showing the method of division.



The ponds were then filled to a depth of 1.2 m with water from University wells. This water had a pH of 8.3 and a total alkalinity in excess of 200 mg/l as $CaCO_3$.

Treatments were assigned to ponds by numbering the ponds and then drawing numbers from a random number table. Fertility levels were assigned to the six whole ponds first, then fish populations were assigned to the half ponds within each fertility level.

Biweekly applications of 250 g urea (45% N) and 110 g super-phosphate (20% P) were made to the high fertility ponds during the ice-free period of the experiment. This was approximately 3.5 mg/l N and 0.67 gm/l P, giving an N:P ratio of 5:1. Fertilization was begun one month before fish were introduced into the ponds.

Temperature and oxygen profiles made prior to the introduction of fish and periodically thereafter indicated that the ponds did not stratify. The maximum temperature gradient observed from surface to bottom in the ponds was 1 C; the maximum oxygen gradient was 1.5 mg/l O_2. Therefore, it was assumed that the ponds were uniformly mixed, probably by wind action.

Fish Populations

Smallmouth bass (*Micropterus dolomieui* Lacépède) and carp (*Cyprinus carpio* L.) were selected as the game fish and rough fish species. These species are common to many warm water streams and lakes in Michigan. Attempts

were made to collect native fish for use in this study but were unsuccessful due to weather and equipment problems. The bass were obtained from a private hatchery in Pennsylvania; the carp were seined from a marsh connected to Lake Erie near Toledo, Ohio. The literature was consulted to determine the age structure and standing crop biomasses of these species occurring naturally (Beckman, 1949; Bennett, 1938, 1945; Carlander, 1955; O'Donnell, 1943; Smith, 1942). The standing crops of each species which could be supported in ponds of the size used were then calculated. Since the ponds were small and older fish were difficult to obtain, only age 0 and age I fish were used. This allowed a larger number of fish to be used per pond than if older fish had been present, and these age classes possess all of the feeding habits developed in the ontogeny of these species (Emig, 1966; Burns, 1966). In addition, the literature (Hubbs and Bailey, 1938; Reynolds, 1965; Swee and McCrimmon, 1966) indicated that neither carp nor bass would spawn until age III at the earliest. Thus it was believed that spawning would be eliminated as a variable. This proved to be an erroneous belief. The biomass calculated above was reduced by 20 per cent to allow for error and growth, and then apportioned between the two age classes on the basis of the relative sizes of these age classes in natural populations. The naturally occurring populations used in arriving at this estimate were obtained from mixed species

populations. Therefore the same biomass and age structure were used for a species population regardless of whether the population was to be alone or in combination with the other species in the experiment. Each rough fish population was composed of approximately 80 g of age 0 carp and 100 g of age I carp (180 g total); 20 g of age 0 bass plus 30 g of age I bass (50 g total) constituted a game fish population. The bass were of known age; the carp were aged by the scale method.

Recently hatched bass feed on microcrustaces for the first few weeks, then gradually expand their diet to include larger crustaces and insects. After the first year, their diet consists of insects, crustaceans and fish (Emig, 1966; Lachner, 1950, Wickliff, 1920). Young carp feed in microcrustacea, small insects and plant debris. Older carp feed primarily on insects (especially midge larvae). The amount of plant material found in carp stomachs is quite variable and its importance is unknown (Burns, 1966, Clemens, Rawson and McHugh, 1939, Moen, 1953).

Dissolved Oxygen and Gross Photosynthesis

Continuous 24 hour dissolved oxygen measurements were made on successive days in one high and one low fertility pond, selected at random. Two determinations were made per month during the ice-free period of the experiment. A single mid-depth measurement was made using a Rustrak

Temperature-Oxygen recorder (model 192, Rustrak Instrument Co., Manchester, N. H.). A small (three gallons per minute capacity) submersible pump and a length of garden hose were used to provide a flow of water past the oxygen probe, with the water being returned to the depth from which it was taken. The calibration of the oxygen probe was checked by taking a water sample from the vicinity of the pump, using a Kemmerer water sampler, and determining its dissolved oxygen content with the azide modification of the iodometric method (American Public Health Association, 1965). Gross photosynthesis values were calculated from the diurnal oxygen curves following the single station method of Odum (1956). The values for the gas transfer coefficient (K) were calculated from the rate of change of oxygen concentrations for each diurnal curve as described by Odum (op. cit.).

Temperature

Temperature was recorded continuously in one high and one low fertility pond, chosen at random, using Ryan thermographs (model D-8, Ryan Instrument Co., Seattle, Wash.).

Water Chemistry

Chemical determinations were made biweekly throughout the ice-free period of the experiment and twice during the period of ice cover. The samples were taken on

alternate weeks from those during which fertilizer was added to the ponds. The determinations were made from a single water sample per pond, taken from mid-depth with a Kemmerer water sampler and transferred to a one liter plastic bottle. Duplicate determinations of transparency, ammonia nitrogen and nitrate nitrogen were made the same day the sample was taken; phosphorus measurements were performed the following day. The samples were stored overnight in a refrigerator.

Clarity of the water was estimated by measuring the per cent transmission of the water sample using a filter photometer (Klett-Summerson Photoelectric Colorimeter, Klett Mfg. Co., New York, N. Y.) equipped with a red filter. Nitrate nitrogen measurements were made using the brucine method of Jenkins and Medesker (1964). Ammonia nitrogen was determined with the direct nesslerization test, and total inorganic phosphorus (as phosphate) was measured by the stannous chloride method (American Public Health Association, 1965).

Benthos and Zooplankton

To determine the amounts of food organisms present, and thus possibly detect a competition for food, biweekly estimates of the standing crop biomass of zooplankton and benthos were made. Duplicate plankton samples were taken at night from each pond. A single, vertical net tow (number 20 mesh, 76 u opening) was used per sample.

During 1969 these samples were preserved immediately in 10 per cent formalin. To determine the zooplankton weight the sample was diluted to a known volume from which two subsamples were removed. These subsamples and the remainder of the original sample were filtered onto individually tared glass fiber filters. The zooplankters were removed from the subsample filters by picking them off with forceps. All filters were then dried (105 C) to constant weight and weighed. It was then possible to calculate the dry weight of the total sample and the weight of the sample minus the zooplankton. The weight of the zooplankton then was obtained by subtraction. This method was extremely time-consuming, so a different technique was sought.

During the winter of 1969-70 an easier method was developed for separating zooplankton from debris and most phytoplankton. The samples were collected as before, but now they were carefully transferred to a plastic box filled with filtered water. An amber light was used to draw the zooplankters into a glass vial which was attached to the bottom of the box. After the zooplankters had entered the vial it was removed from the box and the sample preserved as before. The sample was later filtered onto dry glass fiber filters, dried and weighed. In preliminary tests this method was found to be about 85 per cent efficient at removing the zooplankton from the sample, and no difference in response between different species of zooplankton was

observed. On several occasions during 1970 large blooms of Volvox sp. occurred in a few ponds. These organisms were also attracted to the amber light and were highly mobile. When these organisms were present in bloom quantities the previous method of estimating zooplankton weight was used.

Benthos samples were taken with a 15 x 15 cm Ekman dredge. Duplicate samples were taken from each pond and sample sites were selected randomly. The benthic organisms were concentrated by sugar flotation and then sorted from the remaining debris by hand. They were filtered onto dry glass fiber filters, dried and weighed as described for zooplankton. No correction was made for any benthos which may have been present on the walls of the ponds.

Fish Growth Rate and RNA Content

At the end of September, 1970 the ponds were drained and all fish recovered. The fish from each pond were washed, sorted by species and weighed. The growth rate for each species was calculated in terms of grams of weight gained per gram initial weight per 15 months (the term of the experiment).

Four fish of age I and four of age II of each species were randomly chosen from each experimental condition and used in the determination of RNA concentration. About one gram of dorsal muscle tissue was removed from

each fish and frozen for future analysis. Since the amount of deoxyribonucleic acid (DNA) per cell is relatively constant within a species, the ratio of RNA to DNA (RNA per unit DNA) can be used as a measure of the relative amount of RNA per cell (Hotchkiss, 1955). This ratio automatically corrects for differences in numbers of cells per tissue sample. Bulow (1970) has developed a technique for determining RNA and DNA concentrations in fish and applied it experimentally. His method was followed.

RESULTS

Temperature

The temperature data indicate that the average daily summer temperature was about 23 C in low fertility ponds and 20 C in high fertility ponds. The maximum temperature recorded was 26 C; the average daily range was about 2 C. In autumn, winter and spring there was no difference in temperature between low and high fertility ponds.

Dissolved Oxygen and Gross Photosynthesis

Typical 24 hour dissolved oxygen (D.O.) curves for a low and a high fertility pond are shown in Figure 3. The high fertility ponds had wide diurnal variations in D.O. throughout the ice-free period. Afternoon values often reached 18 mg/l O_2 and pre-dawn lows were commonly below 2 mg/l O_2. Low fertility ponds had much less diurnal variation in D.O., the variation seldom exceeding 3.5 mg/l. The D.O. in low fertility ponds was at or near saturation for much of the day, while high fertility ponds varied from 200 per cent saturation to 25 per cent saturation.

The gross photosynthesis values obtained are shown in Figure 4. The first observation was made immediately

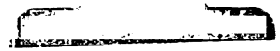


Figure 3. Twenty-four hour dissolved oxygen curves for a low and a high fertility pond on July 8 and 9, 1970, respectively.

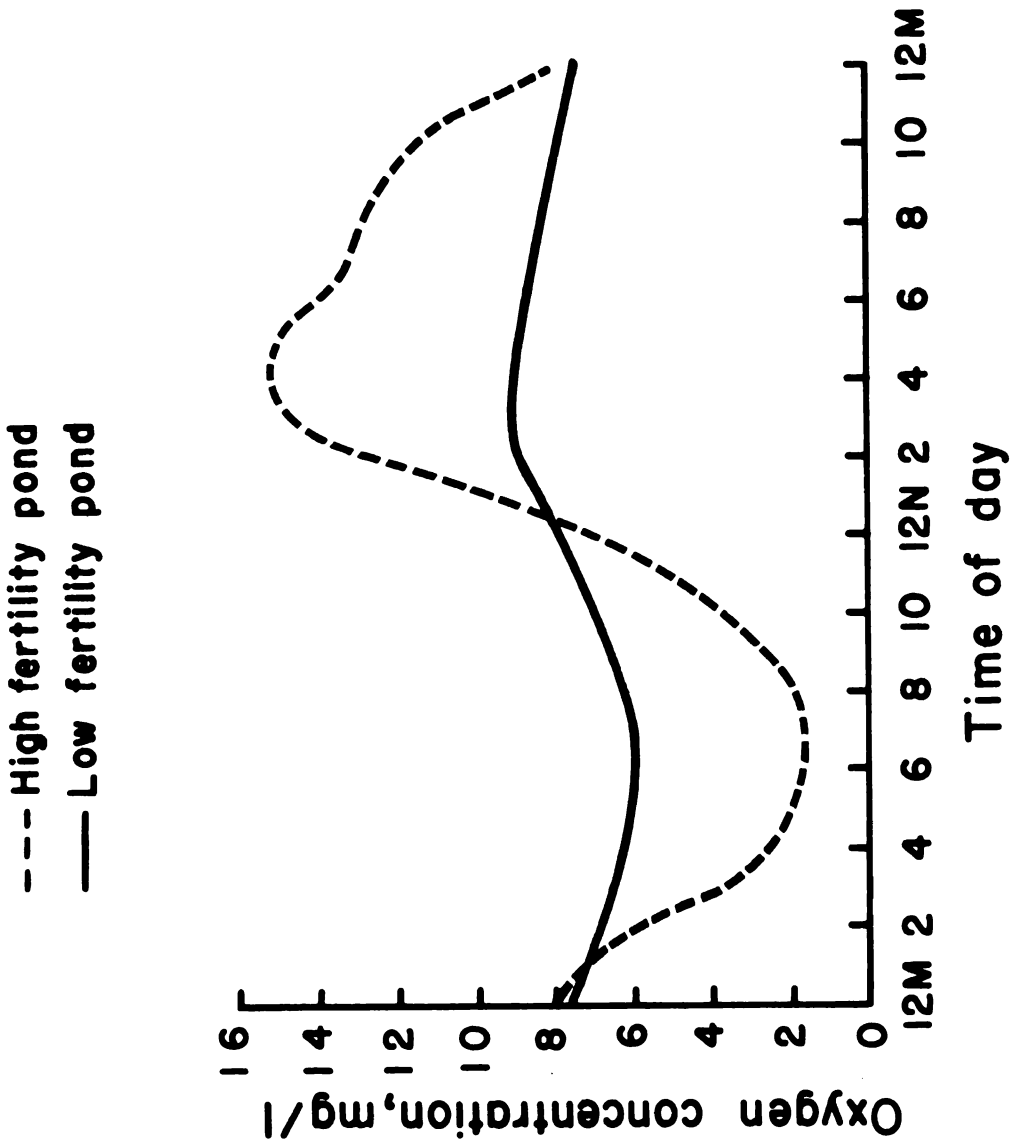
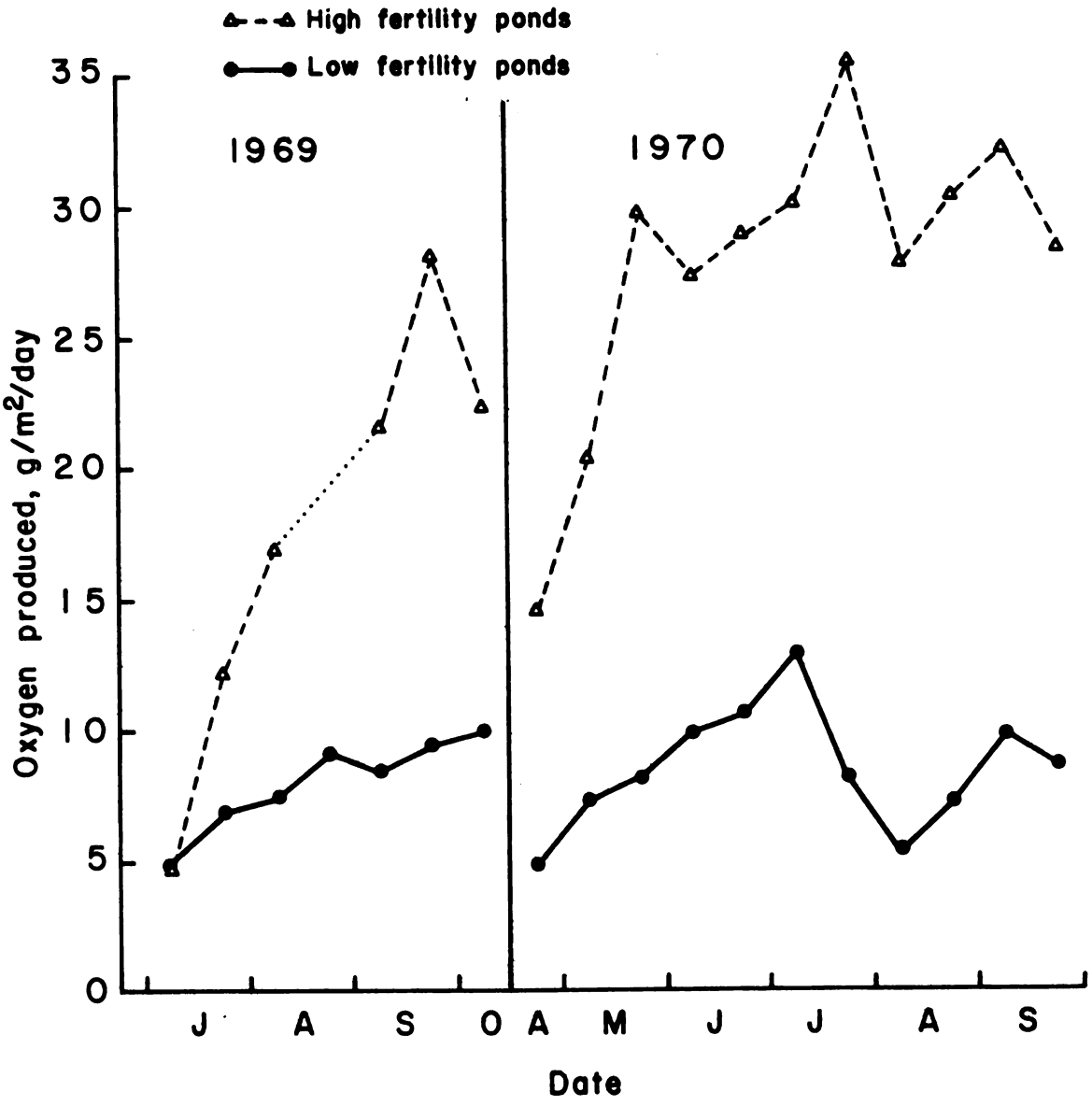

