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SERUM PROTEIN VARIABILITY IN BLUEGILLS, *LEPOMIS MACROCHIRUS* RAFINESQUE, FROM IOWA FARM PONDS

by

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I. INTRODUCTION

On June 1, 1964, the Iowa Cooperative Fisheries Research Unit initiated studies relating to the physiology of farm pond fish. The original objective of the study was to determine if some density dependent physiological parameters could be elucidated. Two phases of the study were undertaken, blood physiology and histology of fish from normal and crowded populations. Results of the histological study were reported by Grover (1966).

One of the basic problems in Iowa farm ponds is overcrowding, which results in poor growing conditions and stunted fish. Stunting is defined by Lagler et al. (1962) as a reduction in specific growth as compared to the average for the area or species. Food supply is probably one of the most important factors affecting the growth of fishes (Brown, 1957). Bennett (1962) considers the food supply to be one of the most important factors to be studied when comparing growth rates of fishes. Therefore, if an adequate food supply is not available, in all probability fish will not attain the desired growth rate suitable for the existing physio-chemical conditions. The degree of crowding might also be a factor in growth rates of fish. Brown (1946) found no apparent effect of absolute tank size on groups of 2 year-old Salmo trutta, but she found an optimum degree of crowding. Still other authors have postulated that growth and reproduction were inhibited by ammonia and other material excreted into the water (Swingle, 1956a, Kawamato, 1961). Although food supply is a very important factor in

fish attaining good growth rates, these other factors should be considered.

A method often used in mammalian populations to determine population stress is based on the concept of the sociophychological-physiological feed-back system of Christian (1959). This technique relies on adrenal hypertrophy as an indicator of population stress. Increased adrenal weights are indicative of stressful condition. Negus (1961) questioned this theory and provided evidence that this theory would not apply in all cases. The greatest disadvantage of using adrenal weights in fish is that in most bony fishes the interrenal tissue (adrenal) is more diffuse and usually found along the post cardinal veins as they pass through the head kidney (Lagler et al., 1962). For this reason adrenal weights were not attempted.

Another possibility related to adrenal function is the assay of adrenal corticosteroids in the blood. The presence of these secretions in fish blood has been demonstrated by Nandi and Bern (1960), Phillips et al. (1959), Hane and Robertson (1959), and Jones and Phillips (1960). Nandi and Bern (1960) stated that no correlation could be made between the secretory pattern of the corticosteroids and the habitat, physiology or taxonomic position of the species. Hane and Robertson (1959) found no qualitative variation in the blood corticoids of salmon under different environmental conditions. Jones and Phillips (1960) reported that 17-hydroxycorticoid levels were altered in carp blood when the fish were removed from water and transported varying distances. Greater differences were also noted in fish that had undergone forced swimming.

After making histochemical determinations of cholesterol and ascorbic acid content of adrenocortical cells of goldfish, Chavin and Kovacevic (1961) concluded that ordinary methods of capturing fish rapidly with a net are sufficient to deplete the adrenals of these substances. These findings were further confirmed with both fresh water and marine teleosts (Chavin and Olivereau, 1961). Because of the lack of correlation between the secretion of corticosteroids and habitat and physiological factors and the apparent sensitivity of these secretions to short term effects rather than to long term effects, adrenal corticoids were not considered in this study. Instead, attention was directed to the blood proteins.

Blood is a complex tissue composed of suspended cells (erythrocytes, leukocytes, and thrombocytes) and a fluid plasma portion, which is made up of water (90%), inorganic constituents, materials in transport and proteins (albumins, globulins, lipo proteins, and fibrinogen). The blood functions principally as a transport system, transporting nutrients, hormones, and oxygen to the tissues and is the media into which waste products are discharged. The blood proteins serve to maintain fluid balances between the tissues and the blood (albumins), as a defense against infection (globulins), for prevention of hemmorrhage (fibrinogen), and as reserve proteins (amino acids) for the tissues.

The biological significance of proteins cannot be overemphasized. They not only form the structural framework of tissues, but also act as controllers of metabolic functions. The protein constituents of the body exist as a dynamic equilibrium (Cantarow and Schepartz, 1962). All

molecules, including structural molecules, are constantly being broken down and rebuilt. In growing organisms the rate of breakdown is exceeded by the rate of synthesis. When organisms are deprived of exogenous protein, the rate of anabolism is exceeded by the rate of catabolism. The blood proteins occupy a central position in protein metabolism because of their close relationship to protein synthesis in the liver as well as their interrelationship with other tissues throughout the body. Therefore it is easy to visualize that an influence on one body function would influence other body functions and be reflected in the blood proteins.

Blood proteins of various animals have been studied extensively for two reasons: (1) they are the most easily attainable sample of protein in the body and (2) the plasma proteins occupy a central position in protein metabolism. Because of the interrelationships between proteins of the blood and tissue, a great deal can be learned about the general status of protein metabolism by examining the blood proteins (Cantarow and Schepartz, 1962). Guyton (1961) states that the plasma proteins function as a labile protein storage medium and represent a rapidly available source of amino acids whenever a particular tissue requires them.

Bier (1959) believes the changes in blood protein are related to the physiological condition of the test organism. Dunn and Pearce (1961) agreed with Bier and suggested that the general increase in low-mobility proteins in human serum represents a non-specific response to stress and termed this a stress pattern.

Booke (1964) states that a close relationship exists among the blood, the protein system, and the physiological state of a fish and presented a comprehensive literature review of fish blood proteins and the factors that cause alterations in them. He cited studies showing that sex, season, hibernation, hormones, oxygen depletion, temperature, and age have an effect on the serum complement of fish. Although the concentration of the serum components may be altered, the molecular structure or species specific nature of these proteins will not change.

Fish blood proteins have been used for taxonomic and comparative studies since the initial study of Nuttal (1901). By necessity the early studies used quantitative chemical methods, Lepkovsky (1929); Vars (1934); Field et al. (1943); and others. It was not until the introduction of the moving boundary electrophoretic method (Tiselius, 1937) that comparative studies of the various protein components could be made.

Booke (1964) in his review of fish serum proteins cited 26 authors that related changes in fish total serum proteins to various physiological influences. The causes of these changes have been listed previously.

More recent studies have made use of electrophoretic techniques to study relationships of fish to their environment. The bulk of these studies is concerned primarily with taxonomic problems and will not be considered here.

The stress pattern of Dunn and Pearce (1961) has been demonstrated in fish by various authors. Neuhold and Sigler (1960) and Fujiya (1961)

found increases in low-mobility proteins and reductions in high-mobility proteins in fish subjected to toxic materials. Bouck and Ball (1965) reported that the proportional amounts of low-mobility proteins were significantly increased in bluegills and largemouth bass when subjected to hypoxic stress, but not in yellow bullheads (Ictalurus natalis). Bouck (1966) found that sublethal conditions which included low diurnal oxygen levels with and without food offered, exposure to divalent nickel, exposure to divalent nickel concurrently with low oxygen, high carbon dioxide levels, and fasting altered the plasma protein composition of rock bass (Ambloplites rupestris).

Fish infected with disease organisms also showed alterations in blood protein patterns. Sindermann and Mairs (1958) found that sea herring (no species given) infected with a fungal disease (Ichthyosporidium hoferi) has reduced amounts of the fastest protein component. Hunn (1964) reported that brook trout (Salvelinus fontinalis) with kidney disease showed increases in the low-mobility proteins with a complete disappearance of the fastest moving fraction.

Diet has also been shown to alter the proportional amounts of the protein bands. Lysak and Wojeik (1960) fed diets containing various amounts of protein to one and two year-old carp. Low protein diets were associated with reductions in the high-mobility fraction. Sorvachev (1957) and Fujiya (1961) reported results similar to those of Lysak and Wojeik in starving carp. Sindermann and Mairs (1961) stated that fraction 1 slumped drastically in serum of alewives (Alosa pseudoharengus) held experimentally in sea water under starvation for

two months.

Ambient temperatures (16° C.) were associated with increased amounts of low-mobility proteins in rainbow trout (Meisner and Hickman, 1962). Various photoperiods were tested in conjunction with temperature but had no apparent effect.

Bouck and Ball (1966) investigated the effects of capture methods on plasma proteins of rainbow trout. Stressful capture methods (angling and electro-shocking) caused reductions in the amount of plasma proteins and caused small but significant changes in the composition of these proteins. The authors felt these changes were due to in vivo coagulation.

Other authors have reported changes in serum protein patterns of fishes caused by natural maturation processes. Moore (1945) reported changed electrophoretic patterns of fishes and other animals as age and development progressed. Vanstone and Ho (1961) found that as coho salmon (Oncorhynchus kisutch) matured sexually, a sixth plasma protein fraction occurred which was admixed with fraction 4. This fraction disappeared from the plasma at about the same time eggs were released from ovarian tissue, and it was absent from spawning or spawned-out fish. Bouck and Ball (1965) observed sexual differences in plasma proteins of spawning pike (Esox lucius). Booke (1965) found that as whitefish grew older, two serum protein fractions increased in amount. These fractions were thought to be in the globulin portion of the serum when compared to human serum.

Inherent individual variations in serum proteins were reported by

Huntsman (1966). He found serum proteins too variable to study the taxonomic relationships of the family Catostomidae and indicated that his method (disc electrophoresis) might have been too sensitive to individual genetic differences and environmental and physiological effects to be used in taxonomy. Tsuyuki and Roberts (1966) reported extremely variable protein patterns in fishes from the Great Lakes region. They felt this variability might be due to the genetic diversity of fresh water fish.

The results of electrophoretic studies of fish serum proteins can be summarized as follows: (1) blood protein patterns are altered by sex, age, thermal stress, diet, disease, toxicity, hypoxic stress, and capture methods, (2) usually the induced changes are similar to the stress pattern of humans reported by Dunn and Pearce (1961), and (3) inherent variation exists in blood proteins of freshwater fish, which might be due to individual genetic variation or environmental and physiological effects.

The specific objectives of this study were: (1) to determine if serum proteins could be used as a tool to elucidate the relative well-being of bluegill sunfish (Lepomis macrochirus) sampled from varying population densities, (2) to determine the effect of age, sex, maturity, season, feeding, and crowding on the serum proteins of bluegill sunfish.

These objectives were tested by selecting study ponds in central Iowa with varying population densities and periodically sampling bluegills from these ponds to make electrophoretic comparisons of the blood proteins. Population densities, ages, and growth rates for fish

from each pond were also determined for comparative purposes. Laboratory experiments were conducted to determine the effect of diet and crowding on the serum proteins under controlled conditions.

II. STUDY AREAS

A. Location

The farm ponds included in this study are located in central Iowa in Jasper, Polk, and Story counties (Table 1).

Table 1. Location and surface area of study ponds

County	Owner	Township	Range	Section	Area (Acres)
Jasper	Sparks	81 N	21 W	19	1.1
Polk	Huffacker	81 N	22 W	9	2.5
	Link	81 N	22 W	15	1.3
Story	Kimberley	82 N	21 W	35	0.78
	McLain	83 N	22 W	14	0.97

B. Soils

Huffacker, Link and Sparks ponds are located in Carrington loam soil (Stevenson and Brown, 1922 and Stevenson and Brown, 1926). This soil type is practically always acid in reaction. In topography the Carrington loam is strongly undulating to gently rolling with adequate drainage.

Kimberley Pond is located on Lindley loam, steep phase, soil which ranges from undulating to rolling with a slope greater than 15% (Meldrum and Perfect, 1941). This soil profile has a light colored surface soil with calcareous till near the surface. Gravel is present

throughout the soil and generally more abundant in the lower part. It is generally acid and low in organic matter.

McLain Pond is located in Clarion loam, eroded phase, soil (Meldrum and Perfect, 1941). This soil type occupies irregularly undulating to gently rolling land bordering some of the smaller streams; and in the uplands it is adjacent to larger streams. The soil profile has a 4-6 inches thick layer of dark material on the surface with calcareous materials occurring at a depth from 18-24 inches, which may come to the surface in certain areas.

III. POPULATION ESTIMATES

Preliminary population evaluations were made on the study ponds with a minnow seine or 25-foot bag seine in the shallow water areas. This seining gave an indication of the species present and whether the pond was balanced or unbalanced (Swingle, 1950). The Swingle (1956b) pond analysis procedure was used to determine the condition of the population before study was continued on a particular pond. The condition of balance is determined from the success of reproduction of bass and bluegills and the abundance of intermediate-sized bluegills. If young-of-the-year bass and bluegills are present without an abundance of intermediate-sized bluegills (2 to 3 inches), the pond is considered to be balanced. If only 2 to 3 inch bluegills are found and there is little or no reproduction of bass or bluegills, the pond is considered to be overcrowded with bluegills. When little or no reproduction is noted and no intermediate bluegills are present, the pond is considered to be overcrowded with bass.

If populations were suitable for further study, the pond was seined with a 60-foot bag seine (1/2 inch bar-mesh bag, 1/2 inch bar-mesh wings). All fish above 2.5 inches were marked by removing one or two pelvic fins. It was necessary to remove two fins from fish in ponds that had been estimated the previous year. Additional seining was delayed at least 48 hours to allow the marked fish to become redistributed. Seining was continued until approximately 30% of the fish in a sample were recaptured. A sample in this case

equaled one sampling day rather than one seine haul. It was noted in initial seinings that bluegills were not randomly distributed throughout the ponds but were found in schools; therefore, an attempt was made to sample as much of the pond as feasible in one sample and to capture bluegills from as many areas as possible. In the smaller ponds where different seine hauls might overlap, fish were held after marking until the entire pond was seined. This procedure was also followed whenever a pond was sampled by electro-shocking.

Populations were estimated by Schnabel (1938) and Schumacher-Eschmeyer (1943) formulae. These formulae are:

(1) Schnabel

$$P = \frac{\Sigma\ AB}{\Sigma\ C}$$

Σ = Summation
P = Population estimate
A = Number of fish caught at any time
B = Number of marked fish present
C = Number of marked fish recaptured

(2) Schumacher-Eschmeyer

$$P = \frac{\Sigma\left[(N^2)(M+U)\right]}{\Sigma(MN)}$$

N = Number of marked fish present
M+U = Total number of fish in each sample
M = Number of marked fish taken in each sample

The sample variance and standard error of the estimate were calculated as follows for the Schumacher-Eschmeyer estimates:

$$s^2 = \frac{1}{K-1} \left[\sum \left(\frac{M^2}{M+U} \right) - \frac{\sum (NM)}{N} \right]$$

K = Number of samples taken

$$S.E. = \sqrt{N^2 \frac{N}{\sum} \frac{s^2}{NM}}$$

Using the same data, the two estimates gave similar but different population estimates. Therefore, a choice was made between the two estimates. Ricker (1948) pointed out that the efficiency of the Schumacher-Eschmeyer estimates is at a maximum when N/P is equal to 0.5 whereas Schnabel's formula becomes more efficient as B/P approaches zero, and the two formulae are of equal efficiency when N/P = B/P = 0.25.

Since the proportion of marked fish to the total population in Sparks, Link, Kimberley, and McLain ponds ranged above 0.25, the Schumacher-Eschmeyer estimation was used on these ponds. The proportion of marked individuals to the population estimate in Huffacker and Link ponds in 1964 was below 0.25; the Schnabel estimate was used in this case (Table 2).

Table 2. Comparison of Schnabel and Schumacher-Eschmeyer estimates

Pond	Year	Estimates fish/acre		Ratio	
		Schumacher-Eschmeyer	Schnabel	N/P	B/P
Sparks	1965	2904	2218	0.58	0.45
Link	1964	3265	3400	0.23	0.22
	1965	3042	3041	0.27	0.27
Huffacker	1965	3750	3895	0.17	0.18
Kimberley	1965	5932	5311	0.29	0.26
McLain	1964	4234	4138	0.23	0.23
	1965	4186	3563	0.35	0.35
	1964	6445[a]	6290[a]	0.42	0.41
	1965	5081[a]	4056[a]	0.39	0.31

[a]Green sunfish. All others are bluegill estimates.

In each case, where one population estimate was assumed to be more efficient than the other, this estimate was higher than the other. Since there appears to be a greater chance to underestimate populations (Buck and Thoits, 1965), this was another reason for using the Schumacher-Eschmeyer and Schnabel estimates on the above mentioned ponds.

Buck and Thoits (1965) examined 20 published records of fish populations estimated by seining which were checked by poisoning or draining censuses. The range of errors within these data was considerable; the average error for the 20 estimates was approximately 38%. Errors of overestimation were few (5) and relatively minor in extent

of error (average about 13%); errors of underestimation were more common (15) and larger (average about 44%). Buck and Thoits found in their own study that the preponderance of underestimation to overestimation for bluegill populations was 39-3.

Certain basic assumptions must be true before marking and recovery methods can be used to estimate total fish populations (Ricker, 1948).

(1) Marked fish must become randomly distributed throughout the population.

(2) Marked fish are as vulnerable to the sampling methods as the unmarked.

(3) The marked fish suffer no greater mortality than the unmarked fish.

(4) Marked fish do not loose their marks.

(5) All marks are recognized and reported on recapture.

(6) Recruitment to the population is negligible during the time recoveries are being made.

To meet the assumption that marked fish were randomly distributed, fish were marked and a period of at least 48 hours elapsed before additional seining was attempted. Since four ponds were no greater than 1.3 acres in size, approximately 80-90% of the pond could be seined. The largest pond was 2.5 acres but due to its shape much of the pond could be seined. On two occasions a 220 volt, A.C., boom, electric shocker was used on the larger pond to collect fish. This method was more efficient for collecting bass, but not as efficient

for bluegills, especially after they had left the spawning nests. By allowing ample time for the marked individuals to redistribute and by seining as much of the pond as possible during each collection period, it was assumed that random distribution of marked individuals was achieved.

The second assumption that marked fish are as vulnerable to capture as unmarked fish is open to question. It might be possible that some individuals may become more adept at avoiding the seine than others (Fredin, 1950, Buck and Thoits, 1965). The less adept fish would have more likely been captured for marking and subsequently recaptured in the sample. Lawrence (1952) used three methods of capture in a 0.7-acre farm pond to determine if the bluegill estimate was biased by using one type of collecting gear. He made no estimate by poisoning or draining but felt that mixed procedures worked better at estimating largemouth bass populations. Westers (1963), studying two Michigan ponds, advocated capturing fish for marking by one method and using another method to obtain recaptures. Since only a seine was used to capture fish for marking and subsequently used to recapture fish for determining the estimate, I cannot say with certainty that this method was not biased. However, if the marked fish were more susceptible to capture after the first sample they should appear in subsequent samples more often and the population estimate should be decreased with time. However, in each case except one (Link pond) the initial estimate was lower than the final estimate. This might indicate that marked individuals were not caught in any

greater proportion than unmarked individuals.

Before and after each collection, the margin of the pond was checked for dead individuals to determine mortality due to marking; nevertheless, dead fish might have sunk to the bottom and escaped notice. Dead bluegills were found only in Link Pond; however, both unmarked and marked fish were found indicating that the mortality was not due to the marking procedure but to some unknown cause acting on the entire population. Bluegills which were not in good physical condition were not marked. Ricker (1949) demonstrated that fin clipping did not cause mortality in bluegills.

Since the population estimates were made in a short period (2-4 weeks), it is doubtful that fins were regenerated in this time. The same field investigators who did the marking also checked for marks, so it is improbable that marks were not recognized.

