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POTOMAC ESTUARY BIOTA: AUFWUCHS MICROFAUNA

PHASE II

WATER RESOURCES

SURVEY, ECOLOGY, AND SYSTEMATICS OF THE ZIPPER POTOMAC ESTUARY BIOTA: AUFWUCHS MICROFAUNA PHASE II.

by

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FOREWORD

Due to an unavoidable delay in funding, the senior investigator was forced to curtail the expensive survey work until early in 1976. Nevertheless, the senior investigator was able to use, when necessary, his departmental research funds to pursue the other objectives of the project. Throughout the whole year, the senior investigator has invested more than his allotted weekly hours on reaching the objectives of this grant proposal. More importantly, the work on the keys for the fauna of the Potomac River was pursued and will be completed and presented to the Department of Interior during the Phase 3 year. Drawings, photographs, and permanent slides were made of the protozoa and micrometazoa of the Potomac River. Several extensive literature searches, vital to this project, were completed. A new method for studying and counting the activated sludge community was discovered and tested. A radioactive tracer method for studying food chains was perfected. Extensive ecological and physiological studies were made on select suctoria and peritrich ciliates. The large culture collection of protozoa and metazoa were maintained and expanded in scope. Several papers were published from the work funded by this grant.

With the initiation of funding in early 1976, the river survey was resumed, and carried out monthly; however, with little success as the floats were routinely cut loose from buoys or removed.By late June, this situation was finally remedied and the monthly survey has continued successfully since then. Probably the major accomplishment of this year's work was the additional study using the two bench-scale experimental Potomac rivers which were improved by addition of peristaltic pumps provided by this grant. Presently, the Phase III of this project is being supported by the Center's allotment funds and the complete 1976-1977 survey data will be made available in the summer of 1977 for comparison to the surveys performed by the senior investigator in 1971 and 1974.

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Mr. Ken Kasper, Mr. Pat Ryan, Ms. Toni Chadwick, Mr. Sam Locatelli, Mr. Mike Phelan, Mr. Phillip Schmidt, and Mr. Peter Eisenhardt receive both praise and appreciation for their collaborative research with the senior investigator. The senior investigator's wife, Jeanette, deserves much credit for editing and typing the manuscript; and of course, our four-year-old daughter, Lora, has done her part.

ABSTRACT

Studies are reported on two model rivers each composed bf four interconnected 24-gallon aquaria representing Potomac River sites at Hains Point, Blue Plains Sewage Treatment Plant, Broad Creek, and Piscataway Creek connected to a common reservoir containing upriver (Key Bridge) water. In one model river at aquarium 2, frozen sewage water was added; in the other, dechlorinated tap water was added. In May of 1974 with a river to sewage flow ratio of 20:1, golden shiner minnows died in experimental aquaria in a manner corresponding to river sites where a fish kill was occurring concurrently. Four attempts to duplicate this fish kill in June 1976, when no fish kill was occurring in the river, all gave negative results with river to sewage flows of 20:1, 5:1, 1:1, and 4:4. In the final experiment, the aquarium temperatures were elevated to 29°C thus reproducing the worst possible river to sewage flow ratio and temperature naturally occurring in the river. A tentative conclusion is that the May 1974 fish kill was caused by a toxin in the sewage and not a heat shock or toxin in the river. The dissolved oxygen sag curves in the river model closely approximated those at Potomac River sites at the same temperature.

A new method using thin plastic film (Handiwrap) coverslips with plastic slides is described for counting and studying the activated sludge community. Studies using this method on a 50,000-gallon pilot activated sludge plant before and after hydrogen peroxide treatment of a bulking condition showed the protozoan community little affected. The oxygen-induced fragmenting of the filamentous bacteria allowed acanthamoeba to feed. Using tritiated thymidine, a food chain from bacteria to Spirostomum to the ostracod Cypridopsis was confirmed. Ecological studies are reported on the suctorian Lernaeo hrya capitata. A list of protozoan and micrometazoan speciecultured in our laboratory is provided. The 1976 physical-chemical survey data is presented.

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Study of Experimental Potomac River

There are several ways to create a model of a flowing system like the Potomac River. The most obvious is to sample the river itself, thus creating a model whose realism is limited by the extent and kind of sampling employed. From such sampling, one can determine which are the most significant parameters to consider as contributing to the general condition or health of the river. With sufficient sampling knowhow, a mathematical model can be devised based on a limited number of parameters. (The Annapolis Field Station of the E.P.A. has made such a sophisticated mathematical model of the Potomac River (Hetling, 1969).

A third modeling method is to create a bench-scale practical model of the Potomac River. The Corps of Engineers has attempted this for the entire Chesapeake Bay including the Potomac River, yet their relatively enormous model is designed for hydrological and not biological studies. A major advantage of studying and sampling a bench-scale model over the real river is that a control and experimental bench-scale model can be operated concurrently and select parameters can be isolated and their influences determined.

The obvious disadvantage of the bench-scale river model is that, like the mathematical model, it must be less complex than the real river. The section of the Potomac River from Key Bridge to Piscataway Creek has a complexity of interacting inputs including the main channel flow, Anacostia River, numerous side streams, storm sewer runoff, power plant coolant water, and numerous sewage effluents including the giant Blue Plains Sewage Treatment Plant. From the aspects of hydrology and biology, the main channel flow and the Blue Plains sewage treatment plant are the most significant in formulating the design of a bench-scale experimental river. The main channel flow varies enormously from a low of about 388 million gallons per day (mgd) to a high of 100's of billions of gallons per day. The average flow is about 7 billion gallons per day. The total effluent of the 18 sewage treatment plants in the Washington area is over 350 mgd with over 70% coming from the Blue Plains Sewage Treatment Plant.

The Potomac River (Figure 1) widens below Key Bridge and further widens below Woodrow Wilson Bridge. The average depth is 10 feet, with a euphotic zone of less than 2 feet due to sediment, organic content, and plankton. Thus the majority of the flow is out of contact with the surface where re-aeration can occur, as well as beneath the euphotic zone where more oxygen can be generated than is required for respiration. It was decided to create this condition of lowered re-aeration by joining 24-gallon all-glass aquaria containing 10 gallons of aerated water by two 50-foot lengths of 1/2-inch Tygon tubing and a half-filled unaerated 5-gallon Nalgene bottle (Figure 2). A 40-gallon common aerated reservour represented Site 1 at Key Bridge and four interconnected aquaria represented the downriver sites which were approximately 3-1/2 miles apart: Site 2 at Hains Point, Site 3 at Blue Plains, Site 4 at Broad Creek, and Site 5 at Piscataway Creek. The common

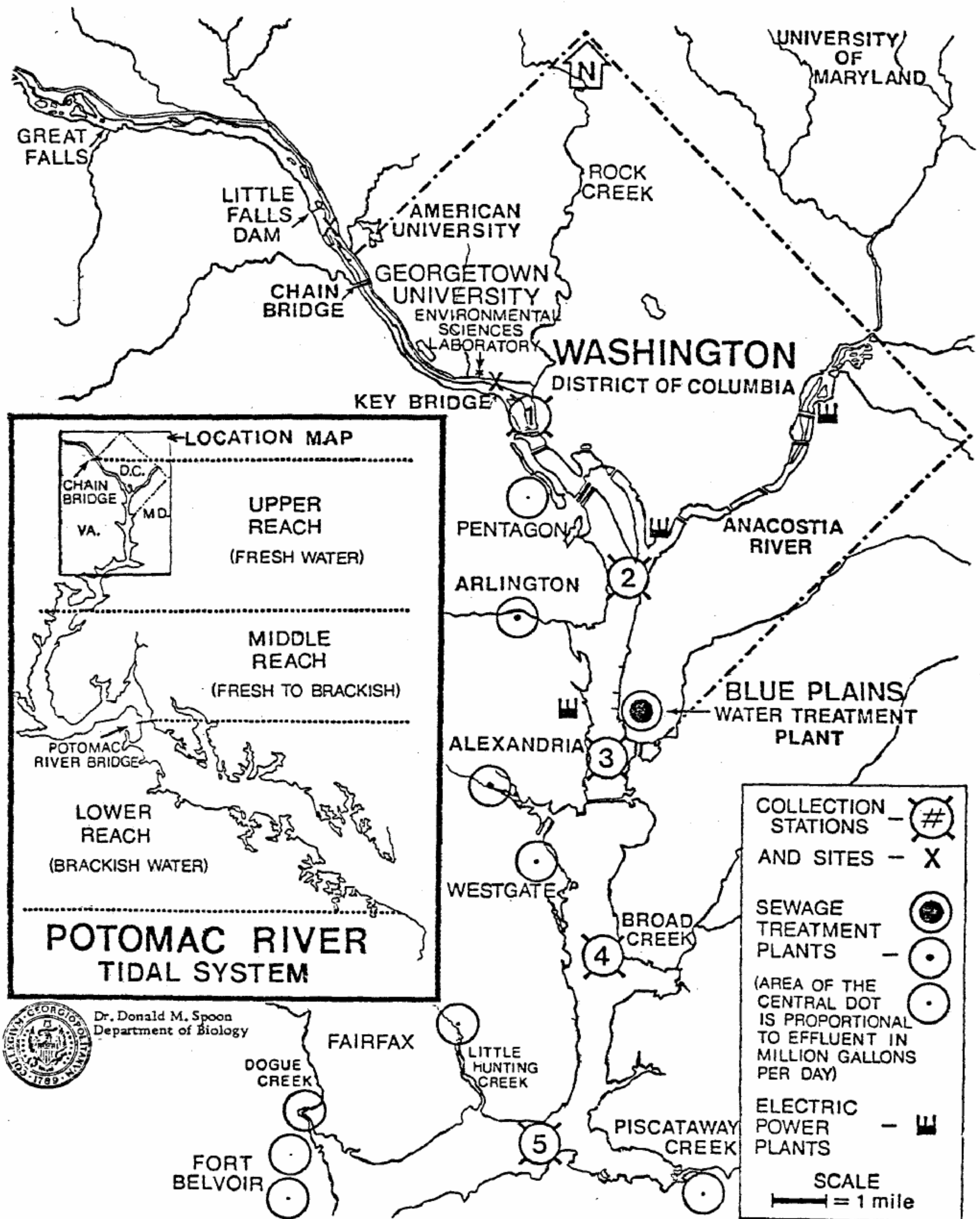


Figure 1. Map of the Upper Potomac Estuary showing project sampling sites.