High fertility pond
Low fertility pond
Oxygen concentration, mg/l
0
2
4
6
8
10
12
14
16
12M 2 4 6 8 10 12N 2 4 6 8 10 12M
Time of day

Figure 4. Gross photosynthesis values for the experimental ponds.


High fertility ponds
Low fertility ponds
1969
1970
35
30
25
20
15
10
5
0
Oxygen produced, g/m²/day
J
A
S
O
A
M
J
J
A
S
Date

after nutrients were first added to the ponds. However, high fertility ponds quickly reached very high levels and stayed high throughout the study, commonly exceeding 28 mg O_2/m^2/day and reaching a maximum of 35.5 mg O_2/m^2/day. Low fertility ponds never exceeded 13 mg O_2/m^2/day. Ratios of photosynthesis to respiration (P:R ratios) are shown in Figure 5.

Water Chemistry

The water chemistry data are presented in Figures 6 through 9. The standard errors shown are the mean standard errors for all points indicated. Since there were two observations per pond and two (or more) ponds per treatment, these standard errors represent variation due to sampling error and differences between ponds treated alike. No attempt was made to separate these sources of error. The data indicate that widely different conditions were established in the low and high fertility ponds.

The per cent transmission (water clarity) data were arranged to show differences between fertility level and presence or absence of carp. The values plotted were obtained by averaging values from all ponds which contained carp and from all ponds in which carp were absent within each fertility level. High fertility ponds were lower in clarity than low fertility ponds in all cases. There was no consistent difference between ponds which contained carp and those which did not at either fertility level.

Figure 5. Photosynthesis to respiration ratios for the experimental ponds.

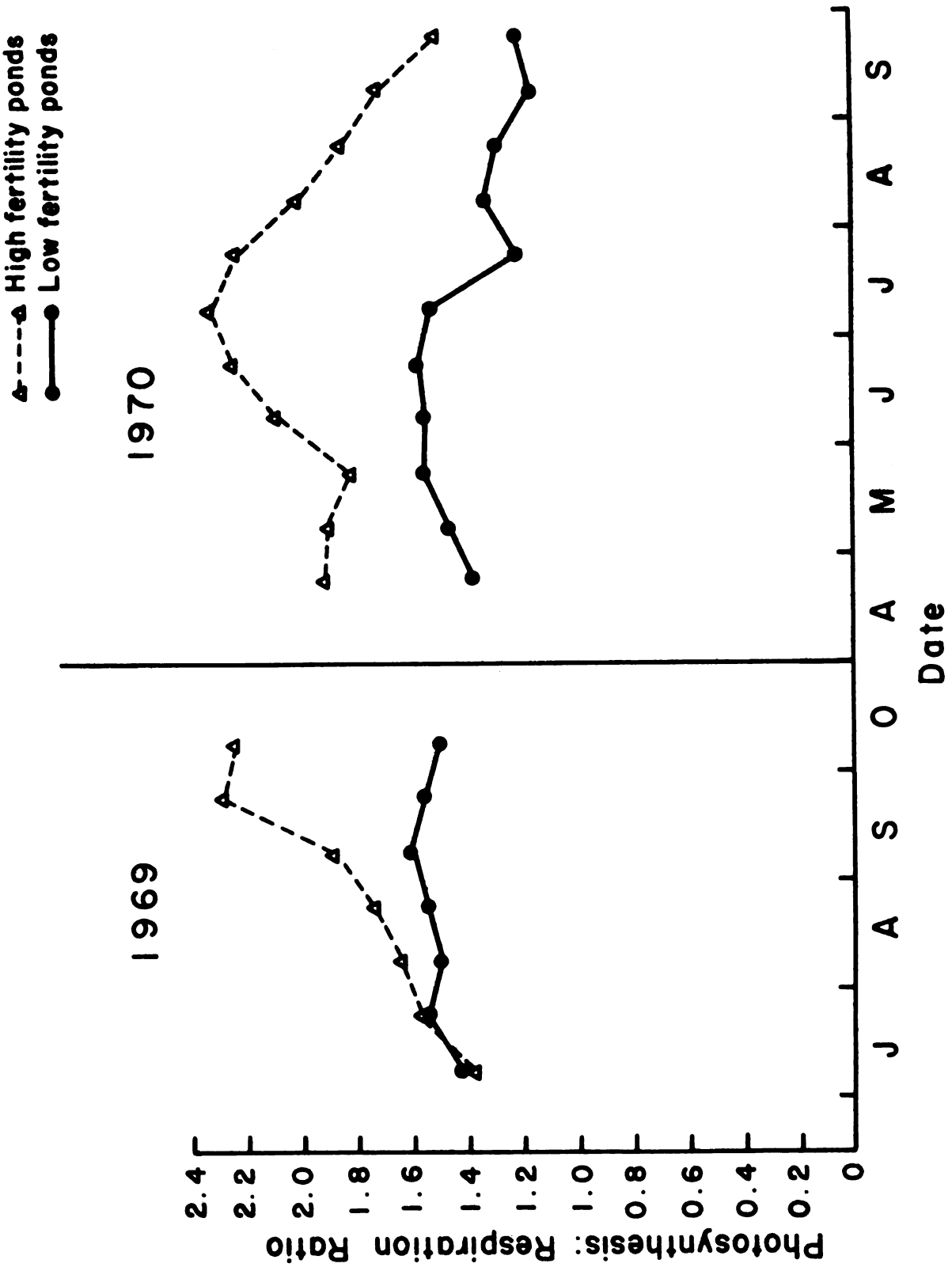

High fertility ponds
Low fertility ponds
1969
1970
Photosynthesis: Respiration Ratio
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
J
A
S
O
A
M
J
J
A
S
Date

Figure 6. Mean per cent transmission values for the experimental ponds.

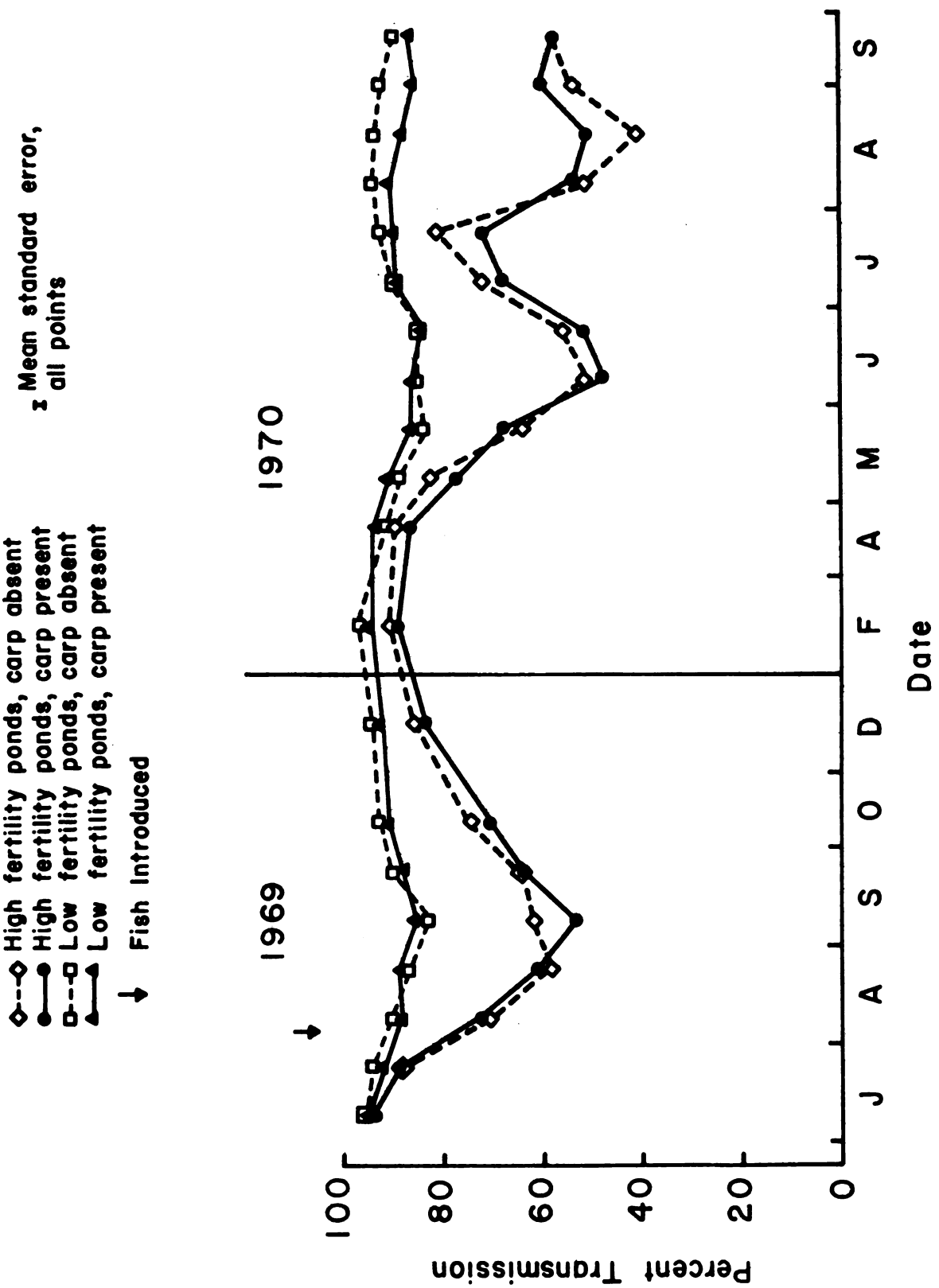

High fertility ponds, carp absent
High fertility ponds, carp present
Low fertility ponds, carp absent
Low fertility ponds, carp present
Fish introduced
x Mean standard error, all points
1969
1970
Percent Transmission
100
80
60
40
20
0
J A S O D F A M J J A S
Date

Figure 7. Mean total phosphorus values (as PO_4) for the experimental ponds.