Recruitment to the population was avoided by setting a lower size limit of 2.5 inches. Individuals of the immediate age class could not attain this size during the duration of the marking and recapture period.

Pounds per acre were obtained by weighing each fish in a sample (one day), adding the weights and dividing by the number of fish to obtain the average weight. The average weight was multiplied by the number of fish per acre to get pounds per acre (Table 3).

Table 3. Average weights of bluegills from the five study ponds

Pond	Species	Number weighed	Average weight (grams)
Sparks	Bluegill	155	38.04
	Bass	37	127.94
Link	Bluegill	163	30.02
Huffacker	Bluegill	333	25.81
Kimberley	Bluegill	143	21.97
	Bass	81	22.97
McLain	Bluegill	153	27.60
	Green sunfish	181	8.67

Population estimates and total pounds per acre are listed in Table 4. Link and McLain pond populations were also estimated in 1964. Link Pond estimate for bluegills in 1964 was 3400 bluegill per acre. McLain pond estimates for bluegill and green sunfish were 4234 $\pm$13.2% bluegills per acre and 6445 $\pm$13.1% green sunfish per acre. In both cases the estimate for 1964 is higher than the 1965 estimate.

Cooper and Lagler (1956) considered a good estimate as one within the limits of approximately $\pm$5 percent of the actual population size and they felt this level of accuracy could be achieved. Fredin (1950) accepted $\pm$10 percent as desirable, but concluded that it was impractical to collect the number of samples needed to attain this goal. Robson and Regier (1964) suggested that $\pm$10 percent level of accuracy was the minimum that should be accepted in careful research into population

Table 4. Population estimates and pounds per acre for the five study ponds 1965

Pond	Size acres	Species	Estimate	Percent error of estimate	Pounds per acre	Total pounds per acre	Total fish per acre
Sparks	1.10	Bluegill	2904	4.7	243	243	3059
		Bass	155	59.7	b		
Link	1.30	Bluegill	3042	3.6	201	222	3227
		Bass	185	51.0	21		
Huffacker	2.50	Bluegill	3895[a]		221	221	3972
		Bass	77[a]		b		
Kimberley	0.78	Bluegill	5932	3.0	321	321	6613
		Bass	341[a]		b		
McLain	0.97	Bluegill	4186	3.0	254		
		Bass	341[a]		b		
		Green sunfish	5081	4.0	129	383	9608

[a]Schnabel estimate.

[b]Present but representative weights were not obtained.

dynamics. Buck and Thoits (1965) found that only in highly restricted categories did they find errors averaging less than 10 per cent.

In the present study the largest per cent error of the estimate for bluegills was 4.7 per cent using the Schumacher-Eschmeyer estimate (Table 4). The bass estimates were nowhere near this level of accuracy and are not considered reliable estimates.

Although the mathematical procedures may indicate that the estimates are reliable, they are no better than the sampling procedures followed. If the assumptions were not met that were described previously, the sample may not be a good estimate of the population present.

Bluegill standing crops from ponds in the present study averaged 241 pounds per acre, ranging from 201 to 287. The mean standing crop is greater than that reported by Carlander and Moorman (1956), but within the range reported by them. McLain Pond, which was overcrowded with bluegills and green sunfish, had standing crops of 254 pound of bluegill per acre and 129 pounds per acre of green sunfish. Carlander and Moorman found that ponds overcrowded with bluegills usually had a small standing crop and attributed this to the fact that bluegills in these ponds were usually below 3 inches and were not included in the estimate. Bluegills in McLain Pond were stunted and all were greater than 2.5 inches total length; therefore, all were included in the estimate. Green sunfish were also larger than 2.5 inches and were included. For this reason the pounds per acre of forage fish were greater in this pond than in the balanced ponds (Table 4).

IV. AGE AND GROWTH

General considerations and assumptions of the scale method are discussed by Van Oosten (1929). Evidence for validity for age determination by the scale method in the family Centrarcidae was given by Creaser (1926) and others.

A. Collection and Preparation of Scales

Age and growth determinations were made for 638 bluegills collected from five study ponds. All fish were collected with a 60-foot bag seine having 1/4-inch bar-mesh bag and 1/2-inch bar-mesh wings.

Several scales were removed from the right side of each fish at a point adjacent to the posterior margin of the depressed pectoral fin. Scale samples were stored in coin envelopes upon which were recorded total length, weight, date of capture, sex, and maturity stage. Total lengths (tip to snout to end of caudal fin with lobes of caudal fin parallel) were measured to the nearest one-tenth inch. Weights were obtained on a 500 gram spring platform balance to the nearest gram.

Impressions of uncleaned scales were made on clear plastic strips using a roller press (Smith, 1954). Impressions were analyzed at a magnification of 44X, with a scale projector similar to that described by Van Oosten, Deason, and Jobes (1934).

Criteria used for the identification of annuli were: (1) crowding of circuli, (2) crossing over of circuli in the lateral field, (3) wider spacing of circuli distal to a suspected annulus, and (4) erosion

of ctenni in the posterior field with more distinct ctenni distal to the annulus. Crossing over of circuli in the lateral field and erosion of ctenni were the most consistent features.

The positions of the focus, annuli, and anterior margin of the scale were marked on paper tab strips, which were used on a nomograph as described by Carlander and Smith (1944). Each scale was read at least twice without knowing the length of the fish or until agreement as to the position of the annuli was reached.

B. Body-Scale Relationship

To derive the body-scale relationship, it was assumed it could be adequately represented by a straight line (Ricker, 1942a, Lewis, 1950). Sprugel (1954) found that a curvilinear regression line fit his data better than a linear regression line; however, calculations based on linear regression appeared to be satisfactory for describing the growth history of McFarland Pond bluegills.

The linear distance between the focus and the anterior scale radius was measured from tab strips and was recorded as the anterior scale radius. A linear regression line was fitted to these data by the least squares method. The general formula for the regression line was:

$$L = a + bS$$

where L = body length (total length)

S = anterior radius of scale inches (X44)

a = intercept on the body length axis

b = slope of the line, a constant

The mathematical expressions for each pond are as follows: Sparks, $L = 0.66 + 1.1096\ S$; Link, $L = 0.65 + 1.4235\ S$; Huffacker, $L = 0.42 + 1.0956\ S$; Kimberley, $L = 1.00 + 0.9513\ S$; and McLain, $L = 1.9 + 0.7292\ S$.

The intercept on the body length axis seems to be overestimated for the McLain Pond data. Data for this pond were concentrated on one part of the curve (4.3 to 5.3 inches). Regier (1962) found that body scale relationships based on little data, or data poorly distributed over the range of total lengths, gave variable intercepts and slopes. Since no data were available for fish less than 4.3 inches total length, it is possible that the "a" intercept was not described accurately. Bluegill lengths covering a wider range were available from the other study ponds; therefore, the "a" intercept is probably described more accurately for these ponds.

Regier (1962) calculated the body-scale relationship for 24 New York farm ponds. He eliminated seven of these relationships because of few or poorly distributed data. An F-test indicated there were greater differences in intercepts than would be expected by chance; however, he felt the practical significance of these differences was negligible and he used the mean intercept for his calculations.

All growth calculations in future discussions will be based on a mean "a" intercept of 0.7 inches.

C. Growth Analysis of Bluegills

Data from all collections made in 1965 from the five study ponds were used for the calculation of average growth. These data are

summarized in Tables 5, 6, 7, 8, and 9. Problems encountered in aging fish in each pond will be discussed separately followed by a summary and comparison of growth rates with other Iowa lakes and ponds.

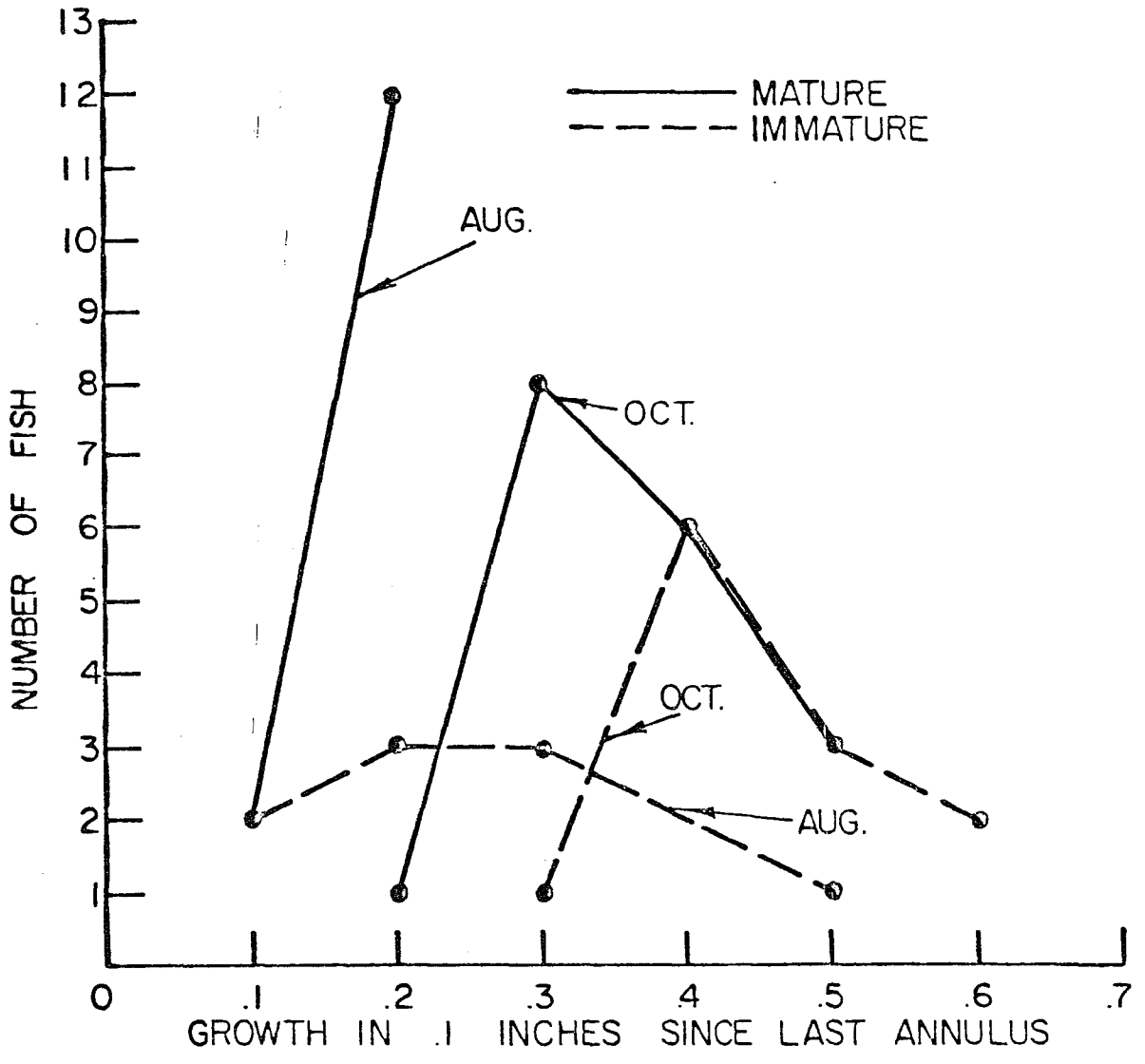
McLain Pond - This pond contained bass, bluegills, and green sunfish, but scales were collected only from bluegills. All fish were stunted with the exception of a few large bass. No reproduction was noted in 1964 and 1965.

No one and two-year-old fish were collected. This may have been due to lack of reproduction in these years or selection for larger fish for blood analysis. Mean calculated total lengths for annuli 1-5 are as follows: 1.5, 2.9, 3.8, 4.4, and 4.7 inches (Table 5).

Annuli occurred near the edge of the scale in the majority of individuals collected in August. This might indicate that a false annulus was being formed; however, when the growth since the last annulus was plotted for mature and immature fish (Figure 1), the immature fish exhibited greater growth from the annulus to the scale margin than the mature fish indicating that an annulus might have been formed earlier in the immature fish and not formed until late July or early August in mature fish.

Bennett (1962) listed factors that must be considered when comparing growth rates of fishes: (1) genetic potential of a given species, (2) a large available food supply per individual fish, and (3) length of the growing season. He stated further that in order to produce fish above average size, a large source of available food per

Figure 1. Frequency grouping of growth since the last annulus in mature and immature bluegills from McLain Pond, 1965


MATURE
IMMATURE
AUG.
OCT.
OCT.
AUG.
NUMBER OF FISH
13
12
11
10
9
8
7
6
5
4
3
2
1
0
.1
.2
.3
.4
.5
.6
.7
GROWTH IN .1 INCHES SINCE LAST ANNULUS

individual fish seems to be more important than the length of the growing season. Since growth rates in McLain Pond were slow, the carrying capacity of the pond was probably exceeded resulting in little food for growth. When little food is available and some or all of this food is channeled into gonad production, it is doubtful that much growth could take place until after spawning in mature fish, thus causing a delay in annulus formation.

Kimberley Pond - This pond contained bass and bluegills. Both had reproduced successfully in 1965. Mean calculated total lengths for annuli 1-4 are as follows: 1.8, 3.1, 4.7, and 6.2 inches (Table 6). The calculated lengths for annuli 1 and 2 are comparable to McLain Pond; however, the mean annual increments increased from the second to fourth annulus. This pond was rotenoned along the edges in the fall of 1963. The owner stated that thousands of small bluegills were killed. The calculated total lengths in Kimberley Pond did not increase after treatment (Table 6). These results are unlike those of Beckman (1941), who reported that rock bass (Ambloplites rupestris) showed increased growth after removal of approximately 50% of the population. However, from the owner's description, it was impossible to estimate the percentage of fish removed from Kimberley Pond.

Sparks Pond - This pond was balanced and contained only bass and bluegills. Mean calculated total lengths for annuli 1-4 are as follows: 1.9, 3.8, 5.0, and 6.0 inches (Table 7).

The average calculated total lengths in this pond are similar to those in Huffacker and Link Ponds (Tables 10, 11), which have slightly

Table 5. Calculated and measured total lengths of 117 bluegills from McLain Pond, Story County, Iowa, 1965

Year Class	Number of fish	Average calculated length in inches at annulus					Length at capture
		1	2	3	4	5	
		-					
		-	-				
1962	10	2.1	3.4	4.2			4.5
1961	90	1.5	2.8	3.9	4.5		4.8
1960	17	1.3	2.7	3.7	4.3	4.7	5.0
Mean weighted total length, inches		1.5	2.9	3.8	4.4	4.7	
Mean annual increments		1.5	1.4	0.9	0.6	0.3	

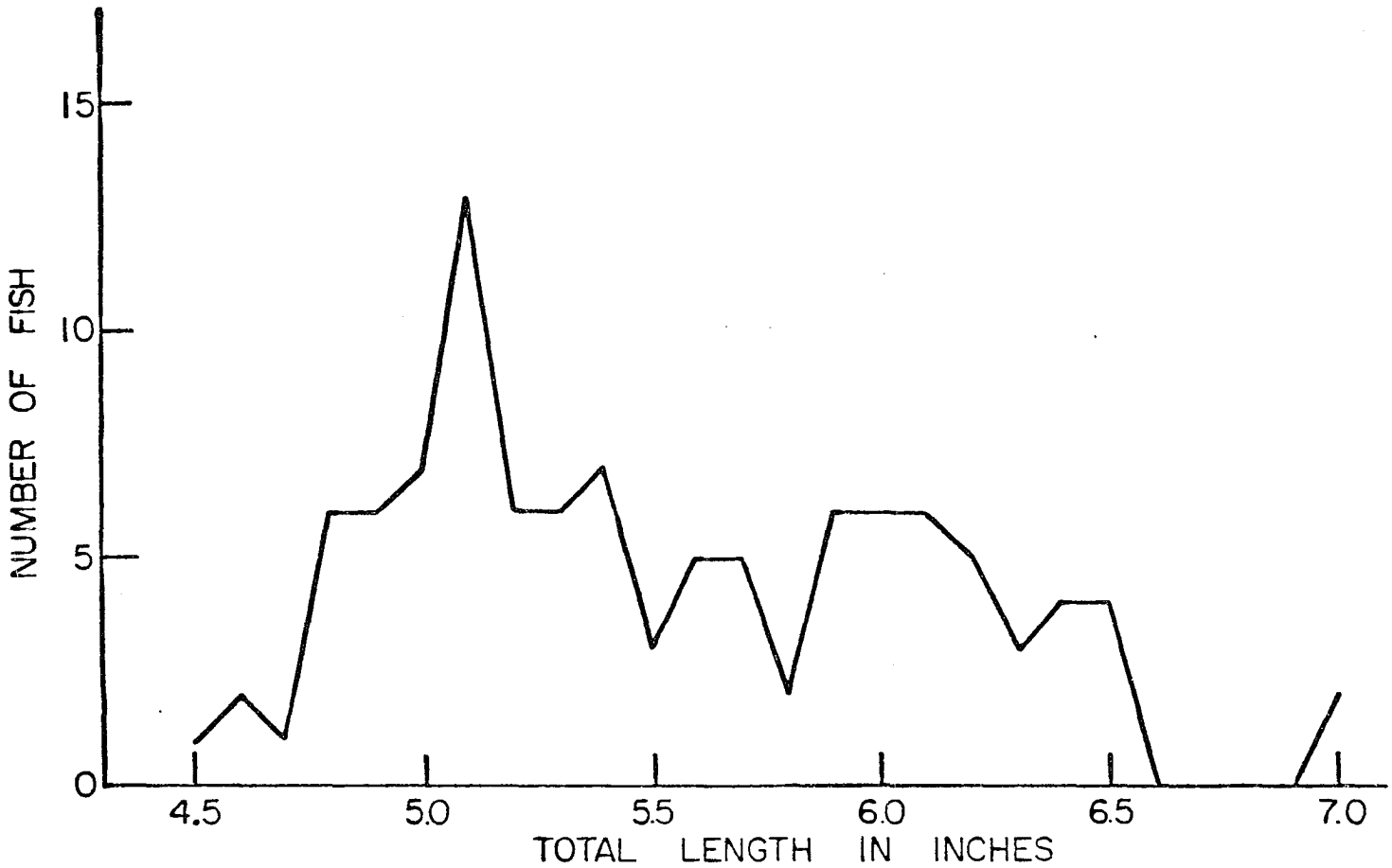
Table 6. Calculated and measured total lengths of 121 bluegills from Kimberley Pond, Story County, Iowa, 1965

Year Class	Number of fish	Average calculated length in inches at annulus				Length at capture
		1	2	3	4	
1963	86	1.8	3.0			4.5
1962	17	2.0	3.2	4.6		5.4
1961	18	1.8	3.2	4.7	6.2	6.8
Mean weighted total length, inches		1.8	3.1	4.7	6.2	
Mean annual increments		1.8	1.3	1.4	1.5	

Table 7. Calculated and measured total lengths of 163 bluegills from Sparks Pond, Polk County, Iowa, 1965

Year Class	Number of fish	Average calculated length in inches at annulus					Length at capture
		1	2	3	4	5	
	-	-					
1963	38	2.1	4.3				5.2
1962	113	1.8	3.7	5.0			5.5
1961	12	2.0	3.8	5.0	6.0		6.4
-	-	-	-	-	-	-	
Mean weighted total length, inches		1.9	3.8	5.0	6.0		
Mean annual increments		1.9	1.9	1.4	0.9		

Figure 2. Length frequency grouping of 1962 year class bluegills in Sparks Pond, 1965


NUMBER OF FISH
15
10
5
0
4.5
5.0
5.5
6.0
6.5
7.0
TOTAL LENGTH IN INCHES

higher population estimates. However, growth data for the 1962 year class were inconsistent. The total lengths of the fish in this year class varied from 4.6 to 7.0 inches. A length frequency grouping was plotted and indicated that two separate groups might be present (Figure 2). The scales from individuals throughout the range contained three annuli. The annuli are distinct and fit the criteria for true annuli (Lagler, 1952, Sprugel, 1950, Regier, 1962). Several possibilities exist for this difference in length within this year class: (1) differential length due to sex, (2) a false annulus was accepted as a true annulus in the smaller size group, (3) the larger fish failed to form an annulus or the annulus was not recognized, and (4) differential length due to intermittent spawning.

