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AN EVALUATION OF FILTER FEEDING FISHES FOR REMOVING EXCESSIVE NUTRIENTS AND ALGAE FROM WASTEWATER

by

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The feasibility of utilizing finfish for the removal and recycling of excessive nutrients and algae from wastewater was investigated. The silver carp (Hypopthalmichthyes molitrix) and the bighead carp (Aristichthyes nobilis) were chosen due to their specifically adapted filter feeding mechanism. An existing wastewater treatment plant with six lagoons served as the project site. No attempt was made to alter or influence the waste load normally received by the lagoons.

The presence of the fish had a beneficial effect on the aquatic system. In all, 14 water quality parameters along with selected heavy metals, pesticides, pathogenic bacteria, and viruses were monitored during the project. Analysis of the data shows that the presence of the fish improves the treatment capability of the conventional lagoon system. There are tradeoffs to be made among some parameters and some liabilities resulting from the presence of the fish. All are within acceptable limits and tip the scales in favor of the benefits gained. In the final analysis, the real determining factor in deciding to use a finfish-aquaculture-treatment system is the capability of using the more than 7,200 kg/ha annual production of fish as a revenue producer.

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FOREWORD

EPA is charged by Congress to protect the Nation's land, air, and water systems. Under a mandate of national environmental laws focused on air and water quality, solid waste management and the control of toxic substances, pesticides, noise, and radiation, the Agency strives to formulate and implement actions which lead to a compatible balance between human activities and the ability of natural systems to support and nurture life. In partial response to these mandates, the Robert S. Kerr Environmental Research Laboratory, Ada, Oklahoma, is charged with the mission to manage research programs: to investigate the nature, transport, fate, and management of pollutants in ground water; to develop and demonstrate technologies for treating wastewater with soils and other natural systems; to control pollution from irrigated crop and animal production agricultural activities; and to develop and demonstrate cost-effective land treatment systems for the environmentally safe disposal of solid and hazardous wastes.

Evaluation of an aquatic treatment process utilizing filter-feeding finfish indicates that such systems are effective for removal of pollutants from municipal wastewater. The experimental system had a very high rate of annual finfish production. Such high yields appear economically attractive, provided acceptable methods can be developed to utilize the product.

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PREFACE

The passage of the Federal Water Pollution Control Act of 1972 (Public Law 92-500) generated considerable interest and concern for the development of wastewater treatment methods that would meet the more stringent standards at a reasonable cost. The emphasis on reuse of wastewater and the recycling of nutrients into useful products brought about a new look at old biological treatment methods. The biological production capability of nutrient laden wastewaters is obvious. However, directing this energy and raw materials into useful products has proven difficult.

Often the emphasis has been on developing new products and uses for the mostly invertebrate species that grow naturally in wastewaters. With an already growing demand and a decreasing world supply of fish and fisheries products, many investigators have attempted to rear fish in wastewaters. This has been largely unsuccessful in the United States due to the lack of a native species with a high production capability utilizing primary production from ponds or lagoons as a food source. The importation of the silver and bighead carp into Arkansas in 1973 by a private concern provided the opportunity for experimentation with fish species uniquely adapted for the job.

Out of a concern for premature and widespread release of these exotic species, the Arkansas Game and Fish Commission began a program to evaluate the possible beneficial uses as well as the dangers of these fish. From observations in hatchery ponds, it quickly became evident that they possessed certain characteristics that could be useful under a number of circumstances. After a review of the state's wastewater treatment facilities, an existing six lagoon facility was located at the Benton Services Center and a cooperative agreement for use of the ponds for testing fish production and water quality improvement was reached.

Initial interest in the silver and bighead carp resulted from an extensive amount of literature reporting the many characteristics they possess that make them a seemingly ideal fish for culture. A fish that could be added to native species in Arkansas' large fish farming industry to increase production was an attractive possibility. It became apparent that these filter feeders had quite an impact on water quality and this became an increasingly important subject of subsequent studies. All preliminary work corroborated reports in the literature concerning production and growth rate potential of these fish. By the time this project was designed and implemented, the major emphasis was on the use of these fish to improve the quality of wastewater. This was somewhat unique in that all previous work had been concerned with the optimum nutrient loads to add to ponds to maximize production. The ability of the fish to withstand heavy wastewater loads and their concomitant impact on water quality is relatively unexplored territory.

ABSTRACT

This study was instituted to determine the feasibility of utilizing certain species of finfish for the removal and recycling of excessive nutrients and algae from wastewater. The silver carp, Hypopthalmichthyes molitrix, and the bighead carp, Aristichthyes nobilis, were chosen as the central species due to their specifically adapted filter feeding mechanism. An existing wastewater treatment system with 6 lagoons served as the project site. Since the results from previous controlled field trials were available, this project utilized the entire facility as a pilot scale system. No attempt was made to alter or influence the daily waste load normally received by the lagoons.

It can be said unequivocally that the presence of the fish had a beneficial effect on the aquatic system. Because of the many variables involved in such a dynamic, stressed ecosystem it is difficult, if not impossible, to quantify a direct relationship between the standing crop of fish and any one water quality parameter. In all, fourteen water quality parameters along with selected heavy metals, pesticides, pathogenic bacteria, and viruses were monitored during the project.

Analysis of the data shows that the presence of the fish improves the treatment capability of the conventional lagoon system. There are trade-offs to be made among some parameters and some liabilities resulting from the presence of the fish. All are within acceptable limits and, when considered, still tip the scales in favor of the benefits gained. In the final analysis, the real determining factor in deciding whether to use a finfish-aquaculture-treatment system is the capability of using the more than 7,200 kg/ha annual production of fish as a revenue producer to sufficiently offset or pay for water treatment costs.

This report was submitted to fulfill the terms of a cooperative agreement between the Arkansas Game and Fish Commission and the U. S. Environmental Protection Agency. This report covers the period from November 1, 1977 to September 30, 1981 with fieldwork being completed as of December 31, 1980.

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INTRODUCTION

Fertilization of fish ponds has long been recognized by the fish culturist as a method of increasing production. The production of finfishes as a method of reducing fertility is a relatively new approach that has been stimulated by the increasing need for effective, low cost treatment of wastewater by small municipalities. The initial emphasis on this and other "alternative" strategies as opposed to conventional methods was largely a result of more stringent effluent guidelines and the high cost of construction and operation of conventional plants. It seems, however, that the even more recent realization of the need to conserve energy sources and to recycle what has previously been discarded as a troublesome waste product has provided the impetus for exploring new technologies. Also, even the remote possibility of producing a useful and/or valuable product from wastewater treatment demands attention.

The Arkansas Game and Fish Commission's interest in this project evolved from the importation into the state of two species of Chinese carps by a private fish farmer. The silver carp and bighead carp were brought into Arkansas in 1973 with initial interest resulting from the fact that they were unknown, exotic species and the possibility of these low trophic level filter feeders being a beneficial addition to fish production ponds. Conversations with Dr. S. Y. Lin who did pioneering work with the Chinese carp species in Taiwan and a visit to the Quail Creek Sewage Treatment Project in Oklahoma during 1973-74 led to the current interest in wastewater aquaculture.

The fact that many finfish species ranging from the lowly esteemed common carp, Cyprinis carpio, to the prize sport fish the muskellunge, Esox masquinongy, have been produced in wastewater ponds attests to the variety of species amenable to production in nutrient rich wastewaters under specific conditions. The fact that X pounds of fish are produced without supplemental feeding obviously shows that in one fashion or another, energy and nutrients are transformed into the very stable form of fish flesh. This is the reasoning behind one of the basic tenets of fish culture and management i.e., that within certain limits the natural productive capacity of a given body of water is increased by increasing available nutrients. The fish culturist may draw on a rather large body of available literature resulting from research and practical experience in determining the proper type and amounts of fertilizer to add to the culture pond.

If, on the other hand, the objective is to utilize available nutrients, little is known about the effectiveness of finfish in general or of any particular species. Common sense dictates that those fishes that have adapted to feeding at the lower trophic levels would be most efficient at converting nutrients. Therefore, those that are able to feed on the primary productivity, the herbivores, should be considered the most likely candidates for acnieving the objective of nutrient utilization. A group of fishes known as the Chinese carps, in particular the silver carp, meets this criterion and is the key species in this study.

CONCLUSIONS AND RECOMMENDATIONS

The addition of silver and bighead carp to a lagoon wastewater treatment system increases the efficiency of that system. Depending on climatic and other operational conditions, the inclusion of these natural filters can increase treatment by as much as 25-30%. From a practical standpoint, this could decrease the amount of land area needed or improve the quality of water leaving the facility or both. When used as the sole method of treatment, an aquaculture system using silver carp is limited in capability. Properly designed and operated, the system could provide advanced secondary treatment and consistently meet discharge requirements of 10 ppm BOD_5 and 20 ppm total suspended solids. Though nutrient removal is improved and both total phosphates and nitrogen levels were decreased by more than 90% in this system, total removal would require such a lengthy retention time as to be impractical. However, where treatment level requirements do not exceed the capability of the system, finfish aquaculture in wastewater lagoons is a viable and reasonable method of upgrading treatment and recycling wastes into a stable and useful form.

By making the assumption that they are properly designed and operated and comparing other similar sized Arkansas municipal treatment plants using lagoon systems, it can be seen that this finfish system out performed other conventional plants by 30-50% in the critical areas of BOD_5 and TSS. While this is obviously a rather loose comparison, it is also obvious that much could be gained by utilizing this method in Arkansas alone.

Aquaculture treatment systems are competitive with other conventional methods from a cost effectiveness standpoint at the present time. Recycling wastes into useful products is certainly the ultimate goal of waste disposal. This method achieves that goal in theory since fishery products have a high demand. In fact, product utilization ranges from being limited to impossible. The development of quality control standards to allow the use of fish products grown in wastewater is the most pressing need. If that could be accomplished, there is little doubt that a treatment system that could potentially produce a profit would be available.

Aquaculture as a method of wastewater treatment has been shown to work in a variety of ways within inherent limits. There are many applications where this system of treatment is acceptable and the design criteria only needs refining. So long as the fish production from such a process is considered a liability rather than an asset, pursuing further development is rather pointless. The perplexing problem of how to safely and effectively use these recycled products deserves the greatest attention at the present time.

SILVER AND BIGHEAD CARPS

The silver and bighead carp are native to the Amur River basin along the Sino-Soviet border (Figure 1 & 2). Stocks of these fish have been propagated by the Arkansas Game and Fish Commission since 1973 for use in this and other research projects. Both are filter feeding fishes that feed on free-floating or free-swimming planktonic organisms throughout their life. These fishes are capable of reaching a size of 18-23 kg (40-50 lbs.) in four to five years.

The silver carp exhibits certain characteristics that make it more desirable for this type of program than native filter feeding species. The specially adapted gill rakers that have evolved as the filter for this species are somewhat unique and are very efficient at filtering extremely small particles from the water that passes through them. The gill rakers of the silver carp are similar to a sponge-like plate and are capable of removing particles as small as four microns in size (Figure 3). The diet of the silver carp is composed primarily of phytoplankton. The gill rakers of the bighead are filaments that widen at the distal end and overlap to form a more or less solid filtering surface (Figure 4). The filter of the bighead is comparable to many native filter feeders and is not as efficient at removing the smaller particles as is the silver carp. The majority of the bigheads diet consists of zooplankton and the larger phytoplankton species. Both the silver and the bighead are capable of rapid growth, are not particularly susceptible to common fish diseases, and are capable of withstanding relatively low dissolved oxygen levels. For these reasons mentioned above, it is believed by the author that the silver carp should be the central species in a finfish treatment system. The bighead has certain desirable attributes but could be replaced by other native fishes.

The bighead and the silver carp are members of the Cyprinid family. Their food consists of a microscopic organisms that are free-floating and free-swimming. They are particularly adapted to this type of feeding because of their very specially adapted gill rakers which are capable of filtering large volumes of water and thereby concentrating tremendous numbers of microscopic organisms that serve as their only food. Due to this specialized feeding mechanism, their natural habitat is in fertile bodies of water which support large populations of planktonic organisms. More specifically, they occupy open water in the zone of light penetration where their food is the most abundant. Literature reports members of these species as large as 40-60 pounds although a 10-20 pound adult would be much more common.

The silver carp is a deep-bodied, laterally compressed fish. Typical of the minnow family, it has no spines in the fins. Not so typical of this group, however, is the fact that the scales are extremely small, salmonid-like. The silver carp is counter-shaded from olivaceous above to silver below the

FIGURE 1. SILVER CARP

FIGURE 2. BIGHEAD CARP

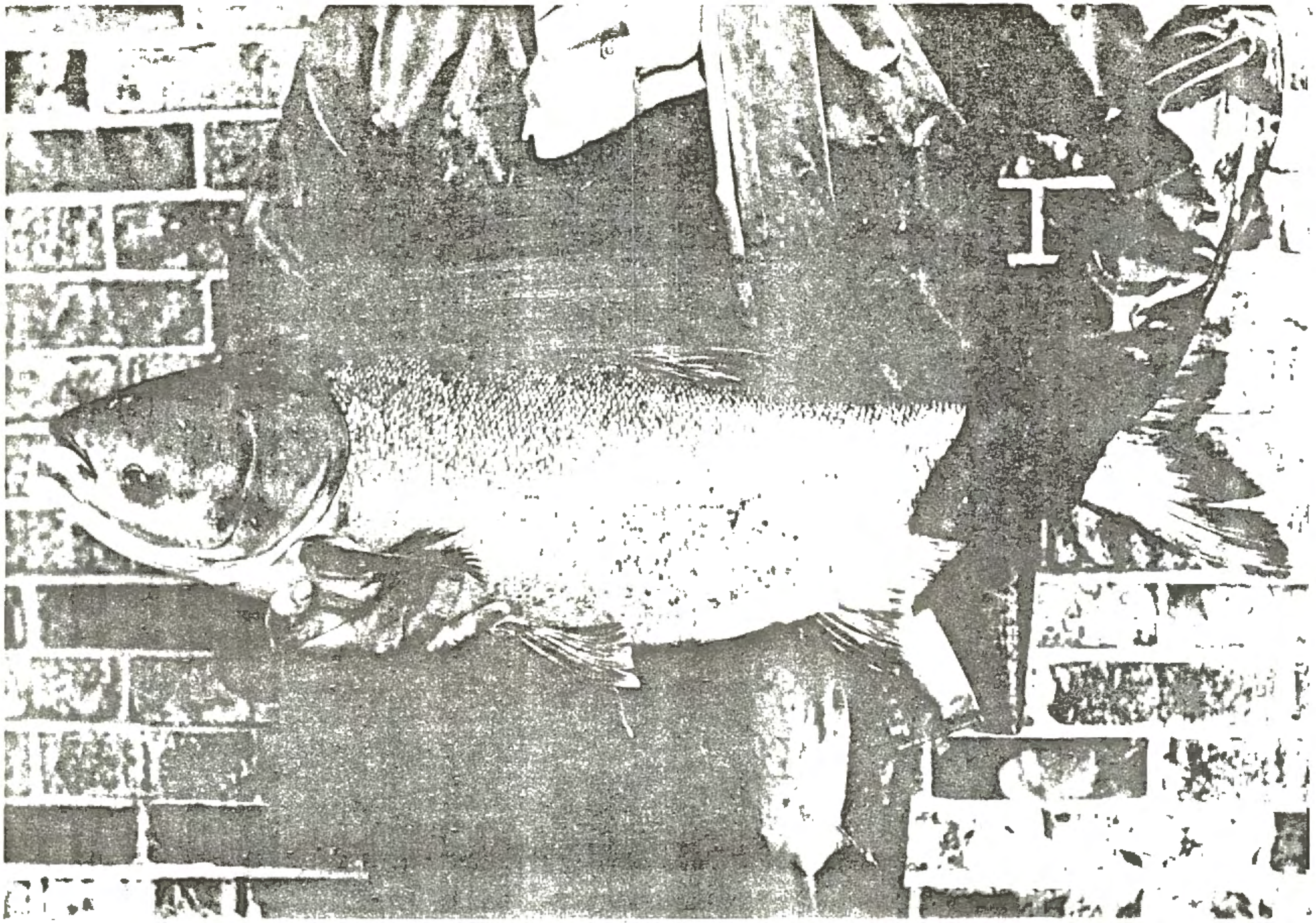


FIGURE 3. GILL RAKERS, SILVER CARP

FIGURE 4. GILL RAKERS, BIGHEAD CARP

lateral line. The mouth is relatively large and contains no teeth. The eyes are located extremely forward along the mid-line of the fish's body and project somewhat downward. The silver carp has a smooth keel that runs from the base of the caudal fin forward to the isthmus.

