

SOLUBLE CARBOHYDRATE
AS AN ENERGY SOURCE FOR
FISH - FORAGE ORGANISMS

by

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ABSTRACT

From November 1965 to April 1967, three experiments in twelve 0.006 acre tanks were performed to evaluate the effects crude beet molasses and crystalline corn dextrose additions might have on the presumed food chain of experimental fish populations. Measurements were made of turbidity, bacterial abundance and metabolism, community photosynthesis, plankton and Aufwuchs chlorophyll concentrations, zooplankton standing crop, sessile and swimming insect populations, and growth of game fish populations dependent on invertebrate production. Carbohydrate additions did not augment the food chain of fish enough to significantly increase growth rates of rainbow trout, Salmo gairdneri (Richardson), the Malacca Tilapia hybrid, and largemouth bass, Micropterus salmoides (Lacepede). Significantly greater phytoplankton biomass, corresponding to carbohydrate additions in one experiment, may indicate utilization of carbohydrate as a source of energy by algae. Increased CO₂ concentrations incident to bacterial metabolism may also have been responsible. Evidence of increases in bacterial numbers following carbohydrate additions were found from plate counts, increase in maximum hydrogen ion concentration, reduction of minimum oxygen concentration,

and possibly from significantly greater turbidity found in tanks receiving the heavier concentration of carbohydrates. Growth of experimental fish populations was probably limited by overpredation on forage invertebrates and possibly by reduction in dissolved oxygen concentrations. Additions of carbohydrates on a schedule corresponding to the planktonic bacterial pulses may have some importance in the management of lakes toward greater production of fish food organisms. Significant increases in gross photosynthesis could possibly be produced at a cost of $11\frac{1}{2}$ cents per acre per day. This cost is not economical in lakes where biogenic salt fertilizations are effective.

INTRODUCTION

Sport fishing demands have stressed the need to increase fish production in lakes and streams. In organically poor waters resident reproduction or fingerling plants often cannot support the bulk of the fishing pressure. These conditions often necessitate stocking with catchable sized fish. In a fishery where increased stocking is economically unfeasible, other management techniques must be used if sport fishing demands are to be satisfied.

Direct feeding of fish as practiced in hatcheries and agricultural fisheries is, of course, impractical in lakes and streams. One of the procedures used to increase fish production through the photosynthetic food chain in small impoundments has been the application of biogenic salts, chiefly phosphate and nitrate (Swingle, 1947; Nelson and Edmondson, 1955; and Hickling, 1962). This is not highly effective in soft-water lakes of which Arizona has several.

Alternative possibilities lie in the application of organic material (Smith and Swingle, 1943; Swingle, 1947 and Warren, et al., 1964) to augment the microbial food web of fish-food organisms. Dissolved organic matter has practical advantages over particulate material. It is

assimilated more rapidly, easier to distribute, and will remain in the epilimnion of the lake. Other criteria to be considered are availability in large quantities, low cost, and high energy content. Inexpensive commercial sugars meet these criteria.

The effect of increases in organic materials on aquatic and marine environments are discussed by numerous authors (Gaufin and Tarzwell, 1952; Katz and Howard, 1955; Ellis and Gowing, 1957; Stephens and Schinske, 1961; Warren, 1961; and Warren, et al., 1964). To the point of pollution, organic nutrient additions to water might increase fish production by augmenting the rate of bacterial production (Gorbunov, 1946 and Warren, et al., 1964). A definite relation was shown to exist between the transformation of glucose in water and bacterial multiplication (Waksman and Carey, 1935a). It is manifest from the abundance of bacteria in lake waters (Henrici, 1939) that many bacteria multiply in very dilute nutrient solutions (Zobell and Grant, 1942). Jorgensen (1966) discusses the importance of bacteria as food for suspension feeders. Direct ingestion of carbohydrates has also been shown to occur in a number of fish-food organisms (Conover, 1966 and Jorgensen, 1966).

This investigation is an attempt to examine the effects of soluble carbohydrates (sugars) on fish production and trophic pathways leading to production of

fish. Although weight of fish harvested is a convenient and valuable index of productivity, the end product is not the only criterion. The effect on the fish-food web must also be understood in estimating the value of a new energy source.

Information also is needed concerning the level and cost of carbohydrate additions that might significantly increase fish production in a lake.

METHODS AND MATERIALS

General Conditions

Experiments were conducted on the 3/4 acre off-campus research complex of the Arizona Cooperative Fishery Unit.

Twelve 5½ meter diameter circular tanks (vinyl-lined swimming pools) were filled to a depth of one meter (23.65 M³).

Experimental fish used were fingerling rainbow trout, Salmo gairdneri (Richardson); the Malacca Tilapia hybrid (Hickling, 1960); and largemouth bass, Micropterus salmoides (Lacepede). Rainbow trout were obtained from the State of Arizona Fish Hatchery at Page Springs, Arizona, and maintained in small stock tanks for approximately four weeks before introduction. The Tilapia were from stocks of the Arizona Cooperative Fishery Unit. The largemouth bass were seined from Parker Canyon Lake, Arizona, and maintained for three weeks before introduction.

Carbohydrates used were crude beet molasses and crystalline dextrose. Analyses of these sugars (Table 1) were provided by the manufacturers. Soluble carbohydrates were evenly distributed in the experimental tanks.

Table 1. Typical and chemical analyses of crystalline pure dextrose and beet feed molasses used as water soluble carbohydrates.

	Clintose [®] A	Beet Molasses
Typical Analysis		
Color	White	Brown
Form	Dry crystalline	Viscous liquid
Moisture	8.5%	16.5%
Total solids	91.5%	83.5%
Chemical Analysis (Percent)		
Organic Constituents		
Dextrose	99.7	1
Sucrose		51
Raffinose		1
Glutamic acid	None	3.5
Other sugars	0.3	
Other protein	None	5.5
Other organic	None	10.0
Inorganic Constituents		
Silica	-	0.1
Potassium	-	3.9
Sodium	0.001	1.3
Iron oxide	less than 0.001	0.02
Aluminum oxide	-	0.07

Table 1 Continued.

Calcium	less than	0.001	0.26
Magnesium	less than	0.001	0.16
Sulfate		0.015	0.55
Phosphate	less than	0.001	0.06
Chloride		0.005	1.6
Carbonate		-	3.5
Sulfur dioxide	less than	0.001	-
Ash (sulfated)		0.01	-
	Total	0.03	11.5

® Trade name of alpha-d glucose monohydrate sold by the Clinton Corn Processing Company, Clinton, Iowa.

The investigation was conducted as three experiments corresponding to periods when water temperatures remained within the range tolerated by either trout or Tilapia (Table 2). Additional experimental conditions are listed in Table 3.

The tanks were drained between each experiment, cleaned, and rinsed twice before refilling. Evaporation losses were replaced periodically.

Individual Experimental Conditions

Experiment I

Fifteen trout per tank, ranging from 2.90 to 3.41 grams (mean of 3.03 grams) were supported by the environment for 71 days.

Organisms (phytoplankton, zooplankton, and aquatic insect larvae) introduced from local ponds were given 85 days to establish the necessary food web prior to trout introduction.

Sessile chironomid larvae populations were measured on ceramic dishes (four per tank) for 107 days. Two dishes were suspended adjacent to the side; and two rested on the bottom attached to floats. Plates were periodically removed for visual counts.

Molasses measured volumetrically was mixed vigorously in four liters of water before introduction.

Table 2. Water temperature data for the three experiments.

Experiment	Time Period	Number of Days	Temperature Range (°C)	Mean Temperature (°C)	Maximum Diurnal Range (°C)
I	November 1965-April 1966	156	8.5-20.9	9.8	6.0
II	July 1966-November 1966	104	19.3-28.4	24.0	1.9
III	November 1966-April 1967	144	0 -18.2	10.4	9.2

Table 3. Range of hydrogen ion concentration, total dissolved solids (salts), and dissolved oxygen present in the three experiments.

Characteristic	Experiment		
	I	II	III
Hydrogen Ion Concentration (pH)	7.7 - 8.9	8.1 - 8.4	8.5 - 8.8
Total dissolved solids (ppm)	105 - 179	552 - 690	600 - 645
Dissolved oxygen (mg/l)	5.29- 10.48	4.77- 13.40	11.12- 19.52

The molasses was added three times at concentrations of 2 ppm, 7 ppm, and 15 ppm. Three replicates of each of the above concentrations and three controls were maintained. Application dates were February 18, March 9 and 30.

Experiment II

Largemouth bass (ten per tank) and Tilapia (eight per tank) were introduced nine days after the tanks were filled and inoculated with pond invertebrates. Two nylon mesh ($\frac{1}{4}$ inch) net enclosures ($1.5 M^3$) were installed in each tank; one served as a refuge for invertebrates and the development of Aufwuchs, the other confined the Tilapia. Largemouth bass foraged in the area outside the net enclosures.

Experimental fish were graded into groups, anesthetized in a 3 mg/l solution of MS-222, weighed, and the mean weight in grams calculated. No mortality resulted from sorting and weighing. Representative samples of the range of mean weights were used in each treatment. Largemouth bass and Tilapia were allowed to grow 96 days and 85 days, respectively. Largemouth bass received sub-maintenance feedings of earthworms averaging about 0.2 grams (live weight) per day for each tank.

Snails (Helisoma sp.), introduced as indicators of herbivore production, foraged in the area outside the net enclosures for 72 days.

Aufwuchs growth was measured on ceramic dishes (four per tank) for 50 days.

Black polyethylene sunshades installed one meter above the tanks were used to reduce temperatures and suppress evaporation.

