SEASONAL CHANGES AND TEMPERATURE INDUCED ACTIVITY IN THE CARBOHYDRATE METABOLISM OF THE GOLDEN SHINER, NOTEMIGONUS CRYSOLEUCAS

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by

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ABSTRACT

Nicholas A. Scandale, Jr. SEASONAL AND TEMPERATURE INDUCED ACTIVITY IN THE CARBOHYDRATE METABOLISM OF THE GOLDEN SHINER, NOTEMIGONUS CRYSOLEUCAS. (Under the direction of Dr. Charles W. O'Rear, Jr.) Department of Biology, May, 1983.

The purpose of this study was to obtain data on biochemical activities associated with carbohydrate metabolism in the golden shiner caused by acclimatization and expand present knowledge of the seasonal changes in the carbohydrate metabolism.

Fish were captured using gill nets in a channelized and an unchannelized stream in the Tar River water shed. Seasonal variations in body weights, the weights of the liver, its glycogen content, and two carbohydrate enzymes, glucose-6-phosphatase and glycogen phosphorylase, activity values were studied. The enzymes were assayed at 10°C and 25°C to gain evidence of temperature induced activity.

In general, the liver glycogen content decreased during the study. The phenomenon was accompanied by increased glycogen phosphorylase activity. The fish taken from the channelized stream were often smaller and the livers of these fish contained less glycogen. Some evidence of temperature induced activity was found in the glucose-6-phosphatase values. Some evidence of an inverse relationship in temperature induced activity was found in the glycogen phosphorylase values.

ACKNOWLEDGEMENTS

I wish to give my sincere thanks and appreciation to my thesis advisor, Dr. Charles W. O'Rear, Jr., and my thesis committee, Dr. Sam N. Pennington, Dr. Takeru Ito, and Dr. Everett Simpson, for their advice and criticism of this study. I would also like to thank Joey Poandl for preparing the fine graphs and Barbara James for typing the final draft of this study.

Laboratory facilities and equipment were provided by the Department of Biology at East Carolina University.

DEDICATION

This study is dedicated to two very special people. My friend, Charlotte, and my daughter, Joanie, who gave me their support and understanding while I completed this work.

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INTRODUCTION

Most research on carbohydrate metabolism has been on mammalian species and a number of homeostatic responses to dietary or endocrine imbalance have been reported. Studies on fish are limited to a relatively few species of commercial importance, such as trout, salmon, eel, catfish, and tuna.

Early studies of carbohydrate metabolism, in fish, were mainly concerned with the compositional analyses of the tissue and eggs. Evidence defining the process of carbohydrate metabolism was indirect and, for the most part, inferred from various physiological and environmental factors. The processes of glycogen metabolism are in keeping with that worked out for mammals. Black et al. (1961) reported glycogen levels in both muscle and liver in fish appear to be substantially lower than those recorded for mammals. Real differences do occur and may be related to the lower body temperatures of the fish.

Little has been done to characterize the specific enzyme systems and pathways relating to the intermediate products of carbohydrate metabolism in fish. Macleod et al. (1963) investigated glycolytic enzymes in the tissues of the trout, Salmo gairdenerri gairdenerri, and were the first to assay phosphofructokinase (PFK) in crude extracts of muscle, heart, brain, liver, and kidney. Evidence for the presence of all the enzymes of the Embden-Meyerhoff glycolytic pathway except triose phosphate isomerase and phosphoglycerate kinase was found. The presence of these enzymes could be inferred from the overall glycolysis results. Except for hexokinase and phosphofructokinase, the glycolytic enzymes

of the fish skeletal muscle were found to be three to one hundred times more active than those of the liver tissues examined. Tests indicated that PFK was the limiting enzyme (Macleod et al., 1963). Hexokinase activity was low in all the tissues examined except heart muscle. The results indicated not only that the fish tissues contained the same kinds of glycolytic enzymes as are found in mammalian tissues, yeast, and bacteria, but also that, as in glycolytic enzymes of the other species, ADP and NAD are required. Macleod et al. (1963) also determined that the Embden-Meyerhoff pathway is not the sole pathway of degradation of glucose in these tissues.

Williamson et al. (1967) studied glycolytic control mechanisms in the electric organs of the electric eel, Electrophorus electricus. Their study indicated that the principal sites of control in the glycolytic pathways were glycogen phosphorylase and PFK. Control of hexose monophosphate utilization in the electric organ was found to be similar to that observed with mammalian systems. Their studies also demonstrated few differences between the control sites of the glycolytic pathways of fish and those of mammals.

There have been some recent studies of compensatory patterns of catalytic activity during thermal acclimation. Benziger and Umminger (1973) studied the role of hepatic glycogenolytic enzymes in salt water adapted killifish, Fundulus heteroclitus, acclimated to 20°C and 1.5°C. Their investigation demonstrated that there was in increase in specific activity of glycogen phosphorylase at the lower temperature. This increased activity appeared to be responsible for the cold induced hyperglycemia observed. Cooper and Ferguson (1972) studied the effect of

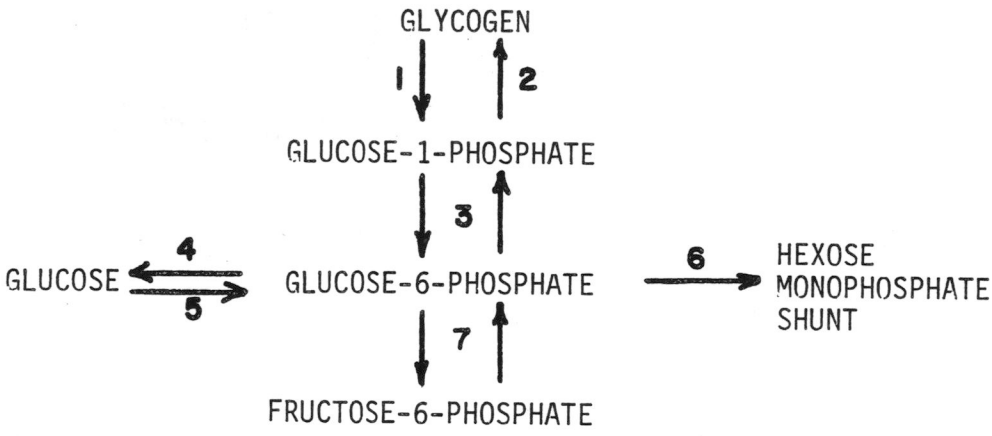
cold acclimation upon glucose-6-phosphatase activity in two free living nematodes. Their work showed that glucose-6-phosphatase activity was greater in the cold acclimated population of Panagrellus redivivus and relatively unchanged in Turbatrix aceti. Low and Somero (1976) studied the adaptation of muscle pyruvate kinase to environmental temperatures and pressures. They found most muscle pyruvate kinases exist in two temperature dependent conformational states. The extent of conformational change which occured during catalysis was positively correlated with all temperatures except for enzymes of deep sea species (Low and Somero, 1976).

The seasonal differences in the carbohydrate reserves of fish, especially those in the liver, have been known for a long time (Plisetskaya and Kuzimina, 1972) although there are very few reports available which concentrate on seasonal observations in nature. A seasonal study allows a comparison to be made between recent results on temperature acclimation and certain acclimatization phenomena (Fry, 1967; Hochachka and Somero, 1971). Recent studies (Bentley, 1976; Carneiro and Amaral, 1983) of the hormonal control mechanism for the carbohydrate metabolism in fish may help in handling these problems.

In this study of seasonal carbohydrate metabolism of the golden shiner, Notemigonus crysoleucas, special attention has been paid to the enzymes, glycogen phosphorylase and glucose-6-phosphatase, at the glucose-6-phosphate branch point because data was available for comparisons with laboratory and field studies (Valtonen, 1974).