The upper size limit of organisms found in the gut is thought to be (at least partially) a result of the action of the pharyngeal teeth and not necessarily the inability of the silver to ingest larger organisms. Both the silvers and the bigheads have been observed to carry out a sort of back-flushing of their filter by shutting the opercular openings and blowing water, clouded with particles, from their mouth. Whether this is an actual rejection of certain organisms either by species or size is not known.

The gill rakers of the bighead are more similar to some native species (shad, paddlefish, etc.). They are comprised of individual filaments rather than being a solid plate as in the silver. At their point of attachment to the gill arch, the filaments are spaced 75-100 microns apart. As they extend outward from their point of attachment, they widen and overlap so that there is no space between them.

PROJECT SITE DESCRIPTION

The wastewater treatment plant of the Benton Services Center was chosen as the site for the study. The primary reasons for its selection were the multiple ponds available, the capability of controlling the pattern of flow through the system, and state ownership which provides greater cooperation and control in operation of the plant.

The Benton Services Center is under the direction of the Arkansas Department of Human Services. The center provides both mental and alcohol rehabilitation programs, a nursing home facility, and serves as a work release center for the Arkansas Department of Corrections. While numbers vary, there are approximately 1,000 persons residing at the center full time. Other than daytime and around-the-clock patient care personnel, the center maintains its own water treatment plant, fire station, laundry, food services department, and a rather large maintenance and grounds staff. There are also several residences for staff members located on the grounds. There are, in all, approximately 1,000 full time employees at the center with some contributing to the wastewater load during working hours six days per week and others around-the-clock.

Other than the collective individual wastes, the biggest contributors of wastewater to the system are the laundry and food services. The laundry is in operation six days per week supplying the needs of the entire Benton facility and food services prepares three meals per day for all residents and at least one for every employee. The character of the raw wastewater is fairly typical of that produced by small municipalities with no major industrial users.

The physical facilities of the wastewater treatment plant include (1) a bar screen and grinder for reducing the size of larger debris entering the system, (2) a clarifier, (3) an aerobic digester (this is a converted anaerobic system providing mechanical aeration to the solids from the clarifier, majority of the water enters the lagoons from the clarifier), and six oxidation ponds with a total surface area of 10.2 ha (24 acres). The average daily flow of wastewater into the system is 1,711 m^3/day (0.45 MGD), the average organic load is 444 kg (977 lbs.) of BOD_5 per day, and 208.6 kg (459 lbs.) of suspended solids per day.

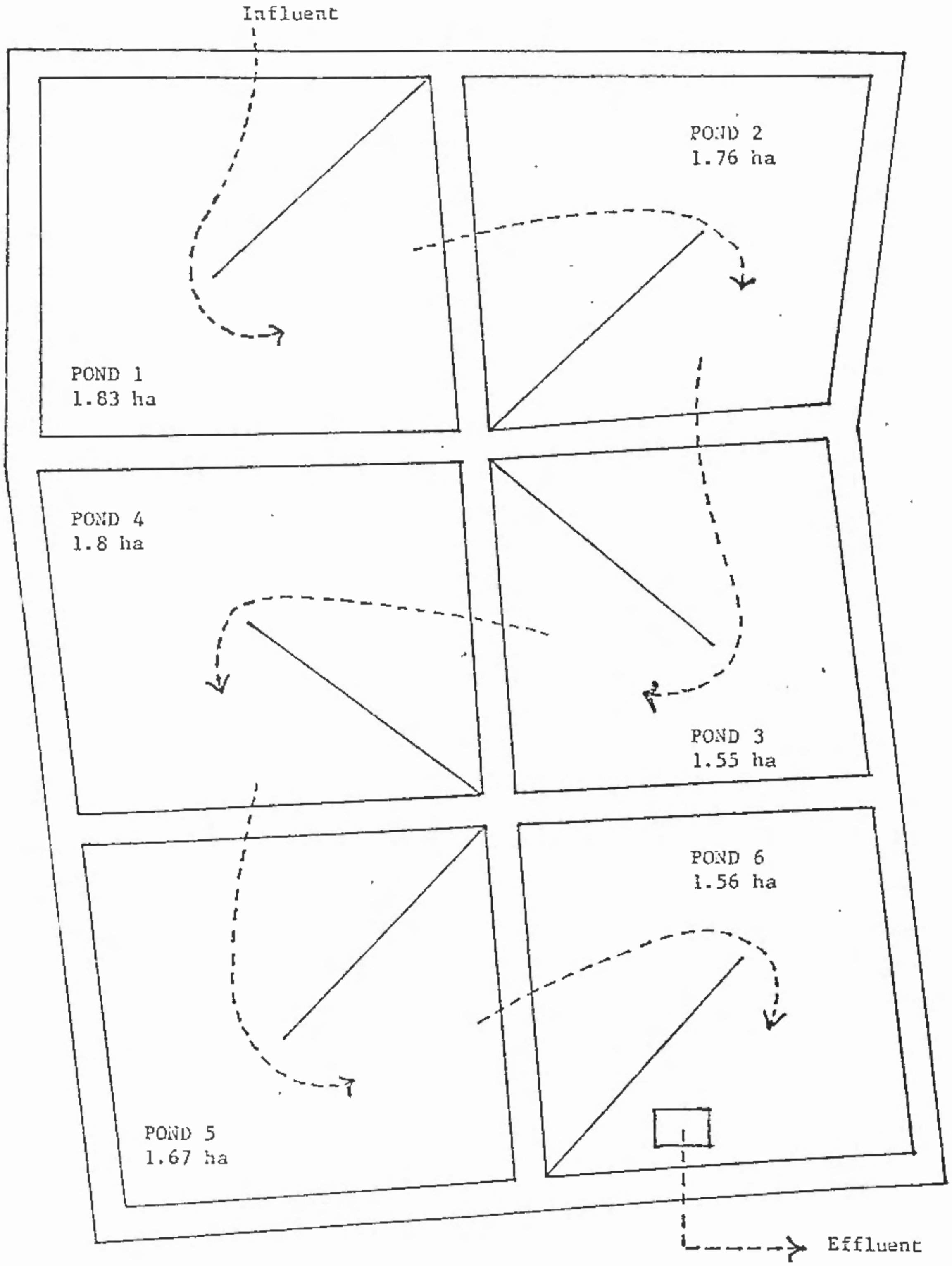
Minor alterations in the existing facility were made prior to stocking the fish and instituting routine water quality monitoring. The existing six ponds were dewatered, sludge buildup was removed and the ponds regraded to their original contour with some minor changes to facilitate the harvest of the fish. All ponds average 1.2-1.3 m in depth with the bottoms being graded to the deepest point of approximately 2 m. The flow pattern was arranged so the wastewater flows through each of the six ponds in series with the ponds numbered one-six in the order they receive the wastewater (Figure 5). All wastewater entering the plant is lifted by pumping into Pond 1 where it travels by gravity flow - drop in elevation of approximately 0.76 m (2.5 ft.) - to the surface discharge from Pond 6.

By utilizing the existing piping system, the water flows into each of the ponds at the midpoint of one levee and out an adjacent side. To prevent short-circuiting and provide maximum retention time, baffles were constructed diagonally, three-quarters of the distance across each of the ponds. The influent flow rate of 1,711 m^3/day (0.45 MDG) allows for a residence time for the water in the entire six pond system of 72 days. The individual ponds are approximately equal in size (range from 1.55-1.8 ha) with a retention time of about 12 days per pond. Four recording flow meters have been installed across the six ponds. One is placed in a six inch Parshall Flume measuring influent, two are placed at the outfall of Ponds 2 and 4, and the last at the end of the system recording effluent flow.

All wastewater flows directly into Pond 1 and then serially through the remaining ponds. Ponds 1 and 2 serve as stabilization and plankton culture ponds and were not stocked with fish. The remaining four ponds were stocked with fish as follows:

Pond 3 (1.55 ha) -	20,270	silver carp (41 g each)
	4,103	bighead carp (32 g each)
Pond 4 (1.8 ha) -	12,198	silver carp (41 g each)
	2,052	bighead carp (32 g each)
Pond 5 (1.67 ha) -	12,070	silver carp (41 g each)
	2,052	bighead carp (32 g each)
Pond 6 (1.56 ha) -	8,100	silver carp (41 g each)
	600	bighead carp (32 g each)
	600	channel catfish (300 g each)
	100	buffalofish (1.6 kg each)
	40	grass carp (0.5 kg each)

FIGURE 5. FLOW PATTERN THROUGH PONDS; PONDS 3, 4, 5, AND 6 STOCKED WITH FISH



MATERIALS AND METHODS

WATER QUALITY

During the full two year sampling period for this study, one liter grab samples were taken from the effluent of each of the six ponds. During 1979, samples were taken weekly and in 1980 sampling was done monthly. All sampling was done between 6:00 a.m. and noon. All sampling and testing was done according to APHA Standard Methods. Water quality analysis was performed by the Arkansas State Department of Pollution Control and Ecology at their lab in Little Rock. Parameters measured during the project were:

Air Temperature	Nitrite - Nitrogen
Water Temperature	Nitrate - Nitrogen
Carbon Dioxide	pH
Dissolved Oxygen	Total Suspended Solids
BOD_5	Total Phosphorus
Turbidity	Fecal Coliform
Ammonia	Plankton Enumeration

Recording flow meters were installed at the influent of the plant and at the outfall of Ponds 2, 4, and 6 for flow data and loading rate calculations.

OTHER CONTAMINANTS

Toxic Substances

Due to the need to utilize the fish produced in this treatment system to provide economic return as well as to maintain an expanding population for optimum treatment efficiency, those contaminants considered most likely to be present were monitored. APHA Standard Methods were used for all testing. Samples were taken of both water and fish flesh and delivered to American Interplex, an independent testing laboratory, and analyzed for:

Pesticide Scan	Metal Scan
Aldrin	Lead
Dieldrin	Copper
Endrin	Cadmium
Mirex	Mercury
DDT (and derivities)	Arsenic
Toxaphene	
Kepone	
PCB	

Biological Contaminants

Bacteriology. Samples of fish gut and skin were blended in a Stomacher 400 (Dynatech Laboratores, Inc.) with 10 ml of 0.1 M phosphate-buffered saline (PBS) per gram of fish tissue. Initially, samples of fish muscle were processed, but due to the low levels of bacteria in the flesh, later samples were blended in only 5 ml of PBS per gram of flesh.

Water samples were tested for fecal coliforms and fecal streptococci using the membrane filter technique. Sediment samples were mixed with 0.01 M PBS for 5 min and immediately assayed for fecal coliforms, fecal streptococci, and salmonella spp. using multiple tube techniques. Dulcitol selenite broth was used for initial enrichment of salmonella followed by streaking on brilliant green agar and identification by biochemical and serological tests.

Lactose broth was used as the initial enrichment medium for fecal coliforms. Cultures showing gas production within 48 hours were transferred to EC broth and incubated at $44.5^{\circ}C$. Gas production in EC broth within 24 hours was considered a positive test for fecal coliforms.

Azide dextrose broth was used to enrich for fecal streptococci. Inocula from tubes showing turbidity within 48 hours were streaked onto PSE agar plates. Formation of black colonies indicative of esculin hydrolysis was interpreted as a positive test for fecal streptococci.

Virology. Samples of fish flesh, skin, or guts were blended in 0.05 M glycine, pH 9.5, using a stomacher. Each 100 ml of homogenate was mixed with 10 ml of a 1% solution of Cat-Floc and centrifuged at 2,500 rpm for 30 min to clarify the suspension. The supernatant was decanted into a dialysis bag and hydroextracted overnight at 4 C. The contents of the dialysis bag were resuspended in 3% beef extract, pH 10.

All concentrates were clarified by centrifugation at 10,000 rpm for 30 minutes and by filtration through positively charged (Zeta-plus type 50S) filters. They were then treated with antibiotics prior to assay.

Samples were inoculated onto monolayers of buffalo green monkey kidney (BGM) cells in 75 cm^2 tissue culture flasks. After a 1.5 hour adsorption period, the sample was withdrawn and saved, and the bottle was rinsed with PBS to reduce cytotoxicity. An agar overlay containing neutral red was applied and the bottles were incubated at $37^{\circ}C$. Plaques that appeared on the monolayer were picked and inoculated into 1-oz BGM tissue culture bottles to confirm them as viral.

Twenty-liter samples of pond water were collected in a stainless steel pressure vessel. The samples were prefiltered, if necessary, through a 142-mm diameter 3.0-micro nominal pore size fiberglass (Filterite) filter. The sample was adjusted to pH 3.5 with 1 N HCl, and AlCl was added to a final concentration of 0.005 M. The sample was then filtered through a 3.0 to

0.45-micro Filterite series. Both filter and prefilter were eluted with 50 ml of 3% beef extract at pH 10. This elute was concentrated to a final volume of 8-12 ml by hydroextraction.

Sediment samples were mixed with 300 ml of 3% beef extract for each 100 g of sediment. This mixture was centrifuged at 1,500 rpm for 10 minutes. The supernatant was adjusted to pH 3.5 with 1 M glycine, pH 1.5. The floc that formed was sedimented by centrifugation and the sedimented material was eluted with 0.05 M glycine at pH 9.5.

RESULTS AND DISCUSSION

WATER QUALITY

The wastewater entering the plant had an average BOD_5 of 251.4 mg/l and a suspended solids concentration averaging 97 mg/l. The loading rate for the initial pond was 234.5 kg/ha/day of BOD_5 and 78.3 kg/ha/day of TSS. When this loading rate is applied to the total surface area of all six ponds, the load rate for the entire facility is 42.06 kg/ha/day of BOD and 14.2 kg/ha/day of TSS. During the two year project period, the system has reduced the BOD_5 by 96.01% and the TSS by 78.22%. Also, the effluent has been within the criteria established for secondary wastewater treatment and many parameters were at levels associated with advanced secondary treatment. A complete listing of effluent quality is presented in tabular and graphic form in Appendix I.

TOXIC SUBSTANCES

With the exception of the metals, copper and mercury, all samples have contained less than the standard detection limits or have been negative. In no instance has any sample contained the listed contaminants at levels above action guidelines established by the FDA or the Arkansas Department of Health (Appendix II).

BIOLOGICAL CONTAMINANTS

With either direct or indirect human consumption being the ultimate use of the fish, special consideration was given to human health hazards by a more intense sampling for pathogenic bacteria and viruses as well as the typically used indicator organisms. During the first year of the study, samples of both water and fish flesh were screened by American Interplex for salmonella, shigella, staphylococcus, edwardsiella, and clostidium. None of the true human pathogens were detected. This first year sampling program was considered limited and the methods used were suspect. During 1980, the sampling was expanded to include enteric viruses as well as bacteria and samples of individual tissue types from the fish along with water and sediment samples. This work was contracted to the Baylor University College of Medicine and performed by Thomas Hejkal.

Viruses and pathogenic bacteria which are present in domestic sewage present a potential health hazard to consumers of organisms grown in wastewater ponds. Vaughn and Ryther conducted studies in a model aquaculture system which used treated sewage as a nutrient supplement for primary production and found enhancement of bacteriophage survival by growing algae. Laboratory studies have shown that viruses may be accumulated by bottom-feeding fish which eat contaminated worms. Recent studies have been conducted by Buras which indicate that fish grown in ponds containing wastewater accumulate fecal bacteria and that above a threshold concentration of about 10^4 bacteria/ml detectable levels of bacteria penetrate into the muscle tissue.