Three applications of molasses at concentrations of 7 ppm and 15 ppm were made with four replicates of each concentration. Four controls were used. Application dates were August 13, September 6 and 28.

Experiment III

Twelve trout per tank were supported for 116 days. Trout were graded into groups, weighed, and the mean weight in grams calculated. Representative samples of the range of mean weights were used in each treatment. Trout were fed earthworms averaging about 0.9 grams (live weight) per day for each tank.

Forage organisms were introduced 27 days prior to fish introduction. Net enclosures (two per tank) were used as a refuge for forage organisms. Artificial aquatic plants used to increase refuge substrate for forage organisms were made of polyethylene strips. These were tied into clumps, weighted, and 36 placed in each net enclosure. Helisoma sp. (100 per tank) foraged in these areas for 140 days. Aufwuchs growth was measured on ceramic dishes (two per net enclosure) for 84 days.

Crystalline dextrose was completely dissolved in four liters of water before introduction. Six applications at concentrations of 15 ppm and 25 ppm were made with four replicates at each concentration. Four controls were used. Application dates were December 22, January 20, February 2 and 28, and March 18 and 28.

Physical and Chemical Conditions of the Environment

Chemical profiles of the water sources are given in Table 4. Introduction and replacement sources are listed in Table 5.

Water temperature, hydrogen ion concentration, and total dissolved solids (salts) measurements were determined periodically in the tanks. Air and surface temperatures were determined with a mercury thermometer. Hydrogen ion concentration was determined with a LaMotte Chemical Company color comparator which gave readings within 0.2 of a pH unit. An approximation of total dissolved salts was obtained using a portable conductivity meter manufactured by Industrial Instruments Inc. (Model RA-2A). The conductivity readings given as micromhos per centimeter were converted to ppm TDS using a chart provided by the manufacturer.

Turbidity measurements were determined from a 1000 ml sample using a Hellige turbidimeter with a B-964

Table 4. Summary of water quality data for the McGee Road Well and the Arizona Cooperative Fishery Unit Compound Well, Tucson, Arizona.

Characteristic	McGee Road Well	Compound Well
Total dissolved solids ppm	110	1309
pH	7.3	7.9
Alkalinity as CaCO ₃ ppm	68	256
Silica (SiO ₂) ppm	42.5	36.0
Iron (fe) ppm	less than 0.1	0.13
Sulfate (SO ₄) ppm	7.0	430.0
Total phosphate (PO ₄) ppm	0.21	0.30
Orthophosphate ppm	0.20	0.02
Nitrate (NO ₃) ppm	0.60	2.10
Tannic acid ppm	-0-	-0-
Calcium (ca) ppm	16.18	147.4
Cobalt ppm	less than 0.1	less than 0.1
Copper ppm	less than 0.1	less than 0.1
Magnesium ppm	3.64	20.6

Table 4 Continued.

Manganese ppm	less than	0.05		0.04
Potassium ppm		0.75		55.7
Sodium ppm		7.00		199.0
Zinc ppm	less than	0.05		0.05
Molybdenum ppm	less than	0.5	less than	0.01
Lithium ppm	less than	0.005		2.00
Silver ppm	less than	0.01	less than	0.03
Nickel ppm	less than	0.05	less than	0.1
Lead ppm	less than	0.04	less than	0.1
Chloride ppm		9.0		-
Arsenic ppm	less than	0.0008		-

Table 5. Water introduction and replacement sources as percent of total volume of the tanks.

Experiment	Introduction Source		Loss due to Evaporation	Replacement Source		
	McGee Rd.	Compound		McGee Rd.	Compound	Rain
I (156 days)	100		41.4	15.4		26.0
II (108 days)	56.5	43.5	25.7		12.8	12.9
III (144 days)	56.5	43.5	16.0		12.8	3.2

bulb and no filter. Both 20 mm and 50 mm viewing depths were employed. Turbidity was determined as ppm SiO_2 .

Other chemical variables of the water were determined according to the methods described in the Standard Methods for the Examination of Water, Sewage and Industrial Wastes (1965), as modified by the Hach Chemical Company. Alkalinity expressed as milligrams per liter CaCO_3 was determined by using 0.020N sulfuric acid. Colorimetric determinations were made with a Bausch and Lomb "Spectronic 20." Nitrate was determined by the phenoldisulfonic acid method; sulfate by the turbidimetric method; orthophosphate and total phosphate by the stannous chloride method; tannic acid by the tyrosine method; silica by the amino acid (solution) method; and ferrous iron by the phenanthroline method. The results of the chemical analysis are indicated in parts per million.

Atomic absorption spectrophotometry analyses for metals were conducted by the Efco Feed Company Laboratories, Tucson.

Criteria for Community Production Differences

Photosynthesis

Photosynthesis measurements were made from estimates of the diel changes in oxygen concentration

using a technique described by McConnell (1962, 1963). Random sampling in the tanks indicated vertical and areal homogeneity. Ten samples within the same tank showed a mean dissolved oxygen concentration of 6.44 with 95 percent confidence limits of 0.04 mg/l.

Water samples were collected in a 500 ml self-filling syringe described by Kemmerer (1965) and transferred to 130 ml reagent bottles. The syringe was designed to collect a distributed water sample which was free of any contact with atmospheric oxygen. Winkler reagents were added immediately from polyethylene automatic dispensing pipettors.

The Alsterberg (Azide) modification of the Winkler Method was used to determine the content of dissolved oxygen in the water samples. All samples were refrigerated at the end of the sampling period and analyzed within 24 hours. Titration was accomplished with phenylarsene oxide (PAO) delivered from a microburet. Replicates were not employed as the precision of the entire procedure using 23 samples from one tank showed a mean concentration of dissolved oxygen to be 6.62 ± 0.02 mg/l (95 percent confidence limits). The elimination of replicates was important in reducing the "instant" of sampling, which ranged from 15 to 20 minutes.

The oxygen content of the water was determined at sunset, and at sunrise and sunset the following day.

Exact sunset and sunrise times were taken from the U.S. Weather Bureau at Tucson. Sampling of the waters always commenced exactly 10 minutes before the given time. Photosynthetic production of oxygen was calculated from graphic determination of gross primary productivity using the technique described by McConnell (1962, 1963).

Chlorophyll

Water samples for chlorophyll analysis were collected in amber glass one-quart bottles which were moved while sampling to insure a distributed sample. If immediate extraction was not possible, samples were refrigerated in the laboratory and extracted within 24 hours. Extraction was accomplished by passing as many milliliters of sample as possible through a 0.80μ AA Millipore^{®1} filter. When possible a second filter was used. The residues were extracted in 20 milliliters of 90 percent alkalized acetone for 24 hours in the dark at 8-10°C. Solutions were retained in 50 ml centrifuge tubes with polypropylene stoppers. Maximum extractions of the solutions were insured by periodic shaking. Optical density of the solutions were determined with a Bausch and Lomb Spectronic 20 photoelectric colorimeter at a wavelength of 663 m μ .

¹Registered trademark, Millipore Filter Corporation, Bedford, Massachusetts.

using the one-inch (2.54 cm) tube. Chlorophyll determinations in mg/l were calculated using the method described in Odum, McConnell, and Abbott (1958).

Bacterial Development

Differences in bacterial development were analyzed using bacteriological monitors manufactured by Millipore Filter Corporation. A pooled water sample from 17 random locations (250 ml each) was collected from each tank. Samples (5 or 10 ml) were pipetted from the pooled sample and drawn through an HA membrane filter with a Millipore all-metal syringe. Effective filtration area of the 47 mm white membrane filter is 9.6 cm^2 with grid marking squares of 1/100th of this filtration area. Nutrient sterile medium (Millipore TGE broth) from 0.8 ml ampoules was placed on the absorbent pad under the membrane filter. Monitors were capped, inverted, and incubated at 25°C for 24 hours in a Model 310 incubator manufactured by National Appliance Company. Counting precision of bacterial colonies was increased by staining with Loeffler's alkaline methylene blue solution. Numerous counts were made of the percent of grid area covered by bacterial colonies to determine mean colonial size groups. Percentage of the 9.6 cm^2 effective filtration area of the membrane filter covered by bacterial growth was determined from this technique.

Aquatic Invertebrates

Twenty-five liter water samples for zooplankton analysis were taken by vertically thrusting a plastic tube (10.2 cm diameter) to within several centimeters of the tank bottom. Samples were retained while lifting by pulling a plastic toilet float into the lower end of the tube with a line. Zooplankton were concentrated on nylon cloth (35 μ mesh openings). Retained organisms were transferred to a Sedgwick-Rafter cell where total counts were made. Portions of water (130 ml) passed through the bolting cloth were then filtered through SM membrane filters (5.0 μ pore size). Organisms retained on the filters were transferred to a watch glass where total counts were made.

Ceramic dishes with a total surface area of 457.5 cm² were used periodically to sample sessile chironomid larvae in Experiment I and the total Aufwuchs community (Ruttner, 1963) in Experiments II and III. Aufwuchs was brushed from the dishes and rinsed into graduated cylinders with acetone. Extraction of Aufwuchs chlorophyll proceeded for 48 hours under refrigeration with periodic stirring. Chlorophyll concentrations of the supernatant were determined as obtained from phytoplankton. Volume of the settled residue was determined in graduated centrifuge tubes.

Swimming insect fauna was observed at regular intervals from the time the tanks were filled. Collections were made regularly and species collected for identification were preserved in five percent formalin. Identification was usually to the generic level. In several groups only family identification was possible (See Appendix N). Keys by Pennak (1953), Usinger et al. (1956) and Needham and Needham (1962) were used.