Fish were grouped as to sex (Table 8). Inspection of the data suggests that males of the 1962 year class were faster growing than females. This difference may have been due to selective sampling of males which were guarding their nests during the spawning season with the smaller males of the year class not nesting as yet. A Student's "t" test was run on each annulus of the 1962 age class to determine if there were statistical differences (Snedecor, 1956). Differences were significant at .05 level for annuli 1, 3, and total length (Table 8). In order to determine if sexual differences existed within the modes (Figure 2), bluegills were divided into 2 groups (4.5-5.8 and 5.8-7.0 inches). Differences in calculated total length were significant at the 0.05 level for all annuli and total lengths at capture within the small group. A significant difference existed at annuli 3 in the

larger group (Table 9).

Table 8. Average calculated lengths in inches for male and female bluegills from Sparks Pond, 1965

Year Class	Sex	Number of fish	Average calculated length in inches at annulus				Length at capture
			1	2	3	4	
1963	M	6	2.0	4.1			5.3
	F	28	2.2	4.3			5.2
1962	M	38	2.1*	3.5	5.5*		5.9*
	F	46	1.7	3.5	4.8		5.4
1961	M	5	1.9	3.7	5.0	5.8	6.2
	F	3	2.2	4.1	5.2	6.4	6.8

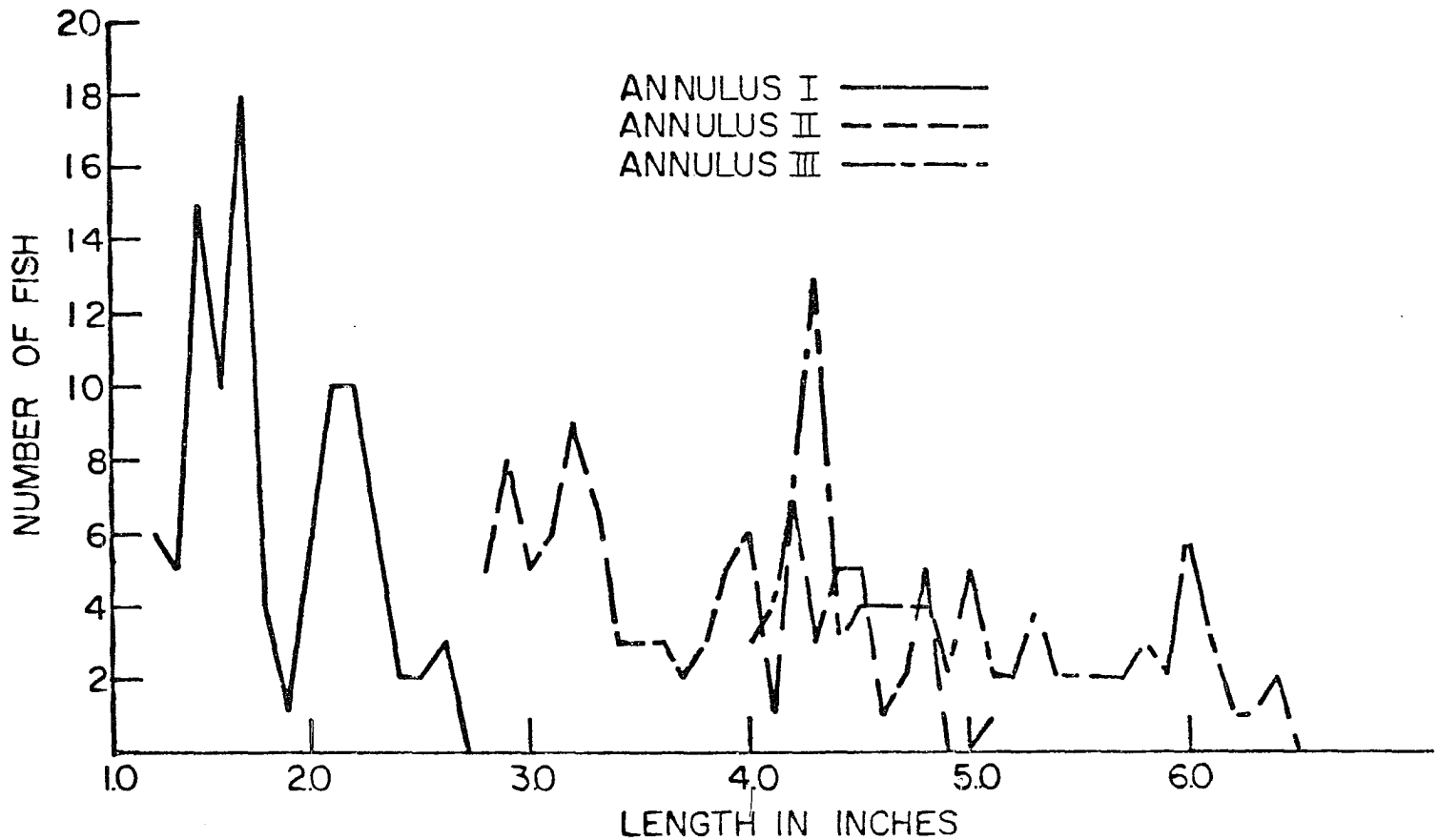
*Significant at .05.

Table 9. Average calculated lengths for male and female bluegills of the 1962 year class in Sparks Pond grouped according to size classes, 1965

Group	Sex	Number of fish	Average calculated length in inches at annulus			Length at capture
			1	2	3	
4.5-5.7	M	17	2.0*	3.9*	5.1*	5.6*
	F	35	1.6	3.2	4.4	5.1
5.8-7.0	M	21	2.2	4.4	5.7	6.2
	F	11	2.1	4.3	6.1*	6.4

*Significant at .05.

Figure 3. Length frequency grouping of calculated total length for each annulus of the 1962 year class from Sparks Pond, 1965


ANNULUS I
ANNULUS II
ANNULUS III
NUMBER OF FISH
20
18
16
14
12
10
8
6
4
2
1.0
2.0
3.0
4.0
5.0
6.0
LENGTH IN INCHES

Another possibility is that a false annulus could have been mistaken as a true annulus; however, all annuli fit the criteria for true annuli. With the available data and without the benefit of known age individuals, it was impossible to state that any one of these annuli were false.

Profitt (1950) stated that small bluegills measuring 1.0 to 1.5 inches at the time of annulus formation might miss their first annulus. Regier (1962) found inconsistencies in his back-calculations of growth and attributed the inconsistencies to missing the first annuli. Burress (1949) found annuli indistinct or absent in small bluegills. In this study, thirty-four of the larger bluegills 5.7 inches or above were grouped. The average length at annulus 1 was 2.2 inches, ranging from 1.3 to 3.2 inches. It is unlikely that an annulus would be present between the focus and 2.2 inches. If so, the growth rate would be extremely slow between the first and second year.

The fourth possibility of differential growth due to intermittent spawning seems to be the best explanation of size differences in samples from Sparks Pond. The larger individuals of the year class may have been hatched earlier in the summer than the smaller individuals. By the start of the second growing season these fish were larger and able to compete more efficiently for food and maintain their superiority. The average calculated total length for fish above 5.8 inches at the first annulus was 2.2 inches and 1.7 inches total length for fish below 5.8 inches in the age class. Sprugel (1950) also noted sizeable variations in growth among individual specimens

of a year class. Sprugel trapped bluegills to learn more about their movement and found that specimens taken in an individual trap at a specific time and place were somewhat uniform in size. This plus the variation in average growth attained by members of the same year class in samples taken at progressively later dates suggested that possibly each individual school of bluegills consisted of the same year class individuals possessing similar growth rates. He stated that sampling different schools with different growth rates could have accounted for some of the variation in his data. This sampling of different schools might have accounted for some of the variation in Sparks Pond, since the majority of the smaller fish were not captured until August and October.

The calculated total lengths for each annulus of the 1962 year class were plotted in frequency groupings (Figure 3). The frequency groupings for each annulus did not show the distinct bimodal relationship as total length; annulus 1 was more bimodal. This might indicate that the growth was variable within this year class and fish at both extremes were actually fish of the same year class rather than two distinct groups.

Huffacker Pond - This pond was balanced and contained both bass and bluegills. Mean total lengths for annuli 1-5 are as follows: 2.4, 3.9, 5.1, 6.0, and 6.6 inches (Table 10).

Link Pond - This pond was balanced and contained bass, bluegills, and black bullheads. The bullheads did not appear to be numerous, for only a few specimens were collected; however, seining may not be a good

method for their capture. Mean total lengths for bluegills for annuli 1-5 are as follows: 2.3, 3.8, 4.8, and 5.9 inches (Table 11).

D. Discussion

Growth summaries for the five study ponds, plus growth summaries for other Iowa lakes and ponds are listed in Table 12. Sparks, Huffacker, and Kimberley pond growth rates were the best of the ponds studied, followed closely by Link Pond. These growth rates compare favorably with growth rates found by Moorman (1953) in unbalanced ponds, Ruhr (1952), Hennemuth (1955), and DiCostanzo (1954) and are slower than growth rates reported by Lewis (1950) and Moorman (1953) in balanced ponds.

McLain Pond bluegills exhibited the slowest growth rates of any of the ponds studied. This pond was judged unbalanced. No bass or bluegill reproduction was noted in 1964 and 1965. Ricker (1942a) reported bluegill growth rates from Bass Lake, Starke County, Indiana, which were similar to McLain Pond. The estimated total length for 1934 for annuli 1-6 were as follows: 1.4, 2.2, 3.0, 3.7, 4.7, and 5.1 inches. Ricker (1942b) found exceptionally slow growth for Springwoood Lake bluegills. The average length after 5 growing seasons was only 3.6 inches, indicating very stunted growth. Although McLain Pond bluegill growth rates were greater than those reported from Springwood Lake, they were below those in the balanced population ponds.

In order to compare the growth of bluegills in ponds within the year fish were collected, the growth increments were plotted for

1965 (Figure 4). The relative ranking of ponds was not identical using this method to the overall average growth rates or to standing crops. Kimberley Pond bluegills exhibited much faster growth; however, the bulk of bluegills collected from this pond were younger (2 years) whereas the bulk of the bluegills collected from the other ponds were 3-5 years-old.

Table 10. Calculated and measured total lengths of 123 bluegills from Huffacker Pond, Polk County, Iowa, 1965

Year Class	Number of fish	Average calculated length in inches at annulus					Length at capture
		1	2	3	4	5	
		-					
1963	32	2.5	4.1				5.1
1962	51	2.4	3.9	5.1			5.7
1961	37	2.3	3.7	5.1	6.0		6.5
1960	3	1.7	3.3	4.7	5.8	6.6	7.2
Mean weighted total length, inches		2.4	3.9	5.1	6.0	6.6	
Mean annual increments		2.4	1.5	1.2	0.9	0.6	

Table 11. Calculated and measured total lengths of 114 bluegills from Link Pond, Polk County, Iowa, 1965

Year Class	Number of fish	Average calculated length in inches at annulus					Length at capture
		1	2	3	4	5	
1964	1	2.3					4.4
1963	13	1.7	3.7				5.0
1962	76	2.4	3.9	4.9			5.5
1961	23	2.4	3.7	4.7	5.5		5.9
1960	1	1.6	2.9	3.8	5.1	5.9	6.4
Mean weighted total length, inches		2.3	3.8	4.8	5.5	5.9	
Mean annual increments		2.3	1.5	1.0	0.8	0.8	

Figure 4. Growth increments in 1965 for bluegills from Kimberley, Huffacker, Link, Sparks, and McLain ponds

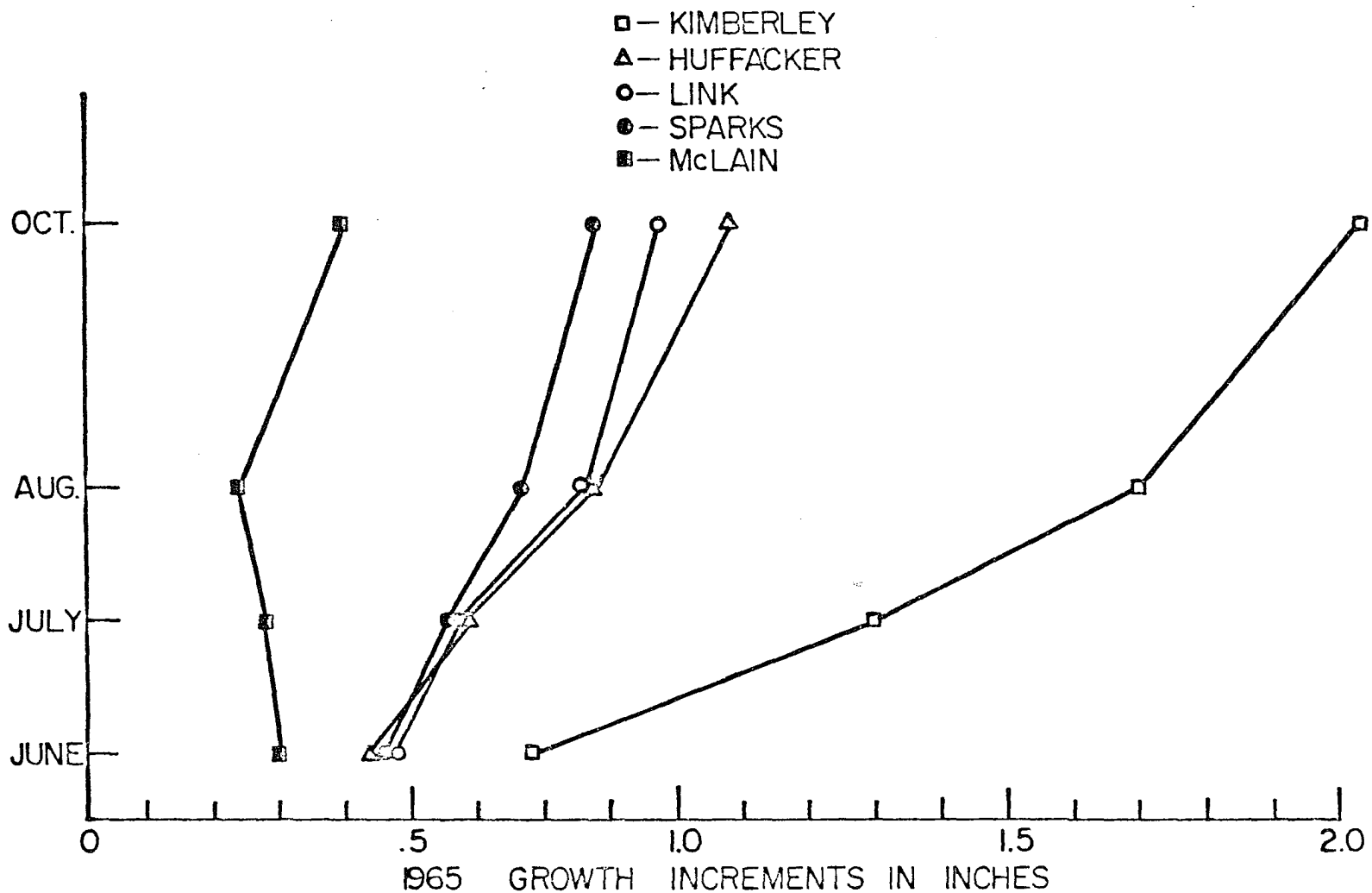

KIMBERLEY
HUFFACKER
LINK
SPARKS
McLAIN
OCT.
AUG.
JULY
JUNE
0
.5
1.0
1.5
2.0
1965 GROWTH INCREMENTS IN INCHES

Figure 5. Electropherogram illustrating the method of separation of protein fractions

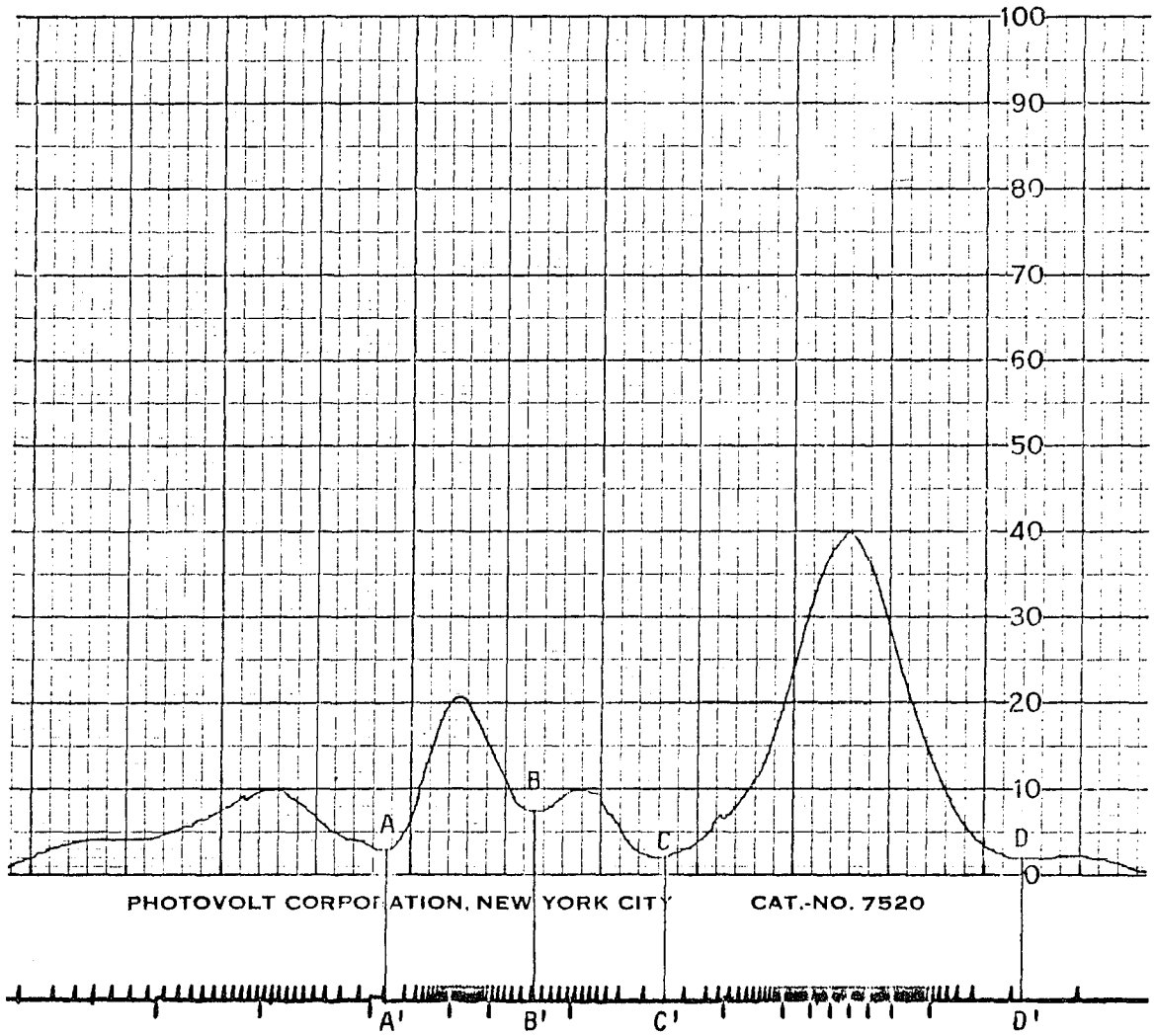

100
90
80
70
60
50
40
30
20
10
0
A
B
C
D
PHOTOVOLT CORPORATION, NEW YORK CITY
CAT.-NO. 7520
A'
B'
C'
D'

Table 12. Average growth of bluegills from Iowa lakes and ponds

Locality	Number of fish	Calculated total length in inches at each annulus					Authority
		1	2	3	4	5	
Sparks	163	1.9	3.8	5.0	6.0		Present Study
Huffacker	123	2.4	3.9	5.1	6.0	6.6	" "
Link	114	2.3	3.8	4.8	5.5	5.9	" "
Kimberley	121	1.8	3.1	4.7	6.2		" "
McLain	117	1.5	2.9	3.8	4.4	4.7	" "
Southern Iowa balanced ponds	374	1.7	4.1	6.1	7.0		Moorman, 1953
Southern Iowa unbalanced ponds	153	1.2	3.0	5.0	5.8		" "
Ike Lake	308	1.4	3.2	4.2	6.4		Ruhr, 1952
Red Haw Lake	133	1.4	3.4	6.1	7.2	8.1	Lewis, 1950
East Lake	145	1.7	3.6	5.6	7.0	7.5	" "
Lake Aquabi	1139	1.9	3.7	4.7	5.6	6.3	Hennemuth, 1955
Clear Lake	1215	2.4	4.2	5.6	6.2	7.8	DiCostanzo, 1954

V. BLOOD ANALYSIS

A. Materials and Methods

1. Field methods

Fish were collected with seines and placed in water-filled wash tubs. Blood samples were taken within 45 minutes after capture. If fish showed signs of distress (gulping for air, raised scales), they were not used but an additional seine haul was made to collect the desired number of samples. Blood samples were collected, from bluegills, in the study ponds in June, July, August, and October, 1965.