Knowledge of the levels of bacteria and viruses which accumulate in fish grown in wastewater ponds and the relationship to levels in wastewater, pond water, or sediment is necessary to evaluate the public health risk. Therefore, studies were performed to determine the levels of bacteria and viruses in the water, sediment, and fish in the Benton lagoon treatment system.

Appendix III, Figure 1 illustrates the decrease in concentration of bacteria in water going from the influent to Pond 6. There was a total decrease of at least 2.5 $\log_{10}$ (99.7%) for fecal coliforms (FC) and a total decrease of 2.3 $\log_{10}$ (99.5%) for fecal streptococci (FS), based on the average concentration in each pond. The decrease was not substantially different from pond to pond. There was an average 2.6-fold decrease per pond for FC and an average 2.4-fold decrease per pond for FS.

Bacterial concentrations in the sediments followed a different pattern than in the overlying waters (Appendix III, Figure 2). There was a substantial decrease in FC from Pond 2 sediments to Pond 6 sediments. A cumulative decrease of 2.7 $\log_{10}$ was observed for FC. This represents an average 4.7-fold decrease per pond for FC in the sediments of the last four ponds.

The concentration of FS in the pond sediments decreased by only 0.4 $\log_{10}$ from Pond 2 to Pond 6. The decrease of FS in the sediments from pond to pond was substantially less than the decrease of FS in the water.

The concentrations of FC and FS in the fish guts were on the average greater than in the surrounding water and sediment (Appendix III, Table 1; Figures 1 and 2). Mean concentrations of FC and FS on the fish skin were lower than in the gut. Mean concentrations of both FC and FS in the gut were correlated with concentrations of FS on the skin with $r = 0.607$ and 0.825, respectively. There was an average 1.5-fold decrease in the levels of FC and FS in the fish from Pond 4 to Pond 6 ($p < 0.14$).

The concentrations of FC in the gut and of FS in the gut and skin were correlated with the concentration of FS in the water (Appendix III, Table 2). Concentrations of FC in the water and sediment and FS in the sediment were not significantly correlated with bacterial levels in the fish. Concentrations in the three types of fish tissue were generally correlated with each other as were concentrations of FC correlated with concentrations of FS in most types of samples (correlation coefficients not shown).

Appendix III, Table 3 shows the levels of bacteria which were detected in the fish flesh. Two methods were used for sampling the fish muscle. The samples in August and September were taken by a normal fillet procedure using a decontaminated fillet knife. Samples taken during these two months yielded sporadically high levels of FC and FS in the muscle tissue, probably due to contamination by bacteria from the fish skin. Beginning in October all muscle samples were taken aseptically to avoid contamination from the skin. Three of nine samples of muscle tissue obtained from October through December were positive for either FC or FS at low levels. Complete bacteriological data are tabulated in the Appendix.

Salmonella spp. was detected in 2 of 4 influent samples at levels of 0.4 and 2.3 MPN (most probable number)/100 ml using dulcitol selenite enrichment. Salmonella spp. was also isolated from a single water sample from Pond 2 in December. No salmonella was isolated from any of the other pond water, sediment, or fish samples.

Six of the 90 samples tested for enteric viruses yielded at least 1 PFU (plaque-forming unit) on the BGM monolayers (Appendix III, Table 4). Three of five influent samples were positive at low levels. Only a portion of each influent sample was tested for virus because of the necessity of dilution to reduce toxicity of these concentrates. The concentrations based on the number of plaques counted ranged from 7.5 to 20 PFU/liter.

Two of 15 sediment samples and one water sample from Pond 2 also yielded 1-2 PFU per sample of 500 g or 20 liters.

A single PFU was detected in the water concentrate from Pond 2 in December. All other pond water samples were negative for virus. No viruses were detected in any of the 45 fish samples processed.

Attempts were made to isolate and identify the virus from each plaque. The plaques recorded in Appendix III, Table 4 produced CPE when inoculated onto BGM monolayers under liquid overlay. However, attempts at additional passages and identification were unsuccessful. Thus, although the original plaques were virus-like, it is possible that they were caused by nonviral agents.

The sewage entering the Benton fish ponds was atypical from a virological standpoint. The levels of virus in the sewage were much lower than would be expected for untreated sewage from a larger and more diverse community. For example, concentrations in raw sewage from treatment plants in St. Petersburg, Florida, averaged 90 PFU/liter and a larger treatment plant in Tampa, Florida, concentrations of over 2,000 PFU/liter were found. The sewage entering the Benton ponds had an average concentration of $<$ 9 PFU/liter for the 5 samples tested.

The low levels of virus in the sewage in this study can be attributed to the population from which the sewage is derived. The population consists of approximately 1,000 persons residing full-time at the Benton Services Center with an additional 1,000 full-time employees. Infants and young children contribute most of the enteric virus to the wastewater of any given community, since they are the most susceptible age group to infection by these viruses. The lack of infants and young children in the population at the Benton Services Center explains the low levels of enteric viruses in the sewage from the center. Additionally, half of the population contributing to the waste load are employees who do not live at the Center. These persons would be less likely to come to work if they had an enteric viral infection and therefore would not be likely to contribute to the viral contamination of the wastewater.

Because of the low levels of virus found in the influent, the results cannot be extrapolated to make conclusions or predictions about the survival and transport of viruses in other fish pond systems that may have a much higher input of viruses. The lack of virus isolates from the fish and pond water in this study does not preclude the possibility of viruses surviving in the fish ponds and being accumulated by the fish if the initial levels of virus were higher. In fact, since relatively high levels of FC and FS were found in the ponds and fish, it is likely that viruses would also be present if the input rate were higher, since viruses generally survive inactivation processes better than do indicator bacteria.

There was little decrease in FS levels in the sediments going from Pond 2 to Pond 6. This is in contrast to the consistent decline in FC in sediment and both FC and FS in pond water. This could be due to extended survival or growth of FS in the sediments.

The levels of bacteria in the fish did not decrease substantially from Pond 4 to Pond 6. The concentrations of bacteria in the gut were highly correlated with the concentrations on the skin and showed some correlation with the levels of bacteria in the water. However, the results did not support the use of water or sediment bacterial levels as good predictors of bacterial levels in the fish. These results indicate that if indicator bacteria, and presumably pathogens, are present in the water column the silver carp and bighead carp are capable of accumulating them in their digestive tract at levels as high or higher than the levels in the water.

Since the muscle tissue is the critical portion of the fish if it is to be used for human consumption, bacterial levels in the fish muscle were a major concern. Even when levels of bacteria exceeded 10^5/100 g in the fish guts, very little penetrated into the fish muscle tissue. However, when the muscle tissue was sampled using normal filet procedures, contamination of the muscle occurred in 8 of 12 samples. The conclusion is that while the fish do not accumulate bacteria in the muscle tissue, contamination of the muscle tissue during processing is difficult to avoid.

Aquaculture-wastewater treatment systems are potentially valuable alternatives to conventional sewage treatment plants. This study shows that while concentrations of indicator microorganisms are reduced by as much as 99.7% they are not eliminated by the fish ponds. Significantly, neither do conventional activated sludge or trickling filter processes eliminate indicator organisms or pathogens. It is clear that fish or other organisms raised in wastewater have a high probability of becoming contaminated with bacteria and viruses and appropriate cautions need to be taken when these organisms are harvested and utilized for human or animal consumption.

However, the public health risk from a microbiological viewpoint may be no greater under these carefully controlled conditions than the risk from the uncontrolled harvesting of fish from waters that are contaminated by effluents from conventional sewage treatment plants.

FISH PRODUCTION

To monitor the growth rate of the fish within the system, monthly samples were taken throughout the growing season and individual fish weighed, measured, and returned to the pond. It was difficult to obtain adequate samples of species other than the silver carp due to relatively low stocking densities and the inefficiency of sampling techniques in the 1.5-1.8 hectare ponds. Ice cover and inactivity of all species also hindered sampling during winter months. As a result, some growth rate projections were made to fill in gaps in the actual sampling data.

Ponds 1 and 2 were considered to be plankton culture ponds necessary to accept the initial stock of the BOD loads and stabilize dissolved oxygen levels. Fish were stocked in Ponds 3, 4, 5, and 6. Other than the initial regrading of the pond bottoms to facilitate harvesting, no supplemental aeration or fresh water was provided to any of the fish ponds. All were left in series accepting the full flow volume and waste load as it passed through the plant. As long as the entire system functioned normally, all four of the fish ponds maintained adequate water quality for survival and growth.

As would be expected, the serial arrangement of the ponds provided increasingly better water quality in each successive pond. Pond 3 was extremely fertile with a heavy plankton bloom and typically minimum dissolved oxygen levels. Pond 4 exhibited wide fluctuations in DO levels and other water quality parameters began to stabilize. Ponds 5 and 6 remained in near optimum conditions for pond fish culture throughout the project period.

In May of 1980 after the system had been operational for 1½ years, a delivery line collapsed necessitating the flow of the total raw waste load directly into Pond 2 until repairs could be made. In the six weeks required for these repairs, the already marginal water quality in Pond 3 deterioriated until a total oxygen depletion and fish kill on July 1, 1980. Recovery of the fish from Pond 3 after the kill showed that the original stocking biomass of 374.8 kg/ha had increased to 7,165.1 kg/ha in the 18 months the fish had been in the pond. One unexplained occurrence in Pond 3 was the appearance of a fleshy growth around the mouth of the fish during the prolonged periods of stress that were prevalent just before the kill. No causative agent for this abnormality has been identified either from a specific disease organism or related uniquely to this species of Chinese carp. Similar growths on bullheads grown in sewage plant effluent have been reported.

Pond 4 was intermediate in water quality between Pond 3 and Ponds 5 and 6. Though there were wide fluctuations, the fish were never in real danger since all critically low oxygen levels were of the transient early morning type. The plant breakdown and resulting short-circuiting of the water also had a visual impact on Pond 4. The period of decreased retention time greatly added to the fertility in Pond 4. In essence, Pond 4 became Pond 3 during that period of time. The diminished water quality coupled with extremely hot, dry late summer weather and a period of cloudy days resulted in a major fish kill occurring in this pond on September 4, 1980. A total of 7,691.9 kg/ha of silver carp were removed from Pond 4 as a result of this kill. This is a considerable production in the 21 months since initial stocking with 40.6 kg/ha. A negligible few hundred pounds of surviving fish were harvested from Pond 4 at the time the entire project was terminated in January, 1981.

Ponds 5 and 6 were the only ones with all the fish surviving the full 24 month project period. Water quality remained good and no problems with the survival and growth of the fish were noted. In January of 1981, all ponds were drained and totally harvested. Pond 5 production amounted to 7,634.4 kg/ha of silver carp and 1,510.4 kg/ha of bighead carp. Pond 6, with the lower stocking rate and nutrient load, contained 4,454.7 kg/ha of silver carp and 589 kg/ha of bighead carp. Including all species of fish stocked in Pond 6 to utilize the variety of food types available, a total of 6,303.1 kg/ha were harvested. Stocking and harvest data for all species and all ponds is listed in Appendix IV.

ECONOMIC CONSIDERATIONS

This type finfish wastewater treatment system has shown the capability of upgrading the effluent of conventionally designed and operated lagoon treatment plants. However, the level of treatment is somewhat limited compared with other types of advanced treatment systems. Only when the fish produced from recycling the waste can be utilized, can the true advantages of this method of treatment be realized. If a true profit or even supplemental income to offset treatment costs can be generated, the production of fish becomes a more attractive treatment method or a viable addition to more advanced treatment practices.

According to EPA Report 600/2-76-293 entitled Economic Assessment of Wastewater Aquaculture Treatment Systems by Upton Henderson and Frank Wort, only when finfish aquaculture was not capable of meeting water quality objectives was it deemed not to be cost effective when compared to conventional systems. The report went further to state that aquaculture wastewater treatment alternatives appear to be economically attractive regardless of the market for products provided water quality goals are met.

Although there are several possibilities and likely many useful fishery products yet to be developed, it appears that the long and the short of the present market lies with the sale of these fish products as a food item or by processing it into fish meal for use as a fertilizer or animal feed supplement. It should be understood that in present day fresh water pond aquaculture the greatest overhead costs are land, feed, fertilizer, and water. By utilizing this systemof wastewater aquaculture, these costs would be borne by the primary function of water treatment. By accepting this and making some other rather basic assumptions within the framework of present markets, some rather cursory economic projections can be made.

Silver and bighead carp from a preliminary hatchery study were rendered into fish meal which assayed at a crude protein content of a minimum 55-57%. This is compared to 62% crude protein for Menhaden meal considered the best product now available. Oil and fat content were not considered. There was an estimated 18% return of meal from fresh fish by weight. Current market prices for pure fish meal, FOB Little Rock, vary from $400-500 per ton in bulk quantities depending on season and harvest source. Based on a price of 7-9 cents per kg (3-4 cents per lb.) for live fish and an annual production rate of approximately 5,000 kg/ha as demonstrated in this study, a gross return of $350-$450 per ha/yr could be realized by processing the fish in this way.

If, on the other hand, human health considerations could be mollified and the product sold for direct human consumption the economic picture could be quite different. Hatchery reared silver and bighead carp have been tested organoleptically for two different methods of preparation. As a fresh fish fillet product, the silver carp has a white, lightly oily meat that is excellent in a variety of preparations with the subjective taste test yielding comments ranging from excellent flavor to barely acceptable. However, the problem is boniness. The silver carp has many small floating bones that do not increase in size proportionately as the fish grows. This is a major problem for American tastes even with larger sized fish.

In an effort to overcome the boniness problem, Dr. Dale Ammerman, a marketing specialist with Mississippi State University, is conducting a nationwide marketing and acceptance survey with a canned silver carp product similar to salmon or tuna. The canning process makes the small bones unnoticeable and the heat involved could overcome some of the health effects problems. The survey is presently in progress and preliminary results are promising. With a 30% return to date, 67% of those responding have rated the flavor in the satisfactory-excellent range, color was rated satisfactory by 65%, and appearance and texture was considered too soft by 60%. The problem with texture is thought to be a processing and packaging problem that could be solved rather easily. A somewhat surprising result of the survey is that almost 60% of those participating have indicated they would purchase the canned product at prices higher than $2.00 per pound, some as high as $3.00 per pound can.

If the fish were marketed in either manner (fresh or canned), a conservative in the round price of 55-65 cents per kg would be reasonable. The gross amount return based on these assumptions and the demonstrated production potential would be $2,750-$3,250 per ha per year. Whatever the market, any income realized would certainly be welcomed to offset treatment costs.

DESIGN CONSIDERATIONS

In general, the factors involved in the selection, design, and construction of a finfish wastewater treatment system are the same as those historically used for conventional aerobic lagoon treatment plants. Prime consideration should be given to climate, availability of land area, and the treatment level desired or necessary. The results of this study have shown that the addition of controlled stocking of certain species and numbers of fish can increase the efficiency of lagoon treatment. Therefore, in instances where conventional lagoon design criteria indicate the system would be marginal either due to space or treatment level, the incorporation of finfish into the design could make this the method of choice.

Since the fish must survive to do the job, the most obvious criteria is that the wastewater contain no contaminants lethal to the organism. This could limit use to specific circumstances or, more likely, require in-house removal of these substances prior to treatment. Because of the flexibility needed to insure proper operation, a finfish treatment system requires a multiple lagoon design with generally a serial flow pattern. The initial impact of the BOD load from raw wastewater must be lessened by some method prior to entering the pond containing fish. Short-term peaks in loading rate are no major problem but generally the concentration of BOD_5 entering the first pond containing fish should be no more than 50 ppm annual average.

There must be the capability of draining each pond individually for maintenance and harvest of the fish while allowing continued operation of the plant. Typical pond construction is applicable with the probable need for a more carefully graded bottom with a catch basin to facilitate harvest of the fish. Little effect on water quality is seen until the standing crop of fish reaches 1,000 kg/ha. Also, larger numbers of smaller, younger fish are more efficient than fewer larger fish even though biomass may be the same. A method of harvest and replacement of the fish should be established to maintain a total standing crop between 1,000-5,000 kg/ha at all times and have a high percentage of small growing fish. Harvest and restocking should be done annually to provide maximum fish production or should be done at least every three years to prevent decreased water treatment capability.