Quantitative sampling of swimming insects was carried out with a 40.6 cm x 26.7 cm dip net of 0.16 cm mesh. A composite sample consisted of a full sweep of 17 meters around the circumference of the tank with vertical sweeps to within several centimeters of the tank bottom. Each composite sample was placed in a shallow white enamel dish, sorted visually, counted, and returned to the tank. Relative population density in this study refers to the mean of the actual number of individuals collected in a composite sample per tank per treatment. Artificial plants were carefully removed and rinsed at the termination of Experiment III to sample insect standing crop. Numbers of insects were so low following fish introduction that sampling became impractical.

Total snail populations (Helisoma sp.) were collected at the termination of Experiments II and III.

Care was taken to prevent loss of snails through the drain hose. Snails collected and counted were graded into two size groups. Large snails were those which would not pass through 8.5 mm square hardware cloth; small snails were those remaining which were retained on 1 mm square mesh after a thorough rinse. Few snails collected passed through this size mesh.

Fish Growth

All species of fish used in this investigation were graded into groups and weighed prior to introduction to the tanks. Individual starting weights were the mean for each group. Fish were weighed individually to within 0.01 grams at the termination of each experiment. Individual biomass increases were calculated from the initial group mean. Growth is given as weighted population mean of individual percent body weight increase.

Statistical treatment of data was computed following methods in Steel and Torrie (1960). Tukey's w-procedure, also called the honestly significant difference (hsd), was chosen for analysis of variance. Each difference is declared significant if it exceeds the corresponding hsd range. The five percent level, which is considered a stringent test, was used at all times.

RESULTS

The experiments were designed to evaluate effects of carbohydrate additions at selected points within the presumed food chain of experimental fish populations. The results of these experiments are, therefore, presented according to the trophic level represented by the parameter measured.

Differences in gross photosynthesis between treatments (Table 6) were significant only during the warmer months. Though some common algae are capable of using glucose as a supplementary energy source (Lewin, 1962), this phenomenon apparently was not reflected as additional capacity for photosynthesis in two of the three experiments.

Increased hydrogen ion concentration after one addition of molasses, during Experiment I (Figure 1), indicates a considerable increase in CO_2 concentration incident to bacterial metabolism of the molasses. This represents another way in which the addition of carbohydrate may have increased photosynthesis in Experiment II. Inadequate CO_2 concentrations often limit photosynthesis of algae (Neel et al., 1961).

Table 6. Mean gross photosynthesis as gms oxygen/M³/24 hours.

Experiment	Number of tanks per Treatment	Number of Measurements	Number Representative days	hsd	Treatment			
					Con-trol	2 ppm	7 ppm	15 ppm
I	3	3	3		1.83	1.51	2.28	1.83
Difference from control				0.84		0.32	0.45	0
II	4	7	49		1.87		2.39	2.34
Difference from control				0.37			0.52	0.47
III	4	10	91		2.02			1.91 1.94
Difference from control				0.36				0.11 0.08

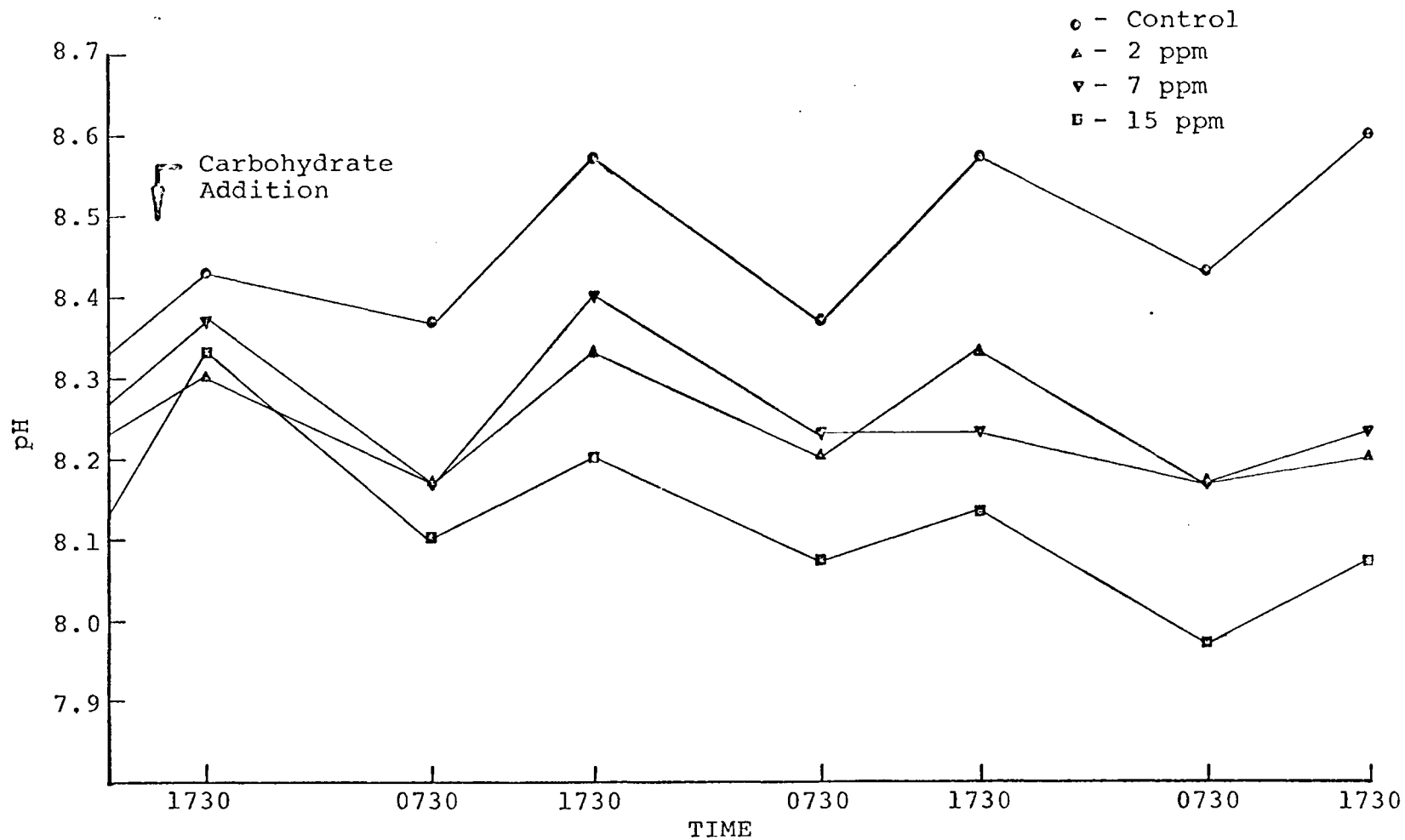


Figure 1. Mean diurnal pH fluctuations following carbohydrate (beet molasses) addition on March 9, 1966 (1430).

Mean plankton chlorophyll concentrations were essentially the same in all treatments within an experiment (Table 7). The heavy concentration (15 ppm) in Experiment II did produce a significant increase; but this concentration and a heavier concentration (25 ppm) in Experiment III did not produce significant increases at lower water temperatures. Phytoplankton biomass and photosynthetic capacity (as indicated by chlorophyll measurements) were increased by the addition of carbohydrates at high water temperatures only. Experiment II, which received less direct sunlight than Experiments I and III, produced the densest mean phytoplankton chlorophyll concentrations. Chlorophyll concentrations of Experiments I and III were about the same despite the use of different kinds of carbohydrates added at different concentrations.

Aufwuchs chlorophyll concentrations were highly variable from tank to tank, but differences were not related to the treatments in any discernible way (Table 8). The bulk of the Aufwuchs biomass (Table 9) appeared to be algae. No evidence of extensive grazing by herbivores was noted. It is therefore probable that Aufwuchs standing crop approached net production.

Obvious bacterial population pulses occurred immediately after the carbohydrate addition of March 9, 1966 (Fig. 2). These were in approximate proportion to

Table 7. Mean plankton chlorophyll concentrations as mg/M³.

Experiment	Number of tanks per Treatment	Number of Measurements	Number of Representative days	hsd	Treatment			
					Control	2 ppm	7 ppm	15 ppm
I	3	10	101		11.45	10.63	12.38	15.15
Difference from control				4.43		0.82	0.93	3.70
II	4	9	71		26.27		36.31	41.35
Difference from control				10.30			10.04	15.08
III	4	10	87		12.73		13.09	13.13
Difference from control				3.60			0.36	0.40

Table 8. Mean Aufwuchs chlorophyll concentration as mg/l.

Experiment	Number of tanks per Treatment	Number of Measurements	Number of Representative days	hsd	Treatment			
					Control	7 ppm	15 ppm	25 ppm
II	4	16	50		0.357	0.463	0.292	
Difference from control				0.151		0.106	0.065	
III	4	16	84		0.107		0.026	0.060
Difference from control				0.069		0.081	0.047	

Table 9. Mean settled Aufwuchs volumes in milliliters.