Blood was drawn from the cuvarian duct (Sano, 1960) using a 1 cc. tuberculin syringe with a 1-inch 21-gauge needle. Sampling by heart puncture was attempted but proved too time-consuming since the heart is small and difficult to puncture on each try. The cuvarian duct sample method allows for less error and contamination since the needle can be placed in proper position by sight. This method has two other advantages: (1) fish can be sampled repeatedly without apparent ill effect and (2) when blood cannot be taken from one side, it is often possible to obtain a sample from the opposite side.

Four-tenths to 1 cc. of blood could be drawn from each fish depending upon its size. The blood sample was ejected slowly from the syringe into 75 x 12 mm. serology tubes. Clotting was rapid and usually complete within 10 to 15 minutes. Only non-hemolyzed samples that were white or pale straw color were accepted. In some instances

it was not possible to obtain the desired number of blood samples due to hemolysis. Serum was removed from the serology tubes with a small pipette and transferred to vials which were then placed in an ice cooler. Serum samples were transferred to the laboratory where they were frozen at -20° C. until analyzed.

2. Treatment of equipment

All glassware that came in contact with serum samples was washed in Alconox detergent, rinsed in tap water, rewashed in detergent, rinsed in tap water, and rinsed three times in distilled water. The sample applicator was dipped in buffer and wiped dry after each application to remove any deposits of serum. After every sixth application the applicator was washed in detergent, rinsed in hot tap water, and rinsed in distilled water. Syringes and needles were handled in the same manner as glassware.

3. Determination of total protein concentration

The biuret method was used to determine the concentration of total protein in the blood serum (Natelson, 1957). The procedure was adapted to small sample volumes as follows: 0.01 ml. of serum was washed into 1 ml. of distilled water, 1 ml. of full strength biuret solution was added and mixed thoroughly and allowed to react for at least 15 minutes, but no longer than 30 minutes. Absorbency was measured in a spectrophotometer at 540 mu. If the sample appeared cloudy due to excessive lipids, 1 ml. of diethyl ether was added; the solution was

mixed and centrifuged. The ether was aspirated and the lower layer read. Grams per 100 ml. of serum were determined from the following formula:

$$\frac{\text{Absorbence unknown}}{\text{Absorbence standard}} \times \text{per cent protein in standard} = \text{per cent protein in unknown (grams per 100 ml.)}$$

Lab-trol (Dade Chemical Co.) was used as the standard solution (7.3 $\pm$ 0.2 grams total protein per 100 ml.).

4. Electrophoresis

Electrophoretic techniques separate protein molecules according to their net charge, size and molecular weight. Since the isoelectric points of individual proteins differ, they will generally bear different net charges at different pH's. When the pH is adjusted by the proper buffer to a value alkaline to the isoelectric point, they will migrate to the anodal side of the field at different rates, depending upon their net charge, size and molecular weight. In zone electrophoresis where an electrical field is set up across a paper strip, cellulose acetate or any other porous medium, saturated with buffer, spatial separation of the individual proteins occurs. The spatial arrangement of these proteins can be determined by staining the proteins upon the support medium and optically scanning them.

Approximately 3-4 μl of sample was applied to Sepraphore III strips, pre-moistened in buffer, with a Gelman sample applicator #51220. Samples were electrophoresed for 45 minutes at room temperature with an

applied voltage of 220 volts. Various voltages and time intervals were tried at the start of the experiment, but the above were the most desirable. Separation was achieved in Gelman high resolution buffer (Tris-Barbital-Sodium Barbital, pH 8.8, ionic strength 0.05).

After protein separation the strips were removed from the chamber and placed in Ponceau S stain (500 mg. in 100 ml. of 5% trichloracetic acid) for at least 5 minutes. Excess background stain was removed by washing the strips one minute each in 3-4 baths of 5% acetic acid. The strips were placed in blotting paper until air dry, then placed in an oven 30 minutes (35°-40° C.) before clearing to assure that all moisture was removed.

The following technique was used to clear strips. They were placed in a solution of 10% acetic acid in methanol (10 ml. acetic acid plus 90 ml. methanol) until completely wetted and transferred to microscope slides. Care was taken at this point to remove all air bubbles between the slide and the strip. When the relative humidity was high, strips were placed in an oven at 35°-40° C. until dry and transparent. Air drying was attempted but proved unsatisfactory, for the strips turned opaque.

After clearing, strips were scanned using the Densicord scanner (505 μm blue filter) with a special cellulose acetate carriage attached, driven by a motor mechanism, which allowed for a 4-1 ratio of graph paper to strip. Scanning produced an absorbency curve of the Ponceau S stain in each fraction, which in turn was proportional to the amount of protein in each band. The integrator measured the areas under each curve

and recorded it on the electropherogram (Figure 4). Protein fractions (I, II, III, IV) were separated for calculations, by differences in relative stain density between areas heavily stained (protein fractions) and the areas between them, which contained relatively little stain (A, B, C, D). Vertical lines were drawn between each protein fraction to the integrator marks (A-A', B-B', C-C', D-D') (Figure 5). When separations were indistinct, measurements of the strips were used to separate bands on the electropherogram. The number of marks were counted under each curve and added to obtain the total number. Each individual number was divided by the total to obtain the percentage of each band.

Cellulose acetate as a quantitative technique has been investigated by Graham and Grunbaum (1963); Grunbaum, Zec, and Durrum (1963); Grunbaum, Lyons, and Zec (1963); and Briere, Golias, and Balzakis (1965). The procedures used in this study follow closely the standards and limitations set forth by these authors.

Using standardized conditions for electrophoresis, the location of each fraction should be consistent as to its position on the electropherogram; however, small differences in sample size, running time, and differences in total serum protein between individuals can alter the density of each band.

In order to determine the variability within the electrophoresis technique, ten replications were made on one sample. Small variations existed between the replications. The largest standard error of the mean for any sample was 0.6%. The influence of the scanner was also

tested by scanning a single strip 16 times. Variations were slight and the largest standard error of the mean for any fraction was less than 0.4%.

5. Laboratory experiments

Bluegills used in laboratory experiments were collected from Sparks Pond by seining. Immediately after capture they were placed in a ten-gallon container filled with pond water and aerated by agitation. At the laboratory these fish were transferred to dechlorinated tap water of approximately the same temperature. Fish were not used in experiments for 2 to 3 weeks or until they would take dry food readily.

6. Crowding experiment

On July 12, 1965, acclimated fish were randomly netted from a holding tank (568 liters). All fish were tagged with a number 1 monel metal strap tag which was attached to the opercle. They were weighed to the nearest gram and total length was measured to the nearest tenth-inch.

These fish were placed in four identical square tanks, each containing approximately 549 liters of dechlorinated tap water. Tap water was dechlorinated by bubbling air and water through glass beads. Water was allowed to flow into the tanks at approximately .95 liters per minute. The excess water left through a stand pipe. The water temperature within all tanks varied from 15.5° to 17.0° C. throughout the duration of the experiment. Ammonia nitrogen levels and dissolved

oxygen levels were checked periodically during the experiment.

Stocking rates were as follows: Tank A - 10 fish; Tank B - 78 fish; Tank C - 40 fish; and Tank D - 25 fish.

On July 15, 1965, a feeding ration of Glenco trout pellets was started. It contained not less than 35 per cent crude protein, not less than 3 per cent crude fat, and not more than 7 per cent crude fiber. Each fish was to receive 3 pellets per day; however, since the pellets were broadcast on the surface of the water, it is probable that some fish received more than 3 pellets while others received less.

During the night of November 15-16 a water hose broke allowing approximately four liters per minute of chlorinated tap water to flow through Tank A. Eight fish died. The remaining two fish were eliminated from the experiment.

All fish were sampled December 1, 1965. Blood samples were drawn and handled as described previously, with the exception that samples were placed immediately into the freezer. Sex, weight, and total lengths were recorded. At the end of the experiment individual fish could not be recognized, since all but two fish had lost their tags.

7. Feeding experiments

In order to determine the effect of starvation on bluegill serum proteins, a feeding experiment was started September 29, 1964. Fish were netted and placed in 4 square tanks (549 liters). Fifty fish were placed in each tank. Treatments were assigned to tanks by a

random number table (Snedecor, 1956). Fish in tanks A and C were fed a ration of 3 pellets each. Fish in tanks B and D received no food ration for the duration of the experiment. Blood samples were taken from 5 randomly netted fish from each tank every sixth day. The last group was sampled November 9, 1964. Total serum protein was determined by the biuret method.

A second feeding experiment was started February 8, 1966, to determine the effect of starvation on the various protein components as shown by electrophoresis. Twenty-seven fish were placed in two tanks. Tank A fish were given a ration of 3 pellets per day. Fish in tank B did not receive a food ration for the duration of the experiment.

The water supply for this experiment was dechlorinated with a model 3 Everpure water purifier containing activated charcoal. Water flow through each tank was approximately 1.9 liters per minute. Tank water was filtered through glass wool at approximately one gallon per minute to remove accumulated organic matter. Dissolved oxygen and ammonia nitrogen levels were measured periodically throughout the experiment.

Bluegills were sampled from both tanks May 13, 1966. Blood was taken from the cuvarian duct. Weight in grams, total length, and scale samples were taken. Sex was checked internally. All fish were immature; therefore, it was difficult to make positive sex determinations. Blood samples were frozen and later analyzed by electrophoresis.

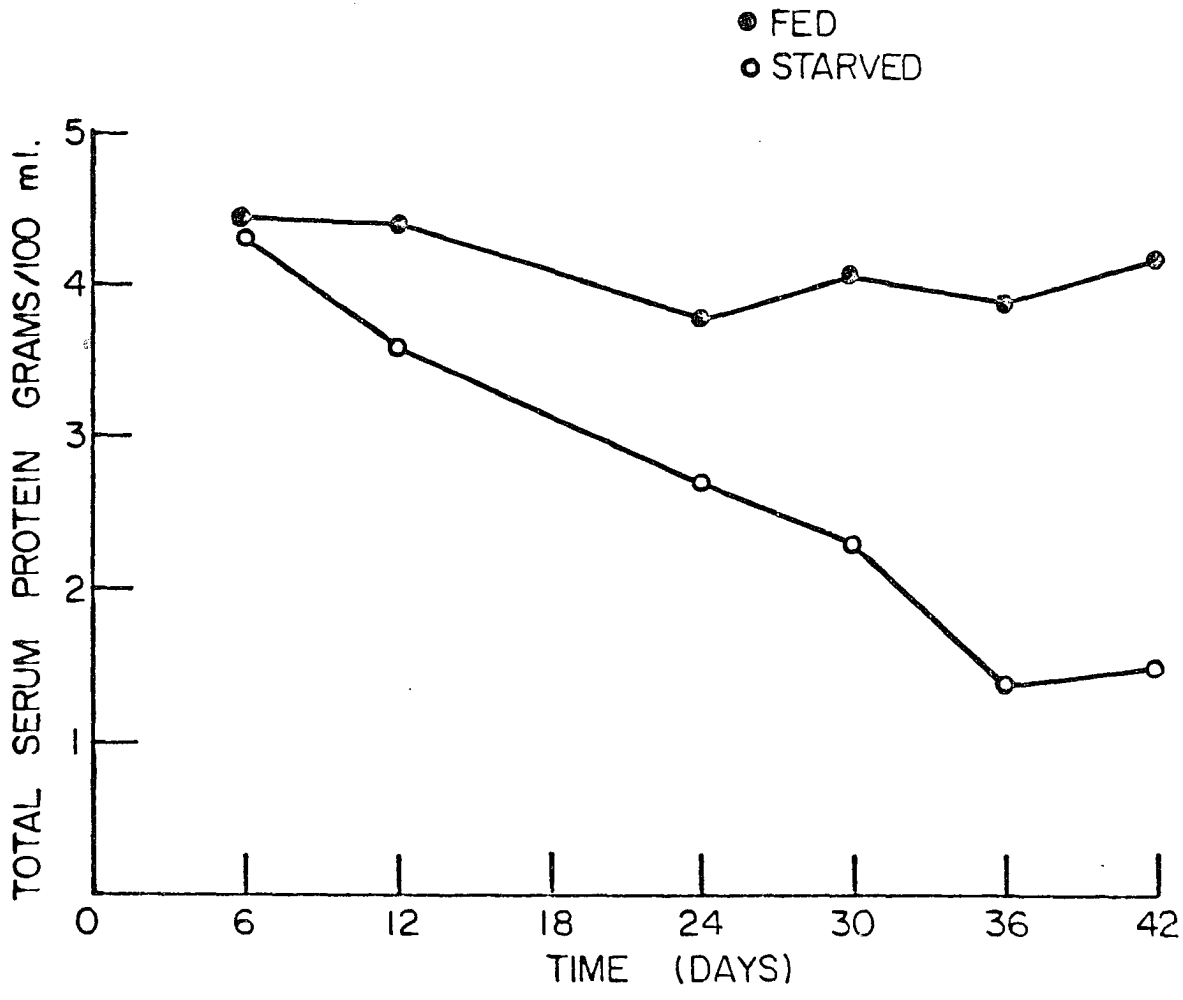
B. Results

1. Feeding experiments

The results of two feeding experiments indicate that food deprivation affects both the total level of serum protein and the proportional amounts of protein in fractions.

Total protein levels varied from 4.77-3.80 gms./100 ml. in the fed group and from 4.33-1.43 gms./100 ml. in the starved group (Figure 6). The total serum protein levels remained fairly constant throughout the experiment in the fed group with slight decreases on the 24th, 30th, and 36th days and elevation to a high level on the 42nd day. A regression analysis was run on total protein levels with time in order to find the "b" value or slope of the line. This "b" (0.0624) was tested to determine if it was significantly different from zero. A Student's "t" test indicated that the calculated "b" value was not significantly different from zero at the 0.05 level of significance. This would indicate that there was no significant change in the serum protein levels in the fed group with time. The starved group total serum protein levels decreased after the 6th day from 4.33 to 1.45 gms./100 ml. at day 42. A regression analysis was run to obtain the "b" value ($b = -0.7095$). This "b" value was tested to determine if it was significantly different from zero. A Student's "t" test indicated that "b" was significantly different from zero at the 0.05 level of significance. The two "b" values were tested to determine if they were significantly different; they were significantly different at the 0.05 level.

Figure 6. Total serum proteins of fed and starved bluegills


FED
STARVED
TOTAL SERUM PROTEIN GRAMS/100 ml.
5
4
3
2
1
0
6
12
18
24
30
36
42
TIME (DAYS)

Plasma (serum) proteins serve as nutrients for all tissues of the body. Food proteins provide amino acids for synthesis of plasma proteins which yield the necessary molecular structures for the synthesis of most tissue proteins (Kugelmass, 1959). The concentration of plasma proteins becomes reduced whenever a sufficient supply of amino acids are not present; however, even during periods of starvation or debilitating diseases the ratio of tissue proteins to plasma proteins remains fairly constant at 33:1 (Guyton, 1961, Kugelmass, 1959). During periods of protein deprivation, the body preferentially uses carbohydrates and fat. Carbohydrate stores will not last more than 48 hours. The fat stores are next to be metabolized and in a healthy individual containing 15 per cent fat it usually takes 5-6 weeks for the fat stores to be used. After the fat stores are depleted, the amino acids of the blood begin to be very rapidly deaminated and oxidized for energy. Guyton (1961) states that the plasma proteins are a labile storage source of protein for the tissues and are oxidized for energy during periods of low protein intake which may account for their decline. However, Kugelmass (1959) states that during periods of deficient protein intake lower levels may be caused by decreased synthesis.

The average total serum protein level of bluegills in this experiment fed daily rations was 4.3 gms./100 ml., which is within the range reported by other authors for various species (Table 13). The average value for starved individuals after 36 days was 1.4 gms./100 ml., which is considerably lower than normal values. The decline in total

serum protein levels appears to be considerably faster than reported for humans.