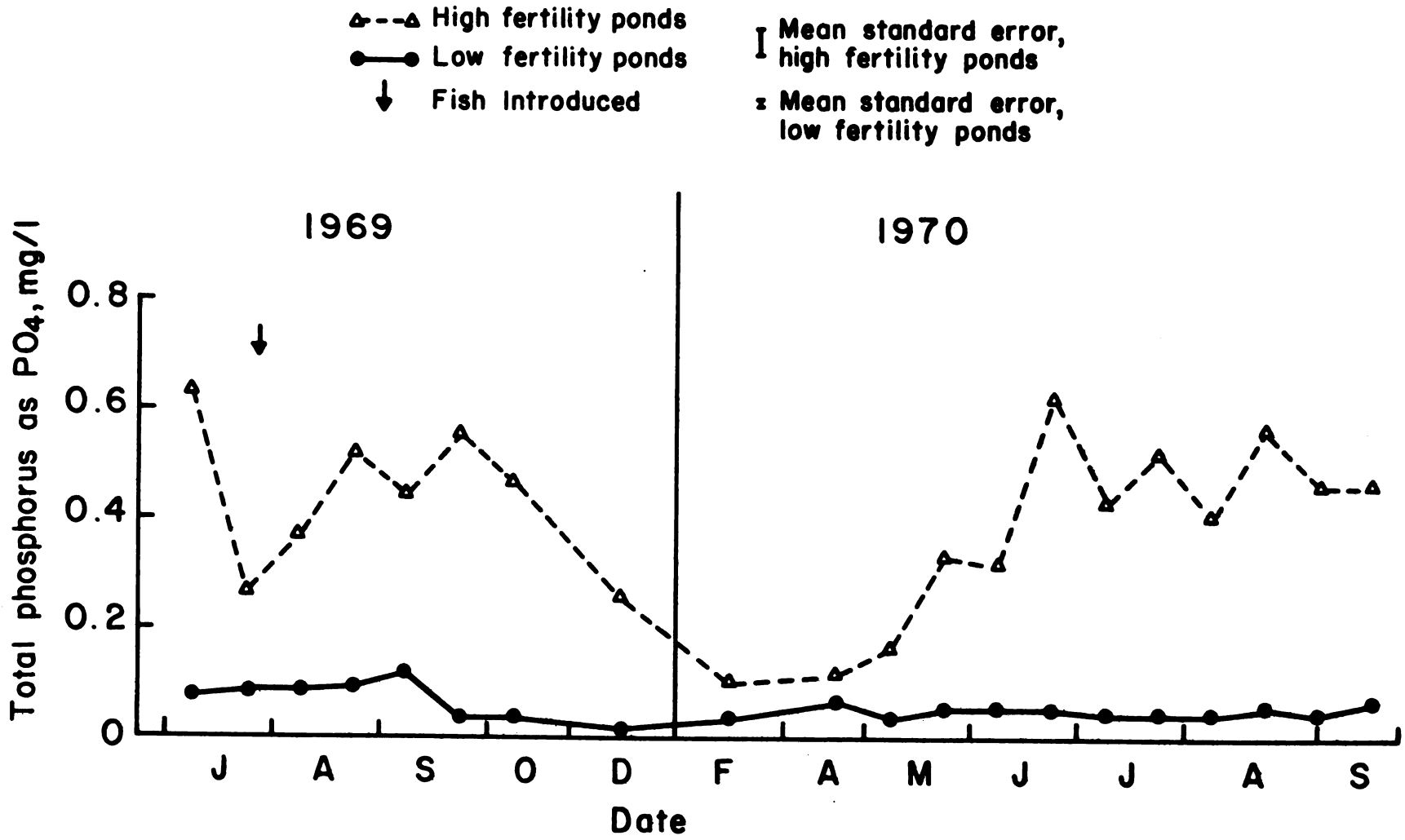
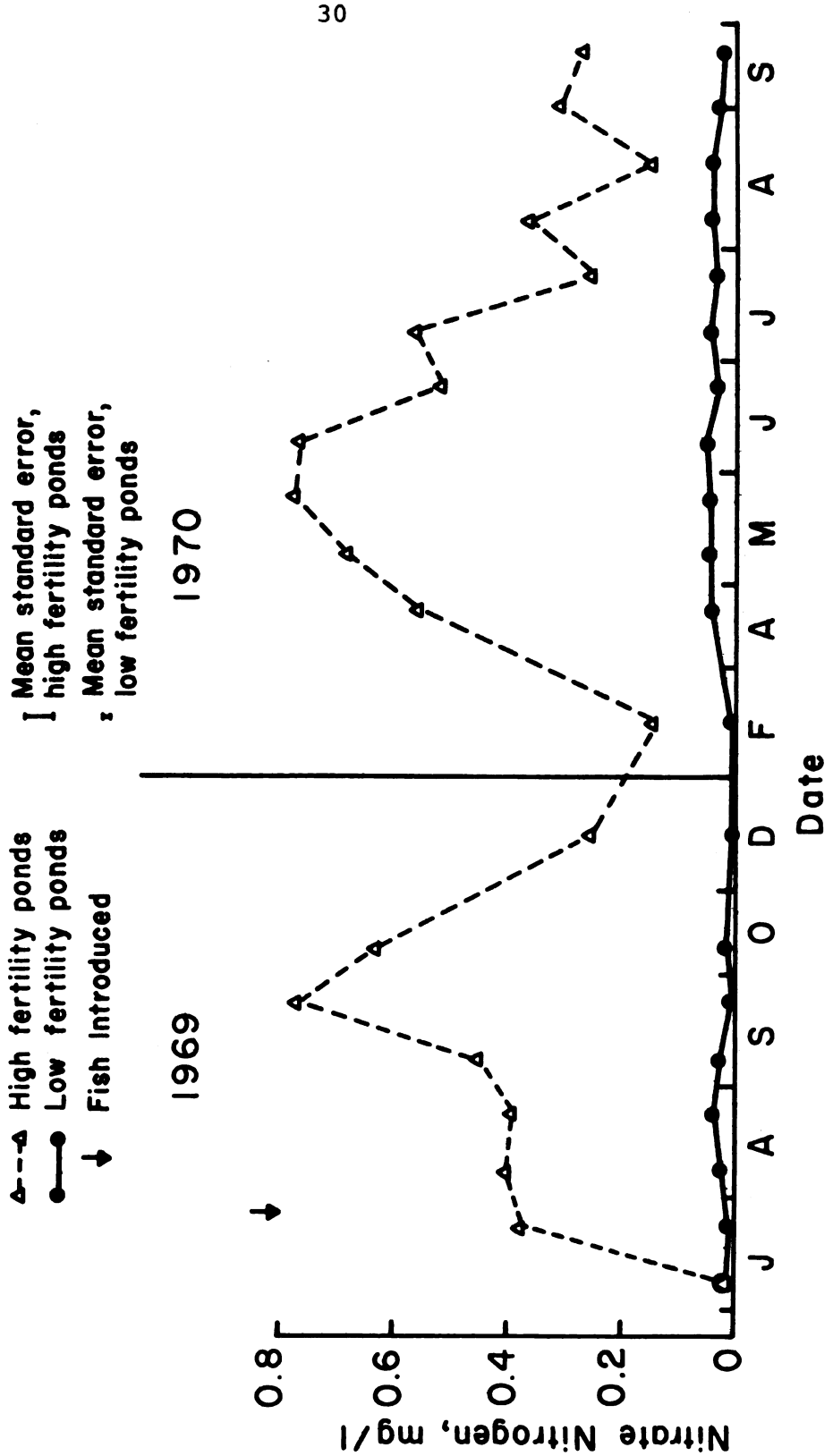

High fertility ponds
Low fertility ponds
Fish Introduced
Mean standard error, high fertility ponds
Mean standard error, low fertility ponds
1969
1970
Total phosphorus as PO4, mg/l
0.8
0.6
0.4
0.2
0
J
A
S
O
D
F
A
M
J
J
A
S
Date

Figure 8. Mean nitrate nitrogen values for the experimental ponds.


High fertility ponds
Low fertility ponds
Fish Introduced
Mean standard error, high fertility ponds
Mean standard error, low fertility ponds
1969
1970
Nitrate Nitrogen, mg/l
0
0.2
0.4
0.6
0.8
J
A
S
O
N
D
J
F
M
A
M
J
J
A
S
Date

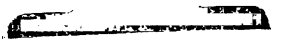
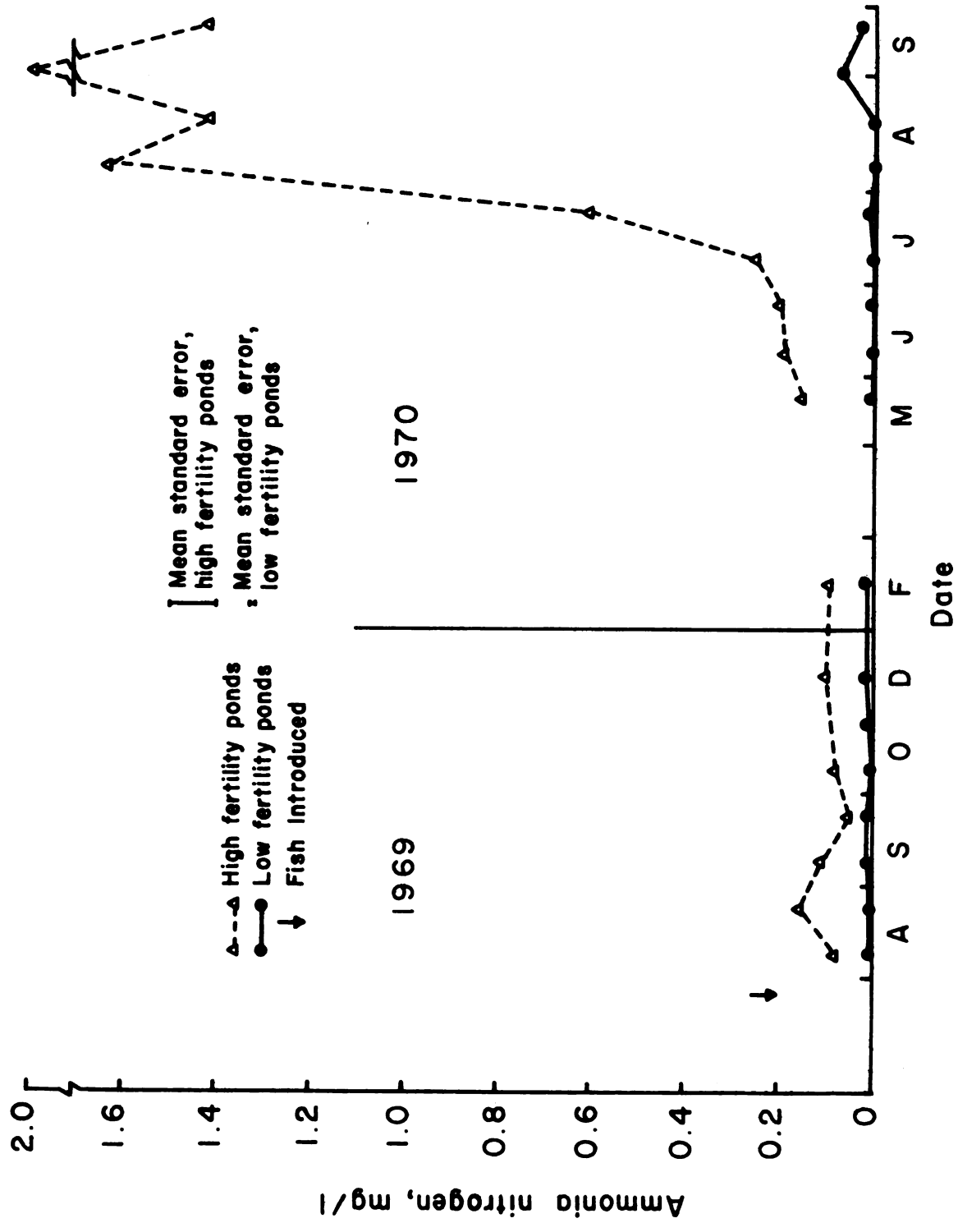


Figure 9. Mean ammonia nitrogen values for the experimental ponds.