Experiment	Number of tanks per Treatment	Number of Measurements	Number of Representative days	hsd	Treatment			
					Control	7 ppm	15 ppm	25 ppm
II	4	16	50		2.3	3.3	3.4	
Difference from control.				1.3		1.0	1.1	
III	4	16	84		1.4		0.5	0.7
Difference from control				0.8			0.9	0.7

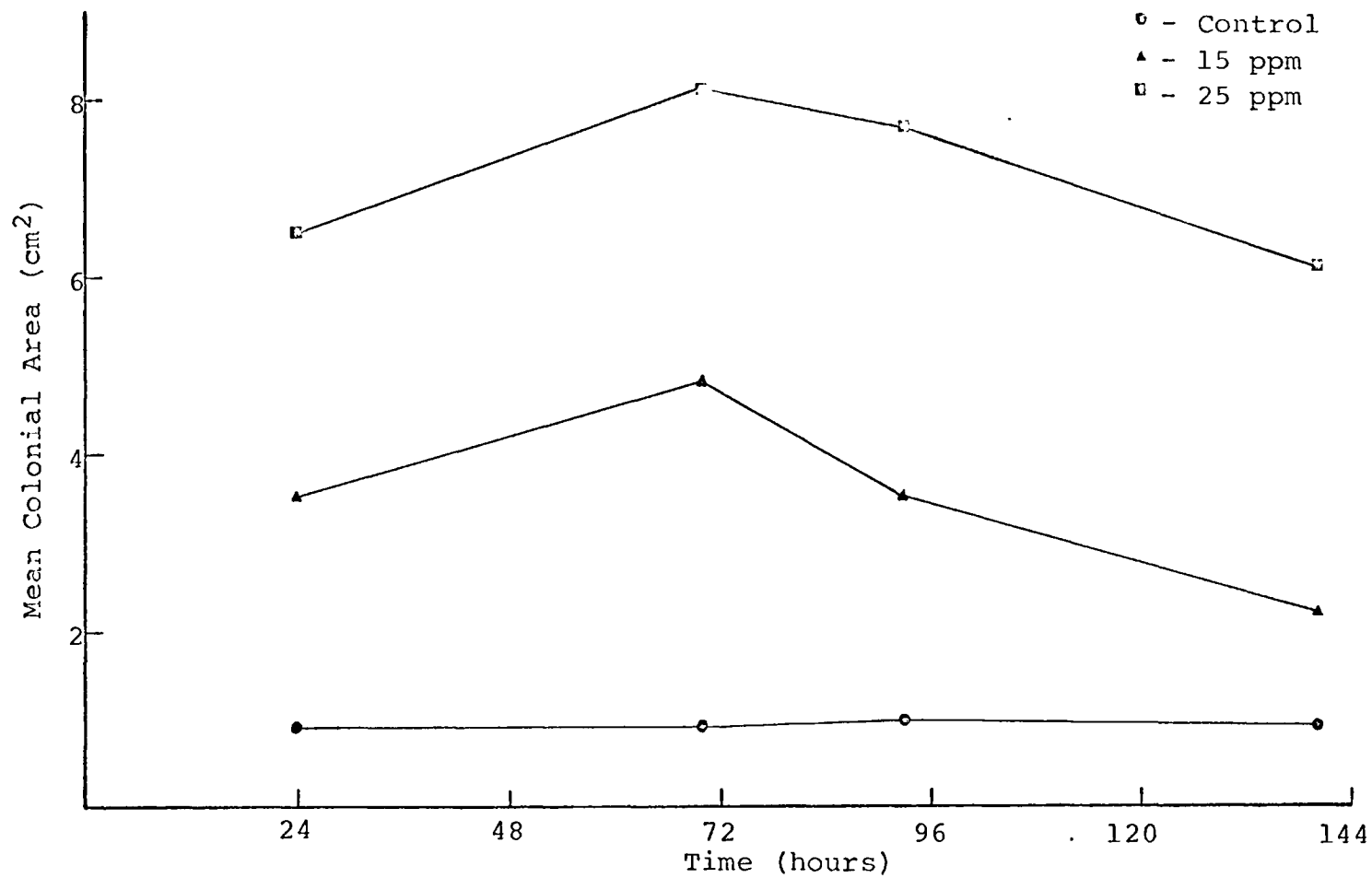


Figure 2. Planktonic bacterial growth as mean plate area covered following carbohydrate addition (0 hours).

concentration. However, bacterial numbers declined greatly within 100 hours. Presumably similar bacterial responses occurred whenever carbohydrates were added.

Increased maximum hydrogen ion concentrations and reduced minimum oxygen concentrations after carbohydrate additions (Table 10) indicated that bacterial numbers were increased at those times also.

Another possible indication of increased bacterial numbers is the significantly greater turbidity at carbohydrate concentrations of 7 ppm and 15 ppm in Experiment I, 15 ppm in Experiment II, and 25 ppm in Experiment III (Table 11). Turbidity and chlorophyll are significantly related in Experiments I and II, therefore, at least part of the turbidity differences may have been due to phytoplankton.

Zooplankton were practically non-existent in all treatments of the three experiments. Comparable data could therefore not be collected. Prior to fish introduction, mean zooplankton (primarily copepods with some cladocerans and rotifers) counts never exceeded one individual per liter.

Snail (Helisoma sp.) numbers increased significantly in all treatments of Experiment II (Table 12, but reproduction and survival was not related to the treatments in any discernible way.

Table 10. Mean dissolved oxygen concentrations (mg/l) taken at sunrise.

Experiment	Number of tanks per Treatment	Number of Measurements	Number of Representative days	hsd	Treatment			
					Control	2 ppm	7 ppm	15 ppm
I	3	3	3		8.21	7.65	7.66	6.67
Difference from control				0.92		0.56	0.55	1.54
II	4	7	49		8.10		7.54	6.77
Difference from control				0.66			0.56	1.33
III	4	10	91		14.50		13.85	14.00
Difference from control				0.81			0.65	0.50

Table 11. Mean turbidity as ppm SiO₂.

Experiment	Number of tanks per Treatment	Number of Measurements	Number of Representative days	hsd	Treatment				
					Con-trol	2 ppm	7 ppm	15 ppm	25 ppm
I	3	9	79		7.2	7.5	9.2	10.4	
Difference from control				2.0		0.3	2.0	3.2	
II	4	8	71		6.2		7.2	8.1	
Difference from control				1.5			1.0	1.9	
III	4	11	79		5.7			6.4	7.2
Difference from control				1.1				0.7	1.5

Table 12. Snail (Helisoma sp.) mean population increase.

Experiment	Number of tanks per Treatment	Number of Representative days	Size	hsd	Treatment			
					Control	7 ppm	15 ppm	25 ppm
II	4	72	Large	347	226	157	149	
			Small	1617	852	280	235	
III	4	140	Large	0	0		0	0
			Small	47	28		13	46

Swimming insect populations in Experiment I were so drastically reduced by the trout that comparable data could not be collected (Fig. 3). Significant populations were not established in Experiments II and III. However, the reduction in numbers of swimming insects may not have been entirely due to trout predation. Decrease in population density of all forms except coleopterans occurred prior to trout introduction in Experiment I.

Sessile chironomid larval populations on the dishes were not as vigorously preyed upon. Populations, which exhibited a range of 9 to 108 larvae per dish, were not increased or reduced by the addition of carbohydrates (Table 13).

Beet molasses apparently inhibited growth of trout in the 15 ppm treatment of Experiment I (Table 14). Mean growth was also less in the 2 ppm and 7 ppm treatments than in controls, but these differences were not significant.

Beet molasses caused no significant reductions or increases in growth of largemouth bass and Tilapia in Experiment II (Table 15).

Trout in both treatments receiving glucose in Experiment III grew at lower average rates than the controls (Table 16). However, the differences were not significant.

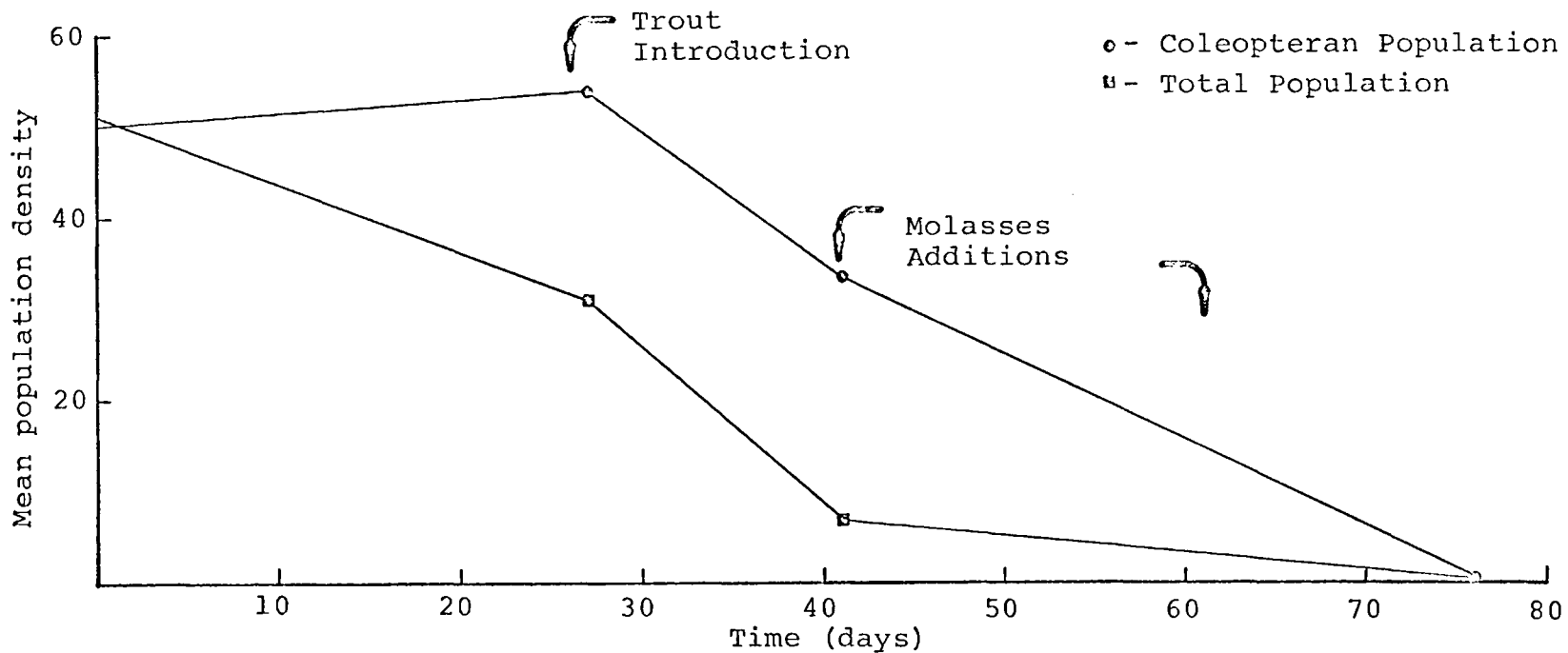


Figure 3. The effect of trout introduction on swimming insect populations (adult and larvae).