The direction of carbohydrate metabolism is determined at the glucose-6-phosphate branch point (Fig. 1). Glycogen is directed toward

Figure 1. Metabolic map of carbohydrate metabolism to and from glucose-6-phosphate



Key: 1 glycogen phosphorylase

2 glycogen synthesis

3 phosphoglucomutase

4 glucose-6-phosphatase

5 hexokinase

6 glucose-6-phosphate dehydrogenase

7 glucose phosphate isomerase

glucose-6-phosphate by the action of glycogen phosphorylase. At the branch point glucose-6-phosphate can be directed toward:

(1) the Embden-Meyerhoff pathway by glucose phosphate isomerase

(2) the hexose monophosphate shunt by glucose-6-phosphate dehydrogenase

(3) glucose production by glucose-6-phosphatase

(4) glycogen synthesis by phosphoglucomutase and glycogen synthase.

Glucose-6-phosphatase catalyses the hydrolysis of glucose-6-phosphate. The enzyme is associated with the microsome fraction of cell homogenates (Ikeda and Shimeno, 1967), although any biological advantage of the association is not clearly apparent (Hers, 1976). Glucose-6-phosphatase functions at an optimal pH 6.0 and is inhibited at high concentrations of glucose (Ikeda and Shimeno, 1967).

Glycogen phosphorylase exists in two forms, active and inactive. Cyclic-AMP stimulates the activity of two protein kinases, one which activates phorylase kinase, and another which converts active glycogen synthase to inactive glycogen synthase. The activated phosphorylase kinase converts the inactive form of glycogen phosphorylase to the active form. This enzyme catalyzes the rate limiting step of glycogenolysis and indirectly controlling the activity of synthase phosphatase thus regulating glycogen synthesis. During glycogenolysis, the glucose produced greatly stimulates the formation of the inactive form of glycogen phosphorylase from the active form. The decreasing concentration of the active form of glycogen phosphorylase activates glycogen synthase which catalyzes glycogen synthesis (Hers, 1976). This antagonistic relationship between active glycogen phosphorylase and active

glycogen synthase permits a very delicate control of glycogen metabolism.

Objectives and study area selection

The objectives of this study were to obtain data on the evidence of temperature induced activities caused by acclimatization and expand present knowledge of the seasonal changes in the carbohydrate metabolism of fish. The study areas, which were in the Tar River watershed, were chosen for their proximity to each other and to Greenville.

STUDY AREAS

The study areas were Grindle Creek, which had been recently channelized, and Chicod Creek, which was in a relatively natural state. Both creeks are in the Tar River watershed.

Grindle Creek

Grindle Creek is located in the northern portion of Pitt County originating east of Bethel and flowing in a southeasterly direction and empties into the Tar River. Grindle Creek was almost completely channelized by a Soil Conservation watershed development project completed in February, 1965. The project modified a total of 28.5 miles of stream channel through snagging and clearing of 2.2 miles, enlarging 23.6 miles of existing channel, and constructing 2.7 miles of new channel. Spoil material was deposited on both sides of the creek.

Chicod Creek

Chicod Creek is located east of Greenville in Pitt and Beaufort Counties. The creek flows north from its origin near Hackney to its confluence with the Tar River about 1 mile north of Grimesland. Only slight watershed development modifications had been made at the time of the study. The flood plains are generally broad, wooded swamps with no distinct channels in much of the upper reaches. Most of the Chicod Creek is considered perennial even though there may be short periods of no flow almost every year.

MATERIALS AND METHODS

Sampling

Sampling sites were located approximately 200 yards upstream from the mouth of each creek. Gill nets used in the sampling included square mesh sizes of 3 inches, 2 1/2 inches, 2 inches, 1 3/8 inches, and 1 inch. The nets were placed face to face in series perpendicular to stream flow the largest mesh downstream.

The samples were taken between 0600 hours and 1200 hours on each sampling day to eliminate variation caused by feeding. To determine sampling days, each day of the week was assigned a number from 1 to 7 from a table of random numbers. Even numbered days were designated as Chicod Creek days and odd numbered days as Grindle Creek days. On the even numbered day the first three hours of sampling time were assigned to Chicod Creek and the last three sampling hours were assigned to Grindle Creek and vice versa on the odd numbered days. Nets were set January through June. The golden shiner was selected for the study because of the number of these fish captured in the creeks sampled. The dates reported are the only dates in which golden shiners were found in both creeks.

Date and time were recorded for all captures. The fish were frozen in dry ice immediately after capture. They were stored in a freezer at a temperature of -25°C. The enzyme assays and glycogen determinations were carried out within one week of sampling. To accomplish this, the fish were thawed just enough to allow the body cavity to be opened. Each specimen was weighed to an accuracy of 0.01 grams and the mass of

the liver determined to an accuracy of one milligram.

Glycogen content

A 10% homogenate of the liver was prepared in ice cold water. A 10 microliter aliquot of the homogenate was preincubated for 10 minutes at 55°C with 0.1 ml of 0.05 M acetate buffer, pH 4.5, 3.5 units of amyloglucosidase and sufficient distilled water to make a total volume of 0.5 ml. After the preincubation period, glucose was determined by the enzymatic-colormetric glucose oxidase GO:PO procedure using a Sigma kit. Four milliliters of this premix was added to each sample, the glucose standards, and reagent blank. The total volume was brought up to 4.5 ml with distilled water. After incubation for 30 minutes at 37°C, the samples were read at 500 nm on a spectrophotometer. The glucose concentration for each sample was calculated from a glucose standard curve which was determined with each set of samples. The glycogen content of the liver was calculated as a percentage of the tissue wet weight.

Glycogen phosphorylase assay

Glycogen phosphorylase activity was measured by the release of inorganic phosphate from glucose-1-phosphate in the presence of glycogen and c-AMP (Hers and Hoff, 1966). Two substrates were prepared for each assay. One substrate contained c-AMP to measure the total amount of glycogen phosphorylase activity. The second substrate contained no c-AMP in order to measure the amount of active glycogen phosphorylase in the liver cells at the time of collection. They were prepared as follows:

(1) substrate A - 0.1 M glucose-1-phosphate, 2% glycogen,

0.03 M c-AMP, and 0.2 M NaF

(2) substrate B - 0.1 M glucose-1-phosphate, 2% glycogen, and 0.2 M NaF

The pH of each substrate was adjusted to 6.1. Four tubes were prepared for each sample. A 1% homogenate, volume of 0.05 ml, and 0.05 ml of substrate were mixed in the bottom of centrifuge tubes and incubated at 25°C and 10°C for 30 minutes and 60 minutes respectively. The reaction was stopped by the addition of 0.5 ml of 1 M trichloroacetic acid at 0 minutes (acid added before extract), 30 minutes for the 25°C assay, and 60 minutes for the 10°C assay.

Glucose-6-phosphatase assay

Glucose-6-phosphatase activity was based on the release of inorganic phosphate from glucose-6-phosphate. Two tubes were prepared for each sample. In each tube, a substrate consisting of 0.1 ml of 0.1 M glucose-6-phosphate, pH 6.5, containing 0.002 M EDTA was mixed with 0.1 ml of a 1% liver extract. The 10°C assay was run for 60 minutes and the 25°C assay was run for 30 minutes. The reaction was stopped by the addition of 0.5 ml of 1 M trichloroacetic acid. The amount of inorganic phosphate was measured at time 0 and the end of each assay.

Inorganic phosphate determination

The inorganic phosphate was determined according to the method of Hers and Hoff (1966) by adding 0.5 ml of molybdate-sufuric acid reagent and distilled water up to a volume of 4.8 ml. The mixture was centrifuged and the clear supernatent was decanted into a colormetric tube containing a 0.2 ml of amino-naphthol sulfonic reagent. A set of

phosphate standards was prepared using 1.3613 g of KH_2PO_4 dissolved in one liter of distilled water. A 1:10 dilution was made so that 1 ml of standard solution corresponded to 1 micromole of phosphorus. The samples were read at 660 nm on a spectrophotometer. The activities of the enzymes were expressed in micrograms phosphate/gram of liver/minute.