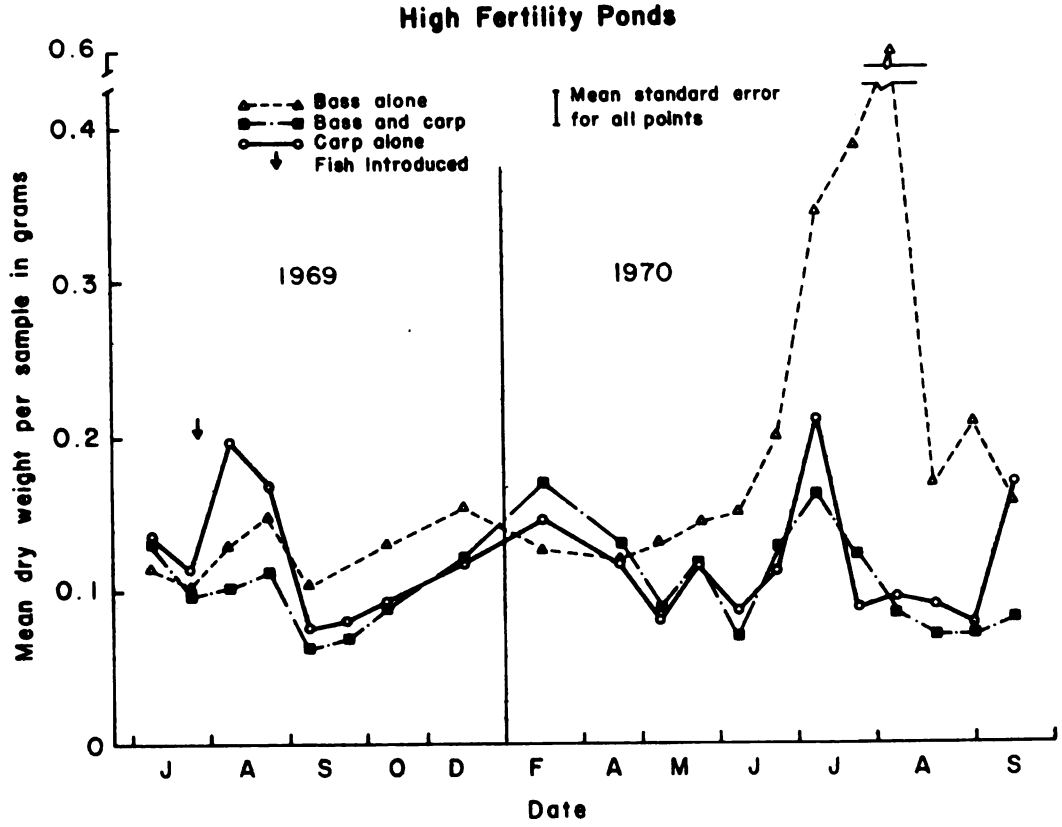

Ammonia nitrogen, mg/l
2.0
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
A S O D F M J J A S
Date
1969
1970
High fertility ponds
Low fertility ponds
Fish introduced
Mean standard error, high fertility ponds
Mean standard error, low fertility ponds

During the growing seasons phosphorus averaged 0.5 mg/l as PO_4 in high fertility ponds and 0.05 mg/l in low fertility ponds (Figure 7). Nitrate nitrogen values were quite variable in high fertility ponds but reached 0.7 mg/l in both years, while low fertility ponds averaged about 0.04 mg/l (Figure 8). Ammonia nitrogen values for high fertility ponds averaged 0.1 mg/l during the summer of 1969 (Figure 9), but were as high as 2.05 mg/l in 1970. This increase was associated with a decrease in nitrate nitrogen values in these ponds. Low fertility ponds rarely had any detectable ammonia nitrogen.

Benthos and Zooplankton

The benthos and zooplankton standing crop biomass data (dry weight) are given in Figures 10 and 11. The standard errors shown were obtained in the same way and contain the same components of error as those given for the chemistry data. In general, higher standing crops of benthos were found in high fertility ponds than in low fertility ponds. The dominant benthic organism was Chironomus sp. The same organism was dominant in both high and low fertility ponds. Species of fish present had little effect on the benthos until mid-summer of 1970. After this time, high fertility ponds in which carp were present had lower weights of benthos than those ponds which contained bass only. The magnitude of the standard errors suggests

Figure 10. Mean standing crop biomass of benthos in the experimental ponds.


High Fertility Ponds
Bass alone
Bass and carp
Carp alone
Fish introduced
Mean standard error for all points
1969
1970
Mean dry weight per sample in grams
0.6
0.4
0.3
0.2
0.1
0
J A S O D F A M J J A S
Date

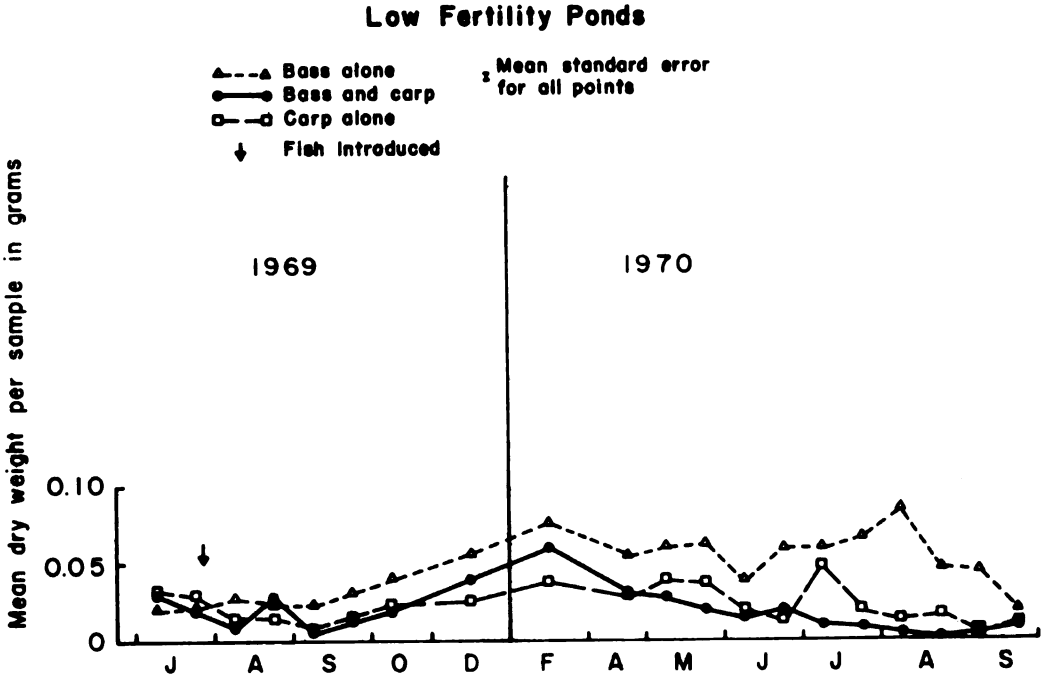
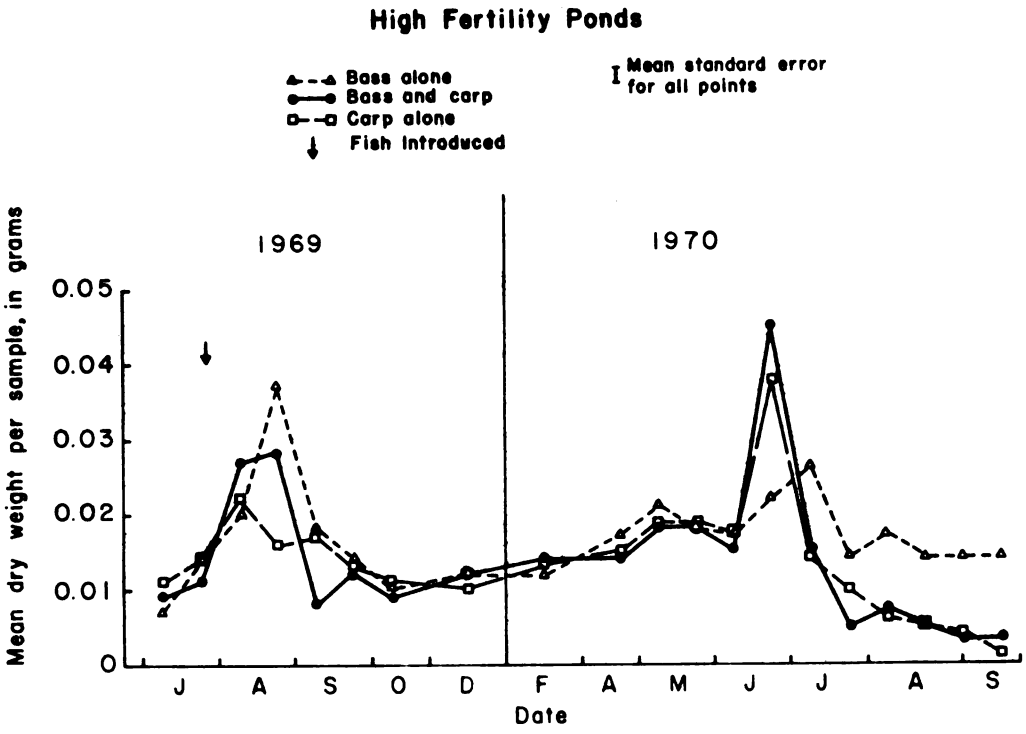
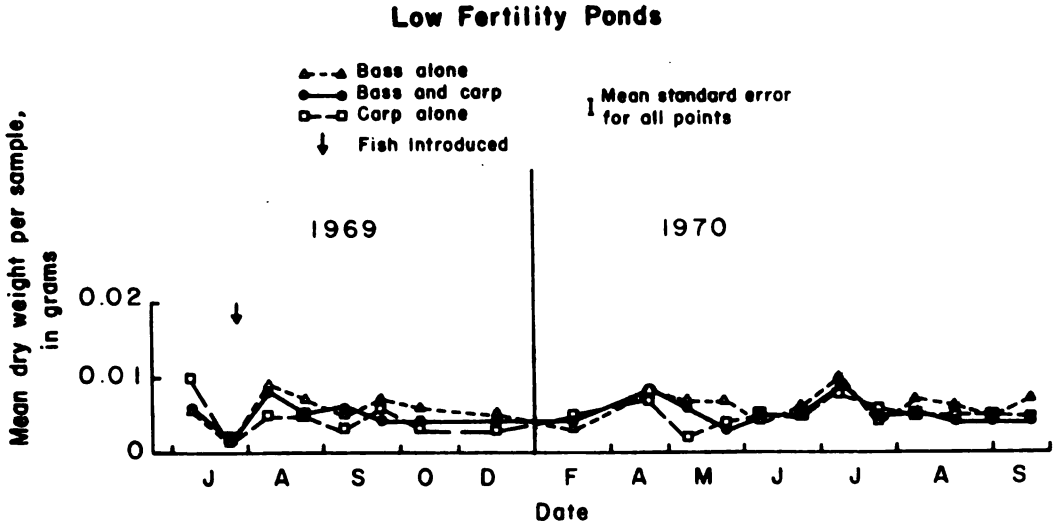

Low Fertility Ponds
Bass alone
Bass and carp
Carp alone
Fish introduced
Mean standard error for all points
1969
1970
Mean dry weight per sample in grams
0.10
0.05
0
J A S O D F A M J J A S

Figure 11. Mean standing crop biomass of zooplankton in the experimental ponds.


High Fertility Ponds
Bass alone
Bass and carp
Carp alone
Fish introduced
Mean standard error for all points
1969
1970
Mean dry weight per sample, in grams
0.05
0.04
0.03
0.02
0.01
0
J A S O D F A M J J A S
Date


Low Fertility Ponds
Bass alone
Bass and carp
Carp alone
Fish introduced
Mean standard error for all points
1969
1970
Mean dry weight per sample, in grams
0.02
0.01
0
J A S O D F A M J J A S
Date

that this may be a significant difference. There was no such difference in low fertility ponds.

Similar results are evident for zooplankton standing crops, except that differences related to the presence or absence of carp were found in low as well as high fertility ponds in late 1970. Again, the weights were lower in those ponds which contained carp.

Fish Growth Rate

The fish growth rate data are shown in Figure 12. These data were analysed using a replicated 2^3 factorial analysis of variance test. The standard error for treatment means shown in Figure 12 was calculated from the error mean square. The treatment sums of squares were partitioned into orthogonal single degree of freedom components. The results of this analysis are given in Table 1. It can be seen that carp had a significantly ($p < 0.01$) greater growth rate than did bass when all treatments are pooled for each species. This is likely a result of the greater growth of carp in the high fertility ponds. Carp spawned successfully in the high fertility ponds, and therefore part of the increased biomass observed was due to reproduction and not to body increment alone. Since it was impossible to determine what the growth rate of the older carp would have been if the age 0 fish had not been present, no attempt was made to separate reproductive increase from body increment. Carp did not spawn in low

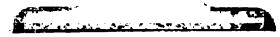


Figure 12. Mean growth rates of the fish populations from the various experimental conditions. Growth rate is expressed as grams of weight gained per gram initial weight per 15 months, the term of the experiment. The legend code is as follows: LF = low fertility ponds, HF = high fertility pond, B = bass alone, B' = bass with carp present, C = carp alone, C' = carp with bass present.