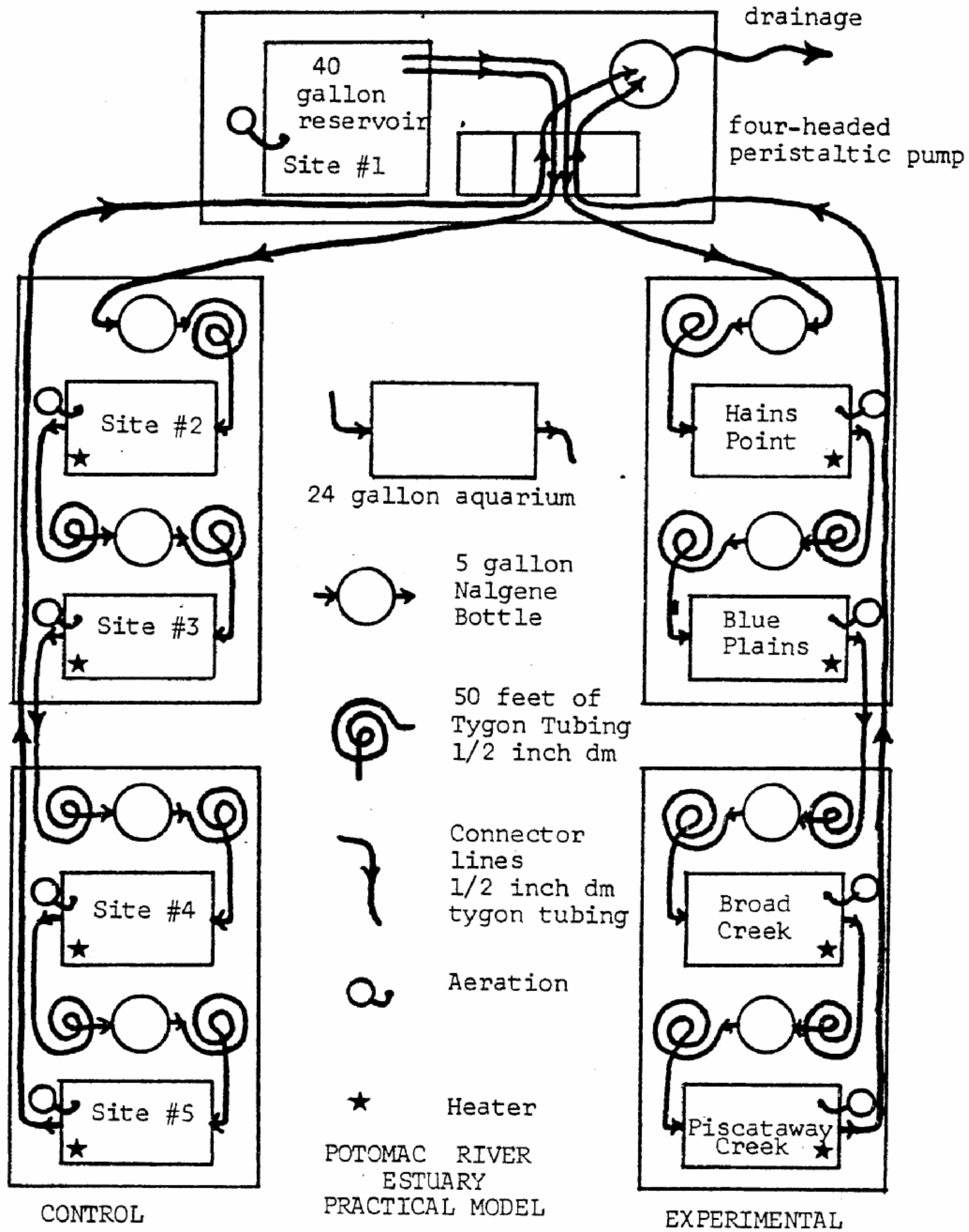


Figure 2. Diagram of the model rivers located in the Environmental Sciences Laboratory at Georgetown University, Washington, D. C.

reservoir supplied identical water at the same flow rate to each of two parallel bench-scale experimental rivers composed of four interconnected aquaria.

Initially, a rather rigorous study of the volumes and flow rates through each of these four realms was considered and plans made to approximate these volumes and flow rates in each aquarium by precise elevation of the aquaria. Consideration of the Jaworsk and Johnson 1969 dye study which indicated the main channel transported pollutants 100 times more rapidly than the embayments led us to discard this complication. Thus each of the four aquari had the same volume and flow rate dictated by gravity flow based on the inflow at Aquarium 1 and outflow at Aquarium 4. To hook up the aquaria and Nalgene bottles, holes were drilled below the proposed water line and glass spouts attached and inserted into the Tygon tubing. The fitting was made tight with high vacuum silicone grease and two thicknesses of twisted wire allowed facility in breaking down and rinsing out the system.

The main reservoir was filled daily with fresh river water collected at Key Bridge located just below the Georgetown University Environmental Sciences Laboratory. The input of sewage effluent was a different problem. The Blue Plains Sewage treatment plant is about a 20-minute drive from the laboratory. It was decided that the only feasible way to add the sewage water in a near-normal organic condition would be to freeze it in set volumes in individual plastic ice cube holders. Thereafter, these plastic holders could be handled safely and a set volume of frozen sewage water added to Aquarium 2, representing the Blue Plains site. This sewage water was collected from the final sedimentation tanks and thus does not receive the final treatment with chlorine which kills certain organisms as does our freezing of the sewage. The fact that the frozen sewage we used contained no chlorine could have altered our experimental results compared to the real river, however we assumed that this difference would be minor. (The addition of the frozen sewage even at the highest level used caused only a small increase in the 10-gallons of wate in the aquarium.)

These two experimental rivers were first set up in the sprinc of 1974. In the first experiment (April 22, 1974) the aquaria of each experimental river were filled with water collected at the corresponding site in the river. After 24 hours of aeration and equilibrium, ten golden shiner minnows were introduced into each of the four aquaria of one river and ten rainbow trout in the oth aquaria. The aquaria were not connected and were equally aerated The minnows remained healthy throughout the 7-day experiment, whe by two days, most of the trout appeared weakened and swam in a disoriented fashion showing abnormal responses to tapping on the glass. After the second day, trout died each day until by the 7t day, 3 had died at site 2, all 10 at site 3, 7 at site 4, and 6 at site 5. Obviously, the trout were unsuitable test animals to determine the effect of the sewage effluent when they could not even tolerate living in the aerated river water when cool enough to allow for their survival.

On April 27, 1974, 20 gallons of water were collected from each of the four sites along with a 2-1/2-quart sample of bottom sediment obtained with an Ekman Bottom Sampler. To each of the aquaria of the two experimental rivers was added 10 gallons of water and half the sediment sample from each respective site. The sediment was allowed to settle in the aquaria until May 3rd when 5 gallons of river water from the respective sites was collected and added to each respective Nalgene bottle and the interconnecting Tygon tubing filled so as to contain no air bubbles. Ten golden shiner minnows were added to each aquarium. A system of valves from the 40-gallon Key Bridge (Site 1) reservoir were opened and adjusted to flow 20 gallons per day to each experimental river. Then to one experimental river was added at Site 3 three times a day at 8 hour intervals, 1275 ml of frozen sewage or a total of 1 gallon. This gave a ratio of river water flow to sewage effluent of 20 to 1. This ratio closely approximates the ratio of the average daily river flow (7,100 mgd) to average effluent (350 mgd) from all 18 of Washington's sewage treatment plants.

By May 5th, the minnows on the experimental side began to show signs of disorientation with about 3 fish per aquarium at sites 3, 4, and 5 swimming on their backs. The minnows began dying on the experimental side on the 5th and by the 7th four had died at Site 3 and 6 at Site 5. Over half of the remaining fish were lying on their backs and swimming little. On May 5th, one minnow died at Site 2, afterwhich the fish in this tank showed marked improvement as the original Hains Point water was flushed out with Key Bridge water. The minnows in the control experimental river were generally in fine condition and responded normally to tapping on the glass. The last sewage was added at 5 p.m. on May 7th. By the following morning, the minnows on the experimental side were showing recovery. This improved as the sewage water was diluted out until the minnows on the experimental side were again swimming and behaving normally.

Physical-chemical parameters were monitored daily throughout the experiments. These included nitrates, and orthophosphate using the Hach Kit, dissolved oxygen with a YSI electrode and Hach Kit, pH by electrode, and air and water temperature with a thermister. Bacteriological counts were also made for total bacteria and total coliforms with millipore filter apparatus. During the experiment, the D.O. (dissolved oxygen) stayed at about 8-9 ppm in all aquaria with water temperatures about 14-15°C, thus dissolved oxygen can be ruled out as causing the fish kill. Nitrate levels followed no set pattern and phosphate was highest in the aquaria with dead fish. The bacterial counts were not greatly increased in the experimental side of the river.

This experiment led to the conclusion that there was either something in the sewage itself that was toxic to the fish or that something in the sewage was allowing some river microbe to produce the toxin. It was of special note that at the same time this experiment was being carried out, there was a large fish kill occurring in the river itself and centered at the same sites, being heaviest at Broad Creek (Site 4). The Potomac River itself had temperatures on May 5th

of 18-20°C with D.O. from 6.4 to 8.0. The experimental river studies rule out heat shock as causing the fish kill. The most striking finding in the experimental river was that the fish in the connected aquaria receiving the sewage revived after the sewage was flushed out.

In the spring of the year, there was a heavy application of methoxychlor to control the beetles that transmit the Dutch Elm Disease. This biocide is especially toxic to fish but less so to mammals. The combined storm and sewage system in most of older Washington would allow this toxin to concentrate in the sewage plant. This is just one possible source of the observed fish kill.

In the spring of 1976, this grant provided the funds to purchase two Sigmamotor peristaltic pumps which allowed a significant improvement in the two bench-scale experimental rivers. Instead of using simple values which varied slightly as the reservoir level changed, requiring numerous adjustments, the peristaltic pumps allowed continuous and equal addition and removal of water from each experimental river. In collaboration with senior thesis student Ken Kasper, the following four experiments were carried out.

First it was decided to repeat the previous experiment using the 20:1 river water to sewage effluent ratio yet with certain modifications and simplifications of the system. The sediment was not added to each aquarium at the onset and all aquaria were filled initially with Key Bridge water. (These changes were dictated by the inavailability of the tug boat used in these collections in the previous experiment.) Easily obtainable and inexpensive goldfish (Carassius auratus) were used in place of the golden shiner minnows (Notemigonus crysoleucas), which have to be netted at Great Falls on the Potomac River.

The physical-chemical data for this first of this new series of experiments are shown in Table 1 . Throughout experiment one (June 3-7), the temperature varied between 17 to 22°C (the cottage housing the experimental rivers was neither heated or cooled). This temperature range was considerably higher than the 14-15°C of the previous experiment. The data is arranged differently with C(control)1, C2, C3, C4, and E(experimental)1, E2, E3, E4, for river sites 2, 3, 4, 5 with aquarium E2 receiving the frozen sewage at 8-hour intervals and C2 receiving dechlorinated tap water. Note that the dissolved oxygen for the aquaria E2 to E4 on the experimental side was slightly lower than on the control side. Table shows the record of fish deaths. There appears to be no pattern related to the sewage input with death occurring in the control and experimental aquaria including E1 and C1, both of which received no sewage. (The goldfish were also dying at a similar rate in the holding tanks.) This experiment indicated that possibly goldfish were more tolerant than golden shiner minnows and that lack of the sediment might have influenced the results.