Table 13. Populations of chironomid larvae in Experiment I from December through March. Means taken from three counts one month apart.

Treatment	Mean per Treatment	Mean per dish
Control	43	17
2 ppm	45	18
7 ppm	34	16
15 ppm	31	16

Table 14. Rainbow trout growth in Experiment I expressed as percent weight gain. Weighted means represent three replicates per treatment.

	hsd	Treatment			
		Control	2 ppm	7 ppm	15 ppm
Initial population		45	45	45	45
Mean initial weight (grams)		3.03	3.03	3.03	3.03
Final population		35	41	44	41
Mean final weight (grams)		8.04	7.63	7.24	5.98
Difference from Control	1.35		0.41	0.80	2.06
Mean percent total biomass increase (grams)		165.35	151.82	138.94	97.36

Table 15. Fish growth in Experiment II expressed as percent weight gain. Weighted means represent four replicates per treatment.

	hsd	Treatment		
		Control	7 ppm	15 ppm
<u>Largemouth Bass</u>				
Initial populations		40	40	40
Mean initial weight (grams)		3.84	3.49	3.67
Final population		28	29	28
Mean final weight (grams)		6.20	5.74	5.84
Mean percent total biomass increase		61.46	64.47	59.13
Difference from control	23.44		3.01	2.33
<u>Tilapia</u>				
Initial population		32	32	32
Mean initial weight (grams)		8.33	10.61	9.87
Final population		28	32	32
Mean final weight (grams)		12.96	15.93	15.93

Table 15 Continued.

Mean percent total biomass increase	55.58	50.14	61.40
Difference from control	25.54	5.44	5.82

Table 16. Rainbow trout growth in Experiment III expressed as percent weight gain. Means represent four replicates per treatment.

	hsd	Treatment		
		Control	15 ppm	25 ppm
Initial population		48	48	48
Mean initial weight (grams)		8.22	8.48	9.09
Final population		48	48	48
Mean final weight (grams)		11.66	11.59	11.95
Mean percent biomass increase		41.85	36.67	31.46
Difference from control	20.31		5.18	10.39

The rates of fish growth in all experiments were lower than are considered adequate in most natural habitats. This was probably due to poor development of forage organism populations.

The high mortality of largemouth bass (Table 15) was probably due to predation of the more rapidly growing bass on the smaller individuals. There is no indication that it was related to the treatments or to starvation.

DISCUSSION AND CONCLUSION

Carbohydrate additions did not augment the food chain of fish enough to significantly increase the rate of growth.

Changes in hydrogen ion concentration and bacterial colony counts indicate that carbohydrate, after it was added, was essentially consumed in a few days. Evidence of increase in bacterial populations may be found in treatments with significantly high turbidity, which were not related to phytoplankton increase. Waksman and Carey (1935b) report a complete utilization of 5 mg/l of glucose in five days. Approximately 2/3 of the glucose was completely oxidized by the bacteria to liberate energy, while the remaining 1/3 was utilized for the synthesis of their cell substances.

There is some evidence of the role bacteria play in the food chain of fish. Bacteria alone can sustain growth of a number of suspension feeders (Freeden, 1960 and Jorgensen, 1966). Provasoli et al. (1959) reports that growth of suspension feeders was strongly enhanced in algal cultures contaminated with bacteria. Suspension feeders living on bacteria-free algal cultures often did not secure optimal growth.

In the present experiments the pulse in microbial activity and numbers was short lived and probably not effectively foraged upon. The large benthic bacteria in streams (Warren et al., 1964) probably provide better invertebrate forage and exist longer than the planktonic forms present in the tanks.

Different species of bacteria (Stuart et al., 1931 and others) as well as algae may vary in nutritional value. Strickland (1960) indicates that carbohydrate content of Chlorella may vary from 6 to 57 percent dry weight depending on the nutrient medium.

The importance of soluble carbohydrates as food for suspension feeders (Conover, 1966 and Jorgensen, 1966) and other aquatic and marine organisms (Stephens and Schinske, 1961 and Haven, 1965) have been discussed. Uptake has been shown to occur in a wide variety of fish-food organisms, but this does not necessarily indicate direct assimilation.

Foraging by dense fish populations was probably responsible for insignificant populations of invertebrates which forage on the bacteria and phytoplankton. Fewer fish per tank would conceivably have allowed invertebrates to maintain denser populations. Unfortunately, if fish numbers had been decreased, significant growth differences could not have been detected.

Fish growth may have been further restricted by reduction in dissolved oxygen (Table 10) due to bacterial metabolism in the treated tanks. Herrmann, Warren, and Doudoroff (1962), in working with juvenile coho salmon, have shown that reduced (though not critical) dissolved oxygen concentrations are related to decreased growth rates.

Warren et al. (1964) demonstrated that the constant maintenance of a 2-4 ppm concentration of sucrose in a small trout stream resulted in a greater than seven-fold increase in fish growth. While the concentration of carbohydrate was low in this case, the total amount available to heterotrophs was about 30 grams/M²/day. Three 15 ppm additions of molasses in the tanks are equivalent to 0.84 grams of carbohydrate/M²/day for 53 days. Warren does not report the uptake of sucrose in his experimental stream sections; but it is probable that the heterotrophic stream community increased its biomass to the point where uptake was significant. Carbohydrates added in the tanks were probably utilized completely.

Thirty grams of carbohydrate per square meter per day would cost more than \$800 per day in a 200 acre lake using molasses at \$30 per ton. Significant increases in gross photosynthesis were produced in the tanks at a rate which would cost only \$23 per day in a 200 acre lake.

This is not economically favorable, however, in comparison to the addition of inorganic salts.

Soluble carbohydrate additions did not increase invertebrate fish forage organisms enough to produce significant increases in fish growth in the present experiments. In more natural lentic conditions zooplankton and other fish forage organisms might show benefit from the bacterial pulses.

Carbohydrates added on a schedule corresponding to the planktonic bacterial pulses, could possibly cause greater production of fish food organisms in natural conditions.

APPENDICES

APPENDIX A. Gross photosynthesis measurements in Experiment I as grams O_2/M^3 at sunset, sunrise, and sunset.

Date	T R E A T M E N T											
	Control			2 ppm			7 ppm			15 ppm		
30 Mar 1966	8.77	8.54	9.85	8.46	8.31	10.31	9.54	8.85	9.08	8.62	6.61	8.31
31 Mar 1966	8.62	8.15	8.62	7.38	7.38	9.23	8.31	8.00	8.00	7.38	5.85	7.39
	8.77	8.69	10.08	7.85	7.85	10.31	9.31	9.00	8.77	8.62	6.38	8.00
1 Apr 1966	8.00	7.69	8.46	7.08	6.77	9.00	7.85	7.46	7.46	7.39	5.54	7.31
	8.62	8.30	10.23	7.62	7.15	9.77	9.00	8.46	8.31	8.62	6.15	7.62
2 Apr 1966	7.97	7.75	8.66	7.08	6.43	8.52	7.66	7.20	7.02	7.31	5.29	6.62
	8.74	8.63	10.48	7.32	6.89	9.12	9.02	8.20	8.00	8.85	6.09	7.46

APPENDIX B. Gross photosynthesis measurements in Experiment II as grams O₂/M³ at sunset, sunrise and sunset.

Date	T R E A T M E N T											
	Control				7 ppm				15 ppm			
18 Aug 1966	8.31	10.88	8.14	10.43	8.46	7.52	8.34	8.00	7.32	8.08	8.25	10.35
	7.66	9.74	7.09	9.17	7.06	6.49	7.12	6.40	6.54	6.83	6.92	8.51
	8.58	10.85	8.42	10.40	8.20	7.48	8.00	7.69	7.46	8.09	8.05	10.46
25 Aug 1966	8.88	11.29	8.46	10.38	8.03	6.62	8.97	8.57	7.40	7.54	8.31	9.40
	8.09	10.00	7.38	9.05	6.86	5.85	7.85	7.00	6.54	6.71	6.97	7.63
	8.60	10.80	8.11	9.74	7.97	6.42	10.63	8.23	7.05	7.72	7.66	8.95
31 Aug 1966	8.63	9.58	8.18	9.05	7.46	6.37	8.63	8.78	7.42	8.42	7.66	8.43
	8.02	8.98	7.29	8.31	6.68	5.85	7.83	7.48	6.57	7.46	6.89	7.18
	9.08	10.20	8.48	9.88	8.15	6.72	9.35	9.15	7.95	9.08	8.38	8.91
9 Sep 1966	8.35	9.23	7.72	9.62	8.03	5.26	8.68	9.14	6.66	7.94	7.77	7.25
	7.69	8.60	7.12	8.75	7.12	4.77	7.63	7.88	5.40	6.80	6.66	6.12
	8.12	9.38	7.88	9.72	8.37	5.12	8.52	9.49	6.58	7.97	7.46	7.15
21 Sep 1966	8.18	9.09	7.85	9.26	8.78	7.49	8.83	11.75	7.75	8.72	6.00	5.82
	7.37	8.48	7.12	8.25	7.82	6.72	8.25	10.09	6.92	7.83	5.40	4.94
	7.91	9.26	7.72	9.12	9.20	7.65	9.08	11.34	7.86	8.82	5.94	5.95
28 Sep 1966	8.12	9.25	8.55	8.94	9.51	8.26	9.54	13.40	8.43	10.06	7.60	7.31
	7.29	8.29	7.58	7.74	8.14	7.35	8.38	11.49	7.31	8.82	6.66	6.08
	8.00	9.11	8.48	8.71	9.46	8.34	9.35	12.26	8.29	9.82	7.82	7.58

APPENDIX B Continued.