Water quality

Water quality was monitored throughout the study for temperature, dissolved oxygen, conductivity, pH, and nutrients. Nutrient analyses included tests for nitrites-dissolved, nitrates-dissolved, ammonia, total organic nitrogen-Kjeldahl, or thophosphate-dissolved, and total phosphorus. Samples for nutrient analyses were collected in one liter Nalgene bottles and stored on ice until arrival at the laboratory. They were then preserved with 1 ml mercuric chloride (40 g/l). Samples to be analyzed for nitrites, nitrates, ammonia, and orthophosphate were filtered through a 0.45 millipore filter. Analyses followed procedures outlined in the Environmental Protection Agency's Methods for Chemical Analysis of Water and Wastes (1971).

Statistical analyses

Data presented in the tables are in terms of means and standard error of individual treatment groups. Statistical analyses were performed using factorial analysis of variance (Sokal and Rholf, 1969).

RESULTS

Weights of the fish

The weights of the N. crysoleacus collected from Grindle Creek were significantly different from those collected from Chicod Creek ($P<0.01$). Generally, the fish collected from Chicod Creek weighed more than the fish from Grindle Creek with the exception of the 4 April collection where the difference appears minimal.

The fish in Chicod Creek showed a decline in mean weight from the 13 March collection to a minimum value on the 4 April collection rising to a near maximum value on the 23 April. The fish from Grindle Creek showed a rise from the minimum value on 13 March to a maximum value on the 4 April followed by a slight decrease on 23 April. The main trends of the fish weights are shown in figure 2.

Weight of the liver in relation to fish weight

The liver weights from Chicod Creek showed a steady decline from a maximum on 13 March to the minimum on the 23 April. The liver weights from Grindle Creek showed a rise from the 13 March collection to a maximum on the 4 April and a decline to a minimum on the 23 April (Table 1, Fig. 3). There was a significant interaction between the collection dates and the liver weights ($P<0.05$).

Glycogen levels

The glycogen levels in the fish collected from Grindle Creek were significantly different from those collected from Chicod Creek ($P<0.01$). The glycogen levels were higher in the fish from Chicod Creek, with the

Figure 2. Body weight of fish collected from Chicod Creek and Grindle Creek.

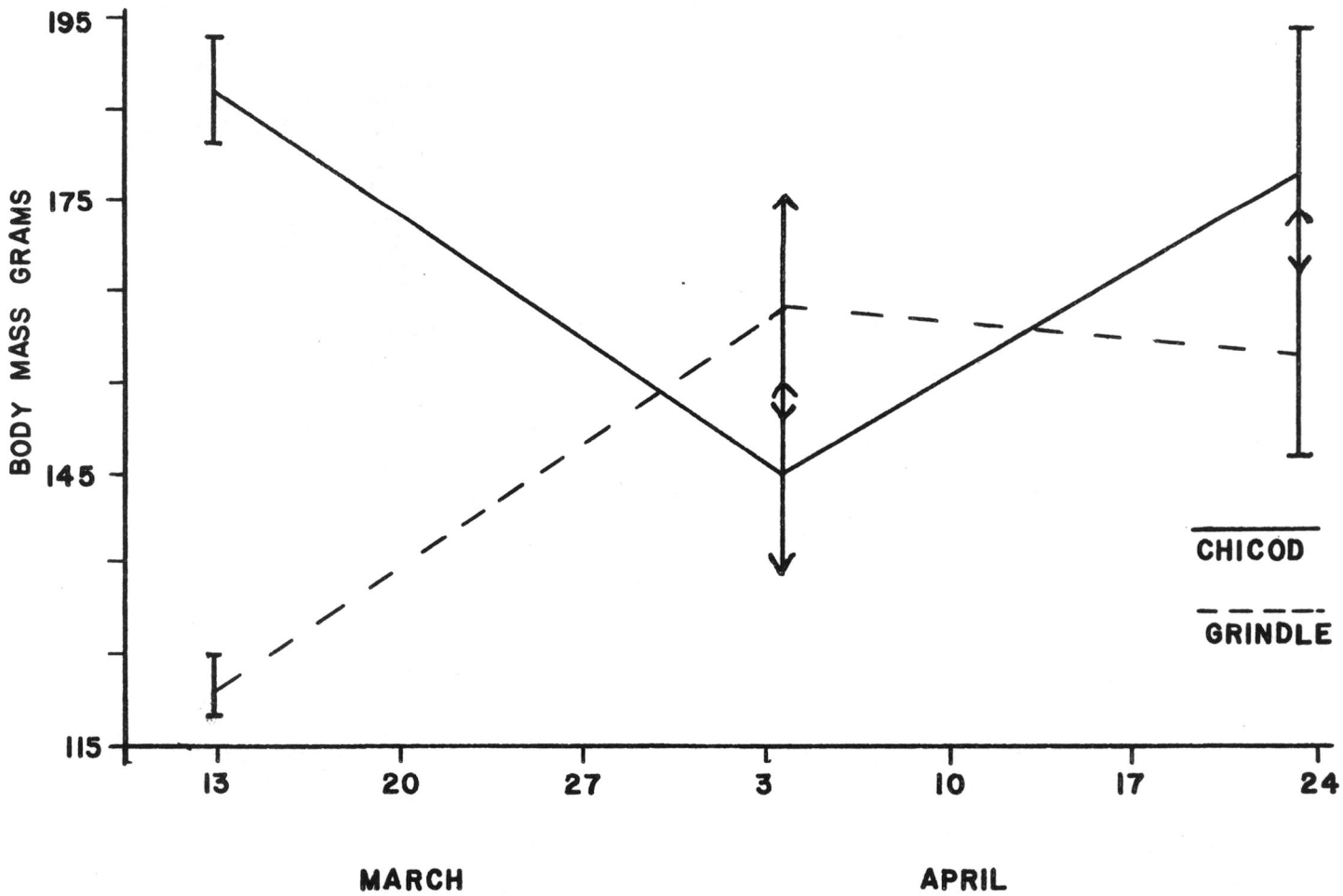
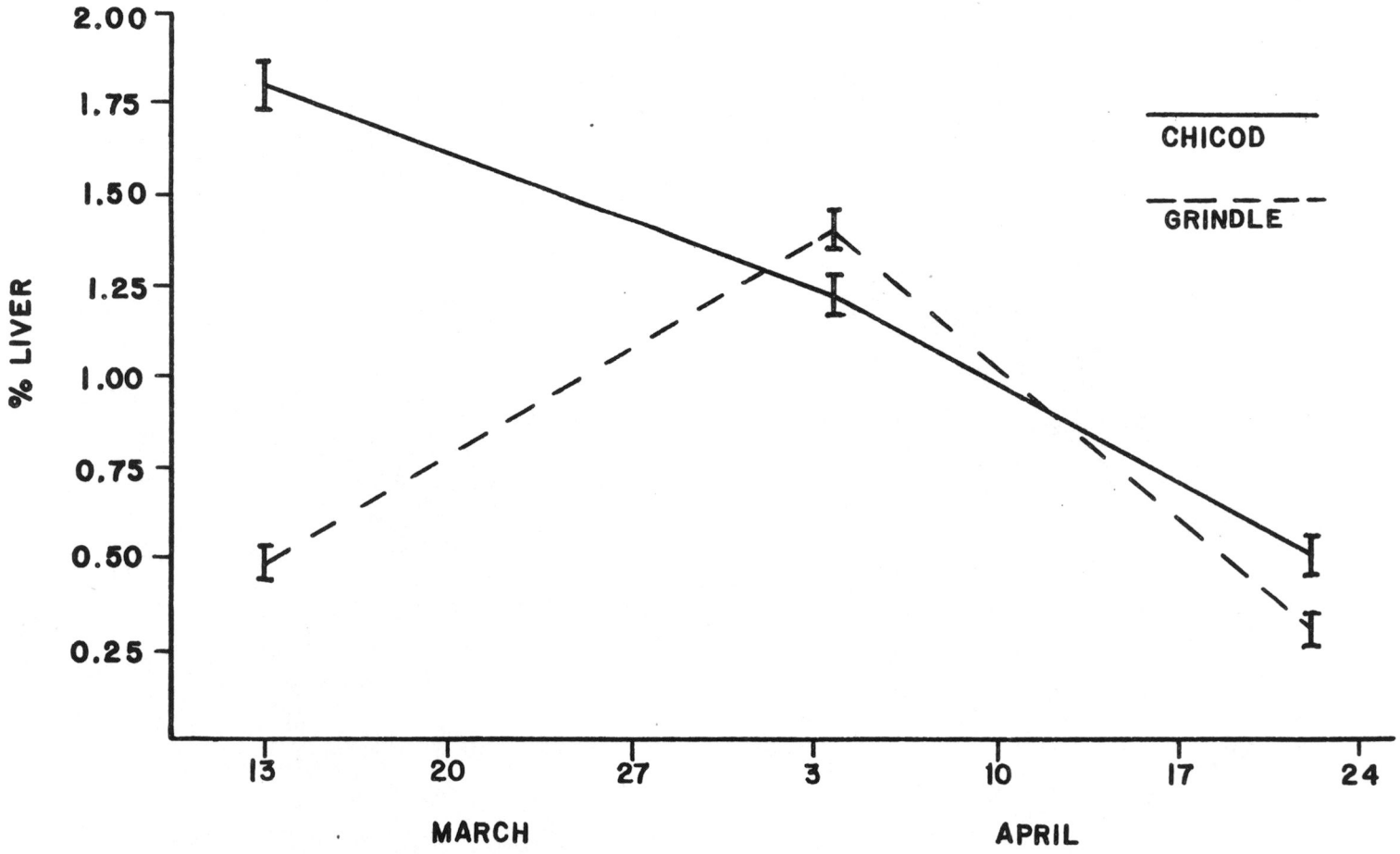