In the second experiment (June 15-18), the ratio of river water to sewage flow was changed to 5:1 by lowering the river flow yet keeping the sewage flow the same. The warmer ambient temperatures brought the aquaria temperatures up to 23.3 to 27.2°C (Table 2).

Table 1. Physical-chemical data for model rivers experiment #1
River flow to sewage ratio 20:1 (June 3-7, 1976)
C = Control aquaria; E - Experimental aquaria receiving sewage.

	Tanks							
	C1	C2	C3	C4	E1	E2	E3	E4
6/3 5:00 PM Air temp. = 21°C								
H_2O temp (°C)	19.5	20.0	20.0	20.0	20.0	20.0	20.0	20.0
pH	6.9	6.9	6.9	7.0	6.9	7.0	6.9	6.9
D.O. (mg/l)	8.1	8.4	8.0	8.2	8.2	7.5	8.5	8.0
6/4 10:00 AM Air temp. = 19°C								
H_2O temp (°C)	18.0	18.0	18.0	17.5	18.0	17.0	18.0	18.0
pH	7.0	6.8	6.9	6.8	7.1	7.1	7.1	7.1
D.O. (mg/l)	7.8	7.6	7.7	7.8	8.0	7.2	7.4	7.6
6/4 2:30 PM Air temp.= 20.5°C								
H_2O temp (°C)	18.5	18.5	18.5	19.0	19.0	18.0	19.0	19.5
pH	7.0	7.0	7.1	7.1	7.0	7.0	7.0	7.1
D.O. (mg/l)	8.0	7.9	7.9	7.8	8.0	7.4	7.7	7.9
6/4 11:00 PM Air temp. = 21°C								
H_2O temp (°C)	20.0	20.0	20.0	20.0	20.0	18.0	20.0	20.5
pH	7.1	7.1	7.1	7.2	7.1	7.1	7.1	7.0
D.O. (mg/l)	7.7	7.5	7.5	7.5	7.6	7.2	7.1	7.3
6/5 10:00 Air temp. = 19°C								
H_2O temp (°C)	18.0	18.0	18.0	18.0	18.0	17.5	18.0	18.0
pH	7.2	7.0	7.1	7.1	7.2	7.1	7.0	7.1
D.O. (mg/l)	8.7	8.8	8.4	8.6	8.6	8.4	8.4	8.4
6/5 6:00 PM Air temp.= 21°C								
H_2O temp (°C)	19.0	19.0	19.0	19.0	19.5	18.0	19.0	20.0
pH	6.8	6.8	6.8	6.8	7.0	6.9	6.8	6.9
D.O. (mg/l)	8.8	8.4	8.5	8.5	8.8	8.1	8.0	7.9
6/6 10:00 AM Air temp. = 19°C								
H_2O temp (°C)	18.5	18.5	18.5	18.5	18.5	18.0	18.5	18.5
pH	7.1	7.1	7.1	7.1	7.0	7.0	7.0	7.1
D.O. (mg/l)	8.0	7.9	7.9	7.9	8.0	7.7	8.0	8.0
6/6 6:00 PM Air temp.= 22°C								
H_2O temp (°C)	20.5	21.0	21.0	21.0	21.0	20.0	21.0	21.0
pH	7.1	7.1	7.2	7.2	7.0	7.1	6.9	7.0
D.O. (mg/l)	8.0	7.3	7.4	7.1	7.5	6.6	7.1	7.2
6/7 10:00 AM Air temp.= 20.5°C								
H_2O temp (°C)	19.5	19.5	19.5	19.5	19.5	19.0	19.5	20.0
pH	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.1
D.O. (mg/l)	8.2	8.2	7.0	7.2	8.2	7.6	7.6	7.4
6/7 4:00 PM Air temp.= 23°C								
H_2O temp (°C)	21.0	21.5	21.5	21.5	21.5	20.5	21.5	22.0
pH	7.1	7.0	7.0	7.0	7.0	7.0	7.0	7.1
D.O. (mg/l)	7.6	7.6	7.2	6.9	7.0	6.7	6.6	7.1

Table 2. Physical-chemical data for model rivers experiment #2
River flow to sewage ratio 5:1 (June 15-18, 1976)
C = Control aquaria; E = Experimental aquaria receiving sewage.

	Tanks							
	C1	C2	C3	C4	E1	E2	E3	E4
6/15 10:00 AM Air temp.= 24.0°C								
H_2O temp (°C)	23.6	23.3	23.5	23.5	23.8	23.8	24.0	24.0
pH	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1
D.O. (mg/l)	7.4	7.0	6.8	7.0	6.6	6.6	6.6	6.6
6/15 4:30 PM Air temp.= 29.5°C								
H_2O temp (°C)	25.8	25.7	26.0	26.0	25.0	25.2	26.0	26.5
pH	7.1	7.1	7.1	7.2	7.1	7.1	7.1	7.2
D.O. (mg/l)	6.8	6.7	6.3	6.5	6.9	5.9	6.1	6.2
6/16 12:30 AM Air temp.= 28.3°C								
H_2O temp (°C)	27.0	26.9	27.0	27.0	27.1	26.5	27.1	27.2
pH	7.0	7.0	7.0	7.0	7.1	7.1	7.1	7.1
D.O. (mg/l)	6.8	6.5	6.4	6.3	6.7	5.4	5.7	5.9
6/16 9:00 AM Air temp.= 26.8°C								
H_2O temp (°C)	26.0	25.7	25.9	25.9	26.0	25.6	26.0	26.0
pH	7.1	7.1	7.0	7.1	7.1	7.0	7.1	7.2
D.O. (mg/l)	6.4	6.6	6.1	6.1	6.5	5.6	5.5	5.4
6/16 4:30 PM Air temp.= 27.4°C								
H_2O temp (°C)	26.5	26.4	26.8	26.7	26.9	25.9	26.9	27.0
pH	7.0	7.0	7.1	7.1	7.0	7.1	7.1	7.2
D.O. (mg/l)	6.2	6.2	6.0	5.8	6.3	5.1	4.9	5.1
6/17 12:30 AM Air temp.= 27.5°C								
H_2O temp (°C)	27.1	27.0	27.2	27.1	27.2	26.5	27.2	27.3
pH	7.1	7.1	7.1	7.1	7.1	7.1	7.2	7.2
D.O. (mg/l)	6.0	6.0	5.6	5.9	6.0	5.2	4.9	4.7
6/17 9:00 AM Air temp.=26.5°C								
H_2O temp (°C)	25.9	25.9	25.9	25.9	26.0	25.6	26.0	26.0
pH	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.2
D.O. (Mg/l)	6.4	6.4	6.2	6.2	6.4	5.2	5.2	5.0
6/17 4:30 PM Air temp.= 28.4°C								
H_2O temp (°C)	26.8	26.7	27.0	27.0	27.0	26.0	27.0	27.0
pH	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.2
D.O. (mg/l)	6.1	6.2	6.0	5.9	6.1	5.5	4.6	4.3
6/18 12:30 AM Air temp.= 27.7°C								
H_2O temp (°C)	26.9	26.8	27.0	27.0	27.0	26.1	27.0	27.2
pH	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.2
D.O. (mg/l)	6.1	6.0	5.7	5.7	5.9	5.0	4.6	4.5

The dissolved oxygen levels were obviously lowered in the E2, E3, E4 aquaria compared to the controls. Note the close correlation in results from aquaria C1 and E1 which serve as comparative controls for the two experimental rivers. Table 5 shows that there was more fish mortality in the E1 to E4 aquaria than in the control aquaria with 3 in E1, 3 in E2, 1 in E3, and 3 in E4 compared to 1 in C1, 1 in C2, 2 in C3 and 3 in C4. The three deaths in E1 and 3 in C4 nullify this mortality as being directly caused by the sewage level. Thus, using goldfish at a 10°C increase in temperature and with one-fourth the main river flow, we could not reproduce the fish kill obtained in 1974 with the golden shiner minnows.

For experiment three (June 22-27), we set up the river to sewage flow at a ratio of 1:1, comparable to the worst possible ratio in the real Potomac River under periods of lowest flow. Six golden shiner minnows (freshly netted from the Potomac River at Great Falls) and six goldfish (from a fresh supply) were added to each aquarium. An air conditioner was installed in the cottage that held the air temperature between 23-24°C so that the water temperature in the aquaria varied from 19.4 to 22.5°C and the dissolved oxygen readings in the E2-E4 aquaria were less depressed over the controls (Table 3). The nitrate values showed little change from the two experimental rivers with the initial aquarium in each usually having the higher value. No mortality occurred and both goldfish and golden shiner minnows appeared normal, although they were obviously hungry as no food was ever added during the duration of these 3-5 day experiments.

For the fourth (June 29-July 2) experiment (Table 4) in this series, it was decided to further stress the minnows and goldfish by using heaters in each aquarium which maintained their temperatures from 28-30°C, which is the maximum level reached in the Potomac River. Also the ratio of sewage to river flow was maintained, yet both flows were increased 4-fold or a ratio of 4:4 over the previous experiments. Each aquarium contained 10 freshly netted minnows and 10 goldfish. No fish died or showed any signs of distress as the dissolved oxygen levels reached a lowest level of 5.3 ppm. (Note that the phosphate levels were higher in the aquaria receiving sewage.) Thus these minnows were exposed to an 80-fold increase in sewage effluent over that used in the 1974 study in which a fish kill was obviously induced in the experimental river receiving the sewage but not the control river. During June of 1976, no fish kill was occurring in the Potomac River while in May of 1974, a massive fish kill was occurring concurrently with our studies of our bench-scale experimental and control rivers. The obvious conclusion is that in May of 1974, there was a fish toxin in the sewage effluent that was lacking in the June 1976 sewage effluent. However, since in the June 1976 study the aquaria lacked sediment, this conclusion is somewhat blunted. Nevertheless, these bench-scale rivers demonstrated their usefulness in eliminating certain alternate conclusions; such as, the fish kill was caused by a heat shock or something in the river water per se. Apparently,

Table 3. Physical-chemical data for model rivers experiment #3
River flow to sewage 1:1 (June 22-27, 1976)
C = Control aquaria; E = Experimental aquaria receiving sewage.