Two explanations are given for this rapid decline. Protein seems to be involved in the principal metabolic pathway in fishes. Phillips et al. (1948) found that trout were relatively inefficient in handling carbohydrate. After feeding glucose to trout, the blood glucose curve resembled that of diabetic man; and he suggested this might be caused by reduced insulin supply. Phillips et al. also reported carbohydrate feeding over extended periods of time caused pathological storage of glycogen and was strongly correlated with increased mortality. Therefore, fish must rely on fats and proteins as sources of energy. Phillips and Podoliak (1957) reported that high dietary levels of soft fat produced edema in trout and caused fatty infiltration of the liver which led to excessive mortality. It appears that both carbohyrate and fat metabolism may be inefficient in fish; therefore, large amounts of protein are used for energy. Phillips et al. (1948) pointed out that apparently trout are poor users of carbohydrate and high-fat diets and use a portion of dietary protein for energy. From the available information, it appears that fish utilize a higher proportion of protein for energy than mammals. Shell (1961) felt this high need for protein would have a profound effect on fish metabolism when a great deal of energy is required for synthesis or increased metabolic rate. During periods of abnormally high energy requirements, it is conceivable that energy intake would not be high enough to meet metabolic demands and tissue catabolism would take place. It is possible, therefore,

that under conditions of food deprivation, endogenous protein sources may be utilized more quickly in fishes. Another explanation is that rapid fluctuations in total serum protein levels in fishes may not be too unusual. Shell (1961) reported cyclic changes in serum proteins with time. He found decreases of 30 per cent in serum protein concentration of small mouth bass over an 8-week period, followed by an increase of 150 per cent in a 12-week period. Sano (1960) reported variations in serum protein levels of brook trout of 2.4 gms./100 ml. within 60 days. Changes of this magnitude in higher organisms are recorded only after severe trauma, malignancy, or other diseases, but appear to be normal in fishes.

Decreases in total serum protein levels due to food deprivation have been reported in fishes by other authors. Robertson et al. (1961) reported that Pacific salmon undergoing prolonged periods of starvation during migration had reduced levels of plasma proteins. Fluctuations in serum albumin levels were reported by Smirnova (1962) and were related to food supply. Sorvachev (1957) studied the protein composition of blood and muscle of carp and found that total serum protein levels dropped from 3.9-2.8 per cent after approximately five and one half months of hibernation. He also found that total serum proteins levels could be brought almost back to normal.

The results of the present experiment along with other experiments suggest that total serum protein levels of fish are reduced drastically by periods of starvation.

Table 13. Total blood proteins in fresh water fish

Family and species	Number of fish	Total protein grams/100 ml. mean	Range	Authority
Aciperseridae				
Rock sturgeon *Acipenser* sp.	2	2.8	2.5-3.1	Duetsch and McShan (1949)
Clupeidae				
Gizzard shad *Dorosoma cepedianum*	20	4.7	3.8-6.3	Hunn and Robinson (1966)
Salmonidae				
Brook trout *Salvelinus fontinalis*	5-19	3.46	2.94-4.12	Field, Elvehjem, and Juday (1943)
Rainbow trout *Salmo gairdneri*	(6) pooled	5.1	-	Deutsch and McShan (1949)
Lake trout *Salvelinus namaycush*	(8) pooled	5.0	-	Deutsch and McShan (1949)
Brown trout *Salmo trutta*	(4) pooled	4.0	-	Deutsch and McShan (1949)
Whitefish *Coregonus clupeaformis*	(25) pooled	5.8	-	Deutsch and McShan (1949)

Table 13. Cont.

Family and species	Number of fish	Total protein grams/100 ml. mean	Range	Authority
Lake herring *Coregonus artedii*	pooled (25)	5.0	-	Deutsch and McShan (1949)
Rainbow trout *Salmo gairdneri*	10	4.66	3.82-5.77	Sano (1962)
Esocidae				
Northern Pike *Esox lucius*	pooled (2)	3.3	-	Deutsch and McShan (1949)
Cyprinidae				
Carp *Cyprinus carpio*	5-19	4.15	3.25-4.75	Field, Elvehjem, and Juday (1943)
Cyprinus carpio	pooled (7)	3.2	-	Deutsch and McShan (1949)
Cyprinus carpio	-	3.84	-	Sorvachev (1957)
Cyprinus carpio	8	3.57	2.74-4.16	Sano (1962)
Indian carp *Catla catla*	12	2.69	0.34*	Das (1961)

*Standard deviation

Table 13. Cont.

Family and species	Number of fish	Total protein grams/100 ml. mean	Range	Authority
Cirrhina mrigala	12	2.87	0.46*	Das (1961)
Labeo rohita	12	3.11	0.45*	Das (1961)
Catostomidae				
White sucker *Catostomus commersoni*	pooled (8)	3.3	-	Deutsch and McShan (1949)
Smallmouth buffalo *Ictiobus bubalus*	1	4.5	-	Deutsch and McShan (1949)
Ictaluridae				
Channel catfish *Ictalurus punctatus*	pooled (25)	5.1	-	Deutsch and McShan (1949)
White catfish *Ictalurus catus*	pooled (3)	3.3	-	Deutsch and McShan (1949)
Bullhead *Ictalurus* sp.	pooled (25)	3.8	-	Deutsch and McShan (1949)

*Standard deviation.

Table 13. Cont.

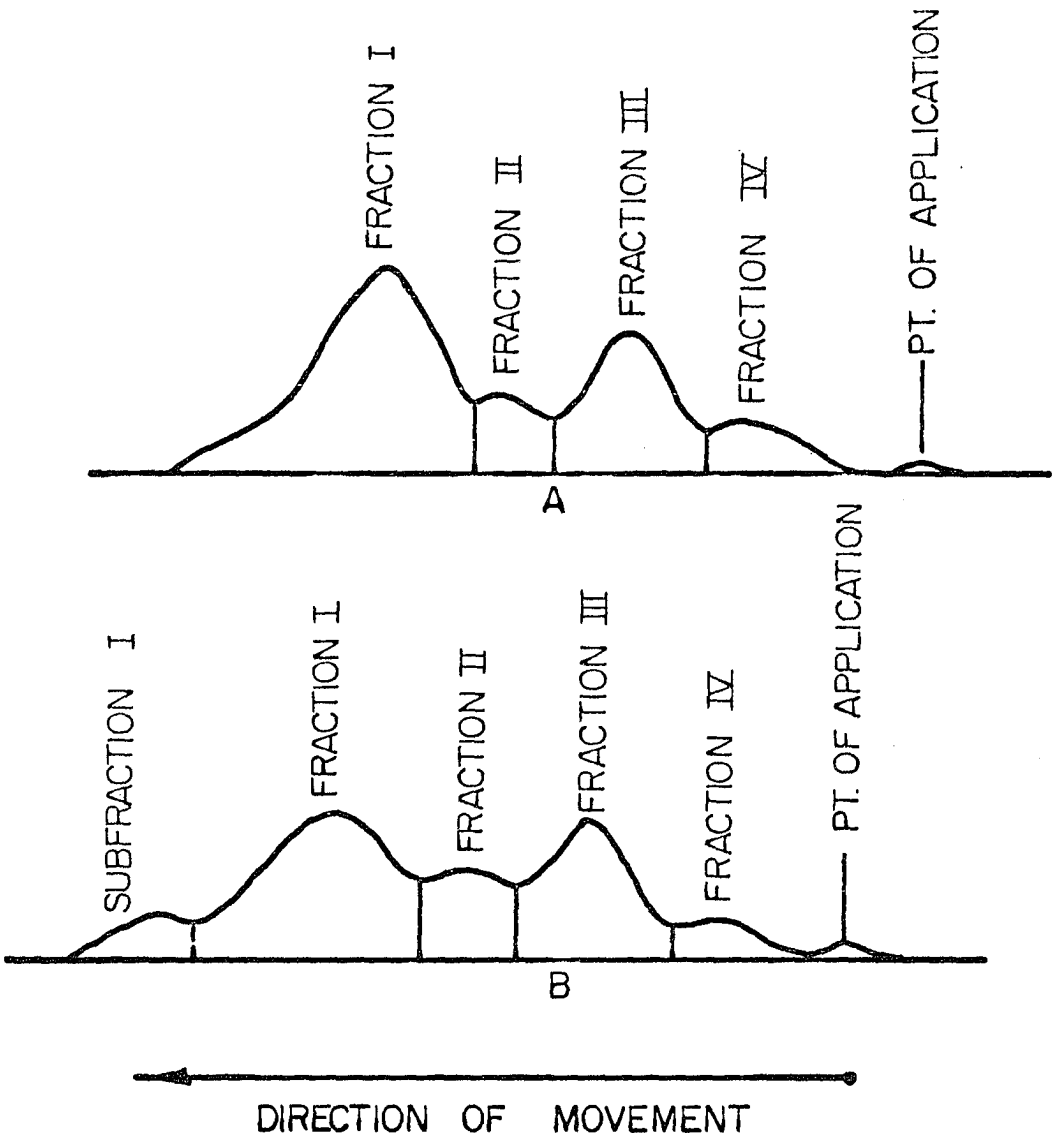
Family and species	Number of fish	Total protein grams/100 ml. mean	Range	Authority
Anguillidae				
Eel *Anguilla japonica*	10	4.5	3.44-4.80	Sano (1962)
Centrarchidae				
Rock bass *Ambloplites rupestris*	pooled (20)	4.0	-	Deutsch and McShan (1949)
Pumpkinseed sunfish *Lepomis gibbosus*	11	5.0	4.6-6.1	Hunn and Robinson (1966)
Smallmouth bass *Micropterus dolomieui*	pooled	2.4	1.8-3.3**	Shell (1961)
Percidae				
Yellow perch *Perca flavescens*	pooled (25)	4.5	-	Deutsch and McShan (1949)
Walleye *Stizostedion vitreum vitreum*	pooled (8)	5.8	-	Deutsch and McShan (1949)

**Seasonal variation.

With the electrophoretic method used, four major fractions were separated. Bouck and Ball (1966) could separate five major fractions with paper electrophoresis in bluegill serum. Summerfelt (1966) separated six major fractions with paper electrophoresis in golden shiners (Notemigonus crysoleucus). When comparing the golden shiner serum protein fractions with those of human serum, he found that his fastest moving fractions, 1 and 2, were in the approximate position of albumin in man. Fraction 1 had a mobility slightly greater than human albumin and fraction 2 had a mobility slightly slower than human albumin. After chemical analysis, Summerfelt designated fractions 1 and 2 as albumins. In the present data, fractions I and II were grouped since in some cases this fraction did not appear as two distinct bands but as one (Figure 7).

Summerfelt stated that fractions 3, 4 and 5 have the solubility of pseudoglobulins and component 6 of a euglobulin. He concluded that although similarities were noted between golden shiner serum protein fractions and human serum protein fractions, and although they may have the same net charge, similar size, and the same relative mobility, they may differ in amino acid composition and biological activity. He recommended using numerals to label protein components, as did Vanstone and Ho (1961), Bouck and Ball (1965), and Booke (1965). He felt a functional approach may supplement physiochemical data. The method of the above authors was used in this study.

Figure 7. Electropherograms illustrating separation of protein fractions. A. Four major fractions separated. B. Four major fractions plus subfraction I


FRACTION I
FRACTION II
FRACTION III
FRACTION IV
PT. OF APPLICATION
A
SUBFRACTION I
FRACTION I
FRACTION II
FRACTION III
FRACTION IV
PT. OF APPLICATION
B
DIRECTION OF MOVEMENT

The second feeding experiment ran for 64 days. Eighteen fish were randomly netted from each group at the end of the period. Protein components were separated by electrophoresis. A general increase occurred in two of the slower mobility fractions, II and IV, with a relative decrease in the proportional amounts of the high-mobility fraction I (Table 14). In this study, and apparently in other studies, high-mobility fractions are usually albumins and low-mobility fractions belong to the globulin portion. In the following discussions reference to high-mobility fractions will pertain to fraction I, low-mobility fractions are II, III and IV.

Table 14. Summary of the influence of starvation on the distribution of protein in the serum of bluegills (expressed as per cent of total serum protein)

	Fractions			
	I	II	III	IV
Fed				
Mean	43.12*	12.86*	33.89	10.10*
Range	30-48	8-24	29-39	7-13
n = 18				
Starved				
Mean	24.05*	30.62*	29.42	15.91*
Range	16-29	21-40	20-39	7-21
n = 18				

*Significantly different at 0.05 by "t" test.

The results in this starved group are similar to those reported in malnutrition studies. Malnutrition from deficient protein intake,

digestion, absorption, or assimilation in gastrointestinal or systemic diseases causes hypoalbuminemia as a result of decreased synthesis (Kugelmass, 1959). Kugelmass also states that globulin synthesis may be stimulated by a depressed protein pool. Thus, in the case of malnutrition where the protein pool is lowered, higher globulin values are likely. In malignant malnutrition of children, the total serum protein level is low with low absolute and relative amounts of albumin and high absolute and relative amounts of gamma globulin (Anderson and Altman, 1951).

This general relative shift in low-mobility and high-mobility proteins has been termed a stress pattern by Dunn and Pearce (1961), and it has been demonstrated in fish by various authors in regards to nutrition. Sindermann and Mairs (1961) found that fraction I slumped drastically in starving alewives after two months. Sorvachev (1957) found that the relative amount of serum proteins in various fractions changed in carp that were kept under protracted fasting. Three times more gamma globulins were present after fasting, the amount of beta globulins dropped by a factor of three, alpha globulins and albumins dropped by a factor of more than five. He assumed that the fractions were the same as those found in mammalian blood. Bouck and Ball (1965) reported general increases in low-mobility proteins, with general decreases in the proportional amounts of high-mobility protein when bluegills were subjected to low diurnal oxygen pulses. Apparently much of the effect could be attributed to disruption of the digestive process. During periods of low oxygen conditions, fish vomited

slightly digested food which they had eaten as much as 12 hours earlier.

It seems apparent from the foregoing discussion that the relative amounts of protein in certain fractions are affected by food deprivation, usually with a reduction of high-mobility fractions and an increase in low-mobility fractions.

2. Crowding experiments

Three levels of crowding were tested in experimental tanks from July 15-December 1, 1965. At the end of the experiment 15 fish were randomly netted from each tank. Blood proteins were analyzed by electrophoresis.

A single classification analysis of variance was used to test differences between the three groups for each fraction. Small but significant differences existed between band I and III. A Tukey's multiple range test (Snedecor, 1956) was used to test differences between fractions that were significant by analysis of variance.

Bluegills were crowded at rates of 21.9, 13.7, and 7.0 liters per fish. The serum protein patterns of fish crowded at 13.7 and 7.0 liters per fish exhibited stress patterns. The relative amount of fraction I from fish stocked at the intermediate density was significantly less than the relative amount of fraction I in fish stocked at 21.9 liters per fish. Fraction III was significantly greater in fish stocked at 13.7 and 7.0 liters per fish (Table 15).

Table 15. Summary of the influence of 3 rates of crowding on the distribution of protein in the serum of bluegills (expressed as per cent of total serum protein)

Density fish per tank		Fractions I	II	III	IV
(21.9 L/fish) 25	Mean	41.94*	27.05	12.43*	18.63
	Range	33-47	23-32	6-15	13-26
(13.7 L/fish) 40	Mean	37.63*	28.23	14.50*	19.64
	Range	26-47	22-32	11-17	16-23
(7.0 L/fish) 78	Mean	41.74	25.72	14.93*	17-60
	Range	33-49	20-32	8-20	8-21

*Significantly different at 0.05 by Tukey's range test.

Brown (1957) states that space factors might affect fish growth in two ways: (1) the total volume of water might influence growth, (2) the degree of crowding of individuals might be important. Brown (1946) found that in 2 year-old Salmo trutta the tank size had no effect on the rate of growth, but there was an optimum degree of crowding. Very crowded fish (3 liters per fish) and uncrowded fish (50 liters per fish) grew more slowly than fish with 12, 23, or 35 liters per fish. Usually crowded fish ate less, used food less efficiently, and disturbed each other more than less crowded individuals. The fish with more space grew erratically and it seemed that a certain amount of social stimulation was necessary.

Peck order has been indicated as a cause of differential growth rates in Kamloops trout by Stringer and Hoar (1955). They found that

dominant individuals grew faster. In the present study, peck orders were established in tanks containing bluegills. Usually the larger individuals would defend a small area of the tank and chase the smaller individuals from this area; however, at feeding time the smaller individuals were as aggressive as the larger fish in seeking food. Brown (1957) suggested that since the smaller individuals feed as readily as the larger individuals that possibly subordinate individuals suffer from "stress" in the presence of larger ones and the production of adrenocorticotropin is increased.

3. Field experiments

Electrophoretic analysis separated four major bands in serum from bluegill collected in the study ponds.

The means, variances, and standard deviations were plotted in different groups to determine if a correlation existed between treatment means and their within-treatment variances. No correlations were apparent which indicated that a transformation of these data was not necessary.

Sums of squares for the analysis of variance were obtained from multiple regression analysis. All main effects (sex, time, and ponds) were significant for each band. The interactions between pond and time, sex and time, and pond and time were significant. The interactions between pond, sex, and time were also significant (Tables 16, 17, 18, 19).

Bluegills were separated into males, females, and immatures for

analysis. Fish were considered immature when the gonads were small and the sex could not be determined macroscopically.

Sex differences were significant for each fraction within specific times and ponds; however, the interactions between pond and sex were also significant, indicating that at different times within different ponds the percentage protein within fractions changed position over time.

Percentages of each fraction showed the same general trends in males, females, and immatures with time (Figures 8, 9, 10, 11). The proportional amount of fraction I in males was consistently higher than females throughout the time periods tested. Immatures had the highest amount of fraction I in June but were below males the remainder of the season, and higher than females until October.

The proportional amounts of protein in fractions II and III were considerably lower for females in June than males and immatures. The most important factor to be considered in June is the relative amount of protein in fraction IV (Figure 11). The absolute percentage of fraction IV was considerably higher in females. The increased percentage of this fraction would cause proportional decreases in other fractions and apparently the decrease occurred in fractions II and III.

Similar observations of an increase in one fraction has been reported in other animals. The appearance of extra fractions in the serum of egg-laying animals has been demonstrated. McKinley et al. (1954) demonstrated the existence of a lipoprotein by electrophoresis in avian serum with the onset of egg-laying and estrogenization.

McCully et al. (1959) tentatively identified two fractions in fowl serum as lipovitellin and lipovitillenin complexes. Two slow-moving fractions in plasma of viviparous snakes were mainly responsible for the increase in plasma protein levels during estrus (Dessauer and Fox, 1959).

The presence of vitellin in fish has been demonstrated by Bailey (1957). Vanstone and Ho (1961) reported that electropherograms of plasma obtained from spawning, maturing and spent male coho salmon were similar to immature fish of either sex. However, as females matured, a sixth fraction appeared, which was associated with lipid staining material and had the same mobility as fraction IV. They reported that this fraction was probably a mixture of lipovitellin and lipovitellenin complexes as reported by McCully et al. (1959). The proposed site of synthesis for this material was given as the liver and higher levels in the serum are measured as this material is being transported from the liver to the developing ova. This fraction was absent from spawning or spent females.

The appearance of lipoprotein components is a probable explanation for the increase in fraction IV in near mature female bluegills. Fraction IV remained slightly high in July in female fish, which was due to a few maturing females at this time. In the August and October collections fraction IV was approximately the same for all fish (Figure 11).

Table 16. Analysis of variance table for protein fraction I (All variables fixed)

Treatment	D.F.	S.S.	M.S.	F.
Main effects	12	15065.62		
Less ponds	8	11628.98		
Ponds within sex, time	4	3436.64	859.16	26.35*
Less sex	10	13040.58		
Sex within ponds, time	2	2025.04	1012.52	31.05*
Less time	9	8514.79		
Time within ponds, sex	3	6550.83	2183.61	66.96*
Main effects plus 2 factor interactions				
Main + 2 factor	38	23259.29		
Main effects	12	15065.62		
Two factor interaction	26	8193.67		
Less ponds and sex	30	22588.85		
Pond and sex	8	670.44	83.80	2.57*
Less ponds and time	26	17026.36		
Pond, time interaction	12	6232.93	519.41	15.93*
Less sex and time	32	22481.63		
Sex, time interaction	6	777.66	129.61	3.97*
Treatment combination	59	27458.92		
Main + 2 factor	38	23259.29		

*Significant at the .05 level.