Table 1. Variation in the percent liver and percent glycogen in fish taken from Chicod Creek and Grindle Creek. (N=5)

Date	Collection site	%Liver Mean	%Liver S.E.	%Glycogen Mean	%Glycogen S.E.
13 March	Chicod Creek	1.770	0.0938	2.670	0.0135
	Grindle Creek	0.047	0.0013	0.196	0.0121
04 April	Chicod Creek	1.230	0.0898	0.236	0.0169
	Grindle Creek	1.350	0.0512	0.646	0.1940
23 April	Chicod Creek	0.472	0.0763	0.622	0.4710
	Grindle Creek	0.286	0.0317	0.184	0.0273

Figure 3. Variation in the percent liver in golden shiner during spring acclimatization. Based on percentage of body weight.



exception of the samples collected on 4 April (Fig. 4).

Glucose-6-phosphatase activity

Glucose-6-phosphatase (G6Pase) activity in the liver (Figs. 5,6, Table 2) as measured at the assay temperatures of 10°C and 25°C is heavily dependent on the collection dates and collection sites ($P<0.005$).

The 10°C assay from Grindle Creek shows a maximum value on the 13 March dropping to a minimum on the 4 April and rising again on the 23 April collection. The 10°C assay from Chicod Creek shows little between the 13 March and 4 April collections with a rise similar to the Grindle Creek data on the 23 April.

The 25°C assays from Chicod Creek and Grindle Creek show little change between the 13 March and 4 April collections with an increase to a maximum for each creek on the 23 April collection.

All of the 25°C assay activities were higher than the 10°C assays excluding the 13 March data for Grindle Creek.

Glycogen phosphorylase activity

The variation in the glycogen phosphorylase activity (Figs. 7,8, Table 2) resemble that of the G6Pase only in that the 10°C and 25°C assay temperatures are dependent on the collection dates ($P<0.05$). There did not appear to be any significant differences between the creeks on any of the collection dates. There were significant differences between the assay temperatures ($P<0.05$) on each of the collection dates.

The 10°C assays from Chicod Creek and Grindle Creek show little change between the 13 March and 4 April collection dates with a rise to a maximum on the 23 April collection.

Figure 4. Variation in the glycogen content of the liver in golden shiner. Glycogen based on percent of liver mass.

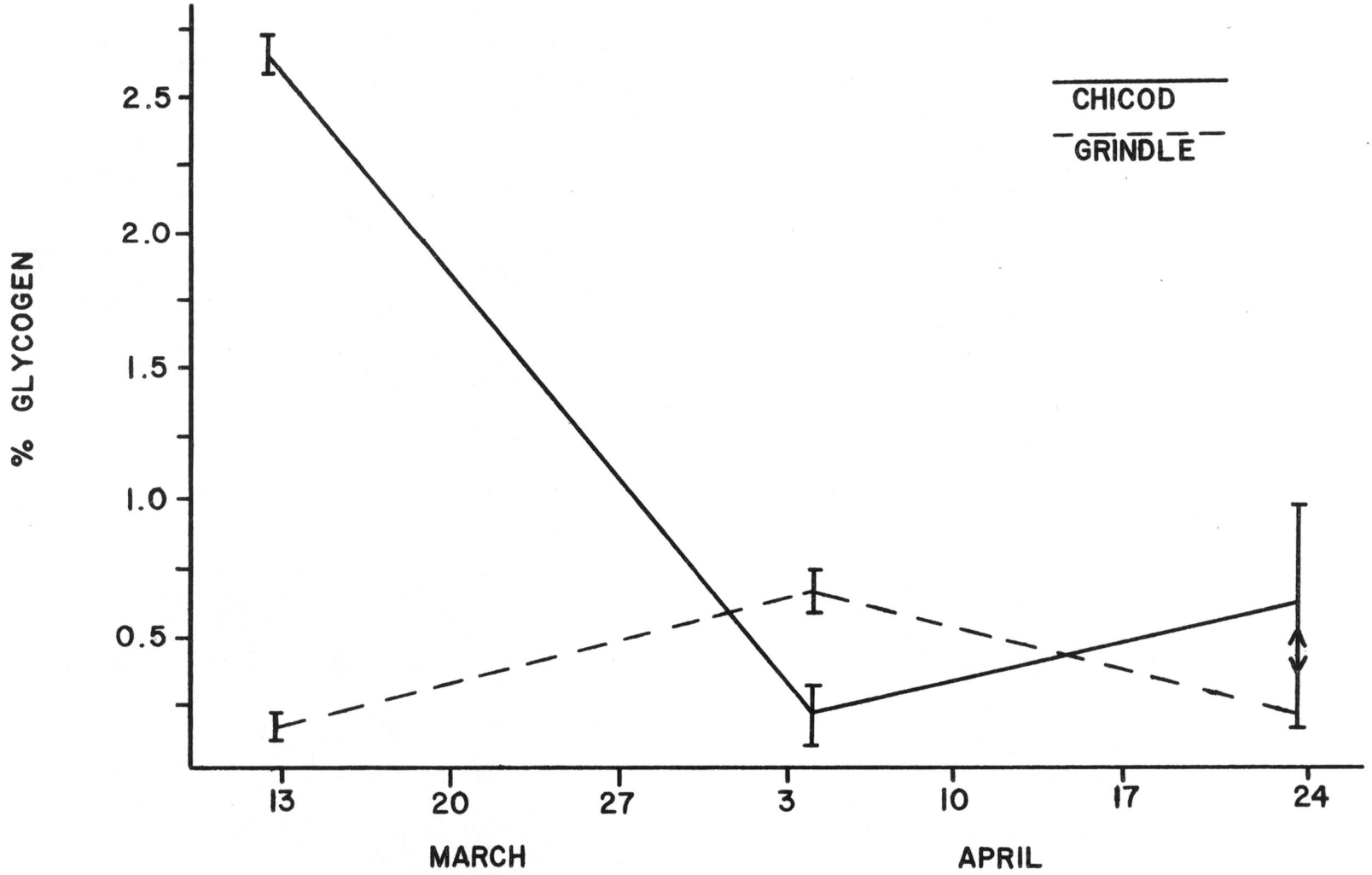


Figure 5. Variation in the 10°C assay of glucose-6-phosphatase activity in the liver of golden shiner. (micrograms PO_4/gram liver/min.)