	Tanks							
	C1	C2	C3	C4	E1	E2	E3	E4
6/22 4:00 PM Air temp.=23.8°C								
H_2O temp (°C)	21.5	22.0	22.0	22.2	21.3	21.8	22.0	22.5
pH	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1
D.O. (mg/l)	7.7	7.6	7.6	7.6	7.8	7.7	7.5	7.6
NO_3 (mg/l)	4.9	4.7	4.6	4.5	5.0	5.0	4.7	4.7
6/22 11:00 PM Air temp.= 23.3°C								
H_2O temp (°C)	21.2	21.8	22.0	22.0	20.9	21.2	21.6	22.0
pH	7.1	7.1	7.1	7.1	7.1	7.1	7.2	7.2
D.O. (mg/l)	7.6	7.5	7.6	7.6	7.5	7.1	6.9	7.2
NO_3 (mg/l)	1.3	0.4	1.0	1.2	1.2	0.8	1.1	1.0
6/23 8:30 AM Air temp.=21.2°C								
H_2O temp (°C)	20.2	20.7	20.9	21.0	20.2	20.0	20.5	20.9
pH	7.0	7.0	7.0	7.1	7.0	7.0	7.1	7.2
D.O. (mg/l)	8.1	7.9	8.0	7.9	8.1	8.2	7.7	7.9
NO_3 (mg/l)	1.1	0.8	1.0	0.8	1.2	0.9	0.8	0.9
6/23 4:00 PM Air temp.= 24.8°C								
H_2O temp (°C)	21.0	21.5	21.6	21.8	20.9	20.7	21.3	22.0
pH	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.2
D.O. (mg/l)	7.7	7.6	7.5	7.5	7.7	7.2	7.3	7.4
NO_3 (mg/l)	1.2	0.8	0.7	0.8	1.1	0.8	0.8	0.8
6/24 9:00 AM Air temp.= 23.2°C								
H_2O temp. (°C)	21.7	21.9	21.9	21.9	21.7	21.2	21.8	22.0
pH	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.2
D.O. (mg/l)	7.9	7.6	7.6	7.6	7.8	7.0	6.9	6.8
NO_3 (mg/l)	1.5	0.7	0.7	0.7	1.3	0.9	0.9	0.9
6/24 4:30 PM Air temp.= 23.9°C								
H_2O temp (°C)	21.2	21.8	21.9	21.9	21.0	21.0	21.8	22.1
pH	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.2
D.O. (mg/l)	7.9	7.7	7.6	7.6	7.9	6.6.	6.2	6.3
NO_3 (mg/l)	0.9	0.4	0.3	0.5	1.0	0.7	0.7	0.7
6/25 9:00 AM Air temp.= 21.7°C								
H_2O temp (°C)	20.2	20.8	20.9	20.9	20.1	20.1	20.7	20.9
pH	7.1	7.0	7.0	7.0	7.1	7.0	7.0	7.2
D.O. (mg/l)	7.9	7.7	7.6	7.6	7.8	6.3	6.2	6.0
NO_3 (mg/l)	1.3	0.5	0.3	0.3	1.2	0.8	0.6	0.7
6/25 4:00 PM Air temp.= 24.8°C								
H_2O temp (°C)	19.8	20.2	20.3	20.4	19.4	19.4	20.1	20.8
pH	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.2
D.O. (mg/l)	8.2	7.9	7.9	7.9	8.1	6.9	6.9	6.0
NO_3 (mg/l)	1.1	0.4	0.4	0.4	1.1	0.7	0.6	0.6
6/26 9:00 AM Air temp. = 22.3°C								
H_2O temp (°C)	20.5	21.0	21.1	21.1	20.5	20.5	21.0	21.2
pH	7.1	7.0	7.0	7.0	7.1	7.0	7.0	7.2
D.O. (mg/l)	8.0	7.8	7.7	7.7	8.0	6.4	6.3	6.1
NO_3 (mg/l)	1.1	0.4	0.3	0.3	1.2	0.8	0.6	0.7
6/27 2:00 PM Air temp.= 21.9°C								
H_2O temp (°C)	20.2	20.7	20.8	20.9	20.2	20.5	20.9	21.0
pH	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.2
D.O. (mg/l)	8.1	7.6	7.6	7.8	8.0	6.6	6.8	6.9
NO_3 (mg/l)	1.1	0.5	0.4	0.4	1.2	0.9	0.8	0.9

Table 4. Physical-chemical data for model rivers experiment #4
River flow to sewage 4:4 (June 29-July 2, 1976)
C = Control aquaria; E = Experimental aquaria receiving sewage.

	Tanks							
	C1	C2	C3	C4	E1	E2	E3	E4
6/29 3:00 PM Air temp.= 23.6°C								
H_2O temp (°C)	24.0	24.8	23.0	23.9	24.8	25.8	25.8	26.0
pH	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.2
D.O. (mg/l)	7.6	7.3	7.3	7.3	7.5	7.2	7.2	7.1
NO_3 (mg/l)	0.8	0.8	0.7	0.8	1.2	0.9	0.8	0.9
PO_4 (mg/l)	0.07	0.04	0.05	0.06	0.18	0.18	0.08	0.04
6/30 8:30 AM Air temp.= 23.0°C								
H_2O temp (°C)	29.3	23.8	24.4	25.3	29.1	30.0	23.9	24.0
pH	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1
D.O. (mg/l)	7.1	7.3	7.3	7.1	7.1	6.3	6.8	6.8
NO_3 (mg/l)	1.2	0.9	0.8	0.8	1.3	1.1	0.9	1.0
PO_4 (mg/l)	0.22	0.07	0.07	0.07	0.28	0.06	0.06	0.08
6/30 4:00 PM Air temp.= 23.3°C								
H_2O temp (°C)	29.8	29.3	29.9	30.0	29.7	29.9	30.0	28.5
pH	7.0	7.0	7.0	7.0	7.0	7.1	7.0	7.2
D.O. (mg/l)	6.9	6.6	6.6	6.5	6.8	6.1	5.6	6.3
NO_3 (mg/l)	1.2	0.7	0.6	0.6	1.3	0.8	0.7	0.7
PO_4 (mg/l)	0.24	0.04	0.03	0.04	0.27	0.12	0.04	0.07
7/1 8:30 AM air temp.= 22.0°C								
H_2O temp (°C)	28.8	30.8	30.9	29.9	28.9	30.2	29.1	28.2
pH	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.1
D.O. (mg/l)	6.8	6.2	6.2	6.2	6.8	5.4	5.5	5.8
NO_3 (mg/l)	1.3	0.7	0.5	0.6	1.2	0.8	0.7	0.8
PO_4 (mg/l)	0.28	0.01	0.01	0.01	0.36	0.48	0.04	0.01
7/1 4:00 PM Air temp. = 23.4°C								
H_2O temp (°C)	29.4	29.6	28.8	28.1	28.5	29.0	28.7	27.9
pH	7.0	7.1	7.0	7.0	7.0	7.1	7.1	7.2
D.O. (mg/l)	6.8	6.3	6.4	6.4	6.8	5.6	5.7	5.9
NO_3 (mg/l)	1.5	0.8	0.7	0.7	1.6	1.1	0.8	1.0
PO_4 (mg/l)	0.39	0.07	0.07	0.07	0.45	0.52	0.05	0.06
7/2 8:30 AM Air temp.= 21.9°C								
H_2O temp (°C)	29.0	28.2	28.2	29.1	28.0	28.0	28.6	29.9
pH	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.1
D.O. (mg/l)	6.9	6.9	6.6	6.3	6.8	5.9	5.5	5.5
NO_3 (mg/l)	1.8	0.8	0.5	0.6	1.7	1.2	0.7	0.8
PO_4 (mg/l)	0.35	0.03	0.03	0.04	0.47	0.35	0.03	0.11
7/2 4:30 PM Air temp.= 22.5°C								
H_2O temp (°C)	28.7	28.8	30.0	28.9	28.0	28.3	30.1	29.1
pH	7.0	7.1	7.1	7.1	7.0	7.0	7.1	7.2
D.O. (mg/l)	6.8	6.6	6.6	6.5	6.7	5.8	5.3	5.5
NO_3 (mg/l)	1.8	1.0	0.5	0.7	2.0	1.3	0.9	0.9
PO_4 (mg/l)	0.25	0.02	0.02	0.02	0.24	0.38	0.18	0.03

Table 5. Data for fish deaths for model rivers experiments 1-4. (Only goldfish were used in experiments 1 and 2; while minnows and goldfish were used in experiments 3 and 4.)

Experiment One

Tank	Date	Time	Number	Date	Time	Number
E1	6/6	6:00 PM	1			
E4	6/5	10:00 AM	1	6/7	2:00 PM	1
C1	6/6	6:00 PM	1	6/8	10:00 AM	2
C3	6/9	10:00 AM	2	6/10	10:00 AM	1
C4	6/8	10:00 AM	1			

Experiment Two

Tank	Date	Time	Number	Date	Time	Number
E1	6/15	4:30 PM	1	6/16	9:00 AM	1
	6/17	11:45 PM	1			
E2	6/15	2:00 PM	1	6/17	10:30 AM	1
	6/17	11:45 PM	1	6/18	9:30 AM	1
E3	6/16	9:00 AM	1			
E4	6/15	4:30 PM	1	6/16	9:00 AM	2
C1	6/16	12:30 AM	1			
C2	6/15	2:00 PM	1			
C3	6/16	4:30 PM	1	6/16	12:30 AM	1
C4	6/16	9:00 AM	2	6/16	12:30 AM	1

Experiment Three

No Fish Deaths

Experiment Four

No Fish Deaths

the sewage water either contains a toxin or is inducing the production of the toxin by a river or sediment organism.

The most enheartening results of these studies was the finding that the bench-scale experimental river could re-create oxygen sag curves which were comparable to that in the river at similar temperatures (Table 6, Figs.3-6). In every case the bench-scale rivers have higher D.O. values than the real river, yet for certain sampling times the curve for the four sites is similar. Obviously we are over-aerating our aquaria compared to the real river's rate or re-aeration. Other refinements under construction are addition of artificial lighting to each aquaria, cooling of the aquaria with circulated water, and addition of two experimental bench-scale activated sludge plants. We intend to place sediment collected from each site in the Nalgene sediment traps where it can best duplicate the river situation. The reader can appreciate the great value of this system for simulating in a simple way, the year-round conditions affecting the Potomac River. For example, a late summer algae bloom can be simulated at any time throughout the year. Furthermore, it may be possible to test with some accuracy the mathematical model now formulated for the Potomac River to determine which factors are limiting in causing the algal blooms for which so much public funds have been committed for tertiary treatment. With the experimental sewage treatment facility added to these bench-scale rivers, one could determine which tertiary method will be best suited for the Potomac River.