5 Oct 1966	8.37	9.23	8.92	8.83	8.80	8.31	8.66	11.68	7.49	8.32	6.74	7.45
	7.51	8.46	7.95	7.86	7.55	7.28	7.75	10.51	6.42	7.29	5.94	6.28
	8.35	9.51	8.86	8.91	9.51	8.46	8.80	11.85	7.40	8.57	7.14	7.95

APPENDIX C. Gross photosynthesis measurements in Experiment III as grams O₂/M³ at sunset, sunrise and sunset.

Date	T R E A T M E N T											
	Control				15 ppm				25 ppm			
9 Dec 1966	19.08	17.82	18.22	17.46	16.95	16.14	18.28	17.43	18.05	18.51	18.40	15.91
	17.51	16.68	16.46	15.91	15.48	14.72	16.80	16.00	16.75	17.23	17.25	14.69
	19.26	18.43	17.49	17.65	16.26	16.48	17.94	17.37	17.40	18.54	18.05	15.60
14 Dec 1966	19.52	17.92	17.26	17.40	16.49	16.66	17.46	17.26	18.03	17.94	18.58	15.88
	18.28	16.78	16.20	15.92	15.40	15.60	16.38	15.94	17.08	16.98	17.28	14.65
	19.37	17.98	17.54	17.46	16.31	16.89	17.71	16.97	18.26	17.88	18.31	15.82
4 Jan 1967	17.32	15.43	14.89	14.03	14.55	15.40	14.63	15.37	16.46	14.83	15.23	13.94
	17.12	15.12	14.54	13.46	14.09	14.80	13.98	14.65	15.92	14.49	14.75	13.60
	17.46	15.42	14.85	14.12	14.34	15.35	14.55	15.15	16.38	14.75	15.18	14.06
11 Jan 1967	16.52	16.08	14.60	13.35	13.78	15.18	13.86	14.98	15.62	13.63	13.63	13.54
	15.42	14.32	13.97	12.62	13.17	14.43	13.05	14.02	14.97	13.20	12.72	12.92
	-	-	-	-	-	-	-	-	-	-	-	-
2 Feb 1967	16.34	15.18	14.05	15.03	12.43	16.23	15.32	14.38	16.52	12.98	13.23	14.86
	15.08	13.95	12.78	13.46	11.51	14.31	14.40	12.68	14.72	12.28	12.31	13.09
	16.46	15.11	13.86	14.97	12.54	15.66	15.25	13.54	16.29	13.31	13.31	14.77
10 Feb 1967	15.06	15.77	14.20	14.92	13.54	16.00	15.62	13.69	16.05	14.51	13.14	15.05
	14.14	14.89	13.20	13.72	12.51	14.68	14.75	12.58	14.48	13.69	12.22	13.85
	14.89	15.82	13.83	15.09	13.35	16.15	15.62	13.60	15.69	14.77	13.23	15.05

APPENDIX C Continued.

17 Feb 1967	14.54	15.17	14.05	14.74	13.69	14.62	15.11	13.25	15.57	15.00	13.54	15.35
	13.54	13.83	12.74	13.38	12.37	13.32	13.85	12.17	13.77	13.77	11.83	13.65
	14.05	14.92	13.82	14.46	13.00	14.22	14.65	13.18	14.49	14.72	12.43	15.18
23 Feb 1967	13.20	15.18	13.46	13.77	12.57	13.06	12.89	12.57	12.98	14.46	11.97	14.26
	12.31	14.34	12.34	13.09	11.94	12.18	12.35	11.85	12.49	13.60	11.12	13.31
	13.42	15.54	13.51	14.42	12.82	13.14	13.14	12.72	13.68	14.77	12.05	14.80
3 Mar 1967	14.35	16.35	15.97	15.66	14.49	13.82	13.86	15.51	13.77	15.57	12.57	15.57
	13.08	15.51	14.72	14.12	13.55	12.77	13.23	13.65	12.98	14.55	11.22	14.18
	13.78	15.94	15.60	16.49	14.18	13.68	15.23	14.49	13.92	16.03	13.08	15.20
10 Mar 1967	13.58	15.02	17.23	15.15	14.52	14.74	14.15	16.77	14.20	15.58	12.78	14.72
	12.86	13.78	14.98	13.85	13.38	13.05	13.02	15.34	13.00	14.37	11.77	13.14
	14.09	14.95	16.28	15.25	14.88	14.92	14.14	17.51	14.25	15.97	12.85	15.20

APPENDIX D. Plankton chlorophyll concentrations (mg/M³) in Experiment I.

Date	T R E A T M E N T												
	Control			2 ppm			7 ppm			15 ppm			
23 Dec 1965	10.97	16.35	15.41	9.20	11.39	1.97	11.78	9.19	5.23	8.26	12.56	8.15	
17 Jan 1966	6.05	5.28	4.17	10.00	9.68	6.25	9.38	4.29	1.56	1.62	9.00	1.92	
4 Feb 1966	6.43	8.65	9.00	10.00	9.05	9.78	17.76	8.33	8.33	10.50	22.73	4.76	
18 Feb 1966	10.86	12.38	11.11	21.74	7.64	8.61	14.00	8.00	20.34	18.00	13.64	14.29	
25 Feb 1966	8.04	13.95	2.34	13.16	10.91	3.66	15.52	1.85	15.63	13.04	7.76	7.69	
9 Mar 1966	10.71	13.33	10.71	11.84	10.55	-	18.75	12.86	13.04	15.79	6.82	20.45	
21 Mar 1966	7.08	3.21	17.53	14.29	11.84	8.04	18.00	4.95	7.32	8.82	9.68	27.27	
31 Mar 1966	8.28	11.30	27.69	7.23	17.21	18.29	22.06	9.09	12.00	32.14	17.14	29.03	
1 Apr 1966	6.97	11.54	27.05	7.32	10.17	13.64	22.06	18.00	16.83	26.67	16.07	22.22	
2 Apr 1966	8.36	13.72	25.00	4.11	17.44	13.16	20.00	9.09	16.22	28.85	14.52	28.85	

APPENDIX E. Plankton chlorophyll concentrations (mg/M³) in Experiment II.

Date	T R E A T M E N T												
	Control				7 ppm					15 ppm			
2 Aug 1966	12.62	12.00	32.50	26.09	30.34	30.50	15.23	19.82	18.83	38.73	27.67	31.25	
10 Aug 1966	21.76	7.78	28.68	45.56	22.54	10.59	10.92	30.26	31.65	32.81	24.22	39.06	
17 Aug 1966	25.20	9.38	23.52	44.42	71.24	68.12	34.35	66.90	18.04	44.57	58.41	94.60	
31 Aug 1966	50.00	20.66	47.55	54.70	69.46	31.47	39.15	70.76	50.66	36.89	85.91	46.88	
7 Sep 1966	41.27	13.71	46.22	48.63	41.17	30.24	34.11	71.86	47.54	40.44	102.84	64.72	
21 Sep 1966	50.00	6.49	9.72	40.43	52.17	16.16	43.69	27.46	45.98	44.91	42.31	36.43	
27 Sep 1966	43.66	14.33	16.25	16.03	65.56	48.54	30.64	26.89	33.71	49.57	29.32	33.87	
4 Oct 1966	18.16	16.13	35.95	16.13	52.54	31.35	18.07	28.85	32.33	25.30	35.58	30.48	
11 Oct 1966	19.36	11.34	9.74	9.79	16.20	16.38	8.06	25.42	24.17	13.95	24.82	50.32	

APPENDIX F. Plankton chlorophyll concentrations (mg/M³) in Experiment III.