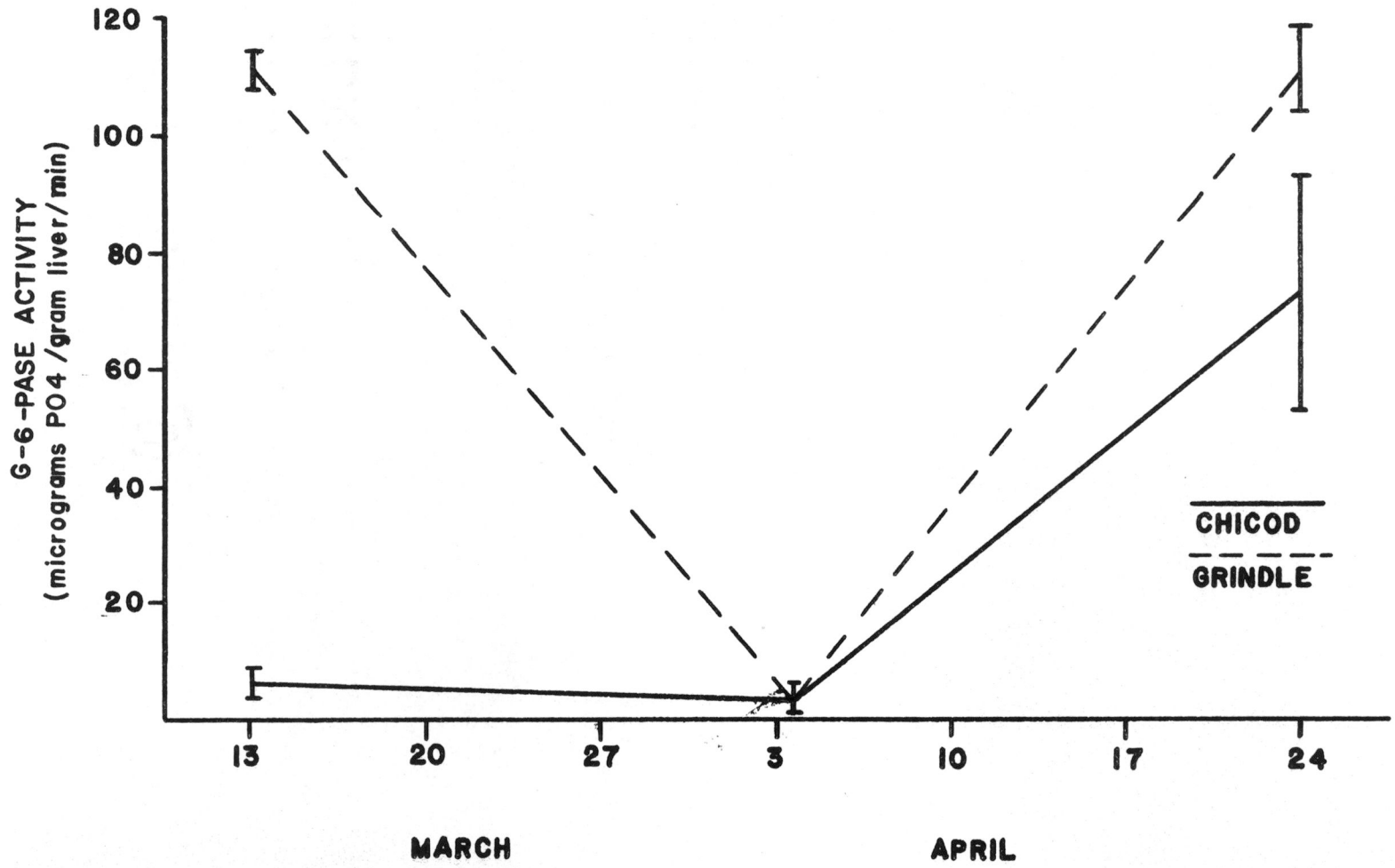


Figure 6. Variation in the 25°C assay of glucose-6-phosphatase activity in the liver of golden shiner. (micrograms PO_4/gram liver/min.)

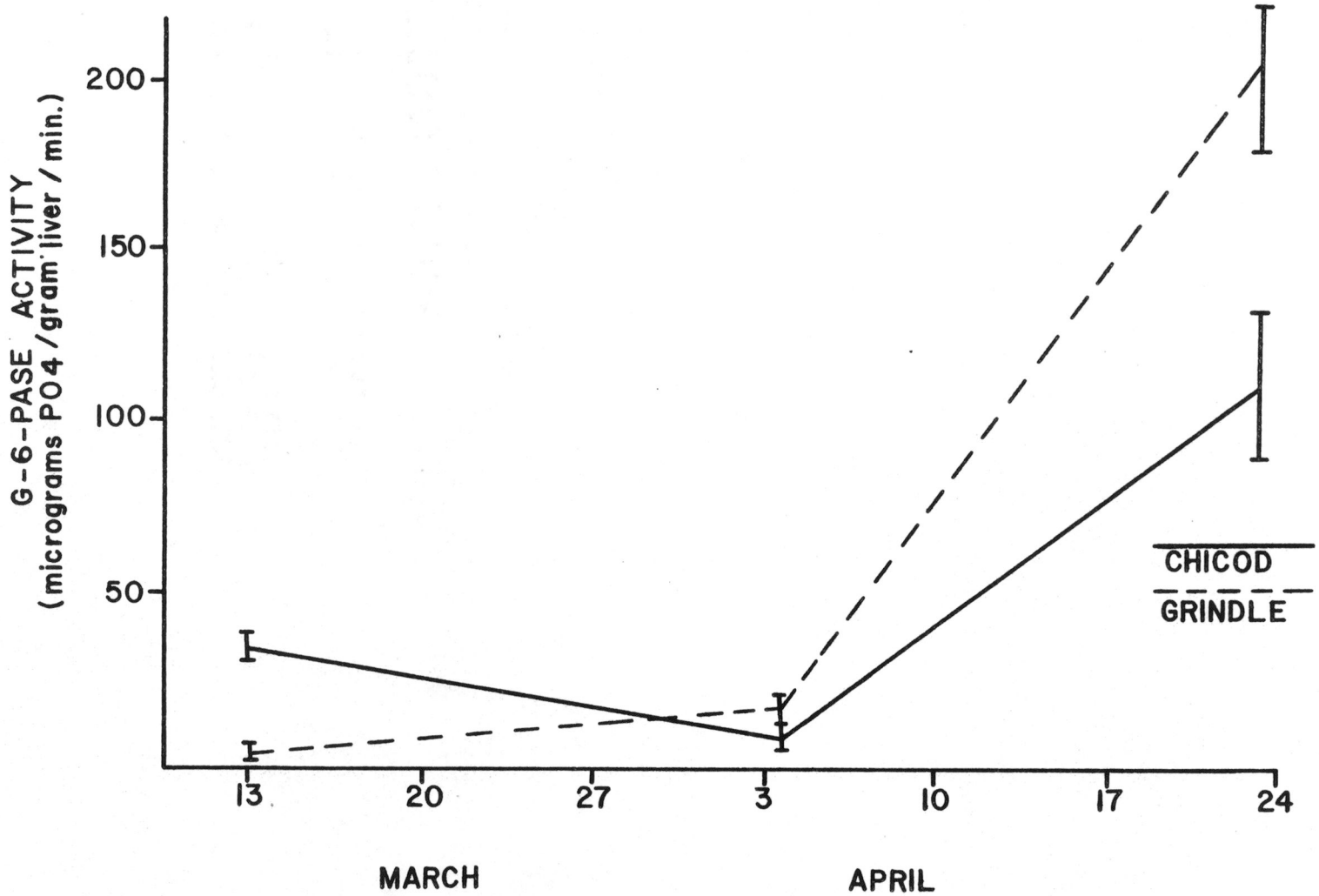


Table 2. Variation in glucose-6-phosphatase and glycogen phosphorylase activity in the liver of the golden shiner. (N=5)