Table 6. Compilation of select data from the model river (experimenta group E1 - E4) and data from the Potomac River at Sites 2 to 5 determined by the senior investigator and the E. P.A. Given as Dissolved Oxygen in ppm (Temperature in °C)

	E1	E2	E3	E4
Experimental Data Summary				
6/6/76 (exp. 1)	7.5 (21.0)	6.6 (20.0)	7.1 (21.0)	7.2 (21.0)
6/16/76(exp. 2)	6.3 (26.9)	5.1 (25.9)	4.9 (26.9)	5.1 (27.0)
6/17/76(exp. 2)	6.1 (27.0)	5.5 (26.0)	4.6 (27.0)	4.3 (27.0)
6/27/76(exp. 3)	8.0 (20.2)	6.6 (20.5)	6.8 (20.9)	6.9 (21.0)
7/1/76 (exp. 4)	6.8 (28.9)	5.4 (30.2)	5.5 (29.1)	5.8 (28.2)
7/2/76 (exp. 4)	6.7 (28.0)	5.8 (28.3)	5.3 (30.1)	5.5 (29.1)
W.R.R.C. Report No. 6 D. M. Spoon				
6/30/71	4.39 (28.0)	2.74 (29.0)	1.78 (28.5)	2.69 (28.0
7/26/71	3.45 (28.0)	2.83 (27.5)	4.66 (27.5)	3.96 (27.0
8/25/71	4.41 (27.5)	2.53 (27.5)	6.46 (27.0)	7.42 (27.0
9/28/71	6.91 (21.5)	6.11 (22.0)	4.54 (22.0)	6.59 (22.5
5/3/74	7.0 (17.0)	8.0 (17.0)	6.4 (16.0)	7.1 (15.0
6/6/74	8.6 (19.0)	8.3 (22.5)	8.2 (19.5)	7.8 (20.0
7/8/74	7.3 (28.5)	4.3 (28.0)	5.3 (28.5)	7.0 (28.0
8/8/74	3.0 (26.0)	2.5 (29.0)	9.7 (27.0)	9.2 (26.0
10/4/74	5.7 (17.0)	4.2 (19.0)	5.6 (16.5)	5.8 (16.0
E. P. A.				
5/1/74	8.23 (20.5)	8.80 (20.0)	7.92 (19.0)	8.08 (21.5
6/18/74	7.76 (24.0)	7.18 (26.0)	4.71 (25.0)	4.94 (25.0
7/15/74	7.46 (29.0)	4.26 (29.0)	3.31 (29.0)	6.92 (28.5
8/5/74	4.72 (26.0)	2.40 (25.0)	2.78 (25.5)	5.06 (26.0

Figure 3

COMPARISON OF EXP. 1 AND W.R.R.C. DATA OF 10/4/74

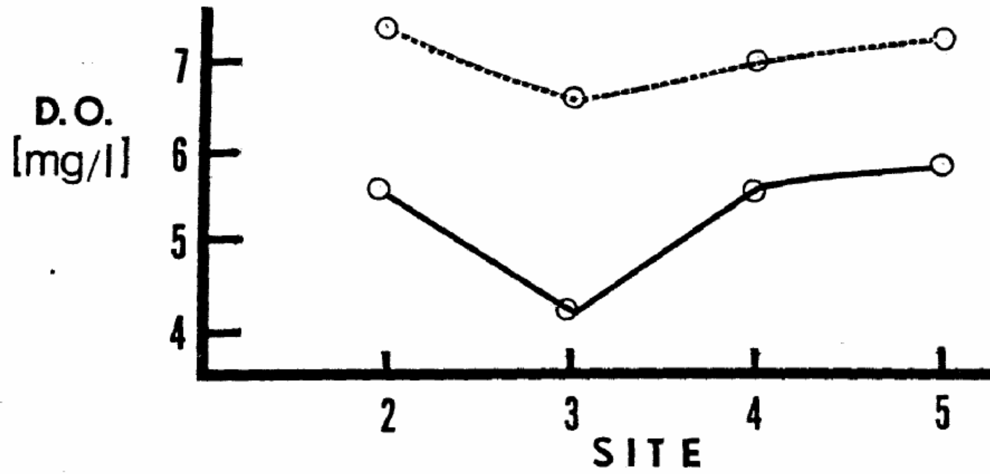


Figure 4

COMPARISON OF EXP. 2 AND W.R.R.C. DATA OF 6/30/71

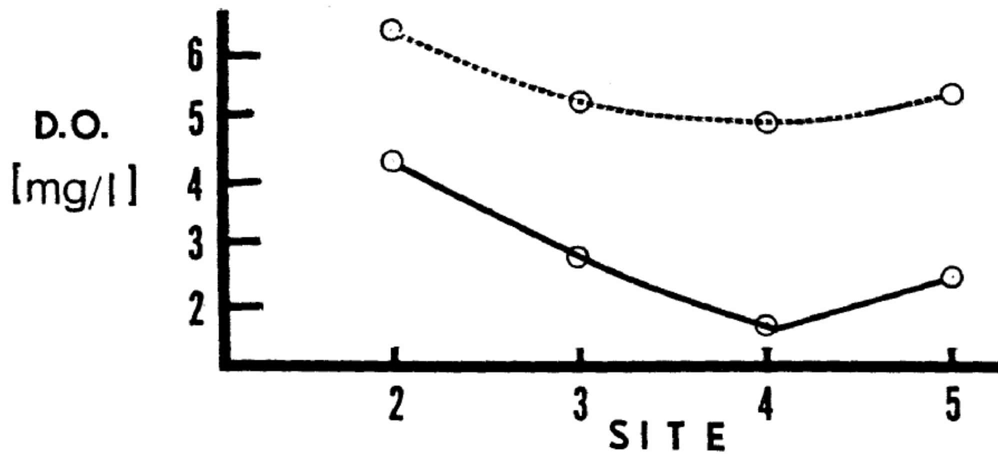


Figure 5

COMPARISON OF EXP. 3 AND W.R.R.C. DATA OF 9/28/7

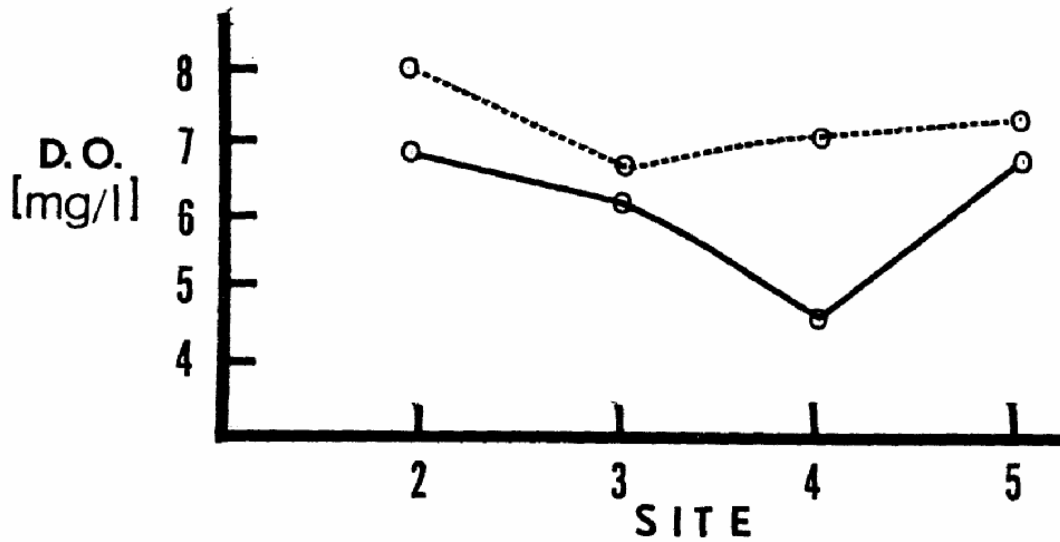
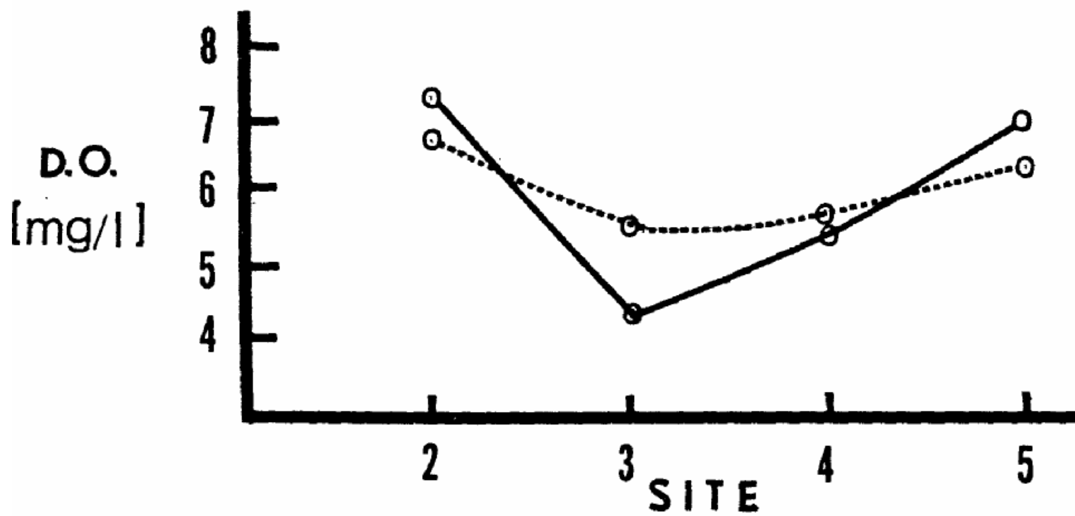


Figure 6

COMPARISON OF EXP. 4 AND W.R.R.C. DATA OF 7/ 8/74



A New Method for Quantitating the Activated Sludge Community Using Thin, Flexible Plastic Coverslips

Slide preparations of samples from activated sludge have high oxygen demands (B.O.D.). In minutes after the cover slip is added, the enclosed organisms cease normal feeding activities. Their morphology and physiology changes rapidly as anoxia brings on death. Even the thinnest commercially available glass and plastic coverslips allow little or no diffusion of gas through them. Thus, gaseous exchange is limited to the edges of the preparation. However, water evaporation occurs continually unless the coverslip is mounted with petroleum jelly, further limiting gaseous exchange. (The author has found that high vacuum silicone grease gives initially firmer coverslip support than petroleum jelly and allows superior prolonged preparations due to improved gaseous exchange.) Glass coverslips are charged surfaces which make them highly wettable as well as adherant to the polyelectrolytic molecules on the surfaces of microorganisms. Most commercially available plastic coverslips, though being nonwettable, usually exhibit a degree of toxicity.

This new technique described here was conceived to solve the special problems of studying the morphology, physiology, and ecology of activated sludge protozoa, especially peritrichs, and making rapid, repeatable counts of the numbers of each species. Its development was prompted by the problems with the standard Sedgwick-Rafter and Palmer counting cell glass preparations (Taras et al., 1971).