Plant maintenance and operation would be essentially the same as conventional lagoon treatment systems. Proper management would certainly increase treatment efficiency, but with fish present, the system still maintains the favorable characteristic of having low technology and manpower needs to function suitably. If the plant is not adequately sized or fish production is of prime concern, then closer supervision would be required. As with any other system, if it is operated at full capacity in a stressed situation, closer management is needed.

There is nothing magical about a finfish system. All the uncontrollable variables of any biological treatment system are still in effect and must be considered. Aquaculture technology and the stabilizing influence of the fish on the phytoplankton populations in nutrient rich ponds actually adds another method of control, not another factor to increase the variability. The ultimate criteria is the usefulness of the fish product resulting from this treatment process. As long as water treatment remains the primary concern, the design considerations are mostly the same as conventional lagoon plants. As the utilization of the fishery products resulting from recycling the nutrients becomes more important, then many other factors affecting construction and operation must be considered.

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APPENDIX I

WATER QUALITY

Parameters Monitored	Units Reported
Water Temperature	F°
Air Temperature	F°
Turbidity	FTU
Dissolved Oxygen	mg/l
BOD_5	mg/l
pH	standard units
CO_2	mg/l
Total Suspended Solids	mg/l
NH_3-NH_4	mg/l
N-NO_2	mg/l
N-NO_3	mg/l
PO_4	mg/l
Fecal Coliform	No./100 ml
Plankton	Total No./l

APPENDIX I

TABLE 1. STRENGTH OF RAW WASTEWATER ENTERING THE TREATMENT PLANT RECORDED AS THE MEAN VALUE FOR THE PROJECT PERIOD.

Parameter	Mean Value
BOD_5	251.35
Total Suspended Solids	97.11
Turbidity	31.0
pH	6.46
NH_3-NH_4	24.24
Phos-Total	22.5

APPENDIX I

TABLE 2. STRENGTH OF INFLUENT AND EFFLUENT FOR POND NO. 1 RECORDED AS THE MEAN VALUE FOR THE PROJECT PERIOD.

Parameter	Influent	Effluent	% inc./dec. Through Pond	% inc./dec. Through System
H_2O Temp		61.593		
Air Temp		57.285		
Turbidity	31.0	39.862	28.58	Same
DO		2.077		
BOD_5	251.348	62.646	-75.07	Same
pH	6.465	7.596	17.49	Same
CO_2		33.877		
TSS	97.111	80.800	-16.796	Same
NH_3-NH_4	24.24	6.221	-74.33	Same
NO_2		0.020		
NO_3		0.018		
PO_4	22.5	3.919	-82.58	Same
Fec. Col		266697		
Plankton		3.8×10^8		

APPENDIX I

TABLE 3. STRENGTH OF INFLUENT AND EFFLUENT FOR POND NO. 2 RECORDED AS THE MEAN VALUE FOR THE PROJECT PERIOD.

Parameter	Influent	Effluent	% inc./dec. Through Pond	% inc./dec. Through System
H_2O Temp	61.593	61.206	-0.62	
Air Temp	57.285	58.410	1.96	
Turbidity	39.862	22.352	-43.92	-27.89
DO	2.077	2.618	26.04	
BOD_5	62.646	30.570	-51.2	-87.8
pH	7.596	7.951	4.67	
CO_2	33.877	20.375	-39.85	
TSS	80.800	46.940	-41.9	-51.66
NH_3-NH_4	6.221	4.956	-20.33	-79.55
NO_2	0.020	0.048	140	
NO_3	0.018	0.026	44.44	
PO_4	3.919	2.954	-24.6	-86.8
Fec. Col	266697	15414.2	-94.22	
Plankton	3.8×10^8	2.36×10^8	-37.89	

APPENDIX I

TABLE 4. STRENGTH OF INFLUENT AND EFFLUENT FOR POND NO. 3 RECORDED AS MEAN VALUE FOR THE PROJECT PERIOD.

Parameter	Influent	Effluent	% inc./dec. Through Pond	% inc./dec. Through System
H_2O Temp	61.206	61.706	0.81	
Air Temp	58.410	57.351	-1.8	
Turbidity	22.352	16.078	-28.06	-48.13
DO	2.618	6.874	139.17	
BOD_5	30.570	24.135	-21.05	-90.39
pH	7.951	8.367	5.23	29.41
CO_2	20.375	9.102	-55.32	
TSS	46.940	47.050	0.23	-51.5
NH_3-NH_4	4.956	3.215	-35.12	-86.73
NO_2	0.048	0.071	47.9	
NO_3	0.026	0.071	173.07	
PO_4	2.954	2.572	-12.93	-88.56
Fec. Col	15414.78	1514.78	-90.17	
Plankton	2.36×10^8	3.5×10^8	48.30	

APPENDIX I

TABLE 5. STRENGTH OF INFLUENT AND EFFLUENT FOR POND NO. 4 RECORDED AS MEAN VALUE FOR THE PROJECT PERIOD.

Parameter	Influent	Effluent	% inc./dec. Through Pond	% inc./dec. Through System
H_2O Temp	61.706	61.741	0.05	
Air Temp	57.351	57.909	0.97	
Turbidity	16.078	12.138	-24.5	-60.84
DO	6.874	5.678	-17.39	
BOD_5	24.135	14.810	-38.63	-94.10
pH	8.367	8.185	-2.17	26.6
CO_2	9.102	9.469	4.03	
TSS	47.050	25.440	-45.92	-73.8
NH_3-NH_4	3.215	2.422	-24.66	-90.01
NO_2	0.071	0.172	142.25	
NO_3	0.071	0.138	94.36	
PO_4	2.572	2.331	-9.37	-89.64
Fec. Col	1514.78	509.102	-66.39	
Plankton	3.5×10^8	1.3×10^8	-62.85	

APPENDIX I

TABLE 6. STRENGTH OF INFLUENT AND EFFLUENT FOR POND NO. 5 RECORDED AS MEAN VALUE FOR THE PROJECT PERIOD.

Parameter	Influent	Effluent	% inc./dec. Through Pond	% inc./dec. Through System
H_2O Temp	61.741	62.137	0.64	
Air Temp	57.909	58.321	0.71	
Turbidity	12.138	8.333	-31.34	-73.11
DO	5.678	6.915	21.78	
BOD_5	14.810	10.754	-27.38	-95.72
pH	8.185	8.185	0	26.60
CO_2	9.469	6.693	-29.31	
TSS	25.440	21.152	-16.85	-78.22
NH_3-NH_4	2.422	1.125	-53.55	-95.35
NO_2	0.172	0.076	-55.8	
NO_3	0.138	0.381	176	
PO_4	2.331	1.980	-15.05	-91.2
Fec. Col	509.102	52.283	-89.73	
Plankton	1.3×10^8	1.25×10^8	- 3.84	

APPENDIX I

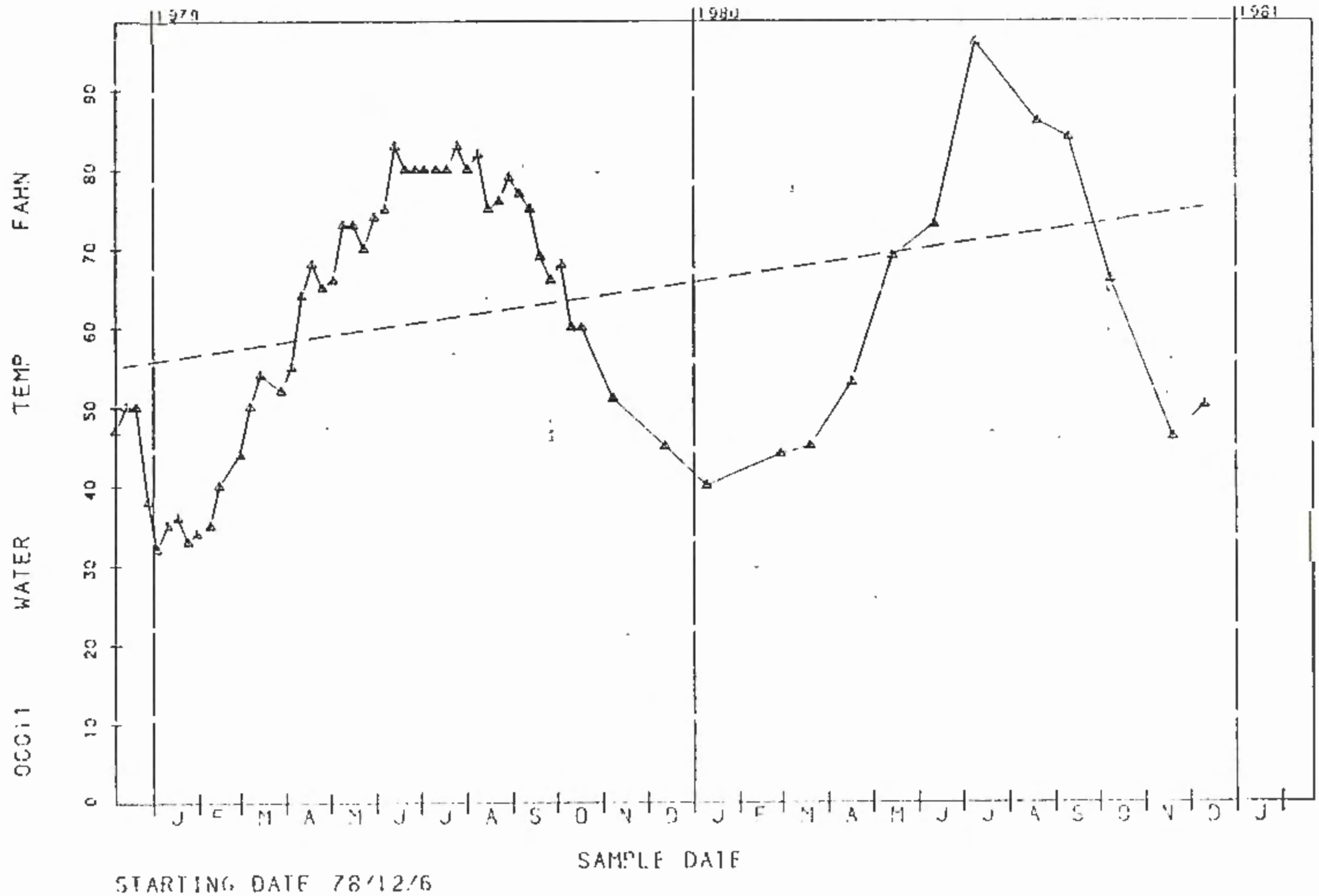
TABLE 7. STRENGTH OF INFLUENT AND EFFLUENT FOR POND NO. 6 RECORDED AS MEAN VALUE OF THE PROJECT PERIOD.

Parameter	Influent	Effluent*	% inc./dec. Through Pond	% inc./dec.** Through System
H_2O Temp	62.137	61.965	-0.27	
Air Temp	58.321	58.163	-0.27	
Turbidity	8.333	8.375	0.54	-72.98
DO	6.915	7.435	7.52	
BOD_5	10.754	10.027	-6.76	-96.01
pH	8.185	8.270	1.03	27.91
CO_2	6.693	6.734	0.61	
TSS	21.152	20.559	-2.80	-78.82
NH_3-NH_4	1.125	1.251	11.2	-94.83
NO_2	0.076	0.092	21.05	
NO_3	0.381	0.326	-14.43	
PO_4	1.980	1.961	-0.95	-91.28
Fec. Col	52.283	68.474	30.96	
Plankton	1.25×10^8	1.23×10^8	-1.6	

*Final plant effluent
**% improvement of final discharge

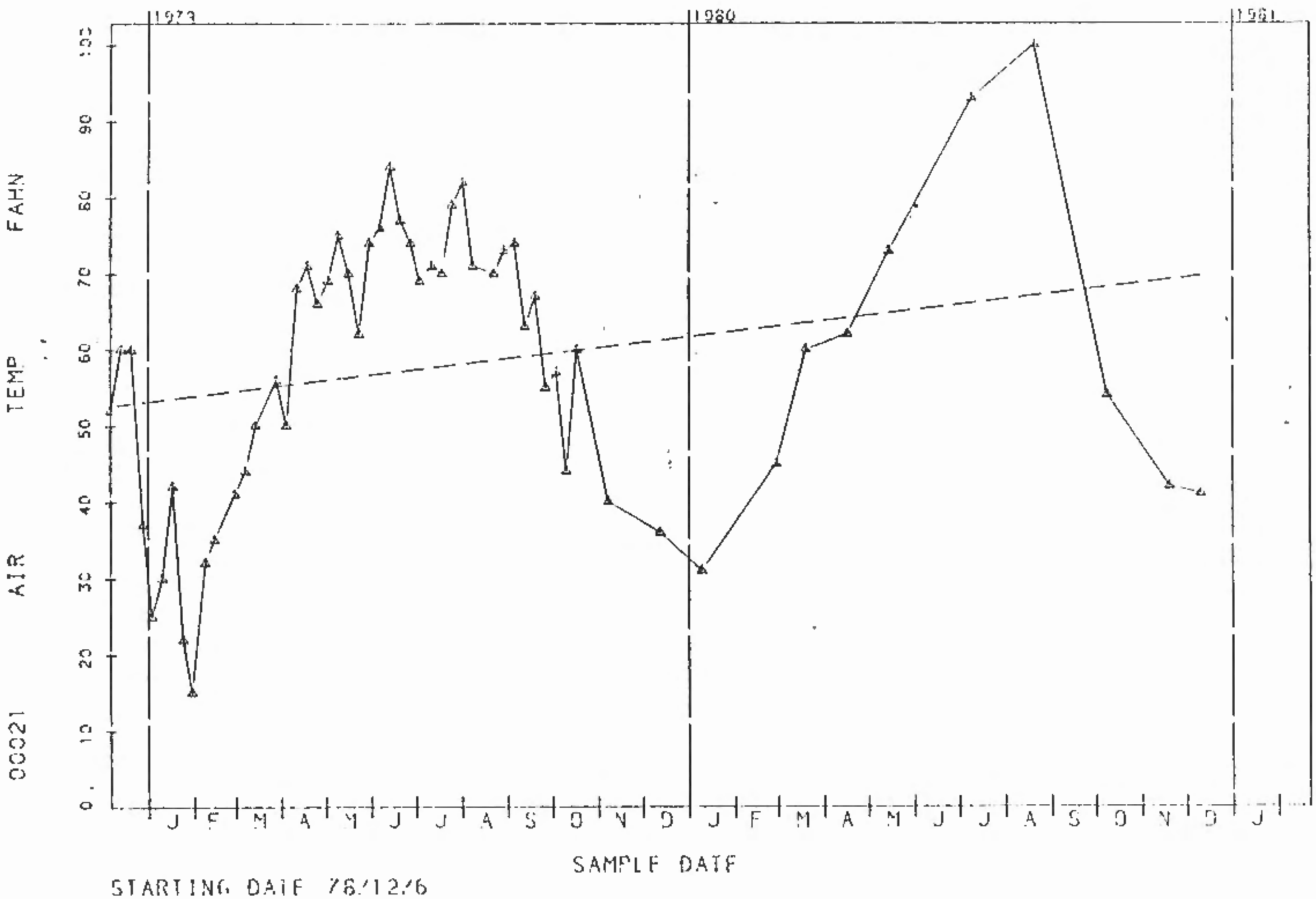
APPENDIX I

Figure 1. Water Temperature vs. time for final effluent from Benton Services Center treatment plant. Broken line represents best fit for the two year sampling period.