Date	Collection Site	Glucose-6-phosphatase 10°C Mean	SE	25°C Mean	SE	Glycogen Phosphorylase 10°C Mean	SE	25°C Mean	SE
13 March	Chicod Creek	5.82	0.09	36.29	3.24	43.75	10.95	28.28	4.09
	Grindle Creek	112.63	0.90	5.31	0.21	46.72	3.49	83.13	5.10
04 April	Chicod Creek	0.26	0.03	8.70	0.03	53.84	7.15	35.90	2.15
	Grindle Creek	0.23	0.08	9.36	2.75	31.49	4.93	2.80	1.41
23 April	Chicod Creek	74.26	20.70	109.43	17.19	195.92	66.00	40.87	19.22
	Grindle Creek	111.31	7.14	207.35	28.21	266.29	77.70	134.83	28.30

Figure 7. Variation in the glycogen phosphorylase activity (with and without c-AMP) of the liver in fish taken from Chicod Creek.

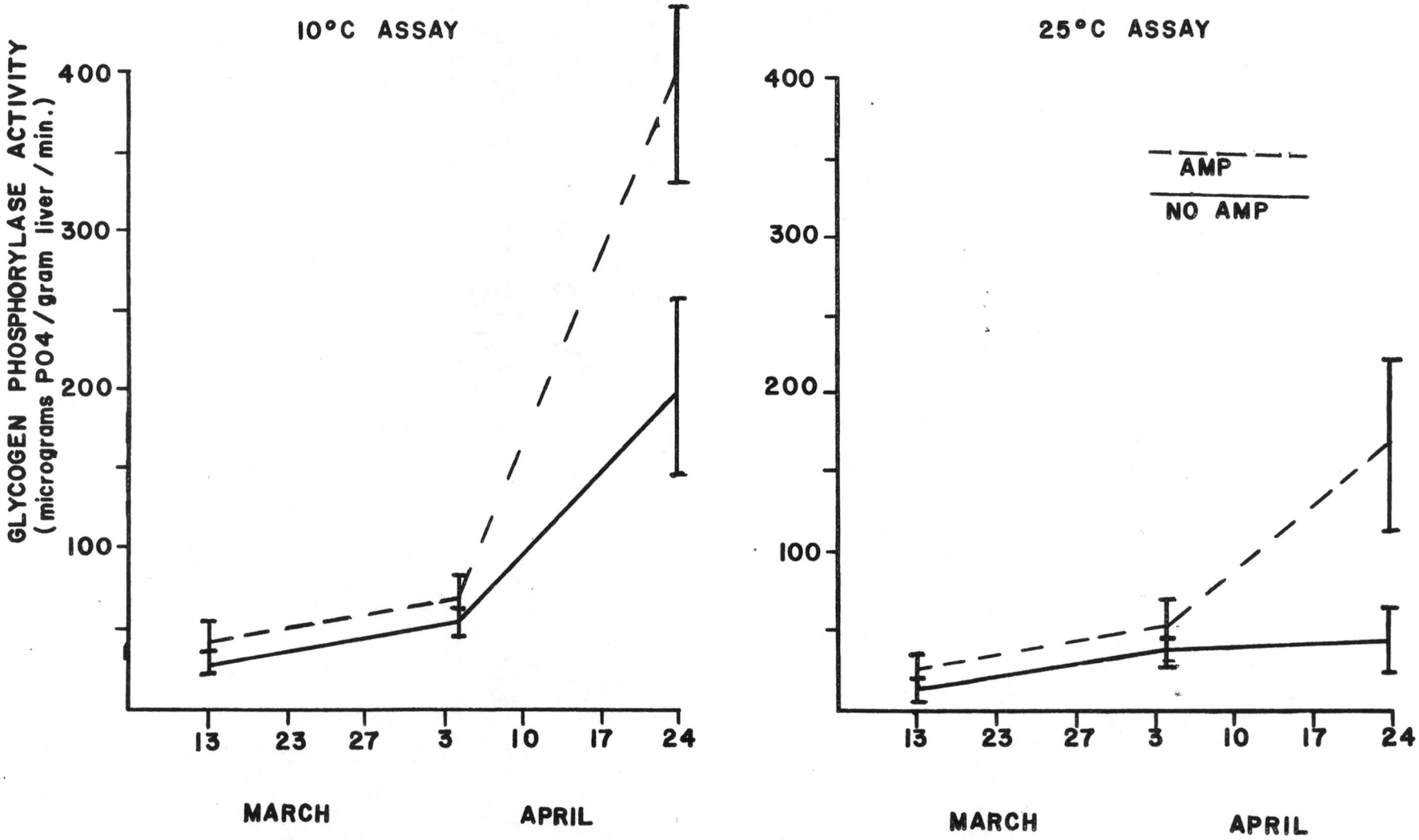
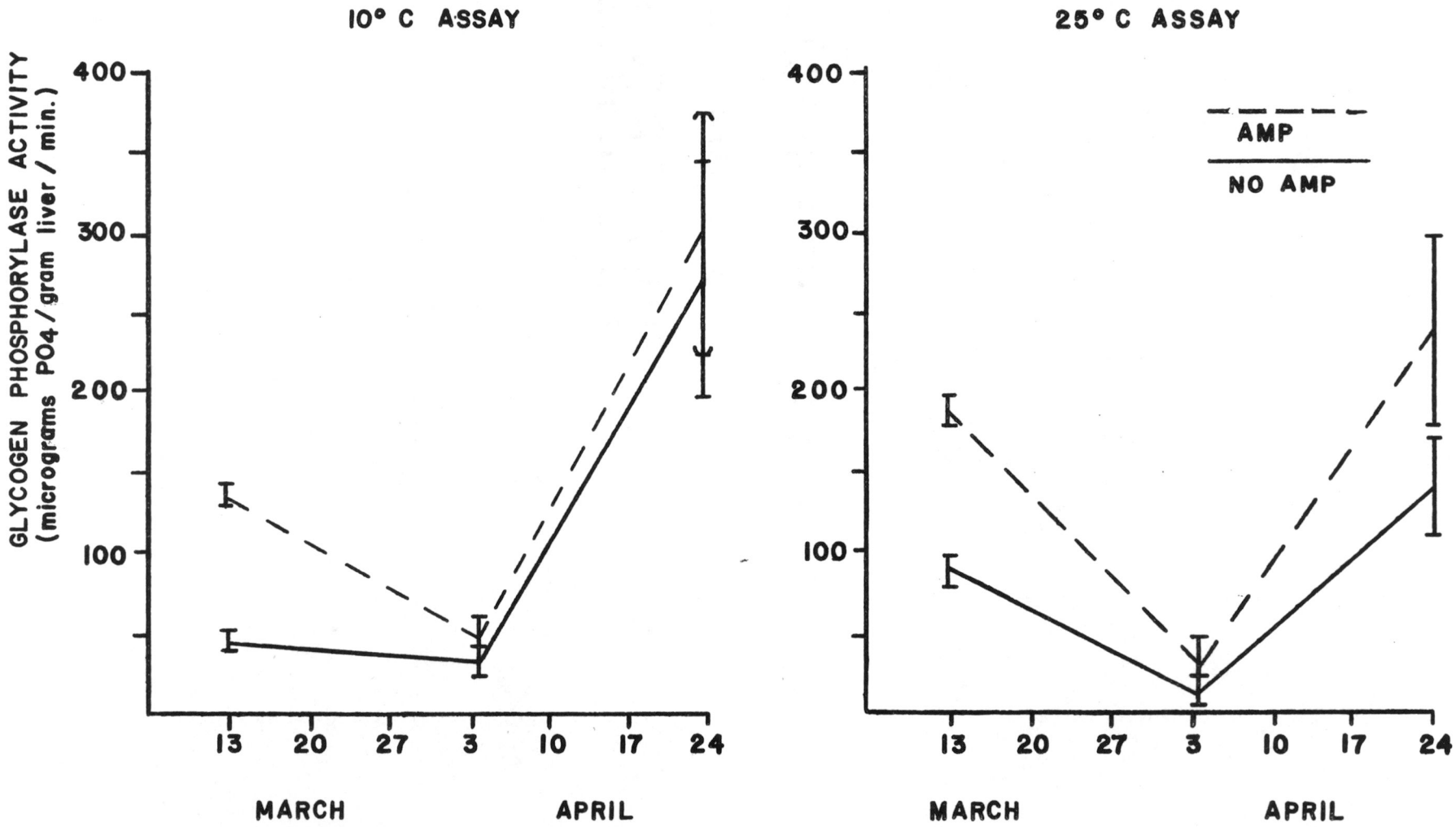