Activated sludge is a man-made continuous flow ecosystem showing a world-wide uniformity in flora and fauna, dominated in biomass by the ciliates in general, and peritrichs in particular (Pike & Curds, 1971; Varma, Finley & Bennett, 1972). Activated sludge is designed to reduce the B.O.D. of waste waters through aerobic respiration stimulated by high levels of aeration and agitation. The resulting biomass, secretion products, and undigested material flocculate in the clarifier. Activated sludge is characterized by its particulate nature making difficult accurate counts using standard counting procedures. Clearly, what was needed was a quantitative sampling method which would sufficiently flatten out these clumps, yet allow adequate gaseous exchange. For this flattening to be non-lethal, both slide and coverslip had to be nonwettable, that is, plastic. To allow sufficient gaseous exchange, the plastic cover had to be so thin it would be highly flexible.

The thin plastic film (Handiwrap®) is placed between a thicker plastic sheet and a sheet of paper and cut into 1 cm squares. The squares of plastic film, stuck to the plastic sheet, are stored in a dust-free container. The squares of film are cut out so that the tiny grid markings (~ 700μm) in the Handiwrap are properly aligned. A drop (0.2 ml) of activated sludge delivered from a Pasteur pipette or a volumetric pipette is dropped onto the plastic slide. A pair of watchmaker's forceps is used to remove the plastic film which sticks to the square of plastic sheet by static electricity. The square of plastic film is placed over the drop on the plastic slide making sure that the side with the square grid markings are on the upper surface. The slightest handling of the delicate square of plastic will mar it

with finger secretions, tiny scratches, or folds. The drop of activated sludge will spread out evenly as the plastic slide is rocked from side to side or tapped on the side. This also spreads out the particles in the drop. A pointed piece of absorbent paper is placed at one corner to pull out the excess water, pulling the plastic film down on the particles to spread them. Microscopic observation during slow removal of water shows that few sludge organisms are lost, depend: on the fauna. This removal of excess water can be omitted if 0.1 ml of initial sample is used under the 1 cm square of plastic film. Counting, which takes from 20 to 40 minutes/0.2 ml sample, is carried out at 100X (10X objective). All protozoans and micrometazoa under the plastic are counted. Using a multiple (24) counter, enumeration can proceed at a rate of better than one count/second as scanning causes no extraneous movements in the particles or organisms. Even though the grid squares in the plastic film are somewhat broad, they can be used by counting down to the top edge of each horizontal line and one edge of each vertical line. While counting, evaporation at the edges requires periodic (every 20 minutes) addition of water to the preparation. The water drop is quickly sucked under the plastic film and spreads out evenly. The thinness of the layer of water reduces the need for wide focusing excursions. When focusing on the organisms, the grid squares are only slightly out of focus. These grid square markings only interfere with the counts of similarly small, irregular and refractile amoeba lying under them. A bright field microscope with achromatic objectives, such as the A. O. Microstar, is ideal for such counting. Apochromatic lenses and especially phase contrast show up the differences in water thickness requiring constant refocusing. The fauna can be studied qualitatively with phase contrast or Nomarski interference phase microscopy by placing a drop of immersion oil on the plastic film, thus allowing 440X observation without touching the oil, or 1000X immersed in the oil. This oil spreads out, filling in the valleys, providing a flat upper surfac and makes the grid squares and most imperfections on the upper surface of the plastic film disappear. However, in about 5 minutes, the oil will seep under the edge of the plastic film, leaving numerous refract islands of water. To avoid this, the plastic film is held down on all four edges by a coat of high vacuum silicone grease (Dow Corning[d]). This will also prevent water evaporation providing a very long-lived (10+ days) preparation as the thin film provides excellent aeration, yet minimal water evaporation. For qualitative study of the activated sludge fauna, plastic film coverslips can be cut into 2 cm squares. When a drop of immersion oil is added onto squares of this size, it usually does not seep under and spoil the preparation. When switching from 44X to the 100X oil immersion lens, there is a rather drastic change in focus level which should be recorded on the fine adjustment knob for future reference.

The plastic film acts as a splendid microcompressor. By periodic water removal, a single free-swimming protozoan can be held immobile, flattened and spread to twice its diameter. Addition of water will again free the protozoan (like a hypotrich) allowing it to spring back into shape and swim away. In the case of peritrichs and amoebae, the recovered shape will appear somewhat crenulated, but the pellicle

will be intact if the compression was not excessive. If a preparation is allowed to dry down normally, the film of water will become only a few microns thick in islands around the particles--yet between islands so thin that even some types of bacteria are compressed and spread. Observations on the feeding of the activated sludge are facilitated by this new method. Because of the good gaseous exchange and lack of toxicity in the plastic film and plastic slide preparation, normal feeding activities are observed. Removing the excess water pulls down the plastic film, compressing the soft-bodied protozoa and micrometazoa, thus facilitating observations on food vacuoles contents. For instance, specific types of bacteria have been distinguished in amoeba food vacuoles, or certain peritrichs have been seen to be feeding only on bacilloid bacteria.

High resolution can be obtained using these thin plastic film preparations with phase contrast and Nomarski interference phase contrast. There appears to be no appreciable reduction in resolution over glass coverslip preparations using 100X oil immersion objectives. For instance, in the suctorian Heliophrya erhardi, the haptocysts in the tentacle knobs are seen, as are the inner tube of the tentacle. In Paramecium, trichocyst tips taper to sharp points, mitochondria and smaller granules, kinetodesmata, and kinetosomes of the cortex are clearly seen, while cilia resolve as two tapering lines; the contractile vacuole nephridioplasm shows vesicles, and the kinetosomes of the peniculus and quadrulus are clearly resolved. In peritrichs, the kinety can be followed down into the infundibulum and the fine pellicular striation of Epistylis are clearly resolved. Peritrichs are especially well-flattened by the thin film and dry down without rupturing. The plastic film comes off cleanly leaving the dried, flattened peritrichs attached to the plastic slide. In an amoeba (such as Cochliopodium), the surface hull units are shown as well as features within the nucleus, such as the endosome. The smallest bacteria show clearly resolved nucleoid regions. The opinion of the author is that tiny features seen with plastic film preparations are better resolved with less distorting refractility than with glass coverslip preparation when using phase contrast. In Nomarski interference phase contrast, there appears to be little difference in the two kinds of preparations.

This method can replace hanging drop preparations as a means of providing good optics with good aeration. Free-moving organisms such as Tetrahymena can be accurately counted in volumetric samples in bioassay or toxicity studies. As drying occurs, no organism is outside the confines of the plastic film as the water does not protrude beyond it. Predator-prey studies are facilitated as the plastic film can be pulled down tightly for superior optics during stages in feeding. Food vacuole contents can be studied closely as microcompression causes good flattening with reduced cell rupture. The squares of plastic film float very well on the surface film of cultures and aquaria. A smaller square of plastic lying on top the larger square will keep off dust particles in prolonged colonizations. Sessile ciliates, such as peritrichs and suctoria, attach readily to the plastic film. The plastic film can be used to collect the neuston in field studies. This thin plastic film method may permit an improvement in direct counts of bacteria and other particulates in set volumes of water.

Unfortunately, Dow Chemical Company has recently decided to discontinue producing this Handiwrap with the embossed grid squares. The new product, now appearing in some stores, has a clinging agent, yet is equally non-toxic and of the same thickness. It should be possible for a firm to manufacture thin plastic film of better optical qualities than Handiwrap® and with or without a fine-lined grid square. These could be provided in boxes with the film on squares of thicker plastic or in easily dispensed rolls.

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Application of the New Method for Activated Sludge Community Identification and Enumeration

In February of 1976, a 50,000-gallon experimental activated sludge system operated by the EPA investigator Mr. Walter Schuck at the Blue Plains Sewage Treatment Plant (EPA Pilot Plant) had developed a bulking condition. The aeration tank was sampled shortly before and two days after treatment with hydrogen peroxide at 50 ppm. Table 7 shows the counts made of all observable protozoa and micrometazoa in four selected 0.02 ml samples of this activated sludge.

Before treatment with hydrogen peroxide, the large floc particles were seen surrounded by long filamentous strands of bacteria. Observations at 1,600X on a Zeiss phase contrast microscope showed about 10 morphologically distinct bacteria with one type dominant, and with a second type representing the majority of the strands. The thin plastic film coverslips flattened these particles allowing observation and counting of the tiny acanthamoeba living in the clumps and feeding on the sparce bacilloid bacteria. After sitting out for one hour, the film flattened, greatly compressing the amoeba and bacteria. Possibly in response to the oxygen diffusion through the thin plastic coverslip, the filamentous bacteria were clearly fragmenting into short segments on which the second smallest acanthamoeba (2) were feeding. Following treatment with hydrogen peroxide, this acanthamoeba (2) nearly tripled in number while the smallest acanthamoeba (1) increased only slightly. The sessile peritrichs and suctoria changed little in number. Most dramatic was the decrease in the larger crawling hypotrichs like *Aspidisca costata*, and to a lesser extent, the smaller *A. sulcata*. The predaceous flagellate *Paranema* and amoeba *Mayorella* decreased while the ciliate predator *Hemiophrys* increased. It should be noted that the suctorian predator on peritrich telotrochs, *Lernaeophrya capitata*, was far more important than their numbers indicate, as each has hundreds of tentacles each terminated by a mouth. Note also the near absence of metazoa from this community.

After treatment with hydrogen peroxide, the particles were smaller and had shorter strands of filamentous bacteria extruding from them. The acanthamoeba (2) was seen feeding on the bacilloid fragments. This study indicates that this bulking, which occurred under conditions of a relatively long retention time, may have been caused by the formation of larger particles in which the bacteria lacked oxygen and were induced to produce filamentous strands on which their grazing amoeba could not ingest. The hydrogen peroxide caused an increase of dissolved oxygen allowing fragmentation of the filamentous bacteria and recovery of the predator-prey relationship between these bacteria and amoeba. In this way, biomass was reduced and a more efficient breakdown of organic substrate resumed through the selective feeding of the amoeba. The hydrogen peroxide treatment did not adversely alter the protozoon community, possibly only causing a decrease in the larger crawling species which may have been stunned momentarily as the hydrogen peroxide was converted to a condition of supersaturation of oxygen.

Table 7.

Four replicate counts of total micrometazoa and protozoa in a 50,000-gallon E.P.A. experimental activated sludge sewage plant before and after treatment with hydrogen peroxide to correct bulking condition.