Date	T R E A T M E N T											
	Control				15 ppm				25 ppm			
13 Dec 1966	12.22	19.92	24.36	37.24	50.51	7.65	10.74	37.84	19.90	22.94	12.86	22.64
20 Dec 1966	13.26	14.59	27.51	38.29	45.11	8.08	11.81	27.08	26.09	20.04	11.85	12.74
27 Dec 1966	12.70	9.76	16.64	26.15	12.07	4.84	13.38	35.11	11.60	17.92	4.39	11.34
3 Jan 1967	12.30	4.86	15.36	13.85	27.42	4.84	3.70	10.69	16.29	18.05	2.11	4.85
10 Jan 1967	6.44	6.47	15.03	10.87	6.52	4.84	9.36	15.04	14.12	7.51	9.13	11.19
19 Jan 1967	26.16	8.11	11.34	0.81	22.46	8.13	6.52	6.30	24.77	8.21	10.42	5.94
31 Jan 1967	17.67	11.27	9.67	11.36	16.48	14.50	19.69	20.09	27.27	9.43	5.59	25.06
9 Feb 1967	14.55	6.64	6.73	7.84	17.04	13.12	12.24	6.61	18.90	6.42	7.40	14.63
16 Feb 1967	25.49	15.07	6.82	3.45	19.23	16.15	20.36	6.06	24.05	8.19	10.26	16.62
24 Feb 1967	17.14	12.04	8.31	5.03	10.07	8.46	3.90	5.08	12.50	6.62	12.74	9.41
2 Mar 1967	25.00	14.53	12.52	8.88	16.10	9.06	14.33	10.98	19.52	10.80	13.16	11.48
9 Mar 1967	31.03	15.02	16.36	9.82	23.67	15.19	20.45	13.60	21.19	12.88	26.09	17.14

APPENDIX G. Trout weights (grams) at termination of Experiment I.

T R E A T M E N T											
Control			2 ppm			7 ppm			15 ppm		
5.11	6.44	5.30	4.89	4.85	4.49	4.33	3.85	5.85	4.16	4.26	3.90
5.32	7.86	5.80	5.65	5.37	4.56	5.22	3.90	5.69	4.28	4.41	4.00
6.28	8.12	6.35	5.79	5.61	5.37	5.67	4.30	6.94	4.40	4.43	4.64
6.57	8.84	6.79	5.93	5.74	5.48	5.67	4.31	7.17	4.62	4.44	5.29
6.78	10.71	6.86	6.20	6.53	5.69	5.74	5.13	7.42	5.17	4.86	5.98
6.91	12.20	7.14	6.51	7.60	6.30	5.88	5.14	7.56	5.27	4.99	6.26
7.49		7.34	6.99	7.67	6.35	6.54	5.31	7.68	5.80	5.17	6.77
7.75		7.46	7.82	8.28	6.43	7.02	6.76	7.97	5.97	5.32	6.89
7.78		7.49	8.27	9.71	6.44	7.19	6.89	7.99	6.11	5.57	6.90
7.97		7.92	9.50	10.98	6.70	7.40	6.99	8.51	6.24	5.67	7.17
8.98		8.19	9.58	11.85	8.40	7.92	7.67	9.12	6.47	6.20	7.28
10.60		8.39	10.22	12.75	8.70	7.95	7.84	9.85	7.65	6.46	7.42
10.65		9.32	11.03	13.07	8.94	8.18	9.50	11.19	8.95	7.43	8.50
12.46		10.21			9.25	8.19	10.55	13.45	10.04	9.98	
		12.17			11.28	9.34	11.76				

APPENDIX H. Tilapia weights (grams) in Experiment II.

	T R E A T M E N T											
	Control			7 ppm						15 ppm		
Mean ini- tial weight	9.70	6.32	9.56	7.59	11.14	12.12	10.10	9.08	19.78	8.38	6.11	5.20
Termination weights	12.02	9.59	9.57	8.35	13.22	13.93	11.83	9.04	13.71	8.70	9.82	8.83
	12.54	9.67	11.88	9.25	14.62	18.23	13.93	10.47	19.87	14.38	11.29	9.18
	14.01	10.58	12.00	9.50	14.83	18.47	14.02	12.76	21.26	17.21	11.38	9.51
	17.63	10.77	13.97	9.93	15.55	18.47	14.34	13.19	21.97	19.91	12.36	9.66
	17.65	11.59	15.67	10.05	16.28	20.87	14.38	13.20	22.96	20.46	12.54	9.83
	20.51	13.03	16.91	10.94	17.36	21.57	15.04	14.11	23.76	20.58	12.85	10.04
			17.64	13.02	18.73	23.32	15.53	14.40	27.81	21.35	13.28	13.21
			20.67	13.94	20.29	24.21	16.60	15.91	28.65	24.51	14.52	14.44

APPENDIX I. Largemouth bass weights (grams) in Experiment II.

	T R E A T M E N T											
	Control				7 ppm				15 ppm			
Mean ini- tial weight	3.37	4.36	4.55	2.59	4.04	2.77	4.13	3.10	3.72	2.80	4.37	3.82
Termination weights	4.39	4.98	5.30	3.04	4.73	4.62	5.16	4.82	3.99	4.98	4.42	5.59
	5.44	6.02	5.91	3.52	4.97	4.79	5.33	5.04	4.43	5.10	4.78	8.58
	5.52	6.05	6.63	3.99	5.75	4.97	5.42	5.17	4.58	5.12	5.21	
	5.80	6.17	6.80	4.19	6.13	5.32	5.43	5.44	5.05	5.44	5.32	
	6.23	7.01	7.30	4.57	6.47	5.35	6.43	5.61	5.76	5.91	5.46	
	7.19	7.06	9.35			5.40	6.59	5.64	5.83	6.12	5.66	
	9.41	7.13				5.69	6.69	5.88	6.83	6.53	5.72	
		7.19				6.52	8.50		7.01	7.02	5.85	
		7.93					8.53		7.02		10.28	
		9.61										

APPENDIX J. Trout weights (grams) in Experiment III.

	T R E A T M E N T											
	Control				15 ppm				25 ppm			
Mean ini- tial weight	5.91	11.03	8.45	7.49	7.76	9.56	7.05	9.56	8.17	5.52	10.69	11.98
Termination weights	6.45	9.42	5.66	8.14	8.22	8.55	7.04	6.40	6.79	5.22	7.41	11.26
	7.73	10.34	7.39	8.35	9.65	8.76	7.12	6.85	7.54	5.63	8.44	11.65
	9.53	10.72	10.07	8.54	9.94	9.90	7.38	8.05	9.39	5.83	10.43	13.18
	9.53	11.37	10.59	8.63	10.69	10.25	8.24	9.63	10.28	6.22	10.82	13.72
	9.98	11.88	12.02	9.23	12.51	10.30	9.13	11.30	10.62	7.27	11.38	13.82
	10.01	12.01	12.38	9.48	12.58	10.74	10.42	11.80	11.77	8.45	11.52	14.18
	11.33	12.13	12.57	9.98	12.63	12.10	10.54	12.36	12.06	8.47	11.91	14.98
	11.65	12.30	12.66	12.21	13.13	12.14	10.70	13.01	14.98	8.92	12.51	15.32
	12.17	12.50	12.79	12.62	13.80	13.03	10.73	13.74	15.54	10.00	13.00	16.56
	12.54	13.94	15.78	13.00	15.71	13.27	12.29	15.12	15.92	12.51	13.18	17.00
	15.46	15.96	16.27	15.37	15.94	14.89	12.43	15.52	16.92	13.10	13.19	18.15
	16.11	16.18	17.44	17.06	16.13	18.64	15.30	17.59	17.88	13.40	13.42	22.13

APPENDIX K. Turbidity measurements (ppm SiO₂) in Experiment I.

Date	Control			2 ppm			7 ppm			15 ppm		
14 Jan 1966	5.5	8.0	7.0	7.0	7.0	5.5	8.0	7.0	5.5	5.5	7.0	7.0
17 Jan 1966	5.5	-	-	-	8.0	5.5	-	-	-	-	-	8.0
4 Feb 1966	8.0	5.5	5.5	8.0	7.0	4.5	8.0	8.0	5.5	7.0	8.0	7.0
18 Feb 1966	8.0	4.5	7.0	10.0	5.5	4.5	10.0	4.5	8.0	7.0	7.0	5.5
25 Feb 1966	8.0	8.0	5.5	8.0	5.5	4.5	8.0	5.5	8.0	7.0	9.0	8.0
9 Mar 1966	7.0	9.0	9.0	7.0	8.0	7.0	7.0	9.0	9.0	8.0	8.0	8.0
21 Mar 1966	8.0	5.5	9.5	9.0	10.0	9.0	9.0	8.0	13.0	12.5	12.0	16.0
31 Mar 1966	7.0	7.0	8.0	5.5	9.0	10.0	11.0	9.0	15.0	15.0	11.0	19.5
1 Apr 1966	8.0	5.5	10.0	7.0	10.0	10.0	12.5	11.0	15.0	15.0	10.0	18.0
2 Apr 1966	5.5	5.5	10.0	7.0	9.0	9.0	12.5	10.0	12.5	13.5	11.0	17.0

APPENDIX L. Turbidity measurements (ppm SiO₂) in Experiment II.

Date	Control				7 ppm				15 ppm			
2 Aug 1966	9.0	4.5	4.5	5.5	7.5	4.5	3.5	7.0	7.0	4.5	5.5	7.0
18 Aug 1966	3.5	3.5	4.5	5.5	5.0	3.5	5.0	7.0	3.5	7.0	7.5	7.0
31 Aug 1966	3.5	4.1	3.9	7.0	3.9	5.1	4.5	9.8	3.5	11.0	9.2	7.0
7 Sep 1966	3.5	4.3	6.1	8.0	5.5	6.0	6.0	10.0	4.9	6.5	9.8	9.0
21 Sep 1966	10.0	3.5	3.9	7.2	5.7	7.5	3.7	7.1	10.4	7.0	7.0	7.0
27 Sep 1966	7.0	8.0	7.3	9.4	5.7	11.0	9.0	10.6	8.0	15.0	9.2	6.0
4 Oct 1966	6.5	6.0	7.5	10.1	9.8	9.0	6.0	12.5	7.0	11.5	9.9	10.1
11 Oct 1966	7.7	6.5	8.0	8.0	7.1	9.4	6.5	15.0	7.5	9.2	15.2	10.4

APPENDIX M. Turbidity measurements (ppm SiO₂) in Experiment III.