Figure 8. Variation in the glycogen phosphorylase activity (with and without c-AMP) of the liver in fish taken from Grindle Creek.



All of the 10°C assays from both creeks were higher than those of the 25°C assays with the exception of the 25°C assay from Grindle Creek on the 13 March.

DISCUSSION

Creek differences

The weights of the fish taken from Grindle Creek indicate that these fish are smaller than those taken from Chicod Creek. This consistent with reports by Bayless and Smith (1964) who reported a 90% reduction in weights of fish taken from channelized streams.

The greatest single factor affecting a fish population appears to be the amount of stream cover (Tarplee et al., 1971). The stream canopy is an important source of organic input into aquatic systems (Hall, 1972). Loss of in-stream shelter and feeding areas may contribute to changes in size characteristics and weights of fish within a population. The loss of in-stream shelter and feeding areas implies a poor nutritional status for fish taken from channelized streams. The low levels of glycogen observed in fish taken from Grindle Creek are similar to glycogen levels in catfish, Ictaluras punctatus, after fasting for thirty days. The glycogen levels of fish taken from Chicod Creek compare favorably to the glycogen levels in catfish which have been feeding (Ottolenghi et al., 1981). It is known that the glycogen content of the liver is influenced by nutritional status (Black et al., 1961; Hochachka, 1961). The low levels of glycogen, observed in Grindle Creek, are consistent with these observations.

Seasonal acclimatization

It has been known for some time that the glycogen content of the liver in fish is maintained at a higher level during acclimatization to cold waters in winter than warm waters in summer (Plisetskaya and

Kuzmina, 1972). DeVlaming and Pardo (1975) reported increased glycogen deposition in liver slices, taken from the golden shiner, maintained at 12°C. High glycogen levels occur in the livers of fish found in Chicod Creek during March. Spawning occured during the April collections because females ripe with eggs were collected in both creeks. The rapid decline in glycogen levels in the fish taken from Chicod Creek agree with the reports by Bullock (1954) and Black et al. (1961) concerning changes in the liver glycogen during spawning. The glycogen decrease is closely connected with the production of eggs and sperm (Bullock, 1954). The fish collected from Grindle Creek did not show the dramatic decline in glycogen levels. In fact, there was a slight increase in glycogen levels during the month of April. This may reflect an increase in food supply in the creek (Mayerles and Butler, 1971), although there is no evidence to support this. It is possible that the fish collected during the spawning period may have moved into Grindle Creek from the Tar River. The phenomena in question are closely connected with acclimatization, though Hochachka and Somero (1971) do not accept high glycogen synthesis as an obligatory feature in acclimatization to cold.

When the enzyme activities studied are considered against the background described above, the changes are most interesting. Glucose-6-phosphatase activity declined during spawning in early April and increased slightly late in April. This enzyme, which liberates glucose into the blood both by gluconeogenesis and glycogenolysis resembles the corresponding enzyme in mammalian liver and is controlled through metabolites (Hers, 1976), glucose (Ikeda and Shimeno, 1967), and hormonally (DeVlaming and Pardo, 1975). The low activity, while there was a

plentiful supply of food in the stomach during April may be understood by reference to the control of insulin on absorbed glucose in the blood. Insulin regulates the carbohydrate metabolism of the teleost by hormonal mechanisms similar to those operating in mammals (Carneiro and Amaral, 1983). Golden shiners adapted to 25°C show a net decline in liver glycogen even though insulin promoted glycogen deposition at the same temperature (DeVlaming and Pardo, 1975). This observation may explain the slight increase in glycogen phosphorylase activity during the same period.

On 13 March, 73% of the available glycogen phosphorylase was active in the fish taken from Chicod Creek. This increased to 90% on 4 April and decreased to 50% by 23 April. This increased activity is responsible for the rapidly declining glycogen content in the livers of these fish (Fig. 4). Only 35% of the available glycogen phosphorylase was active in fish taken from Grindle Creek on 13 March (Table 3). The low levels of glycogen found in these fish indicate that the energy reserve of these fish is nearly depleted. The decrease in the available glycogen phosphorylase in the livers of fish collected on 4 April may be responsible for the increased glycogen levels in the livers of these fish. As spawning activity increased, the total amount of glycogen phosphorylase in fish from Grindle Creek increased. 89% of this enzyme was active causing a decline in the glycogen content in the livers of the fish collected from this creek on 23 April.

Temperature induced activities

The data presented favors the view that temperature induced

Table 3. Variation in the mean percentage of active glycogen phosphorylase in the liver of fish taken from Chicod Creek and Grindle Creek.

Date	Collection Site	10^{o}C Assay	25^{o}C Assay
13 March	Chicod Creek	0.74	0.52
	Grindle Creek	0.35	0.44
04 April	Chicod Creek	0.90	0.69
	Grindle Creek	0.82	0.11
23 April	Chicod Creek	0.50	0.28
	Grindle Creek	0.89	0.58

compensation of enzyme activity takes place in the liver of the golden shiner, Notemigonus crysoleucas. The ratios of enzyme activities incubated at 10°C: incubated at 25°C seems to be connected with regulation of isozyme systems (Hochachka and Somero, 1971). Hochachka and Somero suggested three mechanisms which might be responsible for regulating the rates of enzyme activity after period of acclimation. Organisms acclimate to temperatures by:

(1) increasing the concentrations of certain key enzymes

(2) generating new isozymes ideally suited to function in the new thermal environment, or

(3) altering the microenvironment within which the enzyme functions, thus producing compensated rates of activity by modulation of enzyme efficiency rather than quantity.

The most striking evidence for this temperature induced activity is the data from 13 March. The temperature difference between the creeks was greatest on that day (Fig. 9). The high ratios of the 10°C assays: 25°C assays of glucose-6-phosphatase activity in the livers of fish from Grindle Creek contrast sharply with the low ratios observed from Chicod Creek (Table 3). The low ratios observed in April, when the water was warm in both creeks, adds support to this observation. Cooper and Ferguson (1972) reported the same kind of activity for glucose-6-phosphatase in the nematode, Panagrellus redivivus.

The low ratios of glycogen phosphorylase activities incubated at 10°C: incubated at 25°C in March followed by high ratios during April demonstrate temperature modulated activity (Table 4). In this case, there is an inverse temperature affect. This observation is supported

Figure 9. Water temperatures of Grindle Creek and Chicod Creek.