	BEFORE				2/19/76	AFTER				2/21/76
	9:30 A.M.	10:15 A.M.	11:30 A.M.	12:15	Average	10:00 A.M.	10:45 A.M.	11:30 A.M.	12:15	Average
Flagellates										
Astasia sp.	1	1	0	0	0.5	0	0	0	0	0
Paranema trichophorum	11	8	3	8	7.5	2	5	0	1	2
Amoeba										
Acanthamoeba sp. 1	1422	1457	1571	1323	1443	1284	1366	1388	1230	1317
Acanthamoeba sp. 2	108	215	257	262	210	449	560	740	609	589
Acanthamoeba sp. 3	2	2	0	0	1	0	0	0	0	0
Mayorella sp.	10	13	8	11	10	0	9	3	5	4
Flabellula sp.	0	1	0	1	0.5	1	5	6	0	3
Thecamoeba sp.	0	0	1	0	0.5	0	0	0	0	0
Holotrich ciliates										
Unknown ciliate	3	7	9	7	6	2	0	2	1	1
Hemiophrys sp.	20	22	11	21	18	29	42	29	28	32
Lionotus sp.	0	2	2	2	1	1	0	1	0	0.5
Peritrich ciliates										
Vorticella convallaria	100	68	94	150	103	158	63	95	91	102
V. microstoma	4	6	3	1	3	2	4	3	6	4
V. minuta	1	0	0	0	0.25	1	0	1	0	0.5
Vorticella sp.	0	0	0	0	0	2	1	2	0	1.25
Opercularia coarctica	0	0	0	0	0	0	2	0	4	1.5
Carchesium sp.	8	2	14	5	7	10	14	11	148	45
Epistylis plicatilis	1	2	0	0	0.75	5	0	0	0	1.25
Rhabdostyla sp.	1	0	1	0	0.25	0	0	0	0	0
Hypotrich ciliates										
Aspidisca costata	92	109	89	74	91	16	14	14	12	14
A. sulcata	119	125	144	119	127	80	39	56	62	59
Suctorian ciliates										
Lernaeophrya capitata	2	0	1	1	1	1	2	2	0	1
Podophrya sp.	1	0	2	0	0.75	0	0	0	0	0
Micrometazoa										
Nematode(Rhabditis)	1	1	0	0	0.5	1	0	0	0	0.25
Rotifer	0	0	0	1	0.25	0	1	0	0	0.25

A second study on this 50,000-gallon EPA experimental activated sludge plant was to sample at four-hour intervals for a 12-hour period from 9:00 a.m. to 9:00 p.m. to determine if the activated sludge community changed appreciably, and to determine if four was an adequate sample size (This was tentatively confirmed.). Table 8 shows the total number of protozoa (excluding amoeba which were too numerous to count without using a sampling procedure) and micrometazoa in four 0.02 ml samples taken at 9:15 a.m., 1:20 p.m., 5:30 p.m., and 9:40 p.m. on April 27, 1976. Even though the number of each species varied considerably among the four replicates, the arithmetic means were similar. The average total number of organisms for each of the four time periods was 116.1 with a standard deviation of only ± 2.8. This indicated that little numerical change in total organisms counted occurred during this 12-hour period. Figures 7 to 12 show some representative species in this activated sludge community.

Table 8.

Four replicate counts of total micrometazoa and protozoa (excluding amoeba) in a 50,000-gallon E.P.A. experimental sewage treatment plant at four-hour intervals over a 12-hour period from 9:00 a.m. to 9:00 p.m.

April 27, 1976	9:15 a.m.					1:20 p.m.				
	1	2	3	4	Average	1	2	3	4	Aver
Flagellates										
Peranema trichophorum	3	2	2	3	2.5	0	3	4	6	3
Holotrich ciliates										
Amphileptus sp.	6	7	9	6	7	6	7	12	13	9
Lionotus sp.	2	4	2	1	2	3	2	3	1	2
Hemiophrys sp.	7	2	4	3	4	2	3	1	6	3
Peritrich ciliates										
Vorticella convallaria	42	28	38	47	39	74	10	51	36	43
V. microstoma	11	6	3	3	6	5	1	11	3	5
V. minuta	1	2	4	3	2	2	0	4	5	3
Carchesium sp.	3	7	8	3	5	4	0	1	0	1
Opercularia coarctica	7	2	25	7	10	2	0	5	8	4
Epistylis plicatilis	17	4	7	19	12	33	4	33	13	21
Epistylis sp. 2	3	2	0	4	2	0	0	31	27	14
Epistylis sp. 3	0	8	38	20	16	0	0	2	17	5
Rhabdostyla sp.	0	0	1	1	0.5	0	0	0	0	0
Hypotrich ciliates										
Aspidisca sulcata	0	1	0	2	0.75	0	1	0	1	0.5
Micrometazoa										
Philodina sp.	2	0	1	0	0.75	1	2	2	3	2
Cephalodella sp.	4	0	1	1	1	1	1	1	2	1
Nematodes	0	1	1	0	0.5	2	0	0	0	0.5

	5:30 p.m.					9:40 p.m.				
	1	2	3	4	Average	1	2	3	4	Avera
Flagellates										
Peranema trichophorum	3	2	3	3	3	2	1	1	1	1
Holotrich ciliates										
Amphileptus sp.	3	5	6	7	5	2	10	1	0	3
Lionotus sp.	1	4	5	4	3	4	3	1	2	2
Hemiophrys sp.	9	6	3	7	6	2	3	5	4	3
Peritrich ciliates										
Vorticella convallaria	19	45	44	88	49	68	79	21	30	49
V. microstoma	5	8	5	10	7	9	12	1	8	7
V. minuta	0	3	3	4	2	4	10	1	3	4
Carchesium sp.	0	0	0	0	0	0	8	0	0	2
Opercularia coarctica	4	4	3	0	3	5	28	1	3	9
Epistylis plicatilis	2	2	0	24	7	1	88	0	17	26
Epistylis sp. 2	0	10	38	47	24	0	11	2	18	8
Epistylis sp. 3	0	0	0	1	0.25	0	0	0	0	0
Rhabdostyla sp.	0	2	1	1	1	2	0	0	0	0.5
Hypotrich ciliates										
Aspidisca sulcata	0	0	1	0	0.25	0	0	0	0	0
Micrometazoa										
Philodina sp.	0	1	2	3	1	2	0	1	1	1
Cephalodella sp.	2	0	1	2	1	0	2	0	1	0.75
Nematodes	0	1	0	0	0.25	1	0	0	0	0.25

Figs. 7-12. Photomicrographs of protozoa and micrometazoa of activated sludge (E.P.A. 50,000 gallon, 4/27/76). (Figures 7 & 8 are brightfield; 9-12 are phase contrast.)

Fig. 7. Peritrich ciliates, *Vorticella convallaria*, attached by slender contractile stalks to floc particles. In the center is a single zooid of the colonial *Opercularia coarctata* on its non-contractile cross-striated stalk.

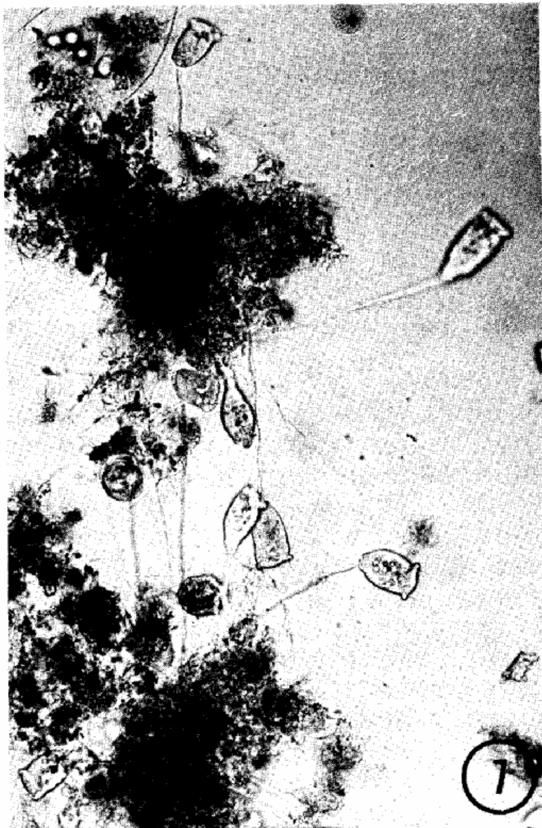
Fig. 8. Long, slender species of Philodina. This rotifer is attached caudally by slender toes and is feeding by beats of its coronal cilia. The fine random pattern in the background is caused by the irregularities in the thin, plastic film coverslips.

Fig. 9. Clusters of zooids of the colonial peritrich *Epistylis plicatilis* with their non-contractile stalks with longitudinal striations.

Fig. 10. Entangled strands of filamentous bacteria showing *Acanthamoeba* species 1 and 2. The smaller species (1) is seen in the lower right corner.

Fig. 11. Two compressed particles of floc showing amoebae feeding on the sludge bacteria.

Fig. 12. *Acanthamoeba* sp. (2) showing large clear vacuoles and nucleus with oval, dark endosome.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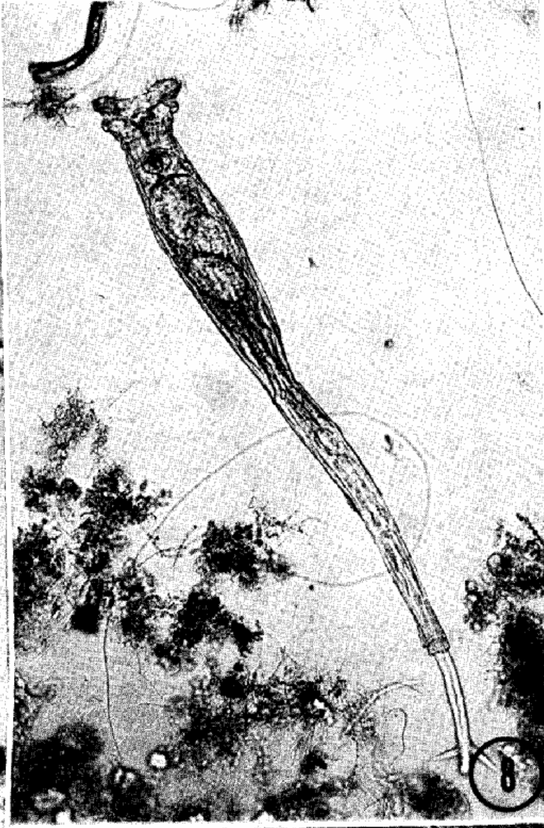
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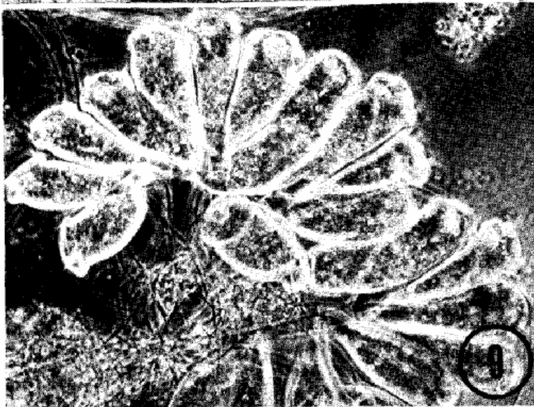
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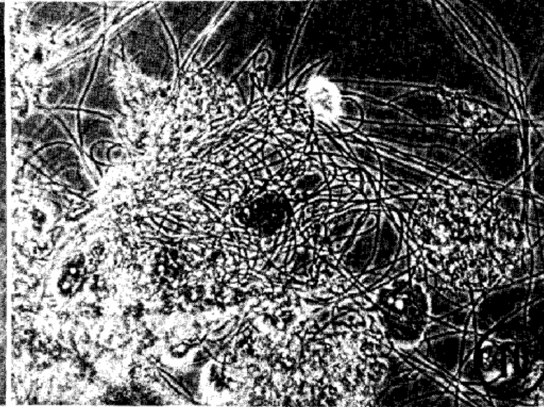