Table 16. Cont.

Treatment	D.F.	S.S.	M.S.	F.
Pond sex time interactions	21	4199.63	199.98	6.13*
Total	446	40077.43		
Treatment combinations	59	27458.92		
Error	387	12618.51	32.61	

*Significant at the .05 level.

Table 17. Analysis of variance table for protein fraction II (All variables fixed)

Treatment	D.F.	S.S.	M.S.	F.
Main effects	12	17995.16		
Less ponds	8	14055.40		
Ponds/sex, time	4	3939.76	984.94	24.95*
Less sex	10	16369.90		
Sex/ponds, time	2	1625.26	812.63	20.58*
Less time	9	10848.41		
Time/ponds, sex	3	7146.75	2382.25	60.34*
Main + 2 factor	38	27983.06		
Main effects	12	17995.16		
Two factor interaction	26	9987.90		
Less ponds and sex	30	26831.84		
Ponds and sex	8	1151.22	143.90	3.64*
Less ponds and time	26	23131.33		
Pond, time interaction	12	4851.73	404.31	10.24*
Less sex and time	32	25422.55		
Sex, time interaction	6	2560.51	426.75	10.81*
Treatment combinations	59	29642.02		
Main + 2 factor	38	27983.06		
Ponds, sex, and time	21	1658.96	79.00	2.00*

*Significant at the .05 level.

Table 17. Cont.

Treatment	D.F.	S.S.	M.S.	F.
Total	446	44919.02		
Treatment combinations	59	29642.02		
Error	387	15277.00	39.48	

Table 18. Analysis of variance table for protein fraction III (All variables fixed)

Treatment	D.F.	S.S.	M.S.	F.
Main effects	12	11571.27		
Less ponds	8	10004.34		
Ponds/sex, time	4	1566.93	391.73	17.48*
Less sex	10	11374.33		
Sex/ponds, time	2	196.94	98.47	4.39*
Less time	9	4134.65		
Time/ponds, sex	3	7436.62	2478.87	110.61*
Main + 2 factor	38	18466.16		
Main effects	12	11571.27		
Two factor interaction	26	6894.89		
Less ponds and sex	30	18070.27		
Ponds and sex	8	395.89	49.49	2.21*
Less ponds and time	26	13870.84		
Ponds and time	12	4595.32	382.94	17.09*
Less sex and time	32	17376.58		
Sex and time	6	1089.58	181.60	8.10*
Treatment combinations	59	19454.09		
Main + 2 factor	38	18466.16		
Pond, sex, and time	21	987.93	47.04	2.10*

*Significant at the .05 level.

Table 18. Cont.

Treatment	D.F.	S.S.	M.S.	F.
Total	446	28125.83		
Treatment combination	59	19454.09		
Error	387	8671.74	22.41	

Table 19. Analysis of variance table for protein fraction IV (All variables fixed)

Treatment	D.F.	S.S.	M.S.	F.
Main effects	12	22962.20		
Less ponds	8	22600.43		
Ponds/sex, time	4	361.77	90.44	4.5 *
Less sex	10	15716.55		
Sex/ponds, time	2	7245.65	3622.83	183.71*
Less time	9	7662.44		
Time/ponds, sex	3	15299.76	5099.92	258.62*
Main + 2 factor	38	36691.17		
Less main effects	12	22962.20		
Two factor interaction	26	13728.97		
Less ponds and sex	30	35998.38		
Ponds and sex	8	692.79	86.60	4.39*
Less ponds and time	26	34819.47		
Ponds and time	12	1871.70	155.97	7.91*
Less sex and time	32	26424.38		
Sex and time	6	10266.79	1711.13	86.77*
Treatment combinations	59	38256.53		
Main + 2 factor	38	36691.17		
Ponds, sex and time	21	1565.36	74.54	3.78*

*Significant at the .05 level.

Table 19. Cont.

Treatment	D.F.	S.S.	M.S.	F.
Total	446	45888.36		
Treatment combinations	59	38256.53		
Error	387	7631.83	19.72	

Figure 8. Percentage protein in fraction I, over time, for male, female, and immature bluegills

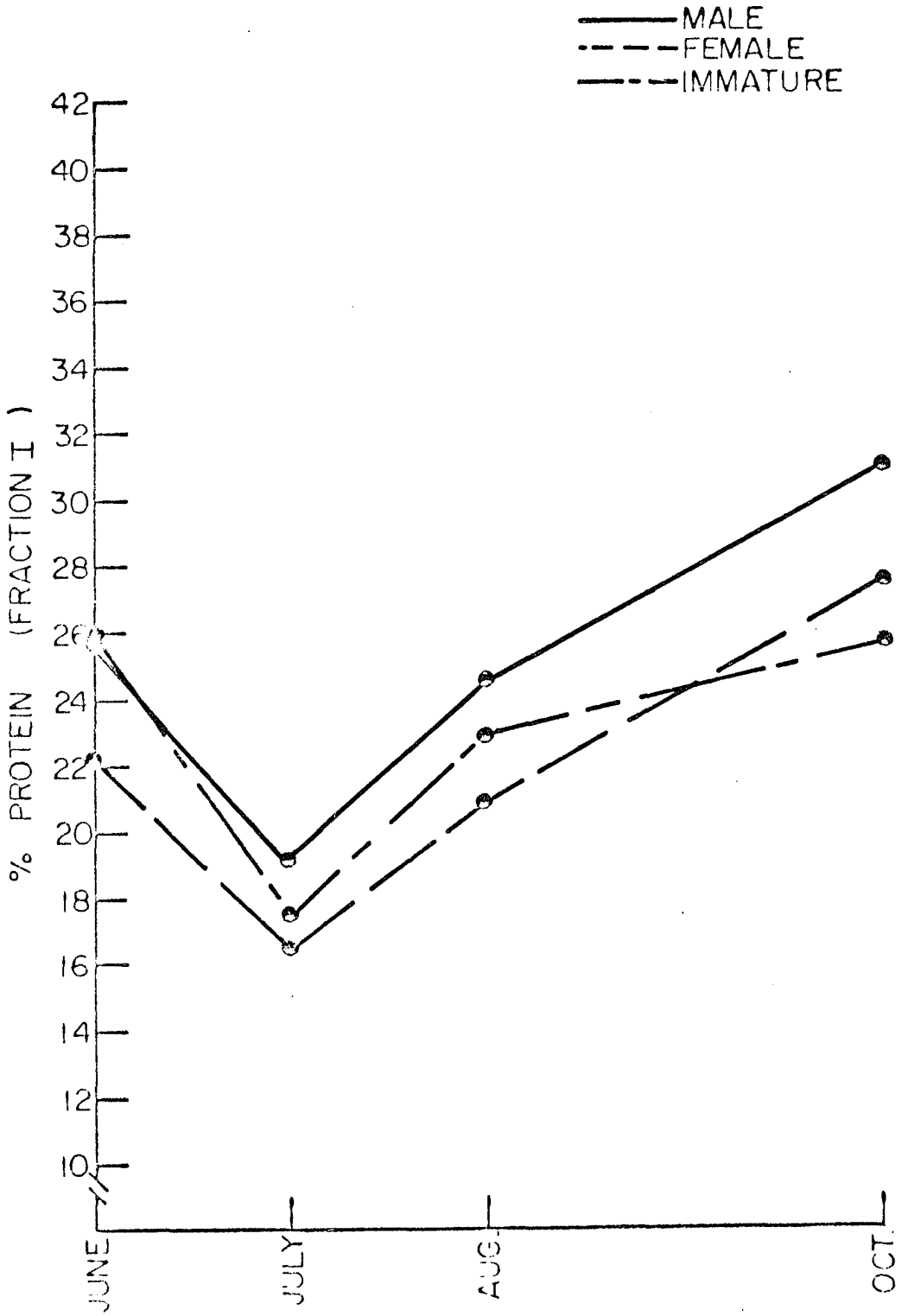

MALE
FEMALE
IMMATURE
% PROTEIN (FRACTION I)
42
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
JUNE
JULY
AUG.
OCT.

Figure 9. Percentage protein in fraction II, over time, for male, female, and immature bluegills

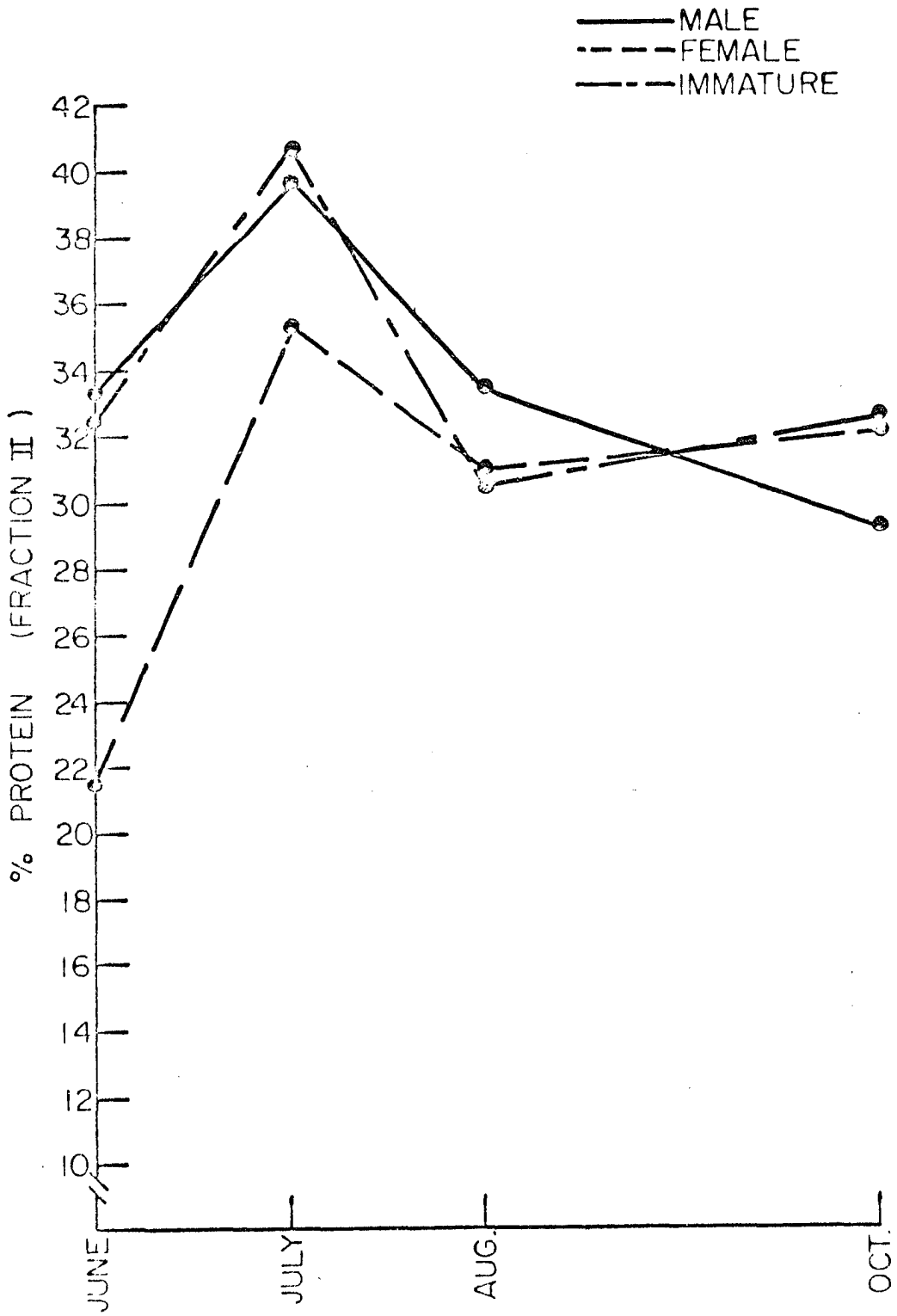

MALE
FEMALE
IMMATURE
% PROTEIN (FRACTION II)
42
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
JUNE
JULY
AUG.
OCT.

Figure 10. Percentage protein in fraction III, over time, for male, female, and immature bluegills

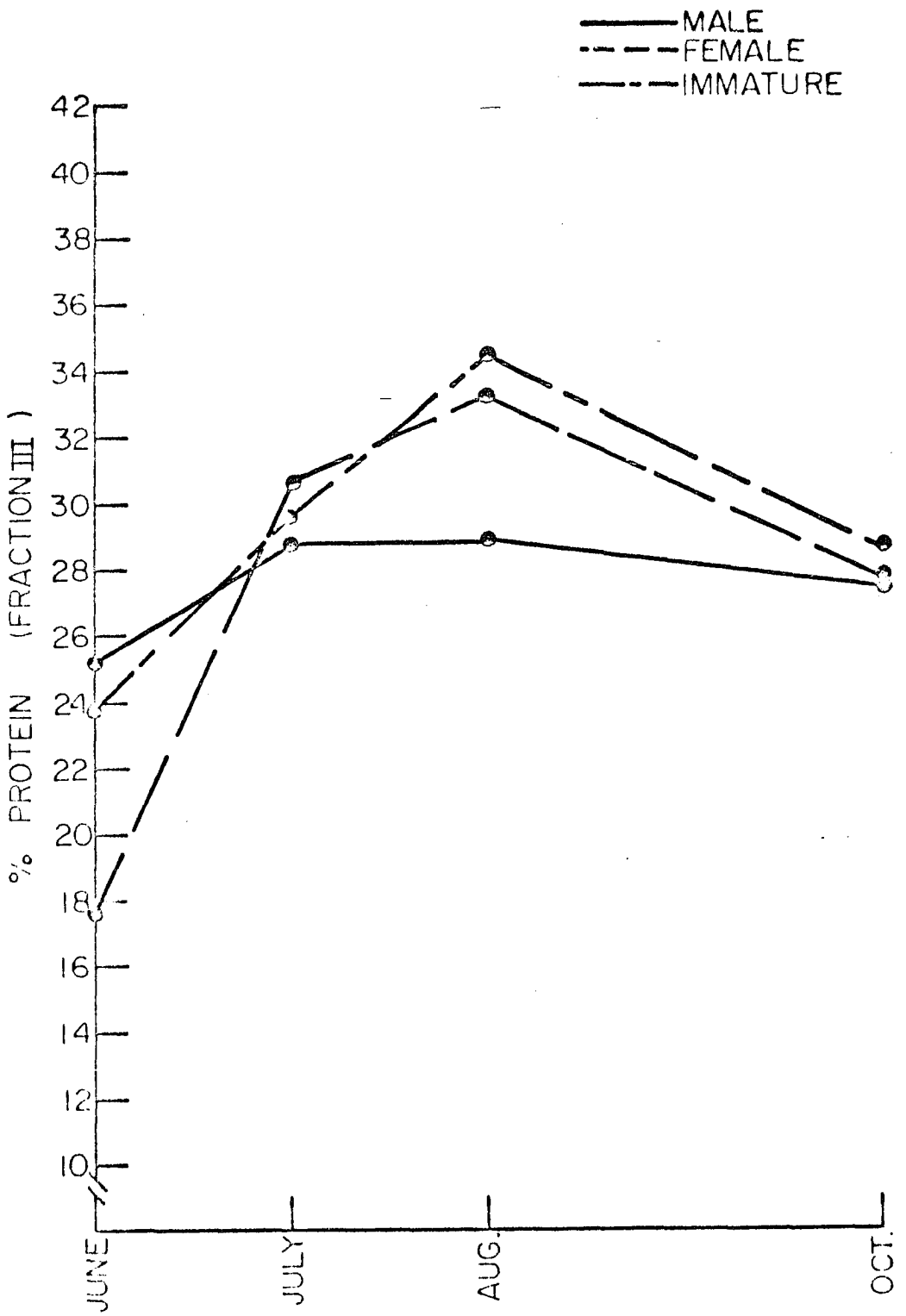
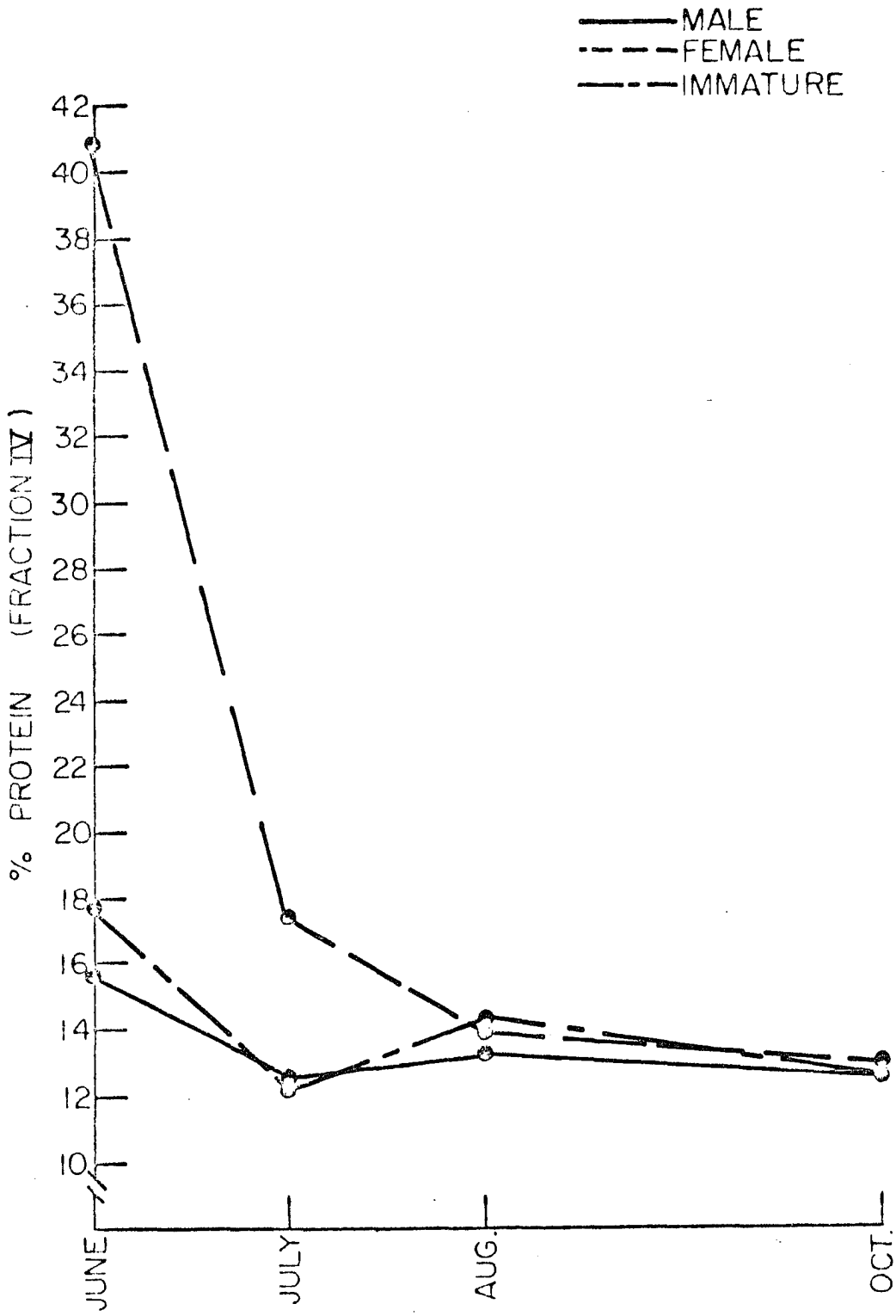

MALE
FEMALE
IMMATURE
% PROTEIN (FRACTION III)
42
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
JUNE
JULY
AUG.
OCT.