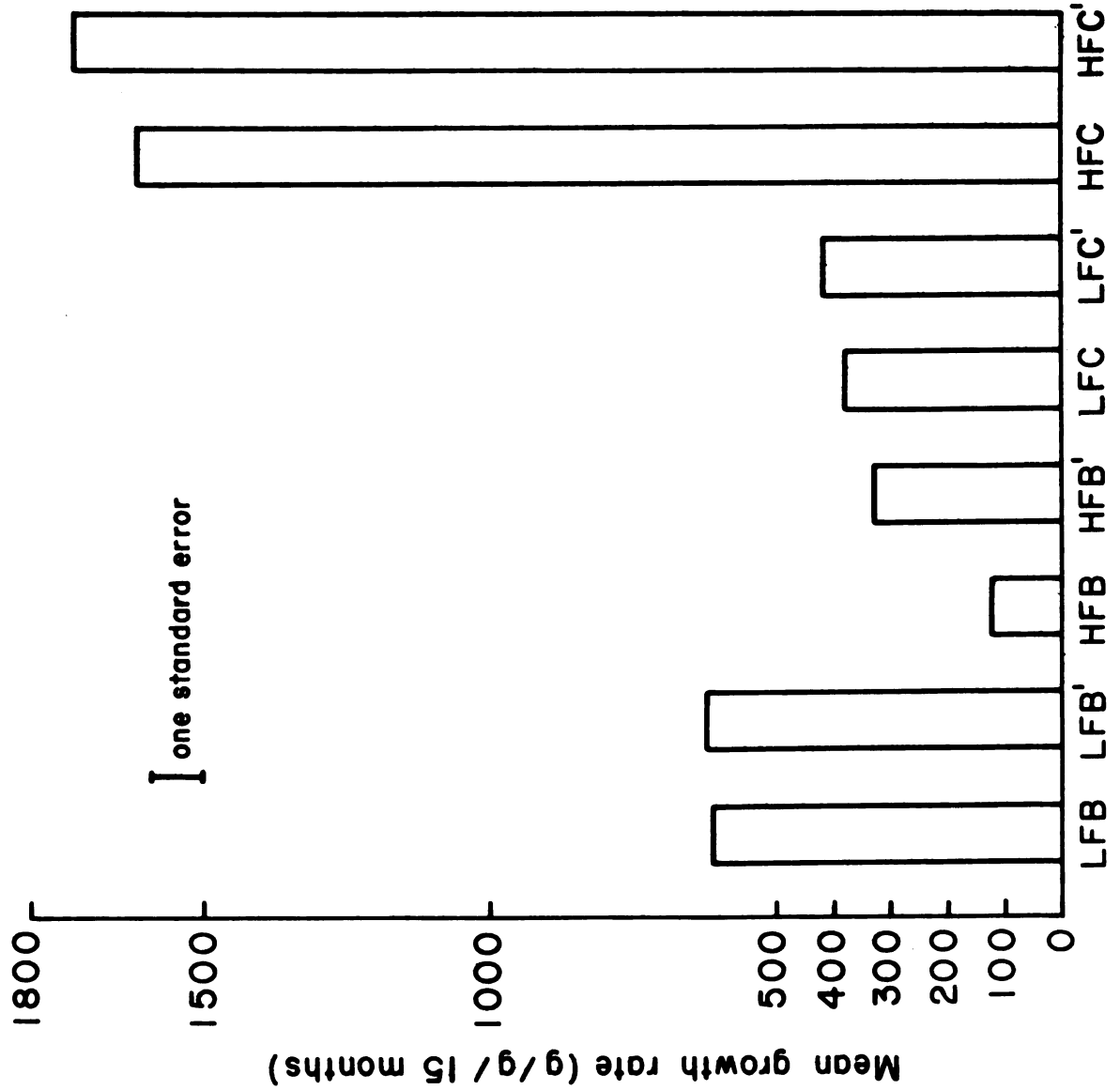

Mean growth rate (g/g/ 15 months)
0
100
200
300
400
500
1000
1500
1800
one standard error
LFB
LFB'
HFB
HFB'
LFC
LFC'
HFC
HFC'

Table 1. Analysis of variance table for growth rate data showing orthogonal comparisons between treatments.

Source	SS	DF	MS	F	$F_{0.95}$
Treatments	5,219,886.98	7	745,698.14	42.92	4.53
Bass vs carp	1,556,692.91	1	1,556,692.91	78.60	8.07
Bass high fert. vs bass low fert.	304,863.36	1	304,863.36	15.39	8.07
Carp high fert. vs carp low fert.	3,297,568.81	1	3,297,568.81	166.49	8.07
Bass alone vs carp present, low fert.	208.80	1	208.80	0.01	8.07
Bass alone vs carp present, high fert.	45,113.76	1	45,113.76	2.28	8.07
Carp alone vs bass present, low fert.	1,432.62	1	1,432.68	0.07	8.07
Carp alone vs bass present, high fert.	14,006.72	1	14,006.72	0.71	8.07
Error	139,001.69	8	17,375.21		
Total	5,358,888.67	15			

fertility ponds and there was no evidence of bass spawning in any pond. It was not possible to design a treatment comparison contrasting bass growth rate with that of carp at each fertility level, which would be orthogonal to the other comparisons desired. This should be considered for future experiments. The magnitude of the standard error assigned to treatment means makes it reasonable to conclude that the growth rates of the two species are probably not significantly different at low fertility levels. The difference in growth rate found between the two species, then, is most likely a difference in response to fertility level and not an intrinsic difference between the species.

When growth rates in low and high fertility ponds are compared within each species, it can be seen that bass have a significantly ($p \sim 0.01$) lower growth rate at high fertility levels than at low fertility levels. The opposite is true for carp ($p < 0.01$).

The presence or absence of carp had no significant effect ($p = 0.5$) on the growth rate of bass at either fertility level. An inspection of Figure 12 shows that the mean growth rate of bass in high fertility ponds is slightly higher when carp are present than when they are absent. There was no apparent difference at low fertility levels. Although not significant, it is interesting to note that this response is exactly opposite to that expected. A slight increase also was noted when the growth

rate of carp alone in enriched ponds was compared to that when bass were present but the difference was not significant ($p = 0.5$).

Fish RNA-DNA Ratios

The RNA/DNA ratios obtained for fish from the various treatments are shown in Figure 13. The standard error for treatment means shown was calculated from the error mean square as found below. These ratios are considerably larger than those obtained by Bulow (1970) for the golden shiner (Notemigonus crysoleucas). However, the RNA and DNA concentrations found in this study, and used to calculate the ratios given in Figure 13, agree well with those reported for a variety of other fish (Table 2). The data were analyzed in essentially the same way as the growth rate data. An additional factor, age, was added to the factorial analysis, and two subsamples were taken from each fish. The analysis of variance table with single degree of freedom comparisons is given in Table 3. With this design it is possible to separate sampling error (among samples within fish) from experimental error (among fish within treatment). These errors are significantly different ($p < 0.01$), indicating that fish subjected to the same treatment did not respond exactly alike. Therefore experimental error (which includes sampling error) was used to calculate all remaining F ratios, and the degrees

Figure 13. Mean RNA-DNA ratios for fish populations from the various experimental conditions. The legend code is as follows: LF = low fertility ponds, HF = high fertility ponds, B = bass alone, B' = bass with carp present, C = carp alone, C' = carp with bass present.

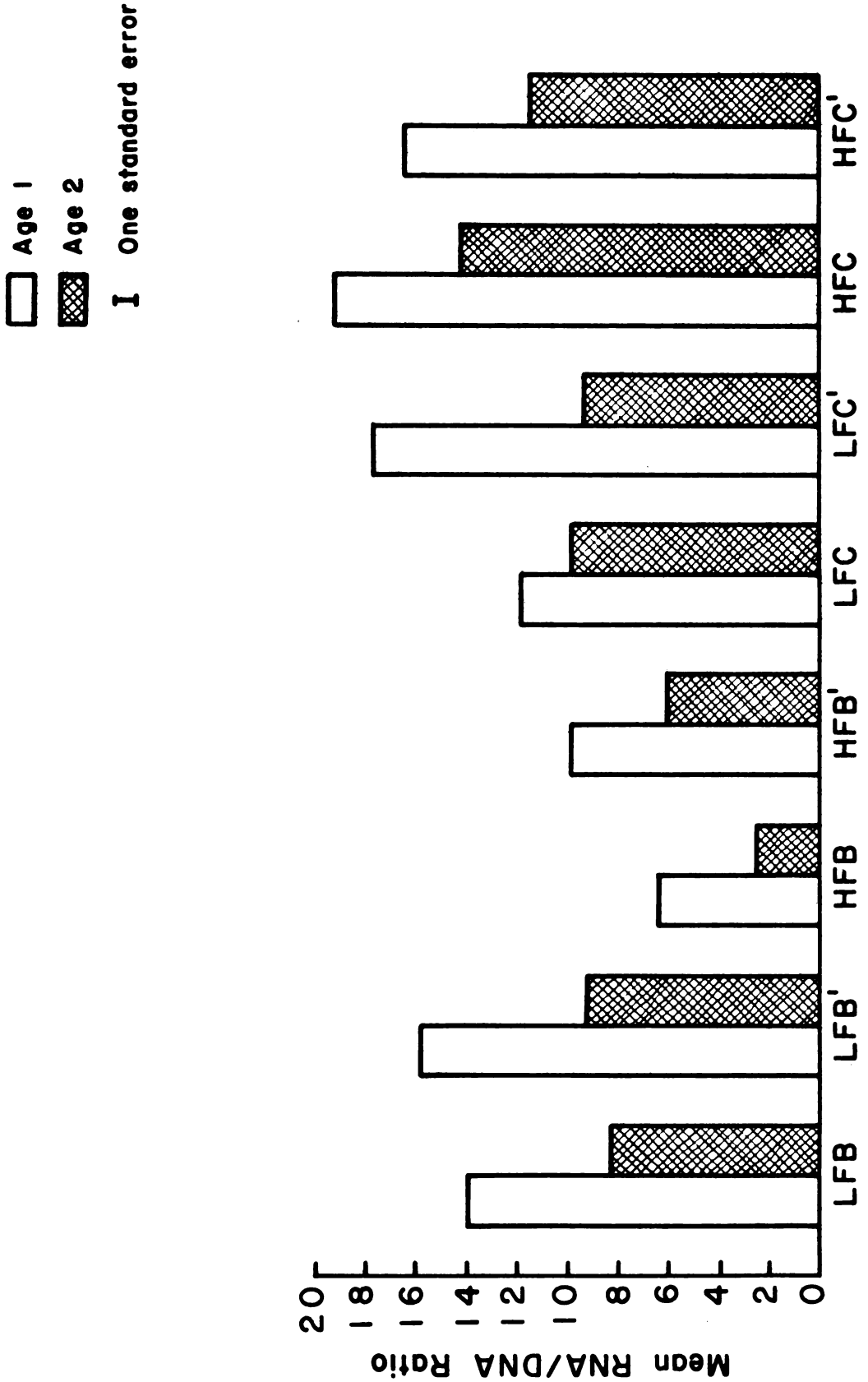

Age 1
Age 2
I One standard error
Mean RNA/DNA Ratio
0
2
4
6
8
10
12
14
16
18
20
LFB
LFB'
HFB
HFB'
LFC
LFC'
HFC
HFC'

Table 2. Comparison of RNA and DNA concentrations in various species of fish. Concentrations are expressed as u g/100 mg tissue (wet weight).

Species	RNA	DNA	Reference
Carp	26.6-103.9	2.4 - 7.9	Present study
Smallmouth bass	14.6- 68.2	1.9 - 4.1	"
Sockeye salmon	47 - 70	21 - 33	Creelman and Tomlinson, 1959
Sockeye salmon	100	2.5	Bluhm and Tarr, 1957
Chinook salmon	142	1.4	"
Pink salmon	42	0.2	"
Coho salmon	123	1.5	"
Ling cod	47.3	1.0	"
Starry flounder	58.0	2.0	"
Pacific herring	10.4- 26.5	1.87- 3.51	Tarr, 1958
Golden shiner	325.6-766.0	119.0 -168.7	Bulow, 1970

Table 3. Analysis of variance table for RNA data with orthogonal comparisons between treatments.

Source	SS	DF	MS	F	$F_{0.95}$
Treatment	2,521.78	15	168.12	24.69**	2.18
Bass vs carp	716.93	1	716.93	105.28**	5.42
Bass low fert. vs bass high fert.	498.35	1	498.35	73.18**	5.42
Carp low fert. vs carp high fert.	167.64	1	167.64	24.62**	5.42
Bass alone vs carp present, low fert.	15.72	1	15.72	2.31	5.42
Bass alone vs carp present, high fert.	98.42	1	98.42	14.45**	5.42
Carp alone vs bass present, low fert.	57.43	1	57.43	8.43**	5.42
Carp alone vs bass present, high fert.	63.31	1	63.31	9.30**	5.42
Bass alone, low fert., Age I vs Age II	125.78	1	125.78	18.47**	5.42
Bass with carp present, low fert., Age I vs Age II	167.51	1	167.51	24.60**	5.42
Bass alone, high fert., Age I vs Age II	62.49	1	62.49	9.18**	5.42
Bass with carp present, high fert., Age I vs Age II	59.91	1	59.91	8.80**	5.42
Carp alone, low fert., Age I vs Age II	14.35	1	14.35	2.11	5.42
Carp with bass present, low fert., Age I vs Age II	279.06	1	279.06	40.98**	5.42
Carp alone, high fert., Age I vs Age II	95.45	1	95.45	14.02**	5.42
Carp with bass present, high fert., Age I vs Age II	99.45	1	99.45	14.60**	5.42
Among fish within treatment	326.91	48	6.81	10.32**	1.74
Among samples within fish	42.34	64	0.66		
Total	2,891.03	127			

of freedom for experimental error were used to determine the critical F values.