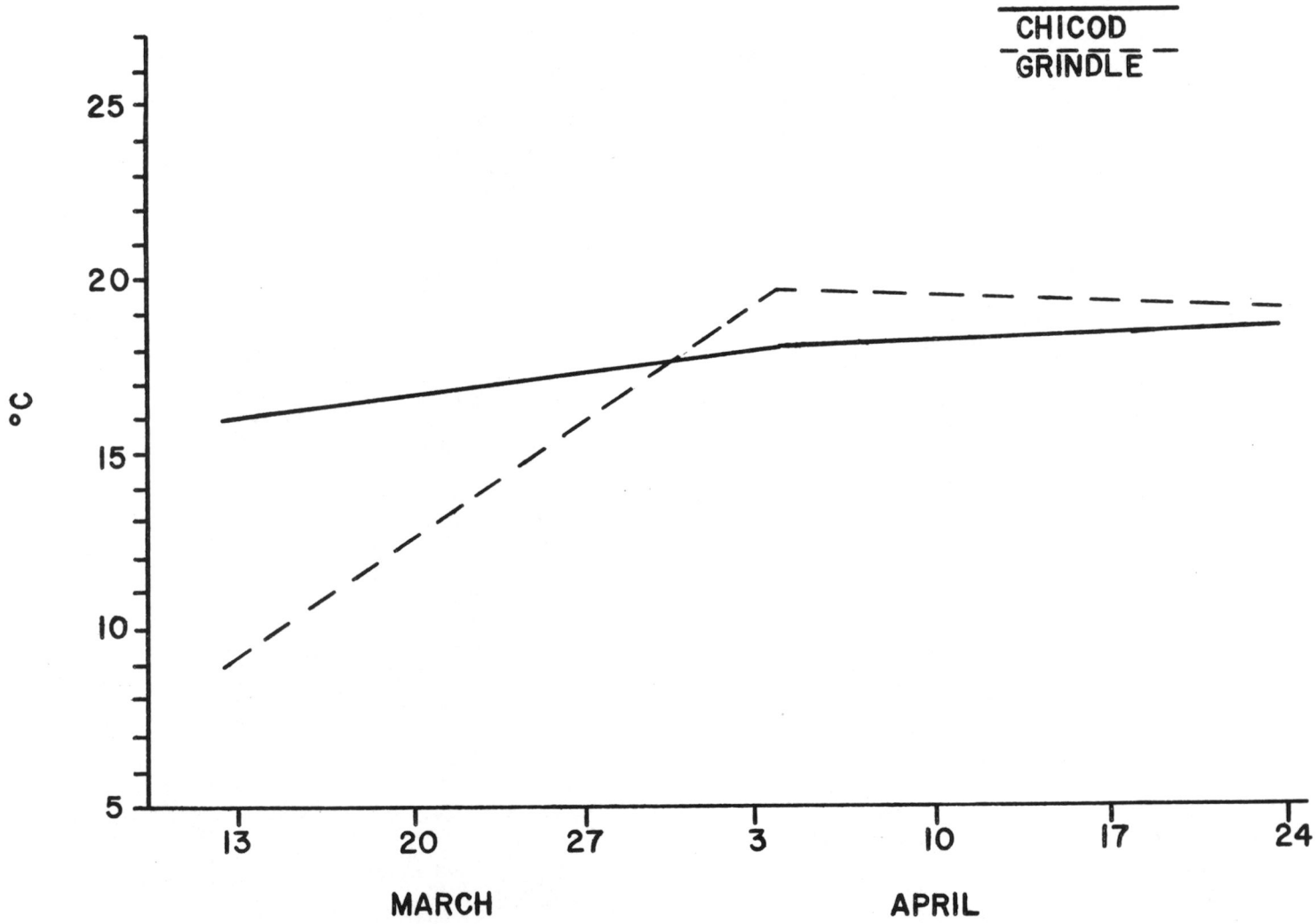


Table 4. Comparison of water temperatures and ratios of $10^{o}C/25^{o}C$ assays of enzyme preparations taken from golden shiners in Chicod Creek and Grindle Creek.

Date	Collection site	Water Temp.(^{o}C)	Glucose-6-phosphatase $10^{o}C/25^{o}C$ assay	Glycogen Phosphorylase $10^{o}C/25^{o}C$ assay
13 March	Chicod Creek	16.0	0.16	1.55
	Grindle Creek	9.0	21.21	0.56
04 April	Chicod Creek	18.0	0.03	1.50
	Grindle Creek	19.5	0.02	11.25
23 April	Chicod Creek	18.5	0.68	4.79
	Grindle Creek	19.0	0.54	1.98

by reports from Hazel and Prosser (1974) and Hochachka and Somero (1971).

RECOMMENDATIONS

The small sample sizes and limited time of this study suggest that it be used as a preliminary report for the further study of the ecology and biochemistry of the teleost, Notemigonus crysoleucas.

Several questions have brought to mind as a result of this study. First, the question of seasonal carbohydrate metabolism. This report cataloged information dealing with only a small phase in the life cycle of the golden shiner, that of spawning and acclimatization from winter to spring. A year long study would give a much clearer picture of the ecological aspects of the life cycle of this fish population.

Secondly, this report indicates the presence of temperature inducible systems for both glucose-6-phosphatase and glycogen phosphorylase. Studies need to be carried out to determine the characteristics of each of the isozyme systems.

It would be advantageous to study the other metabolic pathways leading from the glucose-6-phosphate branch point. This would give a more complete picture of carbohydrate metabolism.

Additional studies concerning the control mechanisms for glycogen deposition and degradation should be carried out. These would shed more light on the importance of glycogen as a source of energy to the golden shiner.

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APPENDIX

Appendix Table 1. Values of dissolved oxygen for Chicod Creek and Grindle Creek on sampling days. (MG/L)

Date	Chicod Creek	Grindle creek
13 March	9.25	10.18
04 April	8.32	10.65
23 April	9.50	10.73

Appendix Table 2. Values of conductivity for Chicod Creek and Grindle Creek on sampling days. ($\mu MHOS/CM^2$)

Date	Chicod Creek	Grindle Creek
13 March	45.16	62.53
04 April	87.50	92.52
23 April	112.43	145.34

Appendix Table 3. Values of pH for Chicod creek and Grindle creek on sampling days.

Date	Chicod Creek	Grindle Creek
13 March	5.0	5.7
04 April	6.5	5.3
23 April	5.6	5.9

Appendix Table 4. Values of dissolved nitrite for Chicod creek and Grindle Creek on sampling days. (MG/L)

Date	Chicod Creek	Grindle Creek
13 March	0.010	0.009
04 April	0.014	0.011
23 April	0.009	0.013

Appendix Table 5. Values of dissolved nitrate for Chicod creek and Grindle creek on sampling days. (MG/L)

Date	Chicod Creek	Grindle Creek
13 March	0.408	0.467
04 April	0.300	2.100
23 April	0.500	2.400

Appendix Table 6. Values of dissolved ammonia for Chicod Creek and Grindle Creek on sampling days.(MG/L)

Date	Chicod Creek	Grindle Creek
13 March	0.017	0.058
04 April	0.233	0.033
23 April	0.795	0.495

Appendix Table 7. Values of Kjeldahl nitrogen for Chicod Creek and Grindle creek on sampling days. (MG/L)

Date	Chicod Creek	Grindle Creek
13 March	1.44	1.46
04 April	2.80	2.00
23 April	4.08	4.71

Appendix Table 8. Values of ortho-phosphate for Chicod Creek and Grindle Creek on sampling days. (MG/L)

Date	Chicod Creek	Grindle Creek
13 March	0.006	0.006
04 April	0.048	0.025
23 April	0.146	0.250

Appendix Table 9. Values of total phosphorous for Chicod Creek and Grindle creek on sampling days. (MG/L)

Date	Chicod Creek	Grindle Creek
13 March	0.138	0.154
04 April	0.183	0.115
23 April	0.215	0.319