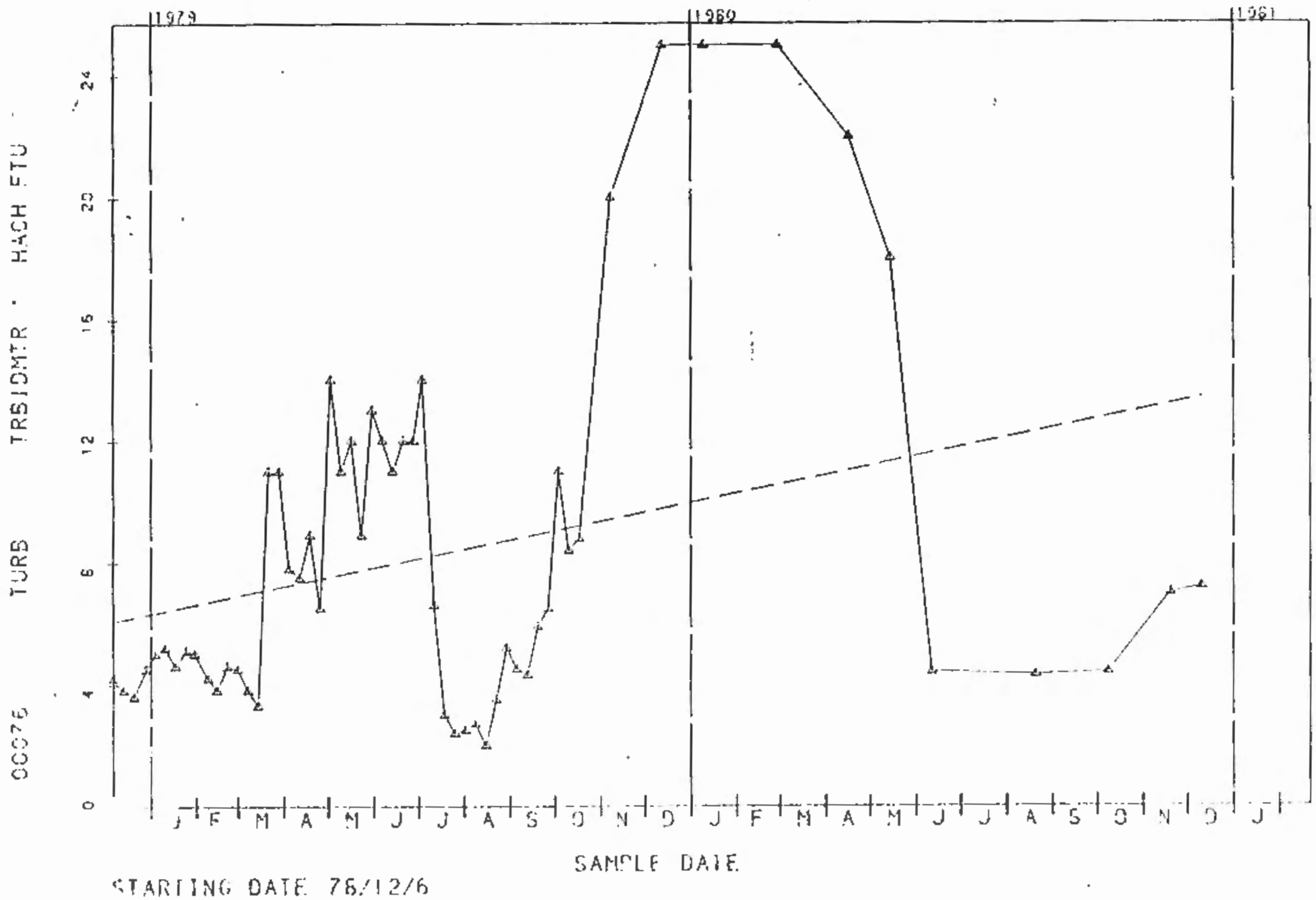
APPENDIX I

Figure 2. Air temperature vs. time for final effluent from Benton Services Center treatment plant. Broken line represents best fit for the two year sampling period.



APPENDIX I

Figure 3. Turbidity vs. time for final effluent from Benton Services Center treatment plant. Broken line represents best fit for the two year sampling period.



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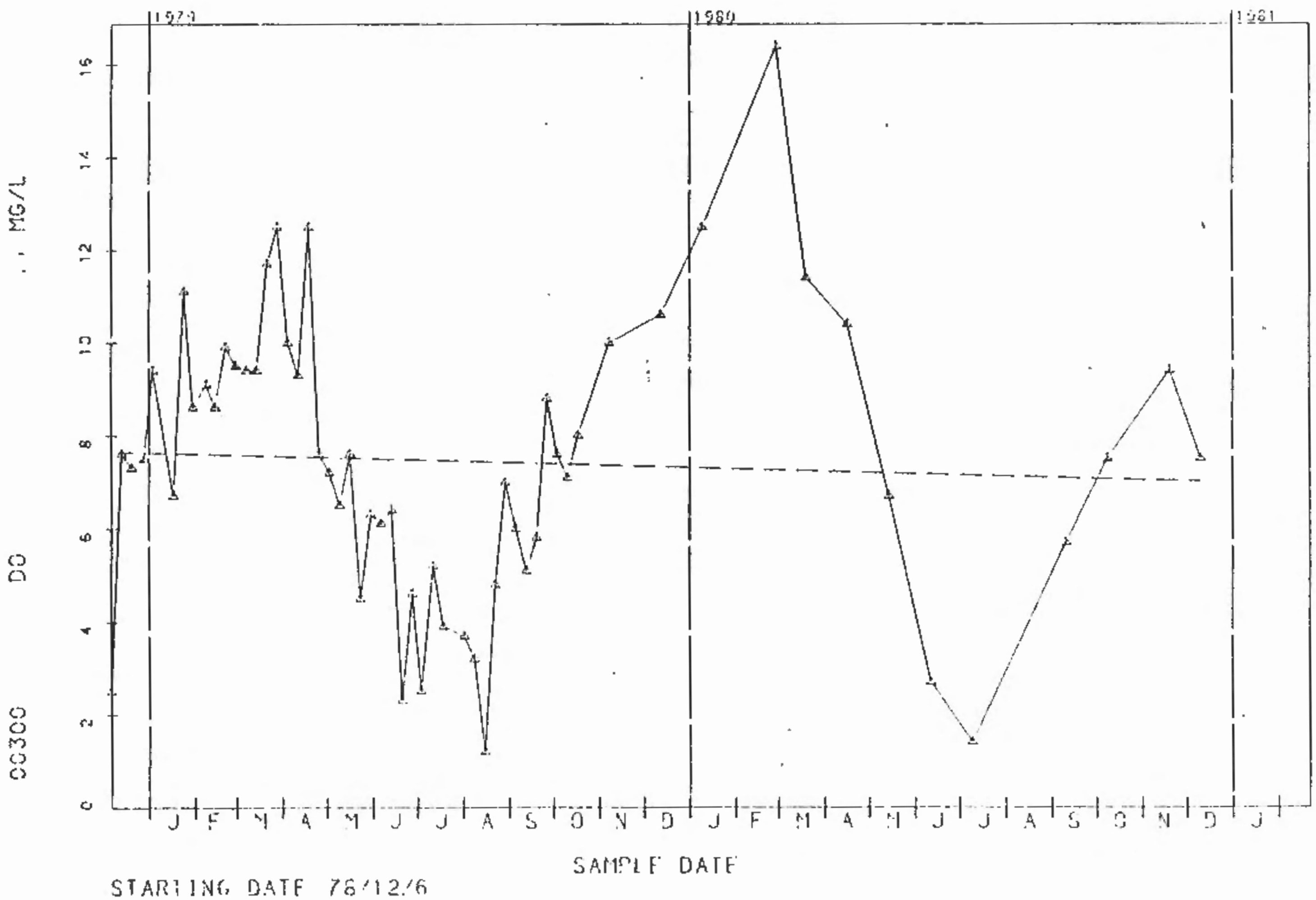
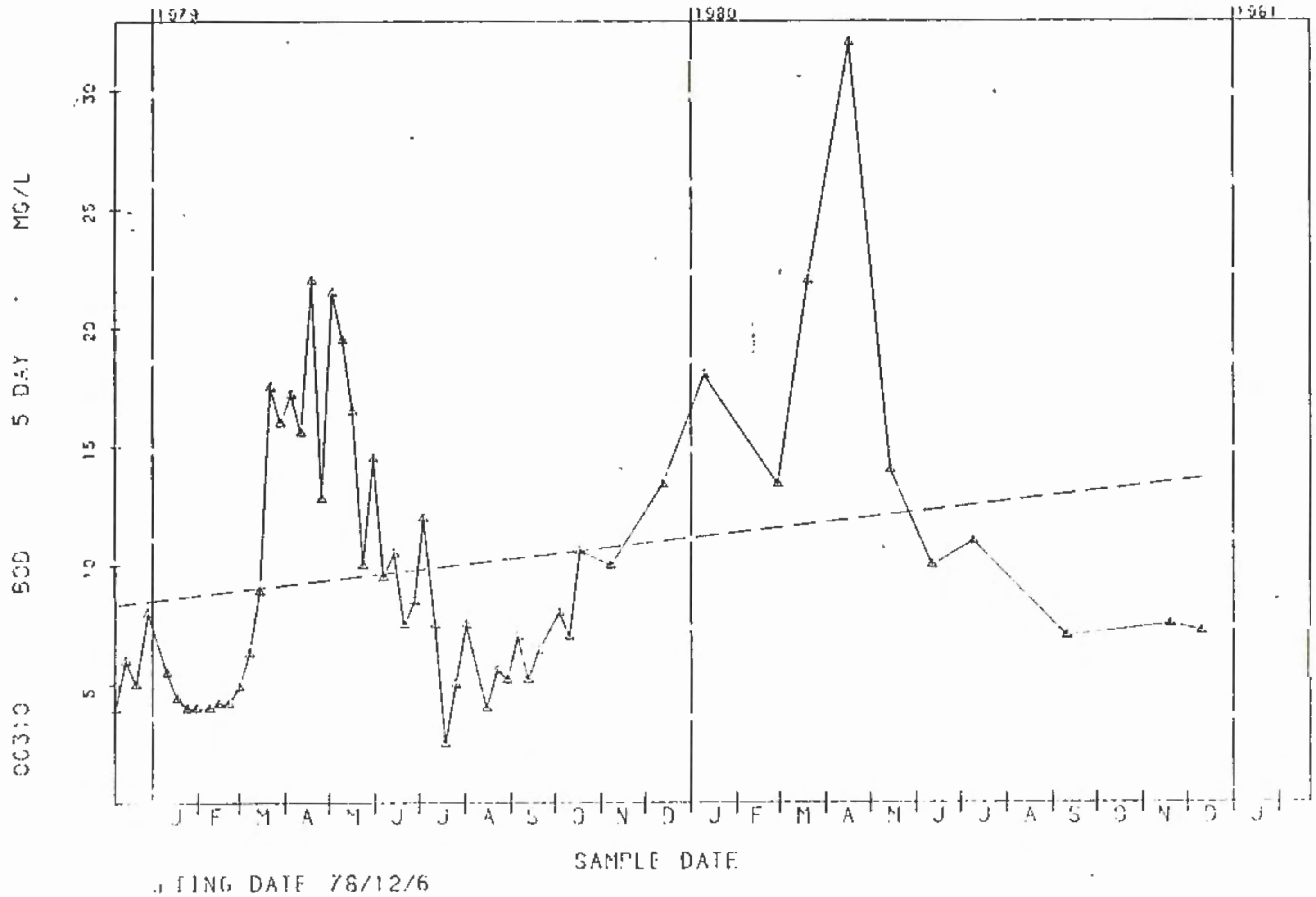
APPENDIX I

Figure 4. Dissolved oxygen vs. time for final effluent from Benton Services Center treatment plant. Broken line represents best fit for the two year sampling period.



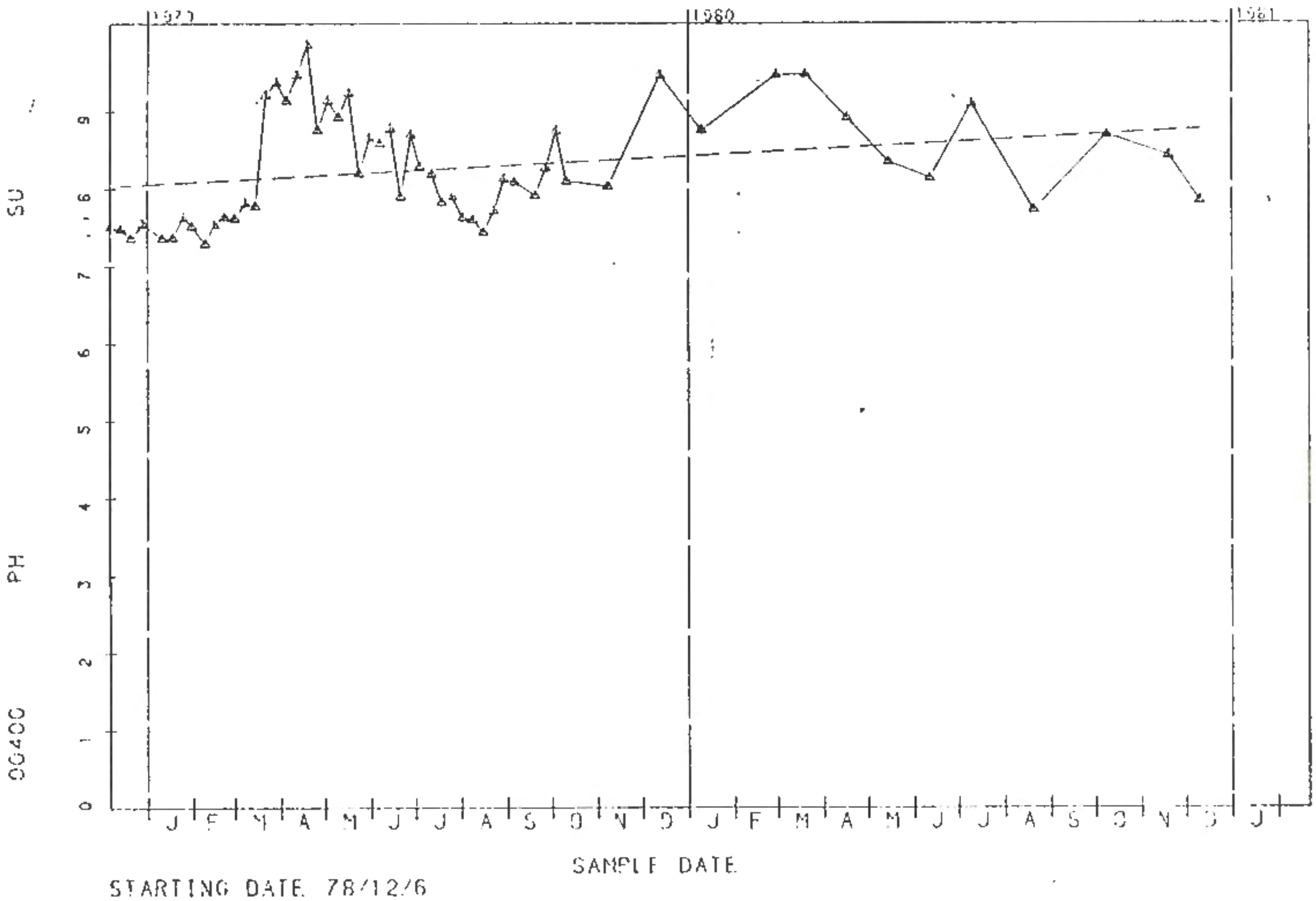
APPENDIX I

Figure 5. BOD_5 vs. time for final effluent from Benton Services Center treatment plant. Broken line represents best fit for the two year sampling period.