All references to RNA amount which follow concern the RNA-DNA ratios. Responses obtained with the RNA data are similar to those obtained with the growth rate data. Carp had a higher RNA value than did bass when all treatments were pooled. Again, this difference probably is due to the large differences between bass and carp from high fertility ponds. In low fertility ponds the RNA values obtained for bass and carp were similar and, judging from the magnitude of the standard error obtained, probably not significantly different. Bass from high fertility ponds had significantly lower RNA values than those from low fertility ponds ($p < 0.01$); carp from high fertility ponds, on the other hand, had significantly higher RNA values than those from low fertility ponds ($p < 0.01$). These are the same responses observed with growth rate data.

Whereas no significant differences were observed in growth rate data with regards to the effect of the presence or absence of one species of fish on the other, such differences do occur in the RNA data. The presence or absence of carp does not result in a significant difference in the RNA level of bass under low fertility conditions ($p = 0.3$). In high fertility ponds, however, bass have a higher amount of RNA when carp are present than when carp are absent ($p < 0.01$). This was observed

as a trend in the growth rate data but was not significant. In low fertility ponds carp had a significantly greater amount of RNA when bass were present than when bass were absent ($p = 0.01$). An opposite response was obtained in high fertility ponds; that is, carp had a significantly lower RNA level when bass were present ($p = 0.01$). The growth rate data for carp from low fertility ponds gave a non-significant trend parallel to that obtained in the RNA data, but no such trend was noted in the carp from high fertility ponds.

A significant effect was also obtained for age differences. Age II fish had lower levels of RNA than Age I fish ($p = 0.01$) except for carp alone in low fertility ponds ($p = 0.3$).

DISCUSSION

Physical and Chemical Conditions in the Ponds

The physical and chemical conditions observed in the high fertility ponds are typical of eutrophic waters. Nutrient levels established are within the range of values reported for enriched waters (Edmondson, 1969; Sawyer, 1965). The decrease in transparency was the result of an increase in the amount of phytoplankton present. The slightly lower temperatures recorded for high fertility ponds probably resulted from the shading effect of the phytoplankton as described by Smith (1934). The diurnal oxygen cycles observed are similar to those observed in other eutrophic waters which do not stratify (Gaufin and Tarzwell, 1952; Odum, 1956; Olson, 1932).

Carp have been reported to be responsible for roiling of the water in some lakes (Black, 1946; Burns, 1966; Sigler, 1955, 1958). This did not occur in the experimental ponds. Although the bottom of the ponds consisted of gravel a considerable amount of fine silt also was present. In addition, much organic debris was quickly deposited on the gravel after the ponds were filled. When

benthos samples were taken with the dredge a large amount of sediment was stirred up. Thus the sediment was there, the carp just did not disturb it.

Gross Photosynthesis

The increased nutrient supply in the high fertility ponds resulted in higher gross photosynthesis values than those observed in low fertility ponds. Compared to the values reported by others (Odum, 1956; Welch, 1968) the gross photosynthesis values obtained in the experimental ponds are quite high, although not unreasonably so. The gas transfer coefficients (K) found ranged from 0.13 to 0.50 and thus fall well within the range of values reported by Odum (1956) for standing waters. The variation in the K values is believed to be due to differences in wind action (Welch, 1968) and surface disturbances by small carp (McConnell, 1962). In any event, the effect of the correction for diffusion on the gross photosynthesis values was relatively minor, never exceeding 10 per cent in my data.

Welch (1968) believes that available carbon may limit primary production in most waters to 12-15 mg $O_2/m^2/day$. Since the water in the experimental ponds was highly alkaline, it is doubtful that carbon limited the production in low fertility ponds. Phosphorus was probably not limiting, but nitrogen may have been. It is difficult to determine what may have limited production in high

fertility ponds. Phosphorus was probably not limiting, but nitrogen, carbon or a micronutrient may have been.

The water in high fertility ponds had a lower transparency than that of low fertility ponds throughout the winter. This indicates that the enriched ponds had higher standing crops of phytoplankton. Since light and temperature conditions were the same for all ponds, they could not have been limiting in low fertility ponds. It is more likely that the loss of nutrients by the deposition of dead plankton organisms was an important limiting factor.

Since all P:R ratios were greater than one, it is apparent that the communities in all ponds were autotrophic and not heterotrophic, by Odum's (1956) definition. The decline in P:R ratios in the summer of 1970 may be a reflection of increased bacterial decomposition of organic matter deposited in the ponds. This may also account for the increase in ammonia nitrogen observed at this time.

Effects of Fertility Level on Fish Populations

The data for growth rate and RNA-DNA ratio both demonstrate that carp are able to take advantage of the high productivity in the enriched ponds and grow at a significantly greater rate than in the low fertility ponds ($p < 0.01$). The bass, on the other hand, are inhibited by the conditions in the high fertility ponds and grow at a slower rate than they do in the low fertility ponds

($p < 0.01$). The fact that the carp spawned in the high fertility ponds tends to confound the results somewhat, but the magnitude of the differences in response between bass and carp are such that the outcome would probably have been the same had spawning not occurred. English (1951) and Rehder (1959) indicate that carp may spawn as early as age II. This is apparently what happened in the enriched ponds. Successful spawning was observed only in 1970 when the carp introduced into the ponds as age I in 1969 were then at age II. Since these fish were aged by the scale method it is possible that some mistakes were made and fish actually older than age I were put into the ponds. However, verification of the aging technique by experienced personnel and the sizes of the fish used indicate that this was not so. Carp in high fertility ponds reached a much larger body size than those from low fertility ponds (maximum total length 35 cm vs 22 cm). This may be an important factor in explaining the lack of spawning in the low fertility ponds.

While gross photosynthesis was higher in high fertility ponds than in low fertility ponds by a factor of three (9 mg $O_2/m^2/d$ vs 30 $mg/m^2/d$), carp growth rate was higher by a factor of four (400 g/g/15 months vs 1650 g/g/15 months) and bass growth rate was lower by a factor of two-thirds (600 g/g/15 months vs 200 g/g/15 months). The carp increase indicates that the consumer food chain

is still functioning efficiently and that there is no imbalance between production and consumption as described for eutrophic waters by Odum (1961) and Ryther (1954). The decline in bass growth indicates that the bass cannot efficiently use the available food in high fertility ponds.

The reasons for the difference in response to high fertility conditions between bass and carp cannot be determined with certainty, but inferences can be drawn from the conditions in the ponds. The high and low fertility ponds differed with respect to nutrient level, transparency, temperature and diurnal oxygen changes.

No toxicity has ever been reported for the concentrations of phosphorus and nitrate compounds which occurred in the enriched ponds (Abegg, 1950; Fuller, 1949; McKee and Wolf, 1963). The toxicity of ammonia nitrogen concentrations as reported in the literature is variable. Ellis (1937) reports that ammonia levels of 2.0-2.5 mg/l were toxic to goldfish. McKee and Wolf (1963) cite references that report 3.4 mg/l ammonia toxic to bluegills in soft water and 24.4 mg/l in hard water. There is no information available concerning the sublethal effects of ammonia on fish. Therefore ammonia levels in the high fertility ponds may be an important factor.

The temperature reached in the experimental ponds were close to the preferred temperatures of both species of fish. Bennett (1965) reports a preferred temperature

of 28 C for smallmouth bass, but acknowledges that this is higher than that normally observed in smallmouth bass streams and lakes. Ferguson (1958) reports that smallmouth bass preferred temperatures of 20.3 to 21.3 C in natural lakes in northern Wisconsin. Beamish (1964) reports that carp reached a peak in spontaneous activity at 27 C, while Pitt, Garside and Hepburn (1956) found a final preferrendum of 32 C. Although the enriched ponds were slightly cooler in summer than the non-enriched ponds, the difference was small (about 2 C), and on the basis of preferred temperature alone it would be expected that bass would grow better in high fertility ponds than in low, while carp would do best in the low fertility ponds. This is the opposite of what occurred.

It has been proposed by Bennett (1965) that since centrarchids are sight feeders, a decrease in water clarity could impair their feeding ability. Since the enriched ponds in this experiment experienced a decrease in clarity, this may have had some effect on the growth rate of the bass. It is difficult to imagine a depression of growth rate as severe as that observed resulting solely from a decrease in water clarity. In addition, several authors (Langlois, 1936; Surber, 1939) have reported higher growth rates for smallmouth bass from turbid environments as compared to relatively clear environments.

A likely reason for the difference in response to high fertility conditions between bass and carp is a difference in response to the oxygen conditions in those ponds. Sustained low D.O. values were never observed in the ponds, but the diurnal variations were quite marked. In high fertility ponds the oxygen content was frequently at or below 2.0 mg/l O_2 for four hours per day, and below 4.0 mg/l for eight hours each day. The oxygen content in the low fertility ponds never dropped below 6.0 mg/l. At average summer temperatures oxygen saturation was about 9 mg/l. Although 24 hour D.O. measurements were not made during the period of ice cover (November to March) spot checks of oxygen content were made on randomly selected ponds at two week intervals. These checks were usually made in mid-afternoon, and the oxygen levels found were usually at or near saturation. No evidence of winterkill conditions was ever observed. The wind kept the snow cleared off of the center of the ponds so that light penetration was not impaired.

Sustained low oxygen levels as the result of organic pollution have frequently been cited as a cause of undesirable changes in fish populations (Ellis, 1937; Hynes, 1960; Thompson, 1925), and the importance of a lethal low point in a diurnal oxygen cycle has been mentioned (Gaufin and Tarzwell, 1952, 1956). However, the effect of a diurnal oxygen cycle with a sublethal low

point has not been investigated in natural systems. Several laboratory studies have been made and are of interest here. Diurnally fluctuating oxygen concentrations have been shown to cause reduced growth rates in brook trout, Salvelinus fontinalis (Dorfman and Whitworth, 1969; Whitworth, 1968), striped bass, Morone saxatilis (Dorfman and Westman, 1970), largemouth bass, Micropterus salmoides (Stewart, Shumway and Doudoroff, 1967) and coho salmon, Oncorhynchus kisutch (Doudoroff and Warren, 1957). The reduced growth rate was correlated with a reduced food intake and not with a reduction in metabolic efficiency (Stewart et al., 1967). Similar data are not available for any species of rough fish. Bouck and Ball (1965) observed a change in blood serum proteins in largemouth bass and bluegills subjected to diurnally fluctuating dissolved oxygen levels. The change, a reduction in the low mobility fraction of the protein, was interpreted as an indication of physiological stress. Such a change was not found in the yellow bullhead, a fish common to low oxygen content waters.

Sustained low but sublethal D.O. concentrations have been shown to reduce the swimming performance in several centrarchids and salmonids (Dahlberg, Shumway and Doudoroff, 1968; Katz, Pritchard and Warren, 1959). Grahm (1949) found a reduced scope for activity in brook trout which were exposed to low oxygen concentrations. Similar

studies have not been done for diurnally fluctuating D.O. concentrations, but it is reasonable to presume that the nocturnal low D.O. values could affect activity levels of some fish and this could possibly reduce feeding ability.

Carp are commonly found in waters with low oxygen contents (Burns, 1966; McCrimmon, 1968). Carp hemoglobin has been shown to have a high oxygen affinity and a small Bohr effect, thus carp are able to saturate their blood with oxygen in water which has a low oxygen and a high carbon dioxide content (Basu, 1959; Fry, 1957; Riggs, 1970). Garey and Rahn (1970) have shown that tissue oxygen concentrations in carp are independent of diurnal fluctuations in the oxygen content of the water in which the carp are maintained. Muennich (1958) found that carp blood sugar levels were not affected by the oxygen content of the water when the D.O. was varied from supersaturation to very low levels.

Smallmouth bass are associated with clean, well aerated aquatic environments (Emig, 1966; Hubbs and Bailey, 1938). Detailed information on their blood oxygen characteristics is lacking but they are probably similar to those found for other centrarchids. In order to be able to saturate their hemoglobin with oxygen, centrarchids generally require water with a higher D.O. concentration than do carp. They also show a significant Bohr effect (Fry, 1957; Riggs, 1970).

Bouck and Ball (1967) determined the blood serum protein distribution in a variety of fish. They found that fish associated with polluted environments had high levels of low mobility proteins, while those associated with clean environments had low levels of these proteins. In their experiment, carp had 20.2 grams of low mobility protein per 100 g of sample protein, while smallmouth bass had 2.7 g/100 g. The biological significance of this protein fraction is unknown, but the amount present in a species appears to be genetically determined.