Date	Control				15 ppm				25 ppm			
20 Dec 1966	4.1	6.1	5.8	5.5	8.4	4.5	5.3	5.1	7.0	4.5	5.1	4.5
27 Dec 1966	4.1	3.9	4.7	4.5	4.5	3.7	4.3	6.1	5.3	3.0	4.9	5.8
3 Jan 1967	4.5	3.3	3.9	4.5	4.1	3.7	3.5	4.5	5.5	5.1	4.7	6.1
10 Jan 1967	4.1	3.7	6.4	6.4	6.1	5.8	5.5	7.2	7.0	4.3	6.7	7.4
19 Jan 1967	4.9	4.1	5.5	4.5	6.1	7.4	5.3	6.4	4.5	4.5	6.7	8.2
31 Jan 1967	6.1	4.9	3.7	6.1	4.5	5.8	5.1	7.8	5.8	6.1	7.4	9.2
9 Feb 1967	10.2	8.2	4.7	6.1	8.6	11.6	4.5	8.8	7.2	8.4	8.8	8.6
16 Feb 1967	8.8	7.2	4.7	3.7	8.4	7.4	7.2	8.8	10.2	8.0	9.4	10.4
24 Feb 1967	11.3	10.0	9.0	7.0	10.0	11.6	10.8	7.2	12.2	9.4	14.1	9.4
2 Mar 1967	8.3	3.8	3.0	3.5	5.7	4.5	5.5	3.2	7.9	4.9	7.6	8.0
9 Mar 1967	11.8	6.9	5.0	4.5	5.7	6.7	6.1	7.4	8.0	5.2	9.6	9.3
30 Mar 1967*	11.7	7.1	6.8	6.7	9.2	8.0	9.1	8.6	11.3	21.5	15.8	13.1

*Taken within net enclosures

APPENDIX N. Swimming insect fauna collected during first 12 weeks of Experiment I.

Order	Family	Genus	Species ₁	Form	Week ₂
COLEOPTERA	HYDROPHILIDAE	<u>Hydrophilus</u> sp.	2	adult	1
		<u>Tropisternus</u> sp.	1	adult	1
	DYTISCIDAE	<u>Thermonectus</u> sp.	2	adult	1
		<u>Thermonectus basillaris</u>		adult	10
		<u>Hydaticus</u> sp.	1	adult	1
		<u>Celina</u> sp.	2	adult	1
		<u>Celina</u> sp.	1	larvae	5
	unidentified		1	adult	12
HEMIPTERA	CORIXIDAE		1	adult	9
			1	nymph	12
	NEPIDAE	<u>Ranatra quadridentata</u>		adult	1
	NOTONECTIDAE		3	adult	5
DIPTERA	EPHYDRIDAE	<u>Notiphila</u> sp.	1	larvae	1
		<u>Ephydra</u> sp.	1	pupa	10
		<u>Brachydeutera argentata</u>		larvae	1
		<u>Brachydeutera argentata</u>		emerg. adult	5
	CULICIDAE	<u>Culex</u> sp.	1	larvae	1
		<u>Culiseta</u> sp.	1	larvae	5
		<u>Wyeomyia smithi</u>		larvae	9.
		<u>Anophelini</u> sp.	1	pupa	5
CHIRONOMIDAE		1	larvae	10	

APPENDIX N Continued.

EPHEMEROPTERA	BAETIDAE	<u>Paraleptophlebia</u> sp.	1	larvae	5
		<u>Paraleptophlebia</u> sp.	1	emerg. adult	12

- 1 Number of probable species.
2 Week of first appearance in the tanks.

LITERATURE CITED

- American Public Health Association and others. 1965. Standard methods for the examination of water, sewage, and industrial wastes. 12th ed., N.Y., Amer. Public Health Assoc. 555 pp.
- Conover, Robert J. 1966. Assimilation of organic matter by zooplankton. *Limnol. Oceanogr.* 11(3):338-345.
- Ellis, R. J., and H. Gowing. 1957. Relationship between food supply and condition of wild brown trout, Salmo trutta Linnaeus, in a Michigan stream. *Limnol. Oceanogr.* 2(4):299-308.
- Ereeden, F.J.H. 1960. Bacteria as a source of food for black-fly larvae. *Nature, Lond.* 187:963.
- Gaufin, Arden R. and Clarence M. Tarzwell. 1952. Aquatic invertebrates as indicators of stream pollution. *Pub. Health Rep., Vol. 67, No. 1.* pp. 57-64.
- Gorbunov, K. V. 1946. Cellulose bacteria as a link in the food chain of sweet water reservoirs. *Microbiology.* 15:149-153.
- Haven, Dexter S. 1965. Supplemental feeding of oysters with starch. *Chesapeake Sci.* 6(1):43-51.
- Henrici, A. T. 1939. The distribution of bacteria in lakes. *Am. Assoc. Advanc. Sc.* 10:39-64.
- Herrmann, Robert B., Charles E. Warren, and Peter Doudoroff. 1962. Influence of oxygen concentration on the growth of juvenile coho salmon. *Trans. Amer. Fish. Soc.* 91(2):155-167.
- Hickling, C. F. 1960. The Malacca tilapia hybrids. *Jour. Genet.* 57(1):1-10.
- _____. 1962. Fish culture. Faber and Faber Limited. London. 295 pp.
- Jorgensen, C. Barker. 1966. Biology of suspension feeding. Pergamon Press, New York. 357 pp.

- Katz, M., and W. C. Howard. 1955. The length and growth of 0-year class creek chubs in relation to domestic pollution. *Trans. Amer. Fish. Soc.* 84:228-238.
- Kemmerer, A. J. 1965. Relation of the chemistry of inflow waters to organic productivity in small fishing impoundments. M.S. Thesis, Univ. of Arizona, Tucson. 124 pp.
- Lewin, Ralph A. 1962. Physiology and biochemistry of algae. Academic Press Inc., New York. 929 pp.
- McConnell, Wm. J. 1962. Productivity relations in carboy microcosms. *Limnol. Oceanogr.* 7(3):335-343.
- _____. 1963. Primary productivity and fish harvest in a small desert impoundment. *Trans. Amer. Fish. Soc.* 92(1):1-12.
- Needham, J. G., and P. R. Needham. 1962. A guide to the study of freshwater biology. Holden-Day, Inc., San Francisco, California. 108 pp.
- Neel, J. K., J. H. McDermott and C. A. Monday, Jr. 1961. Experimental lagooning of raw sewage at Fayette, Missouri. *Jour. Water Pollution Control Fed.* 33(6):603-641.
- Nelson, P. R., and W. T. Edmondson. 1955. Limnological effects of fertilizing Bare Lake, Alaska. *Fish. Bull.* 102, U.S. Fish and Wildlife Service, Vol. 56.
- Odum, H. T., W. J. McConnell and W. Abbott. 1958. The chlorophyll "A" of communities. *Publ. Inst. Mar. Sci., Univ. Texas.* 5:65-96.
- Pennak, R. W. 1953. Fresh-water invertebrates of the United States. Ronald Press, New York. 769 pp.
- Provasoli, L., K. Shiraishi and J. R. Lance. 1959. Nutritional idiosyncrasies of Artemia and Tigriopus in monoxenic culture. *Ann. N.Y. Acad. Sci.* 77(2):250-261.
- Ruttner, F. 1963. Fundamentals of limnology. Translated by D. G. Frey and F.E.J. Fry. Univ. Toronto Press, Toronto. 251 pp.

- Smith, E. V., and H. S. Swingle. 1943. Organic materials as fertilizers for fish ponds. *Trans. Amer. Fish. Soc.* 72:97-102.
- Steel, Robert G. D. and James H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Company, Inc., New York. 481 pp.
- Stephens, G. C. and R. A. Schinske. 1961. Uptake of amino acids by marine invertebrates. *Limnol. Oceanogr.* 6:175-181.
- Strickland, J.D.H. 1960. Measuring the production of marine phytoplankton. *Bull. Fish. Res. Bd. Canada.* 122.
- Stuart, C. A., M. McPherson and H. J. Cooper. 1931. Studies on bacteriologically sterile Moina macrocopa and their food requirements. *Physiol. Zool.* 4:87-100.
- Swingle, H. S. 1947. Experiments on pond fertilization. Alabama Agr. Expt. Sta. Bulletin No. 264. 36 pp.
- Usinger, R. L. (ed.). 1956. Aquatic insects of California with keys to North American genera and California species. University of California Press, Berkeley, California. 508 pp.
- Waksman, Selman A. and Cornelia L. Carey. 1935a. Decomposition of organic matter in sea water by bacteria. I. Bacterial multiplication in stored sea water. *Jour. Bacteriol.* 29:531-543.
- _____. 1935b. Decomposition of organic matter in sea water by bacteria. II. Influence of addition of organic substances upon bacterial activities. *Jour. Bacteriol.* 29:545-561.
- Warren, C. E. 1961. The influence of excessive organic enrichment on the ecology of the Tuolumne River. Ph.D. Thesis, University of California, Berkeley. 267 pp.
- Warren, Charles E., Joseph H. Wales, Gerald E. Davis, and Peter Doudoroff. 1964. Trout production in an experimental stream enriched with sucrose. *Jour. Wildl. Mgmt.* 28(4):617-660.
- ZoBell, C. E. and C. W. Grant. 1942. Bacterial activity in dilute nutrient solutions. *Science.* 96:189.