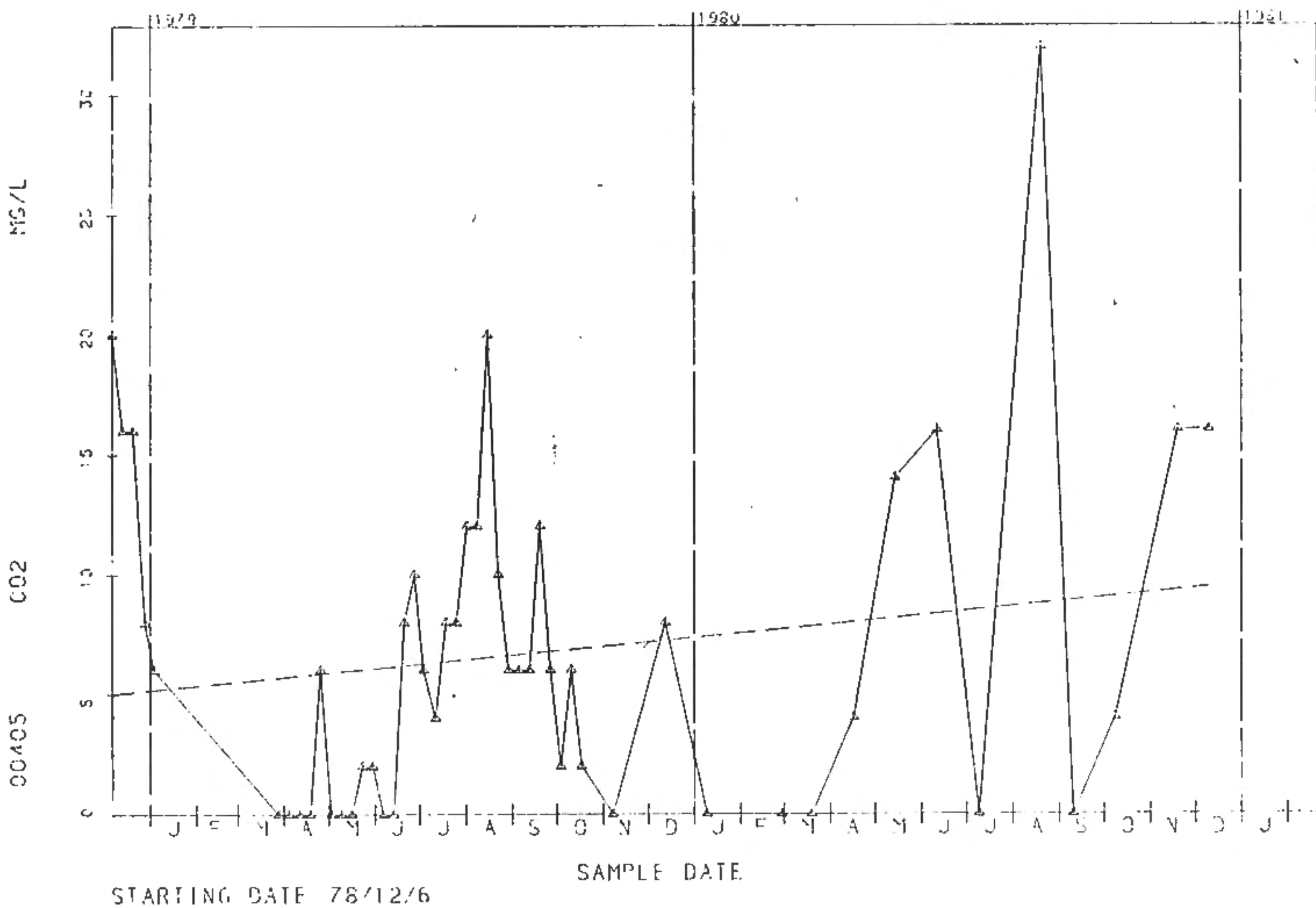
APPENDIX I

Figure 6. pH vs. time for final effluent from Benton Services Center treatment plant. Broken line represents best fit for the two year sampling period.



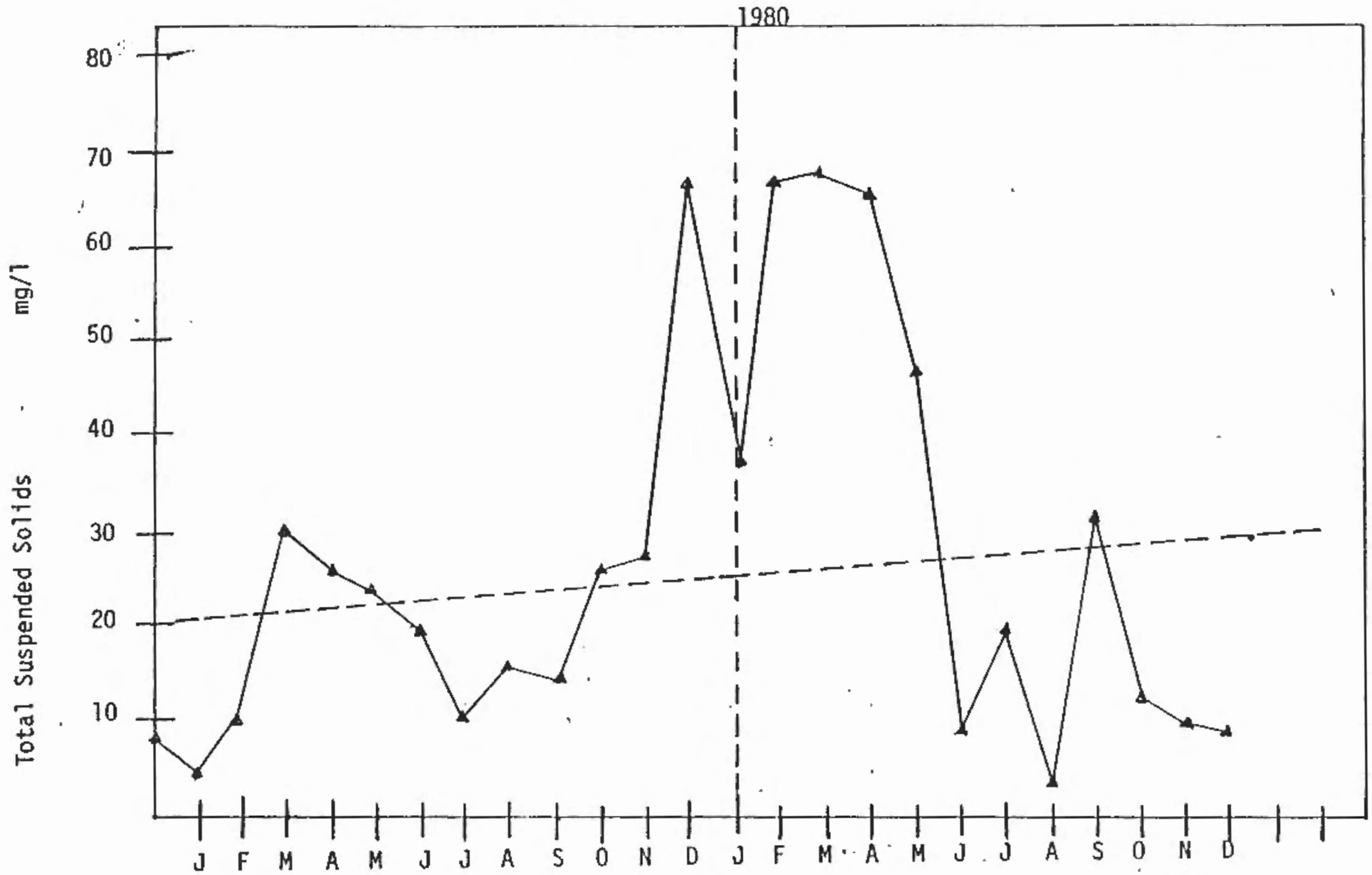
APPENDIX I

Figure 7. Carbon dioxide vs. time for final effluent from Benton Services Center treatment plant. Broken line represents best fit for the two year sampling period.



APPENDIX I

Figure 8. Total suspended solids vs. time for final effluent from Benton Services Center treatment plant. Broken line represents best fit for the two year sampling period.



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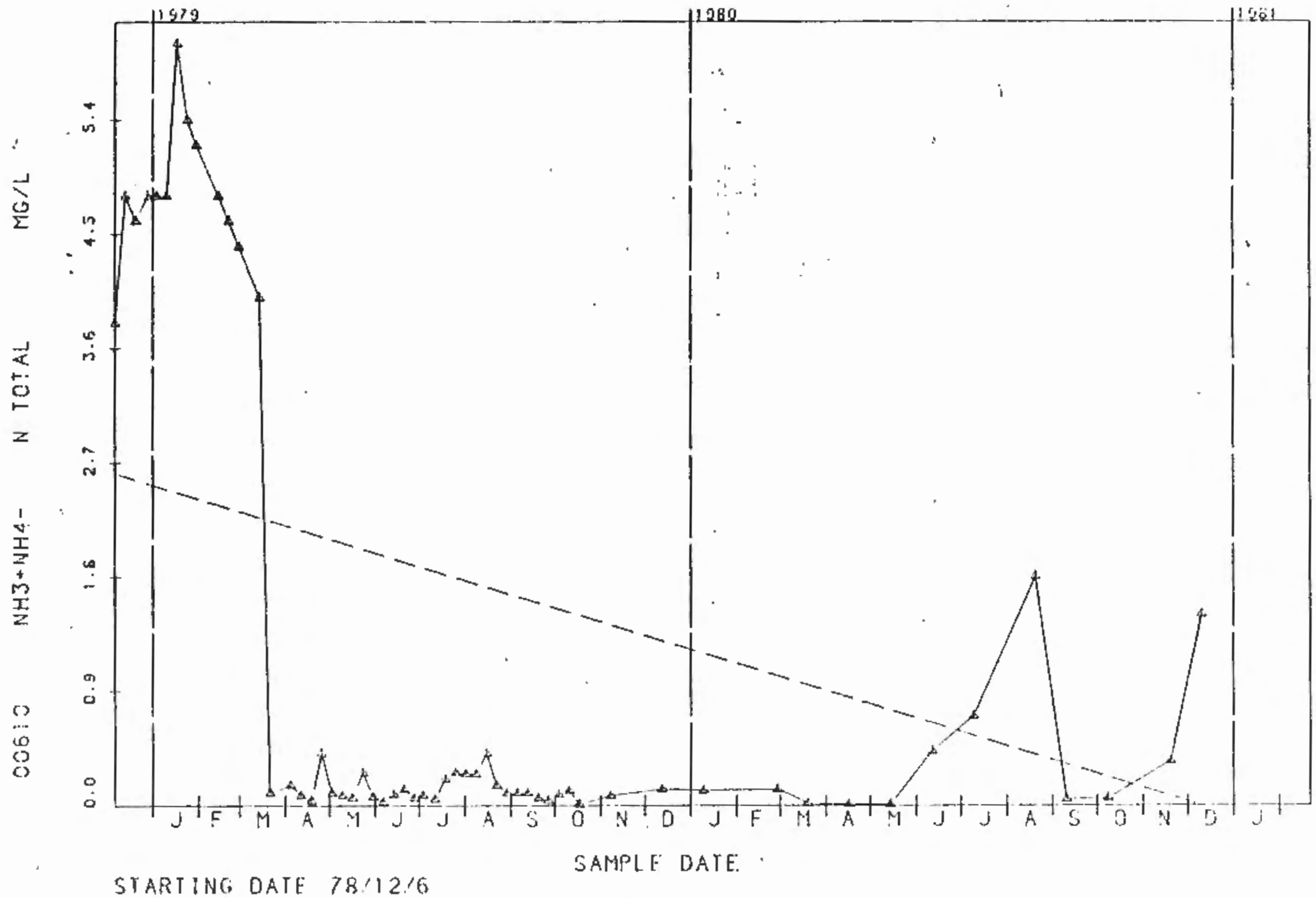
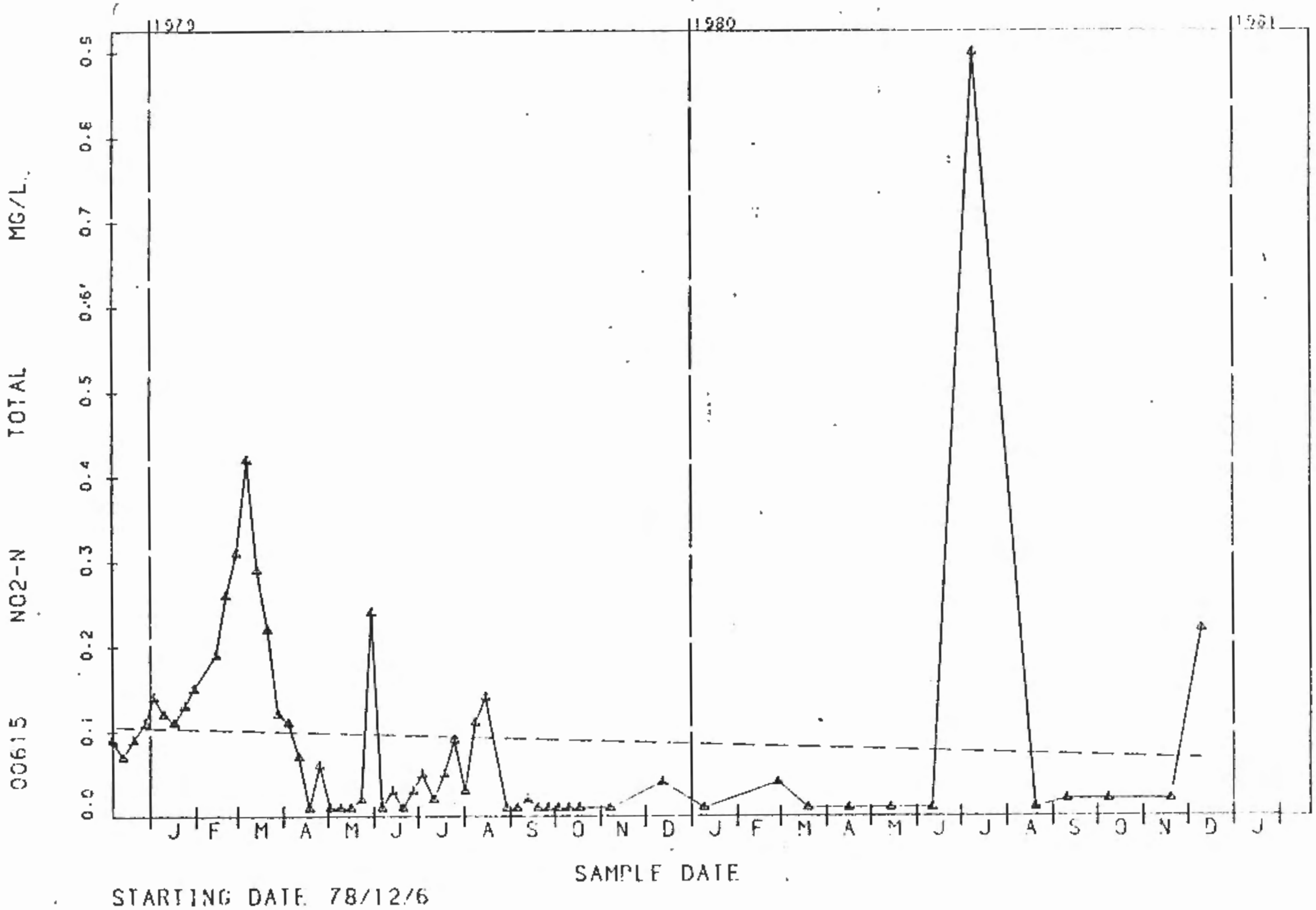
APPENDIX I

Figure 9. NH_3-NH_4 vs. time for final effluent from Benton Services Center treatment plant. Broken line represents best fit for the two year sampling period.