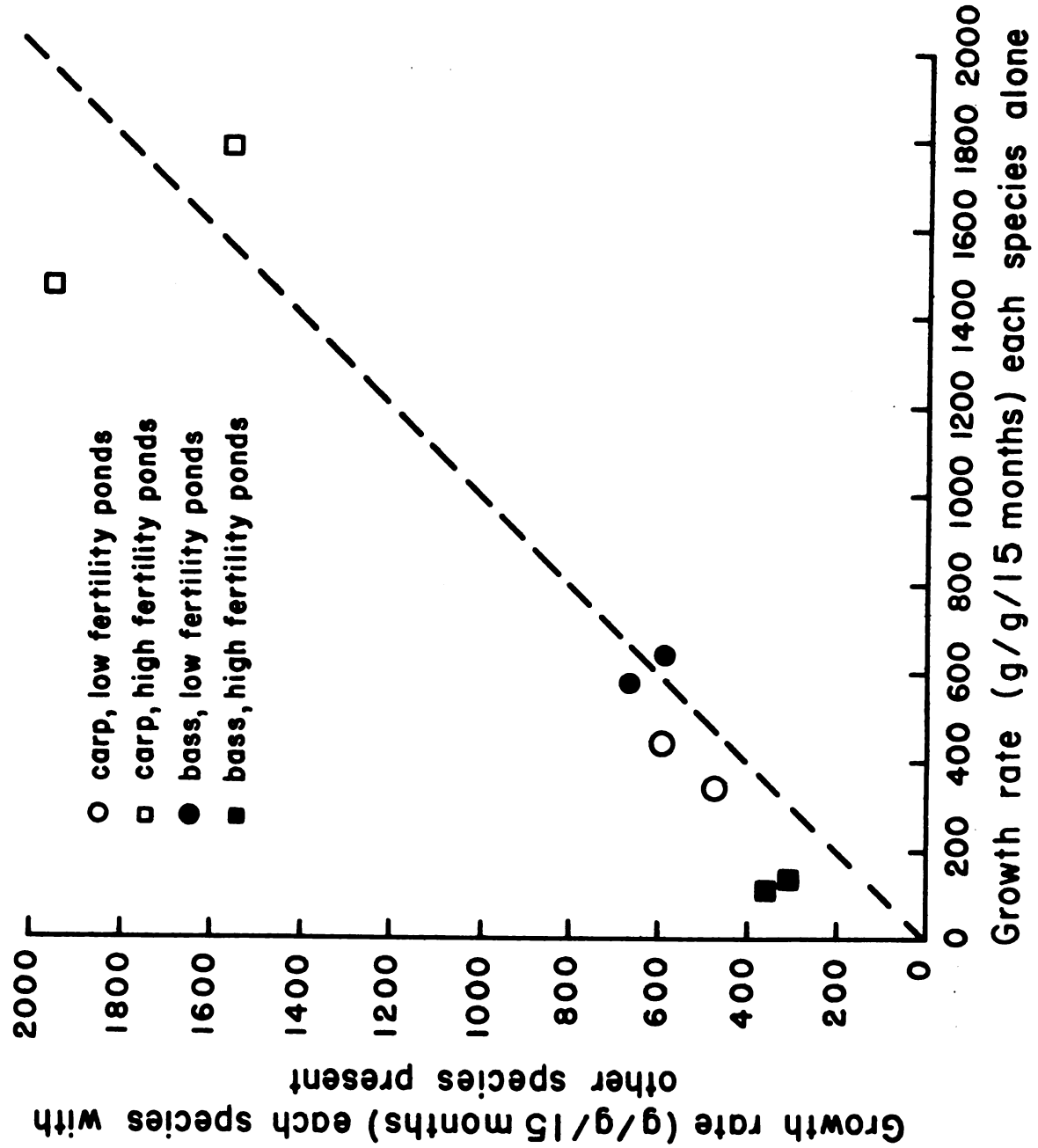
From the above information it can be concluded that the physical and chemical conditions associated with eutrophication, i.e., decreased light penetration, increased diurnal oxygen flux, increased decomposition and ammonia byproducts, are quite likely to have had an adverse effect on smallmouth bass and are unlikely to have affected carp. The reduced growth rate and RNA content of the bass from high fertility ponds was an expression of this effect. Carp, being unaffected, could use the increased production in the enriched ponds to obtain an increased growth rate and RNA content.

Effects of Species Interactions on Fish Populations

The expected inhibition of bass by carp did not occur. There was no significant difference in growth rate of bass when carp were or were not present ($p = 0.5$).

There also was no significant difference in the growth rate of carp when bass were present or absent ($p > 0.5$). There are some trends in the data, however, and these are best seen in an interaction graph (Figure 14). This graph is obtained by pairing the growth rate of a population of one species alone with the growth rate of that species when the other species was present. This was done for both species of fish and for both fertility levels. The populations were paired on the basis of "potential for growth." This potential was estimated from the amount of food organisms present in the experimental ponds before the fish were introduced. An index of the potential for growth was obtained by summing the standing crop biomass estimates of zooplankton and benthos from the July, 1969 samples for each pond. Fish populations from the ponds were then paired on the basis of similarity of this index. If there was no effect on the growth rate of a species due to the presence or absence of the other species, then the points plotted should be equidistant from each axis, falling on a line drawn through the origin at a 45 degree angle. If one species inhibits the other these points would fall below this line, and if a beneficial interaction were present, the points would fall above the line. Distance from the origin is an indication of the magnitude of the growth rate. An inspection of Figure 14 shows that the growth rate of carp in high fertility ponds was not

Figure 14. Interaction graph showing the effect of the presence of the other species on the growth rate of each species of fish.

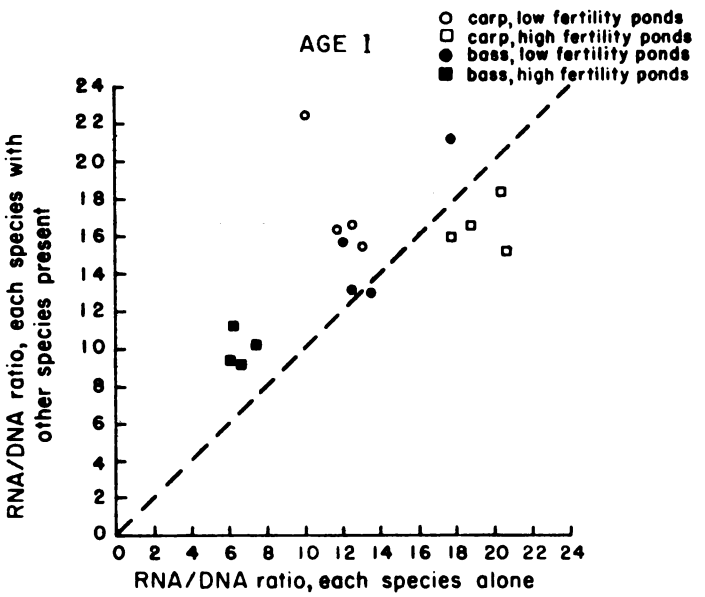

Growth rate (g/g/15 months) each species with other species present
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0 200 400 600 800 1000 1200 1400 1600 1800 2000
Growth rate (g/g/15 months) each species alone
carp, low fertility ponds
carp, high fertility ponds
bass, low fertility ponds
bass, high fertility ponds

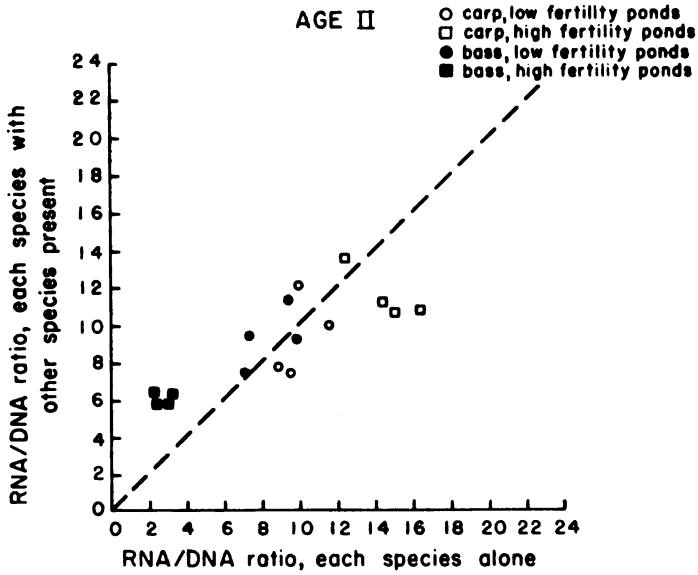
affected by the presence of bass, and that the growth rate of bass in low fertility ponds was not influenced by the presence of carp. However, there is a slight increase in the growth rate of carp in low fertility ponds when bass are present, and a similar increase in the growth of bass in high fertility ponds when carp were present. Since these effects were not significant their importance is doubtful, but it is interesting to see that they are opposite of what was expected. Since growth rate depression due to species combination did not occur, food competition can be ruled out even though the ponds in which carp were present had lower standing crops of benthos and zooplankton in the summer of 1970. An examination of the stomach contents of selected fish at the termination of the experiment indicated that fish of all ages and both species were feeding heavily on midge larvae of about the same size. Midge larve comprised the greatest portion of the invertebrate biomass throughout the study. The fact that the carp did not cause an increase in turbidity of the water could be important. While this factor could explain the absence of a depression of bass growth in the presence of carp, it cannot explain the increase which was found. The lack of any significant species interaction may have resulted because the conditions necessary for such an interaction were not created.

If the RNA data are examined, it is seen that bass in high fertility ponds had higher RNA levels when carp were present than when carp were absent ($p < 0.01$). The same is true for carp in low fertility ponds when bass were present. The same trends were found in the growth rate data but were non-significant. Bass in low fertility ponds still show no species interaction effect ($p = 0.4$). Carp in high fertility ponds show a significant ($p = 0.01$) depression in RNA level when bass are present. An interaction graph shows these effects very well (Figure 15). The graph is plotted the same as that for the growth rate data (Figure 14). Each point represents the mean of the subsamples from a single fish. The fish populations were paired as they were for growth rate data; fish of the same age were paired randomly between the populations.

The increased growth rate and RNA level shown by bass in high fertility ponds when carp were present may be a result of the bass feeding on the eggs and fry of spawning carp. This would be a plentiful and easily available food supply. The feeding actions of carp may also have dislodged benthic organisms, making them easy prey for the bass. A type of social facilitation of feeding could also be operating (Baerends, 1957). That is, the presence of fish that are actively feeding may trigger the feeding response in other fish. The relatively low standing crops of invertebrates in low fertility ponds and

Figure 15. Interaction graph showing the effect of the presence of the other species on the RNA-DNA ratio of each species of fish.


AGE I
carp, low fertility ponds
carp, high fertility ponds
bass, low fertility ponds
bass, high fertility ponds
RNA/DNA ratio, each species with other species present
RNA/DNA ratio, each species alone
0 2 4 6 8 10 12 14 16 18 20 22 24


AGE II
carp, low fertility ponds
carp, high fertility ponds
bass, low fertility ponds
bass, high fertility ponds
RNA/DNA ratio, each species with other species present
RNA/DNA ratio, each species alone
0 2 4 6 8 10 12 14 16 18 20 22 24

absence of spawning carp may have made the effects of these mechanisms unimportant in those ponds and thus resulted in no effect on the growth of the bass.

The increase in the growth rate and RNA level of carp from low fertility ponds when bass were present is more difficult to explain. A social facilitation in feeding response seems unlikely but should not be ruled out. Carp are able to absorb organic molecules from the water (Brown, 1957) and therefore could make use of some of the waste products of the bass plus their own to obtain additional energy.

The decrease in RNA level but not growth rate which was found in carp in enriched ponds when bass were present is unexplained. This is the only experimental condition in which the RNA level and growth rate data do not agree. Creelman and Tomlinson (1959) determined the quantity of RNA and DNA in the muscle tissue of sockeye salmon (Oncorhynchus nerka) at three points in their spawning migration. They found a progressive decline in the RNA content of the flesh. Since carp spawned in the high fertility ponds, the decrease in RNA level recorded might be associated with spawning. However, a similar decline did not occur in the high fertility ponds from which bass were absent, where the carp also spawned successfully. In fact, the carp from every high fertility pond showed increased RNA levels as

compared to all low fertility ponds, regardless of the presence or absence of bass.

When comparing growth rates with RNA changes in the fish, it is important to note that the growth rates were determined over a period of 15 months. RNA ratios, on the other hand, are known to change drastically in a matter of days when fish are fed after being starved or deprived of food after being fed regularly (Bulow, 1970). Thus the RNA ratios probably represent only the growth rate of the last week or so before the populations were censused.

Since spawning was intended to be excluded from this experiment, no information on the effects of eutrophication or species interaction on the reproductive success of either species was collected. Given the high reproductive potential of warm water fish (Carlander, 1953) and the widely different spawning habitats preferred by smallmouth bass and carp (Hubbs and Bailey, 1938; McCrimmon, 1968) a serious effect on population size because of spawning interference is relatively unlikely to occur. Reynolds (1965) correlated annual recruitment in a population of smallmouth bass with mean summer water temperature and not number of adult spawners. There is one recorded case (Latta, 1963) where the activity of carp roiled the water in a shallow cove and spawning smallmouth bass deserted their nests.

The general agreement of responses in growth rates and RNA levels tends to substantiate the hypothesis that RNA level is a reflection of recent growth in fish (Leslie, 1955). The fact that most non-significant species interaction trends seen in the growth rate data were present and significant in the RNA data suggests that RNA level may be a more sensitive indicator of the status of a fish population than is growth rate.