Figure 11. Percentage protein in fraction IV, over time, for male, female, and immature bluegills


MALE
FEMALE
IMMATURE
% PROTEIN (FRACTION IV)
42
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
JUNE
JULY
AUG.
OCT.

The relative proportions of fraction II increased from June to July with lower relative amounts in females. A decrease occurred from July to August in all groups; however, males remained higher than females. Percentage proteins in immatures and females were approximately the same in August and October. Male percentages decreased from August to October and were lower than both females and immatures in October (Figure 9).

The relative proportions of fraction III increased from June to August in females and immatures and decreased from August to October. The increase in fraction III in males was not as abrupt as in females and immatures from June to July and remained fairly constant from July to October (Figure 10).

In order to determine the relationships between ponds and sexes and the proportional amounts of protein within each fraction, the values for each fraction within ponds and sex were plotted (Figures 12, 13, and 14). Several general trends were noted. Usually fractions II and III contained the highest relative amount of protein, except for Link and McLain pond males, where fraction I was greater than fraction III. Females in all ponds had the greatest proportional amount of fraction IV. As discussed previously, the higher value of fraction IV is related to gonadal maturation in the females. The values for fraction II was highest in all ponds except Link Pond, which had a greater amount in fraction III.

Figure 12. Percentage protein in fractions I, II, III, and IV for female bluegills from all study ponds

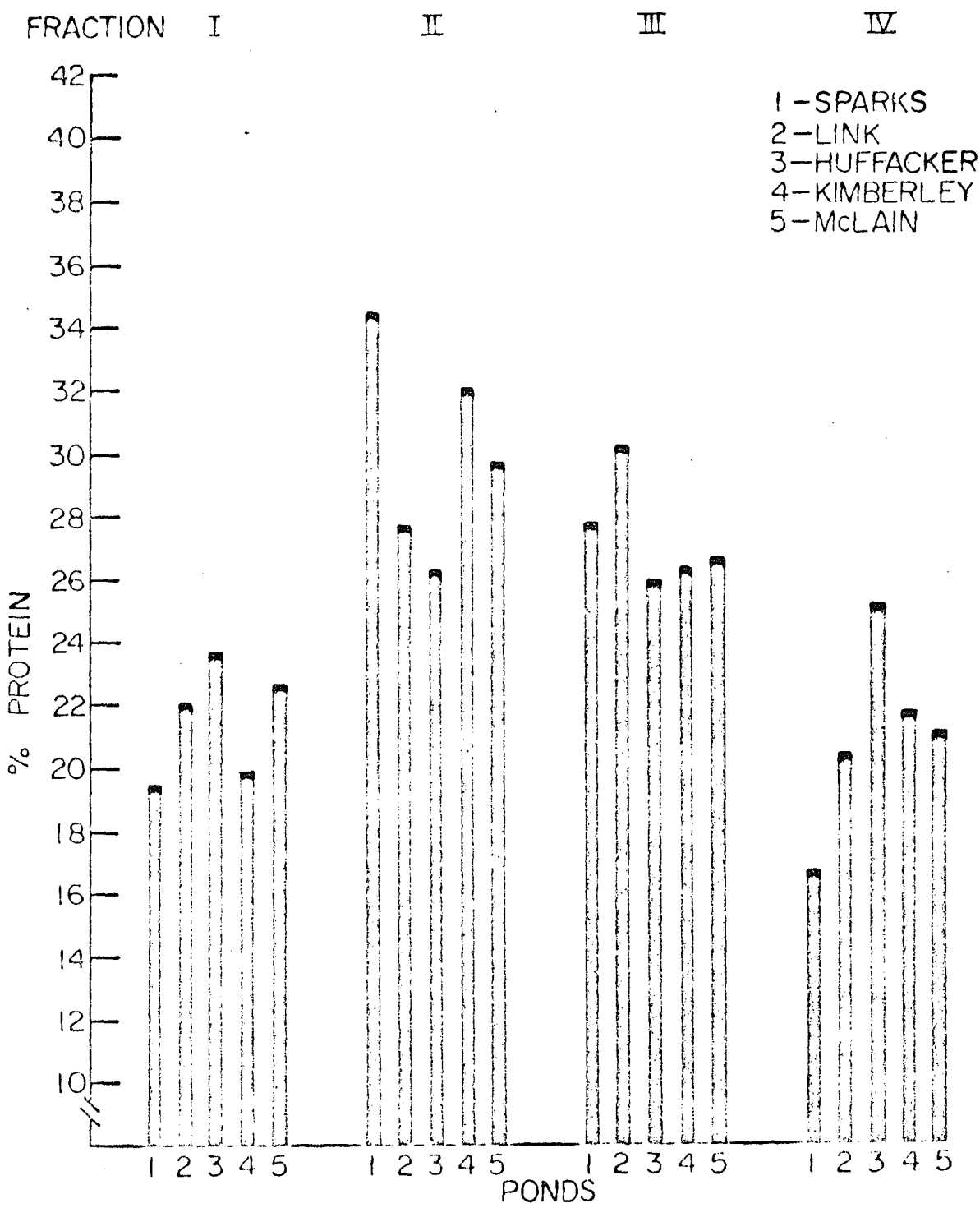

FRACTION I
II
III
IV
1-SPARKS
2-LINK
3-HUFFACKER
4-KIMBERLEY
5-McLAIN
% PROTEIN
42
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
1 2 3 4 5
1 2 3 4 5
1 2 3 4 5
1 2 3 4 5
PONDS

Figure 13. Percentage protein in fractions I, II, III, and IV for male bluegills from all study ponds

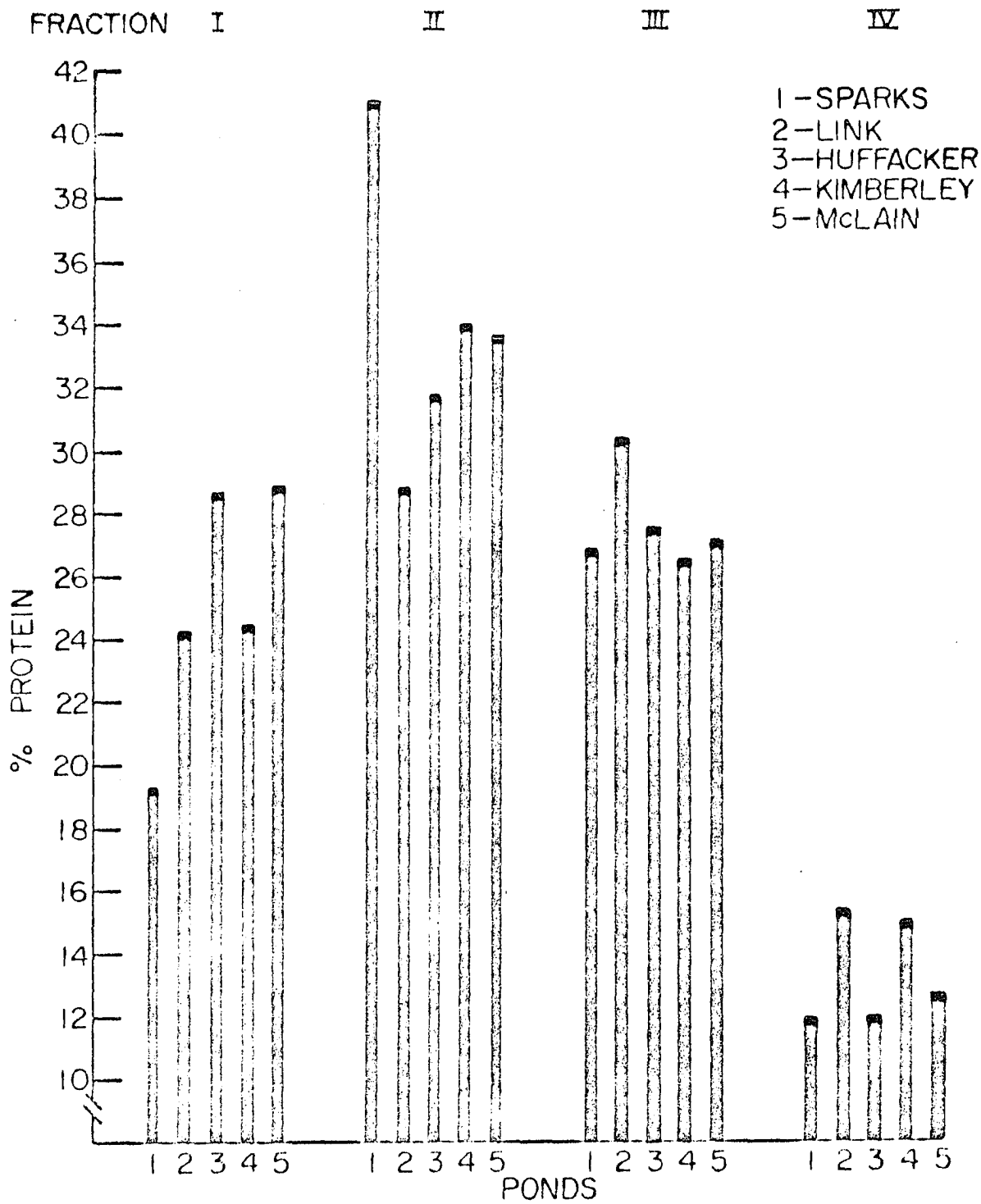
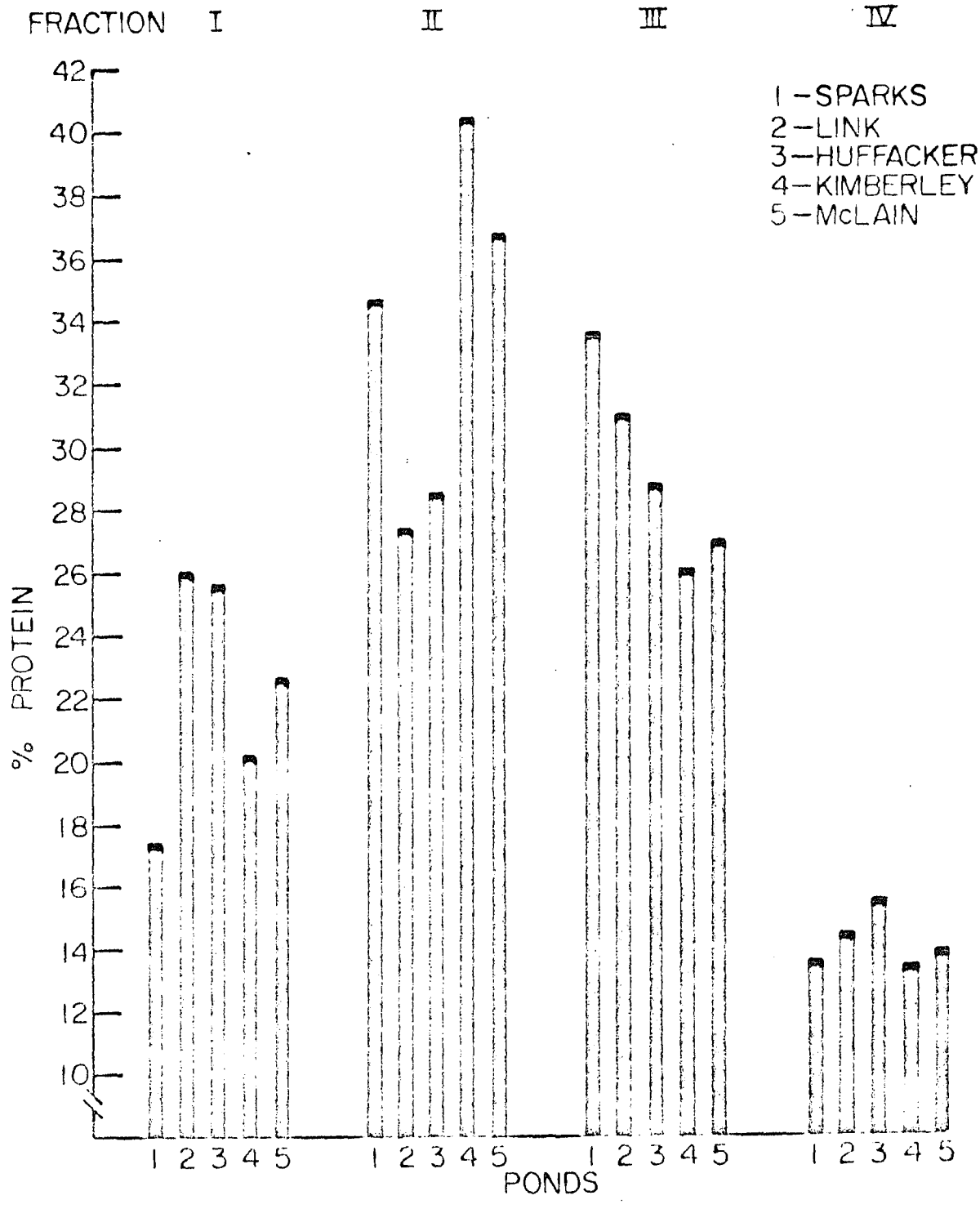

FRACTION I
II
III
IV
1-SPARKS
2-LINK
3-HUFFACKER
4-KIMBERLEY
5-McLAIN
% PROTEIN
42
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5
PONDS

Figure 14. Percentage protein in fractions I, II, III, and IV for immature gluegills from all study ponds


FRACTION I II III IV
1 – SPARKS
2 – LINK
3 – HUFFACKER
4 – KIMBERLEY
5 – McLAIN
% PROTEIN
42
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5
PONDS

Although differences were significant in each fraction between ponds, the percentage protein in each fraction was variable. Sex or stage of maturity caused shifts in the relative ranking of ponds. Bluegills of a particular sex within a pond might have the highest percentage protein within a fraction; however, when the percentage protein of another sex was considered, the relative ranking of the pond might change. It is imperative, therefore, to consider sex as a variable when comparing protein fractions of fish taken from different ponds.

McLain Pond contained stunted bluegills; however, the protein fractions of fish from this pond did not exhibit stress patterns to any greater extent than fish from Sparks and Kimberley ponds (Figures 12, 13, and 14). In all sexes Sparks Pond had lower relative amounts of high mobility fraction I and greater proportional amounts of fraction II in males and females (Figures 12 and 13).

Huffacker Pond, which was intermediate in population density, but had the lowest standing crop, had the lowest relative amount of fractions II and III and the highest proportional amounts of fractions I and IV in females (Figure 12). Using the hypothetical stress pattern as an indication of well-being, females in Huffacker Pond might be considered to be in better physiological condition than females from other ponds. Males and immatures from Huffacker Pond had relatively high amounts of fraction I and were intermediate in the amount of fractions II and III.

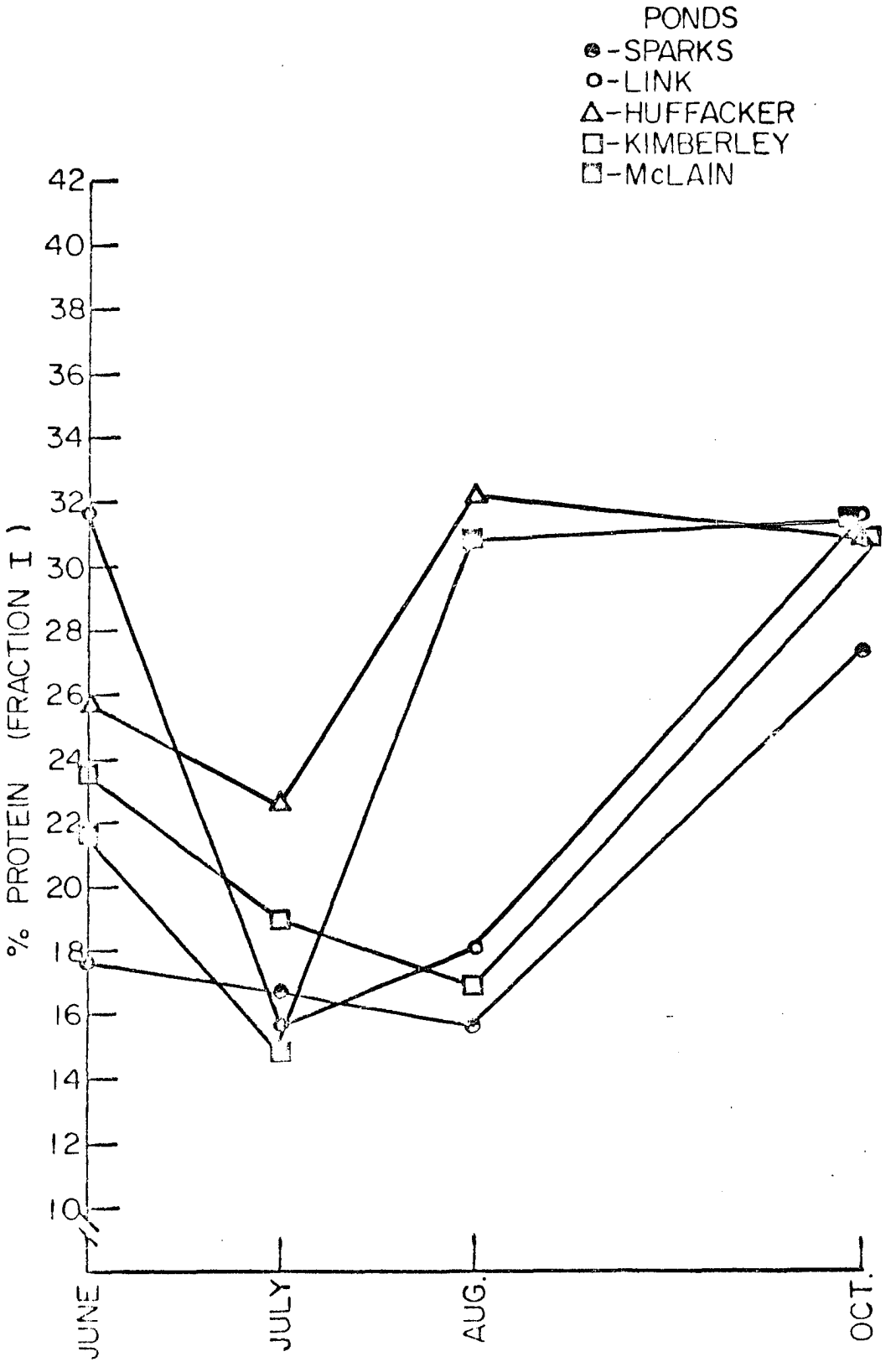
Link Pond was distinctive, since it was the only pond in which fraction III was higher than fraction II in all sexes (Figures 12, 13, and 14).