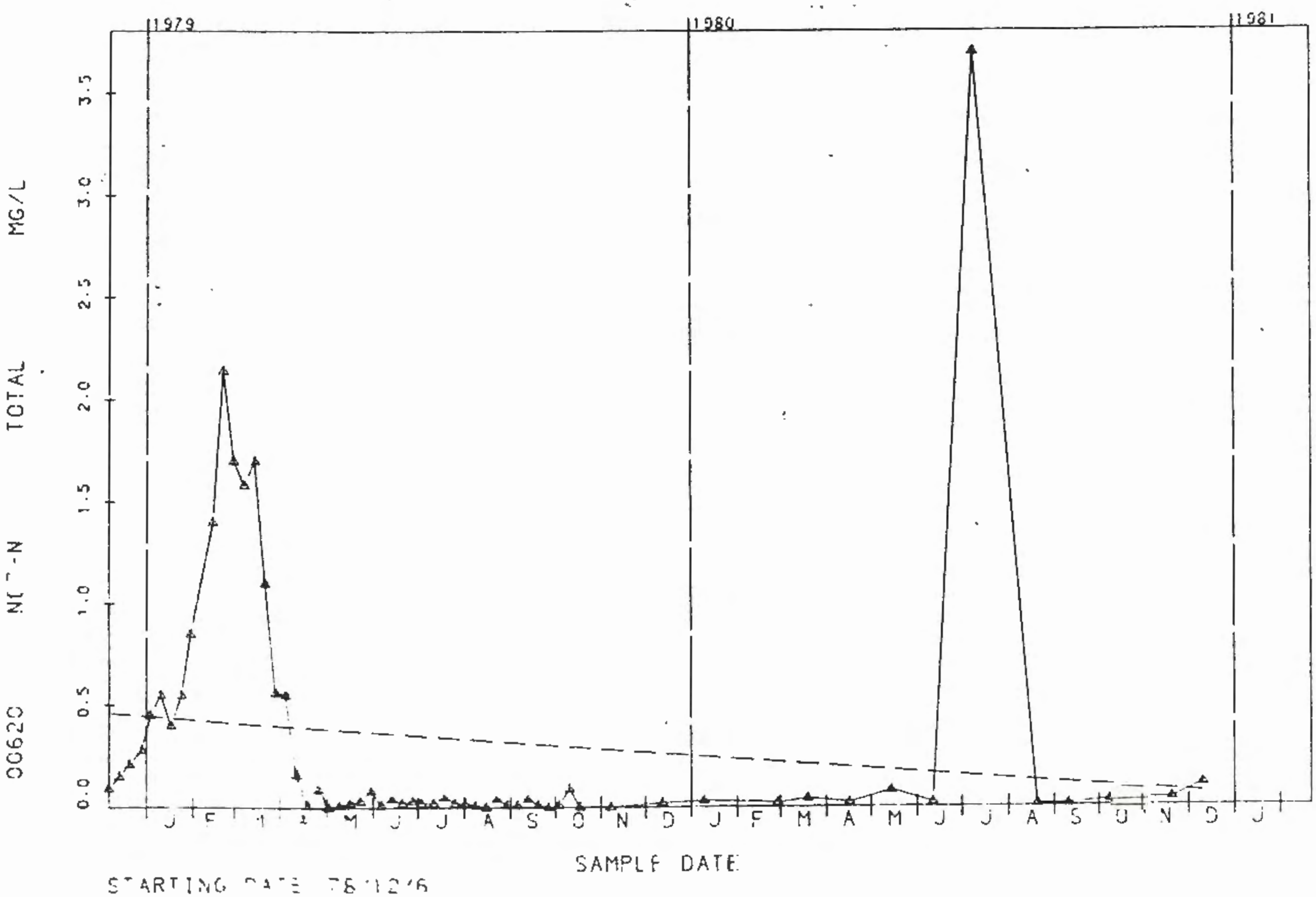
APPENDIX I

Figure 10. NO_2-N vs. time for final effluent from Benton Services Center treatment plant. Broken line represents best fit for the two year sampling period.



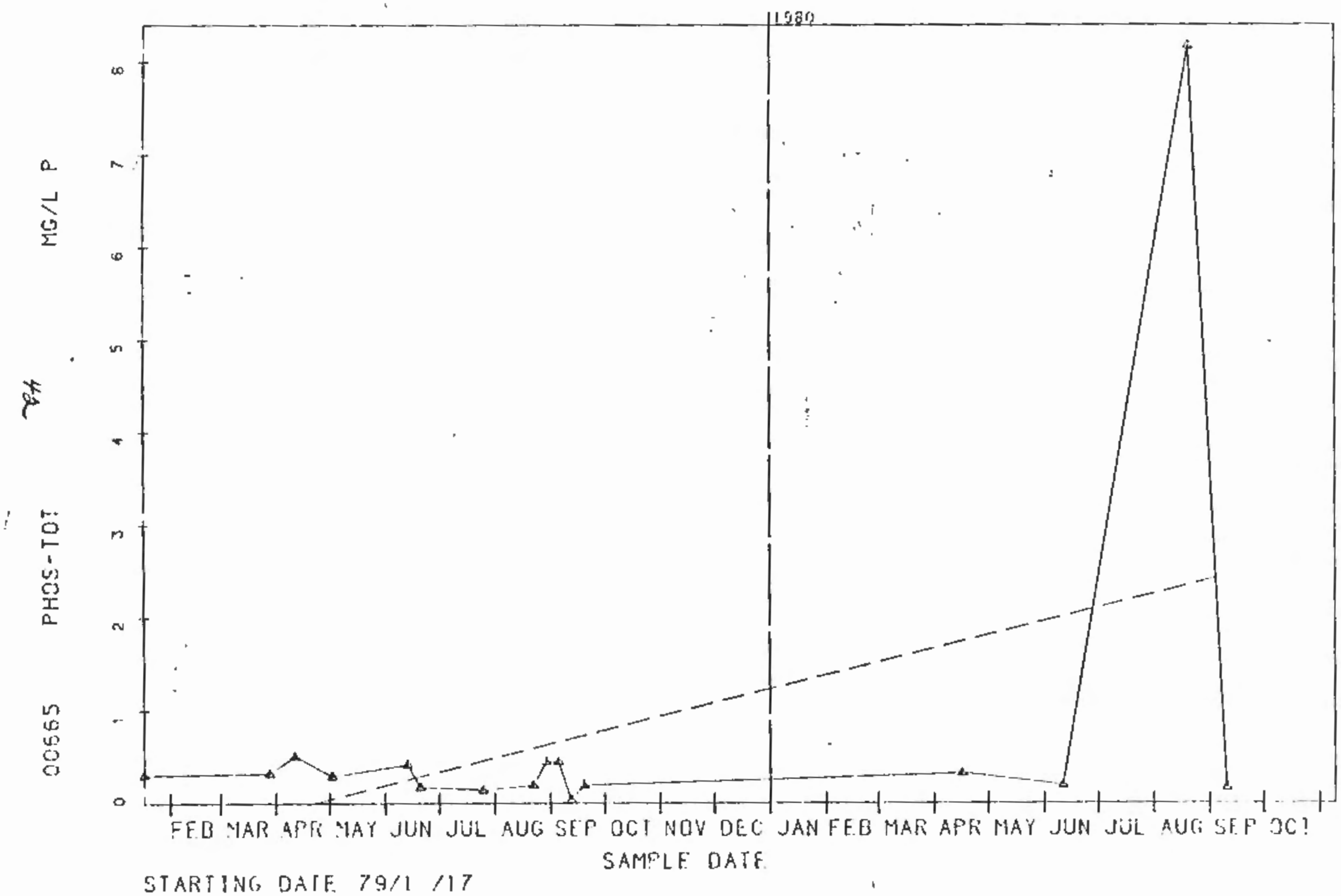
APPENDIX I

Figure 11. NO_3-N vs. time for final effluent from Benton Services Center treatment plant. Broken line represents best fit for the two year sampling period.



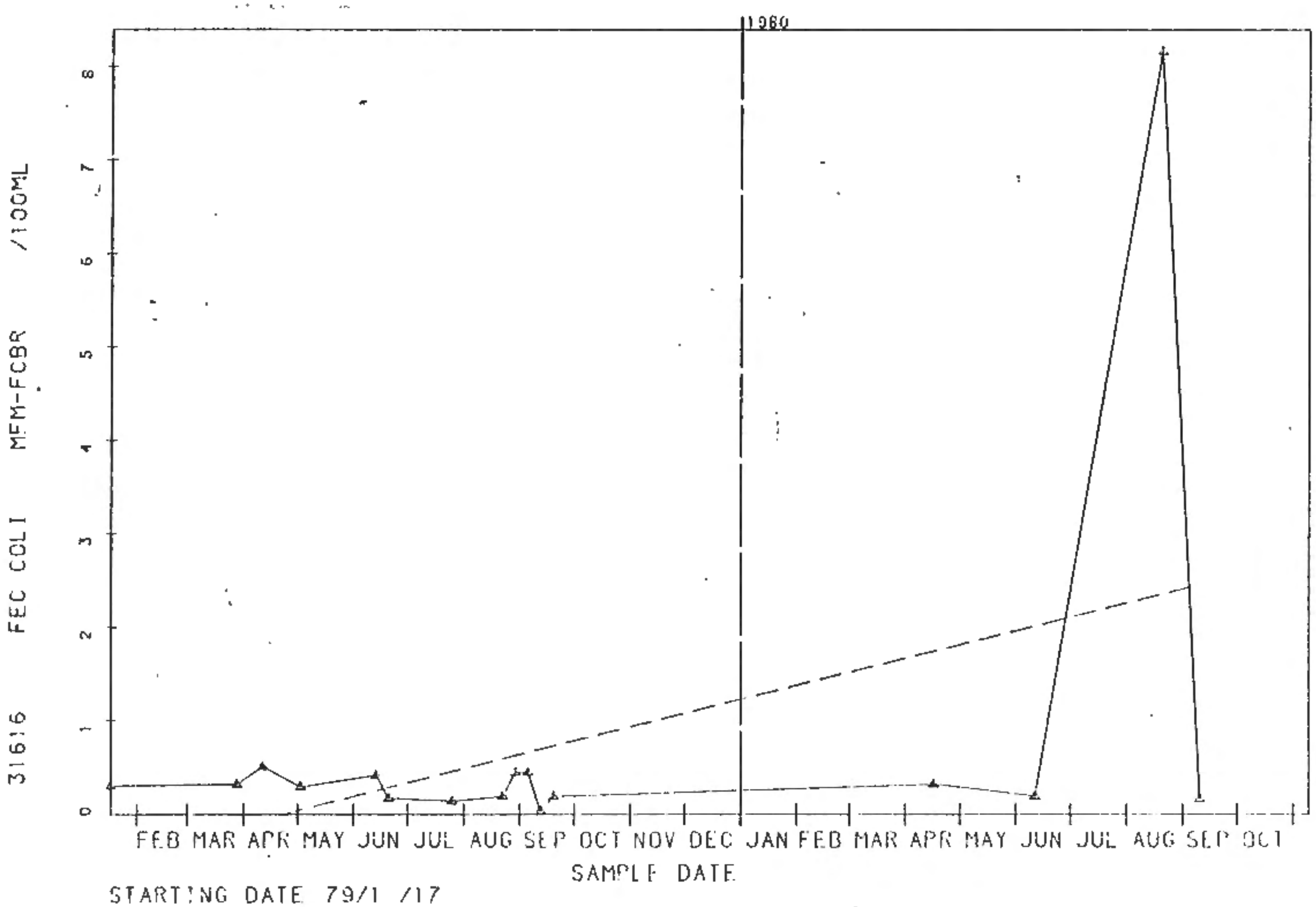
APPENDIX I.

Figure 12. PO_4-Total vs. time for final effluent from Benton Services Center treatment plant. Broken line represents best fit for the two year sampling period.



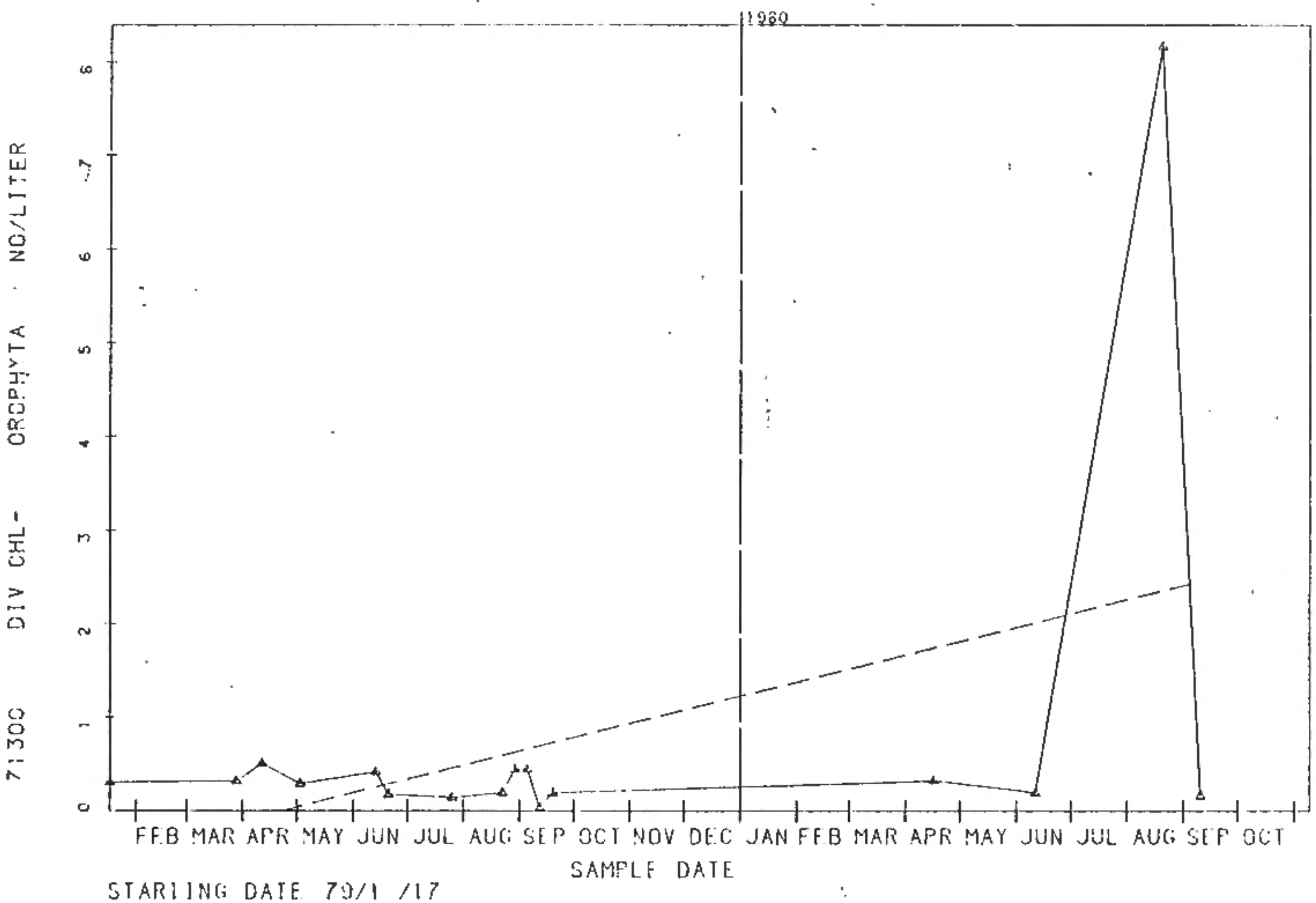
APPENDIX I

Figure 13. Fecal Coliform vs. time for final effluent from Benton Services Center treatment plant. Broken line represents best fit for the two year sampling period.



APPENDIX I

Figure 14. Total plankton organism vs. time for final effluent from Benton Services Center treatment plant. Broken line represents best fit for the two year sampling period.



APPENDIX II

PESTICIDES AND HEAVY METALS

Parameters Monitored	Units Reported
Aldrin	ppb
Dieldrin	ppb
Endrin	ppb
Mirex	ppb
DDT and derivatives	ppb
Toxaphene	ppb
Kepone	ppb
PCB	ppb
Lead	ppm
Copper	ppm
Cadmium	ppm
Mercury	ppm
Arsenic	ppm

APPENDIX II

TABLE 1. MEASURED LEVELS OF SELECTED PESTICIDES FROM WATER SAMPLES TAKEN AT VARIOUS SITES IN THE SYSTEM DURING THE PROJECT PERIOD.