SUMMARY AND CONCLUSIONS

This experiment was designed to test the influence of nutrient level and a rough fish population (carp) on a game fish population (smallmouth bass). Two nutrient levels (high and low) were used, with high levels created by addition of inorganic nitrogen and phosphorus. Growth rate and RNA levels were used as indicators of the status of the fish populations.

Both bass and carp populations had approximately the same growth rates in low fertility ponds. The average RNA concentrations for the two species also were similar under these conditions. In high fertility ponds, both the growth rate and RNA content of the bass declined severely. Under the same conditions carp experienced a large increase in growth rate and RNA level.

The growth rate data show no significant species interactions at any fertility level. Bass had slightly higher growth rates in high fertility ponds when carp were present than when the bass were alone, and carp in low fertility ponds also showed a slight increase in growth when bass were present. These trends were not

statistically significant. The RNA data, however, showed the same trends and here they were significant. There was no effect on the RNA levels of bass in low fertility ponds when carp were present. However, in high fertility ponds, carp showed a significantly reduced RNA level when bass were present. Growth rate data showed no indication of this effect.

The depression of growth rate and RNA level in bass in high fertility ponds was attributed to the large diurnal changes in oxygen content of the water in these ponds, to decreased clarity of the water caused by phytoplankton blooms, and to increased ammonia concentrations due to increased bacterial decomposition of organic matter. The slight increase in growth and large increase in RNA levels noted in bass when carp were present in high fertility ponds may have been a result of the bass feeding on carp eggs and fry. There was no evidence of food competition between bass and carp. The changes in carp growth rate and RNA levels in the presence of bass have not been explained.

It is concluded that, under the conditions which were generated in the experimental ponds, the decrease in smallmouth bass observed in the enriched ponds was the result of the physical and chemical changes in the water due to increased plant and bacterial activity and not to interference by carp. Carp could not be shown to interfere

with the smallmouth bass under any conditions employed. Extensive testing under varied conditions should be performed to determine the validity of these conclusions.

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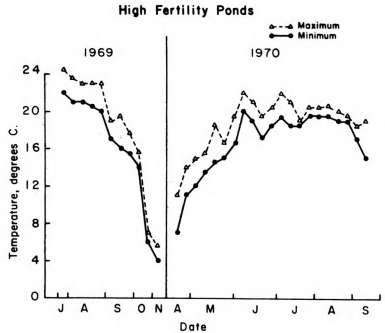
APPENDICES

APPENDIX A

MAXIMUM AND MINIMUM TEMPERATURES RECORDED IN A LOW AND A HIGH FERTILITY POND

Figure A-1. Weekly maximum and minimum temperatures recorded in the experimental ponds.


High Fertility Ponds
Maximum
Minimum
1969
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Temperature, degrees C.
0
4
8
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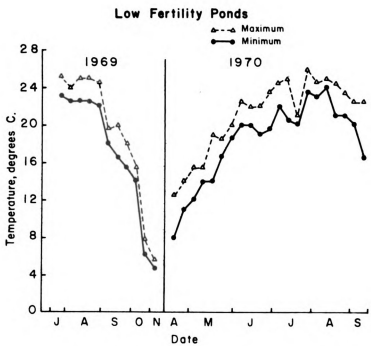
Date


Low Fertility Ponds
Maximum
Minimum
1969
1970
Temperature, degrees C.
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12
16
20
24
28
J A S O N A M J J A S
Date

APPENDIX B

METHOD FOR RNA-DNA ANALYSIS

METHOD FOR RNA-DNA ANALYSIS

The following is the RNA and DNA analysis technique as developed by Bulow (1970). It is reproduced here in detail for the benefit of those who may wish to use this technique.

Preparation of Fish Tissue Samples

About one gram of dorsal muscle tissue was removed from each fish and placed in an individual glass vial of about 50 ml capacity. The vials were capped and immediately frozen. After one week of freezing the fish samples were defatted and dried by a chloroform-methanol-ether process and stored for subsequent nucleic acid analysis. This was done by removing the vials of fish samples from the freezer and adding a 2:1 mixture of chloroform and methanol to each vial. About 35 ml of solvent were used for each extraction. The vials were then capped and agitated periodically with an automatic shaker. After a 12 hour period the solvent was changed and the process repeated for an additional 12 hours. The solvent was then replaced with ethyl ether for an additional 24 hour extraction period. The ether was then poured off and the samples left to air dry for 24 hours. Dry, fat-free samples were ground to a powder in a Wiley mill equipped with a number 40 sieve and stored in capped vials.

Preparation of RNA and DNA Standard Stock Solutions

One hundred mg of RNA powder (Nutritional Biochemicals Corp.) were placed in a large test tube and suspended in 5 ml of distilled water. The mixture was warmed and agitated until it appeared to go into solution. Ten ml of 5 per cent trichloroacetic acid (TCA) was then added and the solution heated at 100 C for 60 minutes. (In heating tubes in the constant temperature bath it was found desirable to cover them with large marbles to prevent loss through evaporation and yet allow for gaseous expansion.) The solution was then cooled and brought to a volume of 200 ml with 5 per cent TCA.

The DNA standard stock solution was prepared in the same way. Stock solutions were quite stable when stored under refrigeration.

Determination of Total Phosphorus Content of Standard Stock Solutions

To accurately establish the concentrations of standard stock solutions of nucleic acids, total phosphorus content was determined colorimetrically using the procedure of Fiske and Subbarow (1925). All colorimetric determinations were made on a Beckman model DK spectrophotometer.

Preparation of reagents and solutions.

Sulfuric acid: Stock solutions of 5 N and 10 N were prepared.

Molybdate reagent I (Acid): Ammonium molybdate salt (25 g) was dissolved in 200 ml of distilled water. The solution was then rinsed into a one liter volumetric flask containing 500 ml of 10 N sulfuric acid, and diluted to the mark with distilled water. The reagent was stored in a plastic bottle under refrigeration.

Molybdate reagent III (Water): Ammonium molybdate salt (25 g) was dissolved to a volume of one liter with distilled water. The reagent was stored in a plastic bottle under refrigeration.

Standard phosphate: In a one liter volumetric flask, 0.3509 g of monopotassium phosphate was dissolved in a small amount of distilled water. Ten ml of 10 N sulfuric acid were added and the solution brought to the mark with distilled water. This standard stock solution contains 80 ug of phosphorus per ml. From this stock solution additional dilutions to 40 ug/ml and 20 ug/ml were made. Since 5 ml of stock solution contains 400 ug of phosphorus, these dilutions became 4, 2 and 1 ug of phosphorus per ml when 5 ml is brought up to 100 ml in subsequent phosphorus analysis.

Amino-napthol-sulfonic acid reagent (ANSA): This reagent was purchased in pre-mixed dry form (Hartman-Leddon Co., Inc., Philadelphia, Penna.). It was prepared according to the manufacturer's instructions and stored in an amber bottle.

Determination of standard curve

Three standards (4, 2 and 1 ug of phosphorus per ml) and a blank were prepared in four 100 ml volumetric flasks. Five ml of standard phosphate solution (replaced with 5 ml of distilled water for the blank), 65 ml of distilled water, 10 ml of molybdate reagent I and 4 ml ANSA were added to each flask. The contents were then mixed, diluted to the mark with distilled water and again mixed. After standing for five minutes the optical density was measured against the blank at 700 mu.

Determination of nucleic acid phosphorus content

The procedure, which was identical for both RNA and DNA standard stock solutions, was as follows:

1. Five ml of nucleic acid solution and 5 ml of 5 N sulfuric acid were added to a Pyrex test tube (200 x 25 mm).

2. Several glass beads were added to the mixture which was then carefully boiled over a microburner. Caution: This procedure should be performed under a hood with the solution being constantly agitated. If not agitated it will superheat and spurt out. When the solution had boiled down to a small amount of fluid, dense fumes appeared and the solution turned a dark yellow. At this time, the flame was turned down and the solution kept boiling. The solution became progressively darker until no further darkening occurred. At this time the solution

was decolorized by letting one or two drops of concentrated nitric acid run down the wall of the test tube. Boiling was continued for about 30 seconds more to remove the nitric acid.

3. The test tube was removed from the heat, allowed to cool slightly and placed under cold tap water to further cool it.

4. Contents of the tube were emptied into a 50 ml volumetric flask. The tube was rinsed three times with about 10 ml of distilled water, the washings being poured into the flask.

5. Five ml of molybdate reagent III and 2 ml of ANSA reagent were added to the flask.

6. The solution was mixed, brought to the mark with distilled water and again mixed.

7. After standing for five minutes, the blue color was measured against a blank at 700 mu. (The blank was prepared by mixing the same reagents, but replacing the 5 ml of nucleic acid solution with 5 ml of 5 per cent TCA. The boiling procedure was eliminated.) Comparison of the optical density measured in the nucleic acid solution with the phosphate standard curve gave the concentration of phosphorus in the nucleic acid standard stock solution. RNA and DNA standards were then prepared by diluting the nucleic acid standard stock solutions with 5 per cent TCA. Standard curves were then run with each batch of unknowns.

Extraction of Total Nucleic Acid

Nucleic acid was extracted from fish tissue samples by the methods of Webb and Levy (1955). The procedure was as follows:

1. Samples of dry, fat-free fish powder were weighed and suspended in 3 ml of 5 per cent TCA in 15 ml centrifuge tubes. Fifty mg of fish powder was used per sample.

2. Suspensions were allowed to stand at room temperature until the powder became saturated and fell to the bottom of the tube.

3. The tubes were covered with glass marbles and placed in a boiling (100 C) water bath for exactly 30 minutes.

4. At the end of 30 minutes the rack of tubes was immediately placed in a cold water bath.

5. After being cooled, each tube received an additional 3 ml of 5 per cent TCA. The tube contents were mixed thoroughly by shaking.

6. Each tube was centrifuged for about 15 minutes and the supernatant fluid carefully drawn off with a pipette, leaving the insoluble tissue protein in the tube bottom.

7. Supernatant fluid, containing the combined nucleic acids, was stored under refrigeration in capped glass vials.

Colorimetric Determination of RNA Content

Analysis of RNA content followed the orcinol method described by Schneider (1957) with a modification by Lusena (1951).

Preparation of reagents

Orcinol reagent: The orcinol should be a perfectly white crystalline product. Orcinol was purified by boiling in glass distilled benzene, decolorizing with charcoal and crystalizing after adding hexane. The process was repeated twice and was performed under a hood using a heater which could be regulated to hold the benzene at boiling temperature (80 C). One gram of purified orcinol was dissolved, immediately before use, in 100 ml of concentrated HCl containing 0.5 g of $FeCl_3$.

Method of color development:

1. Duplicate 0.5 ml aliquots of nucleic acid extract as well as 0.5 ml samples of RNA and DNA standard solutions were transferred to centrifuge tubes.

2. Each tube received 1.5 ml of distilled water and 2 ml of orcinol reagent. The contents were mixed.

3. A blank of 2 ml distilled water and 2 ml orcinol reagent was prepared.

4. The tubes were covered with marbles and heated in a water bath at 100 C for 60 minutes.

5. After cooling the tubes in a cold water bath, optical density was measured against the blank at 660 mu.

Since orcinol reacts with DNA as well as RNA, a correction must be made for the fraction of optical density resulting from the DNA present in each sample of nucleic acid extract. This was done through use of the DNA standards in the orcinol reaction. Calculations were made as described by Schneider (1957).

Colorimetric Determination of DNA Content

DNA was determined using the Burton modification of the diphenylamine reaction (Burton, 1956).

Preparation of reagents:

Diphenylamine reagent: Fisher special indicator grade diphenylamine (1.5 g) was dissolved in 100 ml of glacial acetic acid and 1.5 ml of concentrated sulfuric acid. Just prior to use, 0.5 ml of aqueous acetaldehyde (2.1 ml acetaldehyde per 100 ml glacial acetic acid) was added to the reagent. This reagent was prepared just prior to each use. Acetaldehyde is extremely volatile and extreme care should be exercised in its use.

Perchloric acid (1 N): About 800 ml of distilled water was placed in a one liter volumetric flask and 83.5 ml of concentrated (70 per cent) perchloric acid was added. The total volume was then brought up to one liter with distilled water.

Method of color development:

1. Duplicate 1 ml aliquots of nucleic acid extract were combined with 1 ml of 1 N perchloric acid and 2 ml of diphenylamine reagent. Samples of DNA standards were similarly prepared.

2. A blank was prepared by substituting 1 ml of 5 per cent TCA for the nucleic acid extract.

3. The contents were stirred and the tubes heated at 30 C for approximately 18 hours.

4. Intensity of color was measured at 600 mu against the blank and compared with values obtained with the DNA standards.

Results were reported as concentrations of RNA and DNA. The values obtained in the above analysis are concentrations of RNA and DNA phosphorus. Since the phosphorus content of both RNA and DNA is about 10 per cent (Leslie, 1955), the values obtained are multiplied by a factor of 10 to obtain RNA and DNA values. If only RNA/DNA ratios are desired, this calculation is not necessary.