The most apparent factor is the variability of protein fractions between ponds over sex and the lack of correlation between protein patterns and population density.

The pond-time interactions for each pond were significant (Tables 15, 16, 17, and 18). The percentage protein levels in each fraction in all ponds showed general trends over time. Usually the differences in fractions between ponds were less in July and October, with greater differences in June and August.

The percentages of fraction I decreased from June to July in all ponds. This decrease continued in Sparks and Kimberley ponds until August and increased to high levels in October. In Huffacker, Link, and Kimberley ponds the percentages of fraction I rose from July to August. The highest level of fraction I occurred in August in Huffacker Pond and dropped slightly from August to October. McLain Pond, like Huffacker Pond, showed relatively high percentages in August and rose slightly from August to October. Link Pond percentages increased slightly from July to August and rose sharply from August to October. The percentage difference of fraction I in all ponds was greater between ponds in June and August with smaller differences in July and October. In October the values for Huffacker, Link, Kimberley, and McLain Ponds were nearly the same. The percentages of fraction I in Sparks Pond were lower than the other ponds in October (Figure 15).

Figure 15. Percentage protein in fraction I for Sparks, Link, Huffacker, Kimberley, and McLain ponds over time


PONDS
●-SPARKS
o-LINK
Δ-HUFFACKER
□-KIMBERLEY
□-McLAIN
% PROTEIN (FRACTION I)
42
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
JUNE
JULY
AUG.
OCT.

Fraction II increased in all ponds from June to July. Huffacker, Link, and McLain pond values decreased from July to August and then increased quite sharply in Huffacker and McLain pond from August to October. The values in Link Pond in August and October were almost identical. Fraction II increased in Sparks and Kimberley ponds from July to August and decreased from August to October. Differences between the percentages of fraction II were greatest in June and August (Figure 16).

Percentages of fraction III were lowest in all ponds in June except Sparks Pond, which had its lowest percentage in October. All percentages increased from June to August in Sparks, Huffacker, McLain, and Kimberley ponds, and then decreased slightly from August to October. Fraction III percentages in Link Pond reached high levels in June and decreased continually to October. The differences between percentages of fraction III within ponds were greatest in August and more nearly equal in June, July (except Link Pond), and October (Figure 17).

Percentages of fraction IV were higher in all ponds in June. Since the interaction between ponds, sex, and time was significant, fraction IV over time was similar to the patterns described for sex and time, and pond and sex. Percentages of fraction IV declined in all ponds from June to July. Values in Kimberley and McLain ponds continued to drop slightly from June to October. A slight rise in the percentage of fraction IV was evident in Sparks Pond from July to October. Values in Huffacker Pond decreased from June to July and then

rose from August to October. Percentages of fraction IV were nearly equal in October (Figure 18).

Although differences in percentage protein between all fractions were significant over ponds, they were compounded by sex and time. Ponds could not be ranked according to population density by the relative amounts of protein within fractions, since the relative amount of protein in fractions changed with time.

One variable not considered in the analysis of variance was age of the individuals. The age composition of all ponds was not the same so it was impossible to compare individuals of the same age over ponds. Bluegills of different ages were grouped in McLain and Huffacker ponds within a specific time and sex (Table 20). Differences between each band were tested against age within each of these ponds. There were no significant differences between ages within these ponds at the 0.05 level using a Student's "t" test; however, the sample size was small in each case and may not actually be representative of the entire population.

Figure 16. Percentage protein in fraction II for Sparks, Link, Huffacker, Kimberley and McLain ponds over time

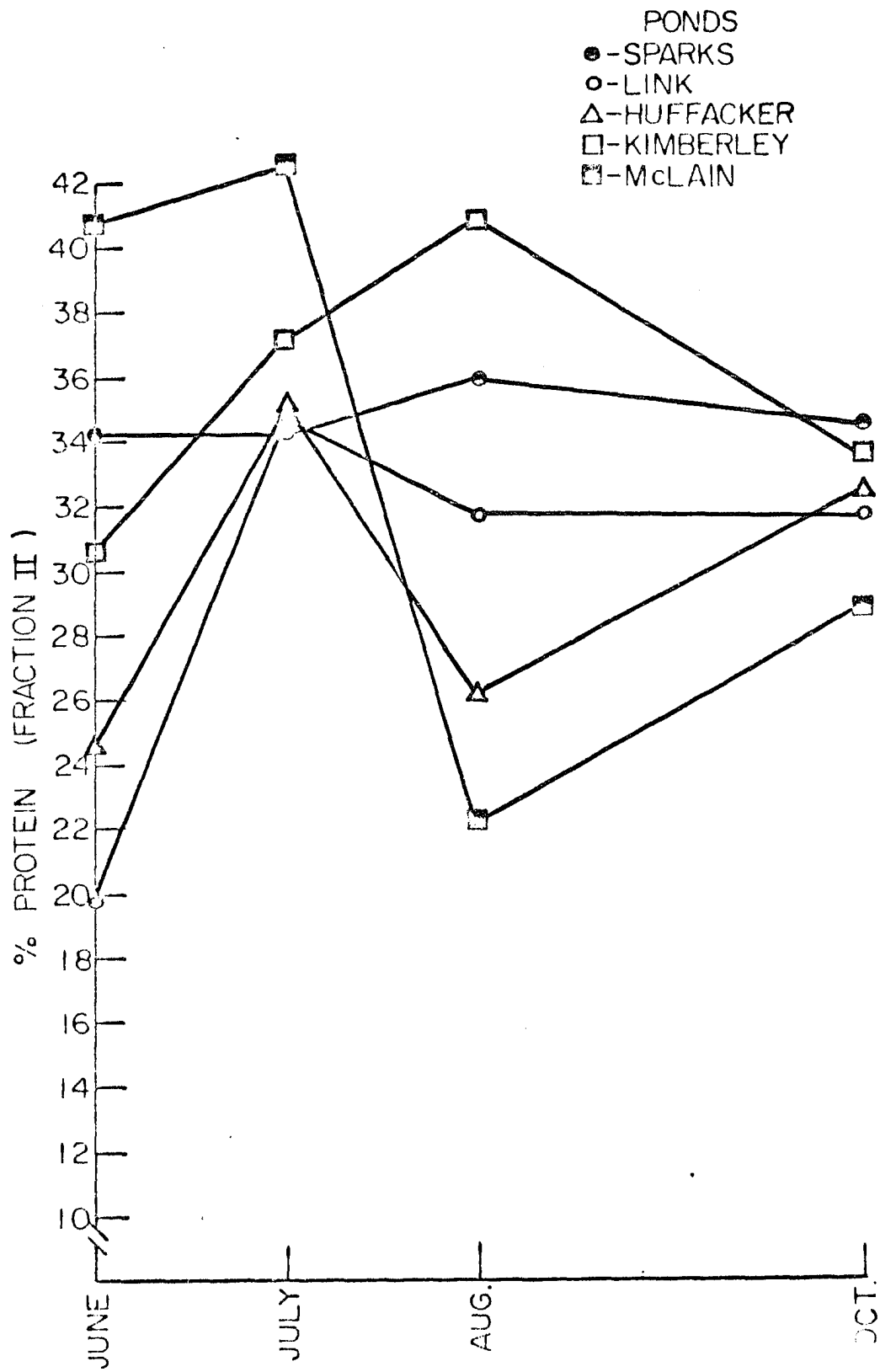

PONDS
●-SPARKS
o-LINK
△-HUFFACKER
□-KIMBERLEY
⊡-McLAIN
% PROTEIN (FRACTION II)
42
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
JUNE
JULY
AUG.
OCT.

Figure 17. Percentage protein in fraction III for Sparks, Link, Huffacker, Kimberley, and McLain ponds over time

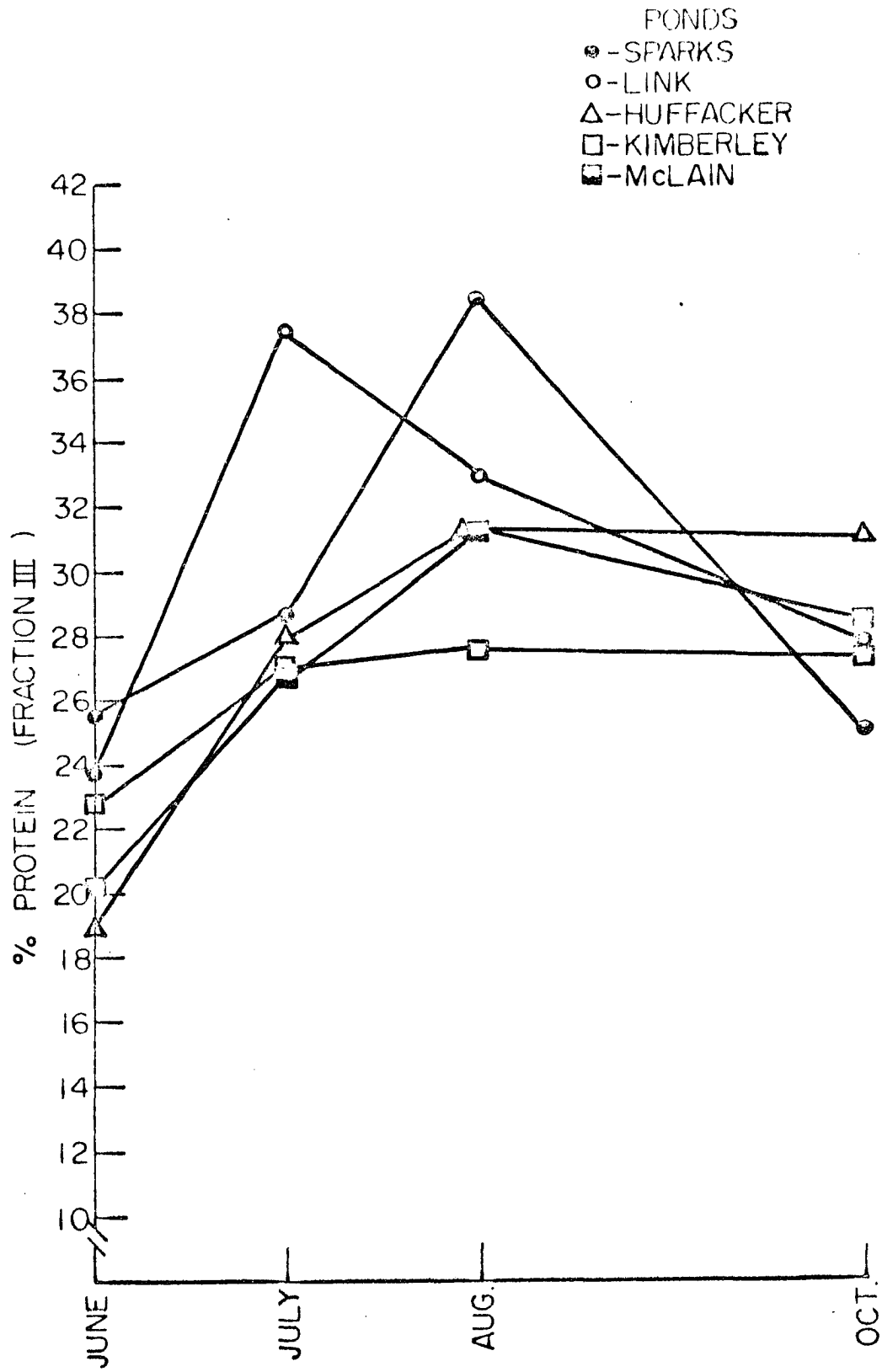

PONDS
●-SPARKS
o-LINK
△-HUFFACKER
□-KIMBERLEY
■-McLAIN
% PROTEIN (FRACTION III)
42
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
JUNE
JULY
AUG.
OCT.

Figure 18. Percentage protein in fraction IV for Sparks, Link, Huffacker, Kimberley and McLain ponds over time

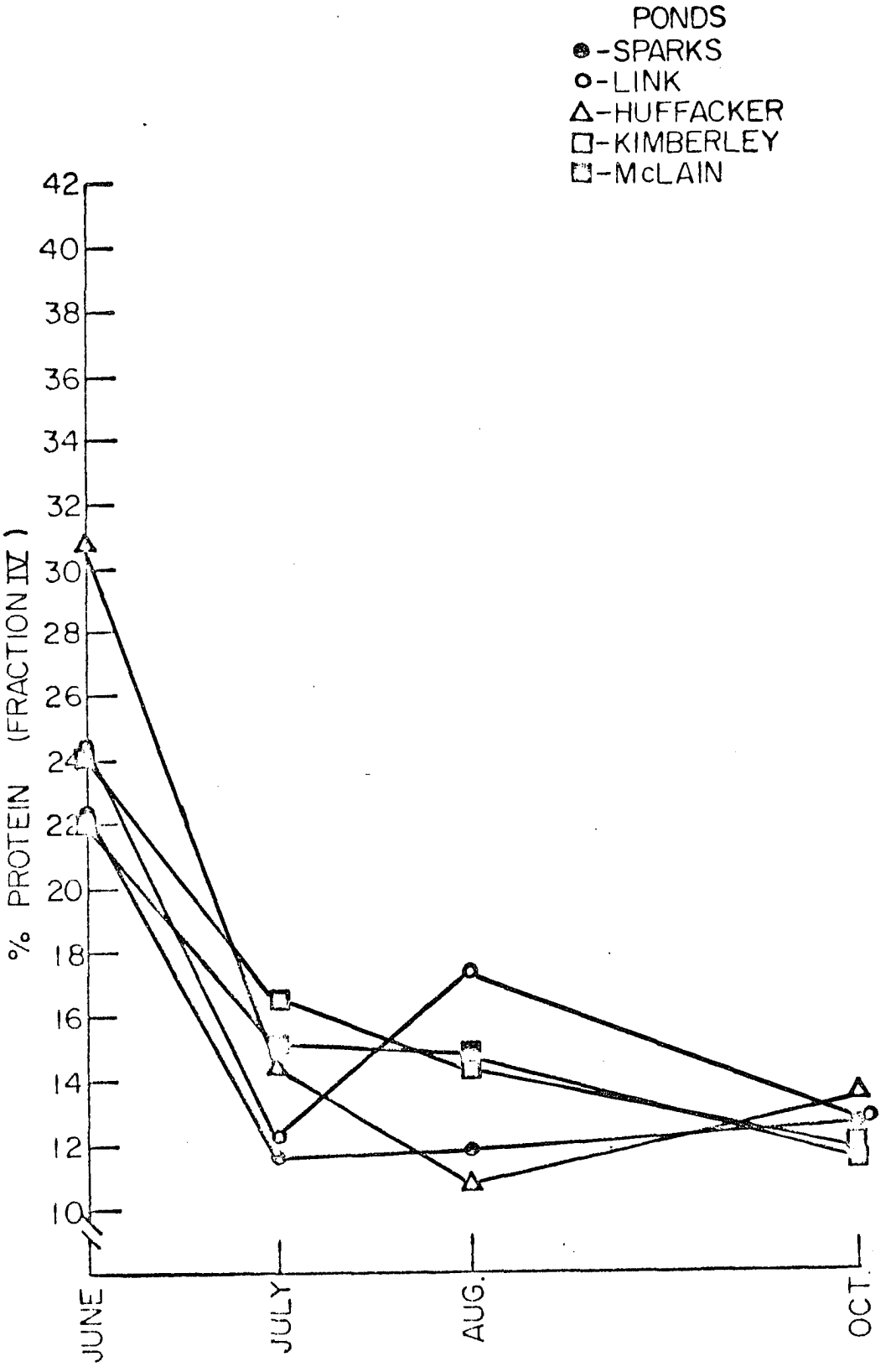

PONDS
●-SPARKS
o-LINK
Δ-HUFFACKER
□-KIMBERLEY
□-McLAIN
% PROTEIN (FRACTION IV)
42
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
JUNE
JULY
AUG.
OCT.

Table 20. Summary of the influence of age in the distribution of protein in the serum of bluegills from Huffacker and McLain ponds (expressed as per cent of total serum protein)

Pond	Age	Fractions			
		I	II	III	IV
Huffacker (male)					
Mean	3	38.88	26.24	22.30	12.58
Range		32-46	20-30	18-32	8-16
n = 8					
Mean	4	36.95	26.63	23.79	12.63
Range		31-44	21-38	17-25	6-18
McLain (female)					
Mean	4	10.06	42.45	26.06	21.29
Range		6-17	28-48	17-31	13-33
n = 10					
Mean	5	13.80	36.61	25.95	23.36
Range		12-18	26-46	21-33	10-33
n = 6					

VI. DISCUSSION AND CONCLUSIONS

A. Laboratory Experiments

Food deprivation for 45 days affected the total serum protein levels in bluegills. A drop of 30 per cent was noted after 36 days in the starved group. The results of this study and others indicate that the amount of total serum proteins are reduced by starvation, and the reduction in total proteins is rapid when compared to man.

Electrophoresis of serum protein of bluegills separated four major fractions. Bluegills subjected to starvation for 64 days had reduced amounts of fraction I with proportional increases in fractions II and IV. This represents the stress pattern found by other workers. Crowding experiments, in which fish were crowded at different rates were inconclusive. Small but significant differences existed between groups in fraction I and fraction III. The percentage of fraction I in the three crowded groups were not greatly different from fraction I in the fed group, indicating that fraction I was not reduced by the rates of crowding tested. The relative percentages of the other fractions (II, III, and IV) were considerably different from the percentages found in the low-mobility fractions of both the fed and starved groups; however, the significance of these changes is not known.

B. Field Experiments

The percentages serum protein in each fraction of wild bluegills from different ponds was variable; however, the differences between ponds were significant. Nevertheless the statistically significant

interactions between sex, time, and ponds made it impossible to determine the relationships between population density and protein fractions. A specific pond did not maintain the same position as to percentage proteins with time or sex. Therefore, it was impossible to state that one pond was constantly better than another.

Since privately owned ponds were used as experimental ponds, the populations could not be altered; therefore, the fish data obtained from these ponds were not always comparable. For this reason it was difficult to rank ponds by comparing population densities, standing crops, and growth rates. Although the ages of fish from all ponds were not the same, the best method of ranking ponds seemed to be by growth increments in 1965.

Electrophoretic analysis of plasma or serum proteins have been studied in conjunction with stressful conditions by other authors. These authors have demonstrated stress patterns which they related to specific stressful conditions. Usually these studies were conducted in the laboratory under controlled conditions. The results of this study indicate that serum protein fractions of wild bluegill sunfish are extremely variable and many factors might be influencing the percentage composition of the protein fractions. Therefore, the value of this technique for evaluating conditions in natural populations is questionable. Until the effects of normal physiological and environmental factors on the serum proteins are known, it seems impossible to attribute stress patterns to specific factors such as pollution or disease.

There are several areas in which further work on fish serum proteins would be useful. First, biochemical analysis and identification of the various fractions are necessary in order to understand the significance of changes in the protein patterns. Secondly, after identification of the fractions, it would be beneficial to measure the actual amount rather than the percentage of protein in each fraction. It would then be possible to determine whether changes in the serum protein components are relative or absolute. Thirdly, although studies have been published illustrating stress patterns, the normal patterns for various species have not been reported. Since protein fractions of control laboratory animals may differ from these of natural populations, it would be desirable to study experimental ponds in which species composition, ages, and numbers of fish could be controlled. In this manner it might be possible to determine normal protein patterns for natural populations.

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