Sample site	Date	Aldrin	Dieldrin	Endrin	Mirex	DDT	Toxaphene	Kepone
Influent	11-78	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<20.0
"	3-79	"	"	"	"	"	"	"
"	6-79	"	"	"	"	"	"	"
"	9-79	"	"	"	"	"	"	"
"	12-79	"	"	"	"	"	"	"
"	9-80	"	"	"	"	"	"	"
Pond 2	9-80	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<20.0
Pond 3	3-79	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<20.0
"	9-79	"	"	"	"	"	"	"
Pond 4	3-79	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<20.0
"	6-79	"	"	"	"	"	"	"
"	12-79	"	"	"	"	"	"	"
"	9-80	"	"	"	"	"	"	"
Pond 5	6-79	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<20.0
"	9-79	"	"	"	"	"	"	"
Pond 6	3-79	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<20.0
"	6-79	"	"	"	"	"	"	"
"	12-79	"	"	"	"	"	"	"
"	9-80	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<20.0
Detection Limit		1.0	1.0	1.0	1.0	1.0	1.0	20.0

APPENDIX II

TABLE 2. MEASURED LEVELS OF SELECTED PESTICIDES TAKEN FROM FISH FLESH SAMPLES FROM VARIOUS SITES IN THE SYSTEM DURING THE PROJECT PERIOD.

Sample site	Date	Aldrin	Dieldrin	Endrin	Mirex	DDT	Toxaphene	Kepone
Composite of fish prior to stocking	11-78	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Pond 3	3-79	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
"	6-79	"	"	"	"	"	"	"
"	9-79	"	"	"	"	"	"	"
Pond 4	12-79	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
"	9-80	"	"	"	"	"	"	"
Pond 5	6-79	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
"	12-79	"	"	"	"	"	"	"
"	9-80	"	"	"	"	"	"	"
Pond 6	3-79	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
"	12-79	"	"	"	"	"	"	"
"	9-80	"	"	"	"	"	"	"
Detection Limit		0.1	0.1	0.1	0.1	0.1	0.1	0.1
Acceptable Limit for edible fish portions		0.3	0.3	0.3	0.1	5.0	5.0	0.3

APPENDIX II

TABLE 3. MEASURED LEVELS OF PCB AND SELECTED METALS FROM WATER SAMPLES TAKEN AT VARIOUS SITES IN THE SYSTEM DURING THE PROJECT PERIOD.

Sample site	Date	PCB	Lead	Copper	Cadmium	Mercury	Arsenic
Influent	11-78	<1.0	<0.05	0.017	<0.002	0.001	<0.002
"	3-79	"	"	0.024	"	0.0007	"
"	6-79	"	"	0.017	"	0.0006	"
"	9-79	"	0.15	0.23	"	0.0157	0.013
"	12-79	"	<0.05	0.021	"	0.0017	<0.002
"	9-80	"	"	< 0.01	"	0.0022	"
Pond 2	9-80	<1.0	<0.05	<0.01	<0.01	<0.0005	<0.002
Pond 3	3-79	<1.0	<0.05	< 0.01	<0.002	0.00038	<0.002
"	9-79	"	<0.13	"	"	<0.0002	0.0077
Pond 4	3-79	<1.0	<0.05	<0.01	<0.002	0.00054	<0.002
"	6-79	"	"	"	"	<0.0002	0.0043
"	12-79	"	"	"	"	0.00078	0.0047
"	9-80	"	"	"	"	<0.0002	<0.002
Pond 5	6-79	<1.0	<0.05	<0.01	<0.002	<0.0002	<0.002
"	9-79	"	"	"	"	0.00043	0.008
Pond 6	3-79	<1.0	<0.05	0.014	<0.002	0.0005	<0.002
"	6-79	"	"	<0.01	"	0.00065	0.0031
"	12-79	"	"	"	"	0.0015	0.0044
"	9-80	"	"	"	"	0.0005	<0.002
Detection Limits		1.0	0.05	0.01	0.002	0.0002	0.002

APPENDIX II

TABLE 4. MEASURED LEVELS OF PCB AND SELECTED METALS FROM FISH FLESH SAMPLES FROM VARIOUS SITES IN THE SYSTEM DURING THE PROJECT PERIOD.

Sample site	Date	PCB	Lead	Copper	Cadmium	Mercury	Arsenic
Composite of fish prior to stocking	11-78	<0.1	0.57	0.77	0.049	0.063	<0.02
Pond 3	3-79	<0.1	<0.5	2.97	<0.02	0.45	<0.02
"	6-79	"	"	0.54	"	0.079	"
"	9-79	"	"	0.60	"	0.64	"
Pond 4	12-79	<0.1	1.7	30.0	<0.02	0.099	<0.02
"	9-80	"	<0.5	0.3	"	<0.01	"
Pond 5	6-79	<0.1	<0.5	0.61	<0.02	0.131	<0.02
"	9-79	"	"	1.12	"	0.77	"
"	9-80	"	"	0.68	"	0.044	"
Pond 6	3-79	<0.1	<0.5	1.55	<0.02	0.263	<0.02
"	12-79	"	"	0.67	"	1.3	"
"	9-80	"	"	0.31	"	0.061	"
Detection Limits		0.1	0.5	0.01	0.02	0.01	0.02
Acceptable Limit for edible fish portion		5.0	---	n/a	1.3-3.0	0.5	10-20

APPENDIX III

BIOLOGICAL CONTAMINANTS

Parameters monitored in water, sediment, fish skin, fish gut, fish flesh (edible portion).

Fecal Coliform
Fecal Streptococci
Enteric Viruses

APPENDIX III

TABLE 1. Average concentrations of fecal coliforms (FC) and fecal streptococci (FS) in fish gut and skin.

	Average concentration (log /100 g)			
	FC		FS	
	Gut	Skin	Gut	Skin
Pond 4	3.07	2.00	4.36	3.57
Pond 5	3.01	2.43	4.03	3.47
Pond 6	2.73	2.21	3.75	2.95

APPENDIX III

TABLE 2. Correlations between bacterial levels in fish and in water or sediment

Variable	FC water	FC sediment	FS water	FS sediment
FC gut	0.559	0.097	0.712*	-0.251
FC skin	0.019	-0.423	-0.029	-0.217
FC flesh	0.357	0.003	0.330	0.099
FS gut	0.307	0.280	0.646*	-0.236
FS skin	0.363	0.525	0.691*	0.370
FS flesh	0.455	0.537	0.375	0.630*

*Significant at $p < 0.05$.

APPENDIX III

TABLE 3. Concentrations of FC and FS in fish flesh

	MPN/100 g fish flesh					
	Pond 4		Pond 5		Pond 6	
Month	FC	FS	FC	FS	FC	FS
Aug.*	<30	140	<30	140	40	860
Sept.*	230	80	430	22,000	<30	<60
Oct.	<11	25	<11	<11	<6.6	15
Nov.	11	<11	<11	<11	<11	<11
Dec.	<11	<15	<11	<15	<11	<15

* August and September samples were taken by a normal filet procedure with possible contamination from the skin. All other flesh samples were taken aseptically.

APPENDIX III

TABLE 4. List of samples which yielded plaque-forming units (PFU) on cell monolayers

Sample	Month	Total PFU counted*	Estimated concentration**
Influent	Aug.	5	20 PFU/liter
Influent	Nov.	3	15 PFU/liter
Influent	Dec.	2	7.5 PFU/liter
Pond 2 sediment	Sept.	2	2 PFU/500 g
Pond 4 sediment	Nov.	1	1 PFU/500 g
Pond 2 water	Dec.	1	0.05 PFU/liter

* Unidentified.

** Dilution factors varied for influent samples.

APPENDIX III

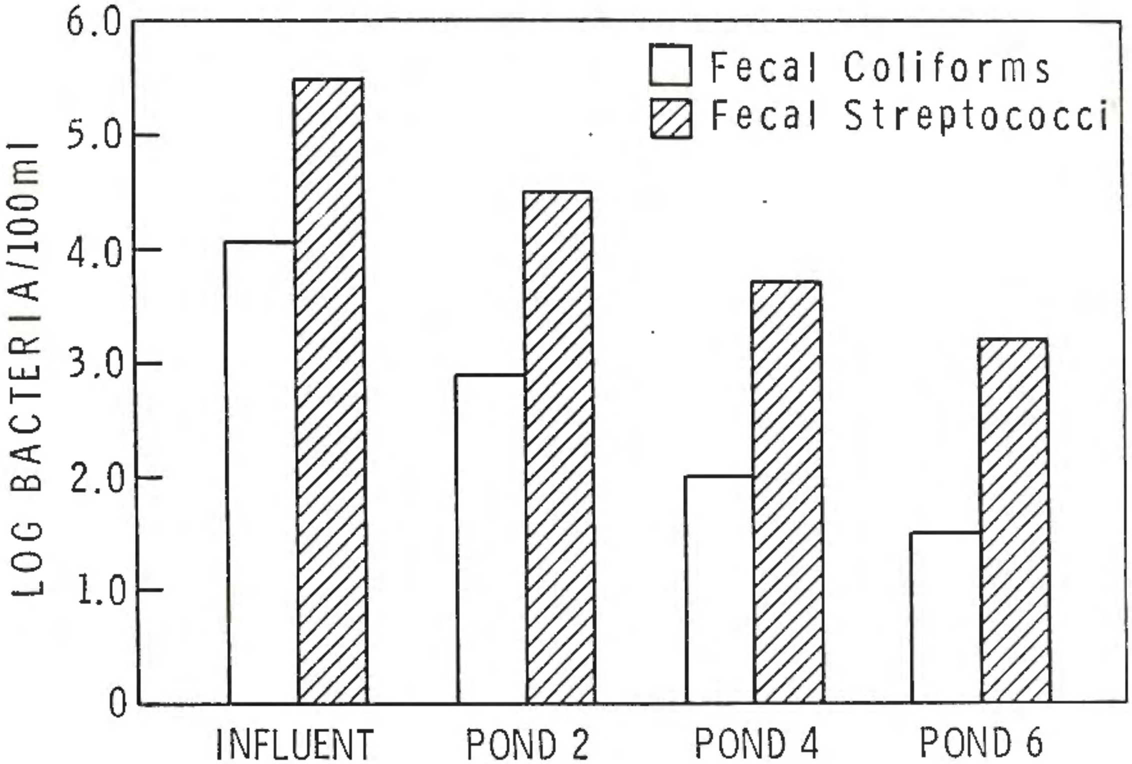


Figure 1. Decrease in concentration of indicator bacteria in pond water.

APPENDIX III

Table A2. Bacterial concentrations in pond sediment

	MPN/100 g sediment					
	Pond 2		Pond 4		Pond 6	
Month	FC	FS	FC	FS	FC	FS
Aug.	92,000	480,000	1,800	480,000	<600	186,000
Sept.	2,300	4,300	90	<600	30	1,860
Oct.	24,000	1,500	380	9,300	90	1,110
Nov.	4,300	400	70	1,500	<30	150
Dec.	--	—	—	—	—	—

APPENDIX III

Table A3. Bacterial concentrations in fish digestive tract

	MPN/100 g fish tissue					
	Pond 4		Pond 5		Pond 6	
Month	FC	FS	FC	FS	FC	FS
Aug.	2,400	≥ 48,000	200	≥ 48,000	<30	1,860
Sept.	11,000	220,000	≥ 24,000	480,000	≥ 24,000	40,000
Oct.	9,300	139,000	15,000	43,000	2,400	93,000
Nov.	300	150,000	230	1,400	430	7,500
Dec.	<30	<30	70	110	60	110

APPENDIX III

Table A4. Bacterial concentrations in fish skin

	MPN/100 g fish skin					
	Pond 4		Pond 5		Pond 6	
Month	FC	FS	FC	FS	FC	FS
Aug.	40	9,200	230	1,860	230	4,800
Sept.	930	4,600	4,600	≥ 480,000	70	4,000
Oct.	40	24,000	<30	930	1,500	2,400
Nov.	230	24,000	1,500	9,300	150	430
Dec.	<30	<30	<30	<30	<30	30

APPENDIX IV

FISH PRODUCTION

APPENDIX IV

TABLE 1. INITIAL STOCKING RATES FOR PONDS 3-6 WITH SILVER AND BIGHEAD CARP. ALL PONDS WERE STOCKED DURING NOVEMBER AND DECEMBER, 1978. INITIAL STOCKING WEIGHTS AND NUMBERS ARE RECORDED AS OF JANUARY, 1979 AFTER EVALUATING POST STOCKING MORTALITY.

	Av. length (cm)	Av. weight (g)	No. of fish per hectare	Total weight (kg/ha)
Pond 3 (1.55 ha)				
Silver Carp	13.8	28.5	13,150	374.8
Bighead Carp	14.5	31.8	2,503	84.2
Pond 4 (1.8 ha)				
Silver Carp	8.6	6.0	6,777	40.6
Bighead Carp	14.5	31.8	1,140	36.2
Pond 5 (1.67 ha)				
Silver Carp	17.0	40.8	7,186	293.2
Bighead Carp	14.5	31.8	1,229	39.1
Pond 6 (1.56 ha)				
Silver Carp	17.0	40.8	5,192	210.6
Bighead Carp	14.5	31.8	385	12.24

TABLE 2. GROWTH OF SILVER AND BIGHEAD CARPS IN POND 3 DURING PROJECT PERIOD.

Date	Time from stocking	Silver carp, standing crop (kg/ha)	Bighead carp, standing crop (kg/ha)
Jan., 1979	0	374.8	84.2
March, 1979	3 mos.	546.0	
June, 1979	6 mos.	1,650.0	1,196.4
Sept., 1979	9 mos.	4,252.7	
Dec., 1979	12 mos.	4,610.0	
March, 1980	15 mos.	5,909.6	
June, 1980	18 mos.	7,164.1*	2,386.4*

* Total fish kill occurred, pond not restocked.

APPENDIX IV

TABLE 3. GROWTH OF SILVER AND BIGHEAD CARPS IN POND 4 DURING PROJECT PERIOD.

Date	Time from stocking	Silver carp, standing crop (kg/ha)	Bighead carp, standing crop (kg/ha)
Jan., 1979	0	40.6	36.2
March, 1979	3 mos.	1,360.0	
June, 1979	6 mos.	2,297.4	493.2
Sept., 1979	9 mos.	4,650.0	
Dec., 1979	12 mos.	6,250.0	
March, 1980	15 mos.	7,107.6	771.1
June, 1980	18 mos.	7,514.0	
Aug., 1980	21 mos.	7,691.9*	931.6

* Total fish kill occurred, pond not restocked.

TABLE 4. GROWTH OF SILVER AND BIGHEAD CARPS IN POND 5 DURING PROJECT PERIOD.

Date	Time from stocking	Silver carp, standing crop (kg/ha)	Bighead carp, standing crop (kg/ha)
Jan., 1979	0	293.2	39.1
March, 1979	3 mos.	900.0	
June, 1979	6 mos.	1,871.9	
Sept., 1979	9 mos.	4,098.9	425.6
Dec., 1979	12 mos.	4,650.0	
March, 1980	15 mos.	5,350.5	560.0
June, 1980	18 mos.	6,075.0	
Sept., 1980	21 mos.	7,260.0	
Dec., 1980	24 mos.	7,634.4	1,510.4

APPENDIX IV

TABLE 5. GROWTH OF SILVER AND BIGHEAD CARPS IN POND 6 DURING PROJECT PERIOD.

Date	Time from stocking	Silver carp, standing crop (kg/ha)	Bighead carp, standing crop (kg/ha)
Jan., 1979	0	210.6	12.24
March, 1979	3 mos.	1,029.6	15.4
June, 1979	6 mos.	1,745.0	
Sept., 1979	9 mos.	2,480.5	
Dec., 1979	12 mos.	2,475.0	
March, 1980	15 mos.	3,441.3	248.2
June, 1980	18 mos.	3,650.5	
Sept., 1980	21 mos.	4,255.0	
Dec., 1980	24 mos.	4,454.7*	589.0

* Channel catfish, grass carp, and smallmouth buffalo were also initially stocked in Pond 6. Due to low stocking rates, difficulty of sampling, etc., no interim growth estimates were made. Also, the buffalo spawned during the spring of 1979 further complicating matters. At harvest, the final standing crop for each species was found to be: channel catfish = 832 kg/ha, buffalo = 562 kg/ha, grass carp = 262 kg/ha.