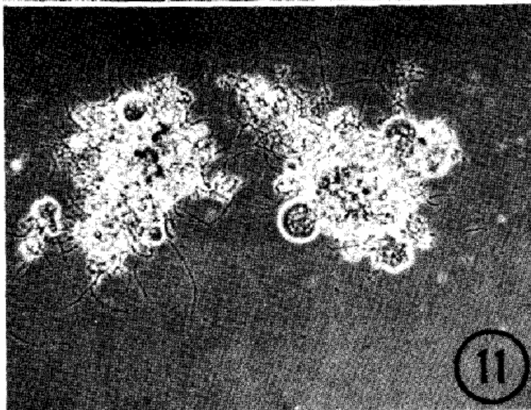
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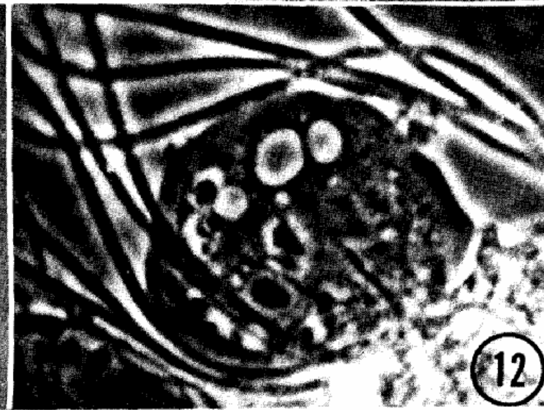
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Studies on Two Varieties of *Lernaeophrya capitata*, A Suctorian Ciliate Protozoan

In August of 1974 we isolated from the Potomac River *Lernaeophrya capitata* and established it in isolated culture, feeding it the non-sessile peritrich ciliate *Telotrochidium*. In February of 1976 we isolated from the E.P.A. activated sludge pilot plant (Tank B) a markedly different variety of *L. capitata*. The Potomac River variety will be referred to as *Lernaeophrya* (PR) and the activated sludge variety as *Lernaeophrya* (AS). Figures 13 to 20 are all of the Potomac River variety.

The literature on *Lernaeophrya* is sparce. It was first reported and named in a short paper by Perez (1903). Gonnert (1935) published a light microscopic study on its morphology, conjugation, and parasites. Batisse (1969) reported an electron microscopic study of the tentacle knob and developing haptocyst. Almost no work has been reported on its ecology or physiology. Yet, our studies indicate it is one of the most highly specialized predators with many specially evolved mechanisms for obtaining large size in microaerophilic environments.

We have found that *L. capitata* feeds almost exclusively on peritrichs, usually the free-swimming telotroch stage. It could not be cultured continuously on the other ciliates given it for food. It has only been found in aufwuchs communities or activated sludge with abundant peritrichs. The Potomac River variety is often seen attached to non-contractile stalks of colonial peritrichs. *L. capitata* is distinguished from the stalkless suctoria *Trichophrya* in that the macronuclear branches extend up into arms bearing the fascicles of tentacles; however, in small stages or when well-fed, the two genera are indistinguishable. Thus *L. capitata* may have been reported as the more commonly seen species of *Trichophrya*.

In 1975, the senior thesis student Ms. Eugenie Shalhoub did a study on *Lernaeophrya* (PR), and in 1976 the senior thesis student Ms. Toni Chadwick made a study of *Lernaeophrya* (AS). Other than these collaborative studies, the senior investigator has also done independent study on the physiology and life cycle of these *Lernaeophrya* varieties. A starved culture of *Lernaeophrya* (PR) with individual suctoria of down to 13 μm dm can grow to over 800 μm dm after five daily feedings of *Telotrochidium*. They will have over 65 fascicles of tentacles each with up to twenty tentacles, some extending up to 400 μm in length. Such a giant *Lernaeophrya* can eat scores of *Telotrochidium* concurrently. After such heavy feeding, the media is exchanged with fresh, aerated water to induce larvae production. Larvae are produced synchronously, with giants producing thirty or more at a time. *Lernaeophrya* (AS) cannot be so overfed without producing larvae. After two to three days of feeding, it will produce larvae without the media being changed with aerated water. Its largest size was 235 μm with up to 10 fascicles with tentacles up to 220 μm. However, the mean size for 50 randomly chosen specimens was 98.3 μm for (PR) variety and 97.2 μm for (AS) variety. The (AS) variety had longer and more slender arms. After forming many larvae, the (AS) variety was usually a clear pentagon; while the (PR) variety was yellowish, elongated or comma-shaped. The major difference was that the (PR) variety did not stick firmly to the substratum, being dislodged by a weak current,

Figs. 13 - 20 . Photomicrographs of the ciliate suctorian *Lernaeophrya capitata*.

Fig. 13. Darkfield view of single *Lernaeophrya* with outstretched tentacles, each tipped by a knob which functions as a mouth.

Fig. 14. Darkfield view of giant *Lernaeophrya capitata* with two newly attached unfed larvae, one on each side.

Fig. 15. Brightfield view of top of giant *Lernaeophrya* individual feeding on numerous free-swimming *Telotrochidium*.

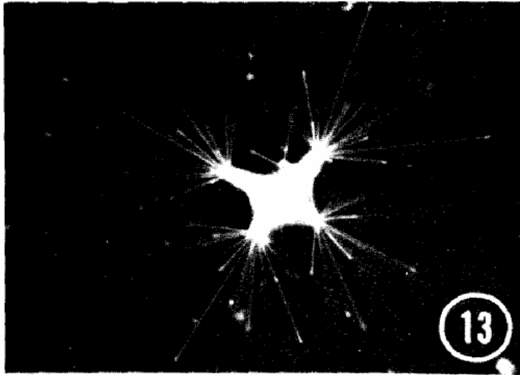
Fig. 16. Brightfield view from side of giant *Lernaeophrya* feeding on *Telotrochidium*. The swollen segments are over 150μ in diameter.

Fig. 17. Phase contrast top view of the tiny larva ringed by the rows of beating cilia.

Fig. 18. Phase contrast top view of two newly attached unfed *Lernaeophrya* showing the separate bundles (fascicles) of tentacles. The one on the left shows clearly the central darker macronucleus.

Fig. 19. Phase contrast view of *Lernaeophrya* showing peripheral contractile vacuoles.

Fig. 20. Darkfield view of *Lernaeophrya* showing the internal strands of the macronucleus here shown lighter.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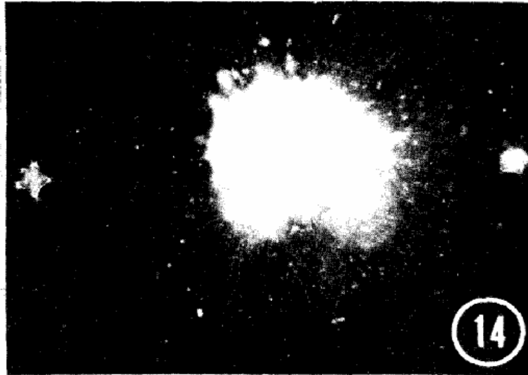
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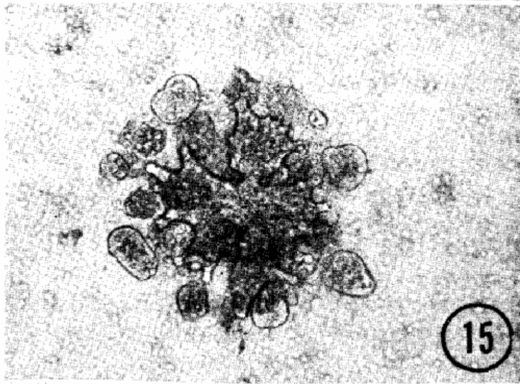
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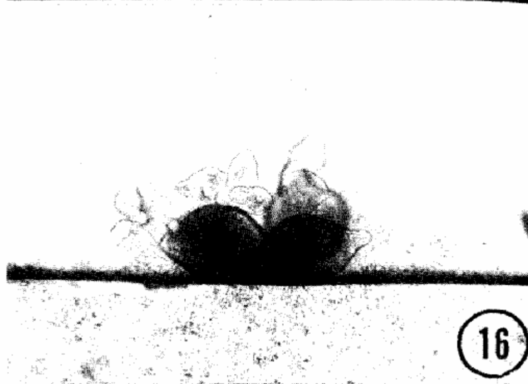
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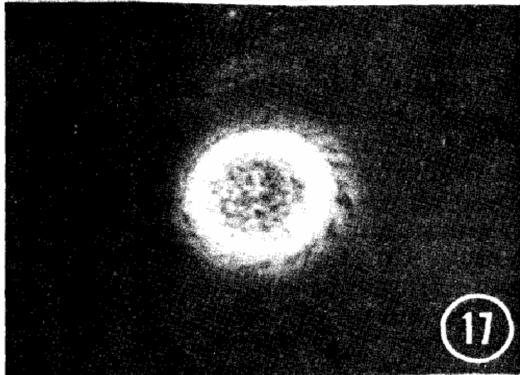
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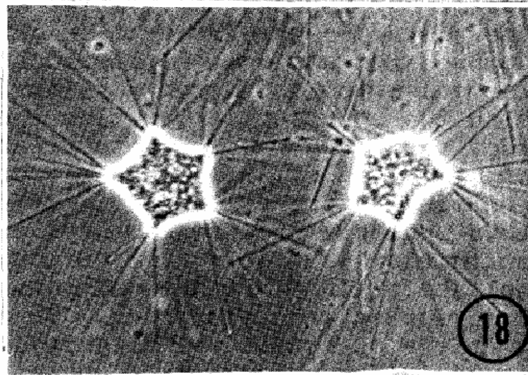
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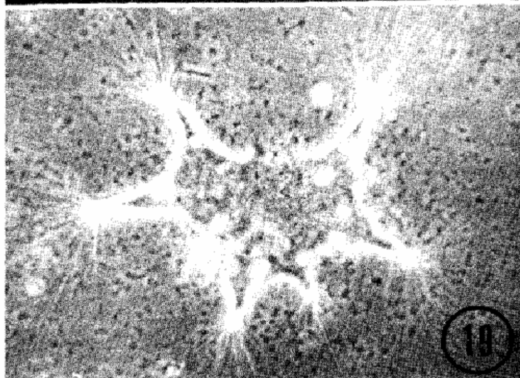
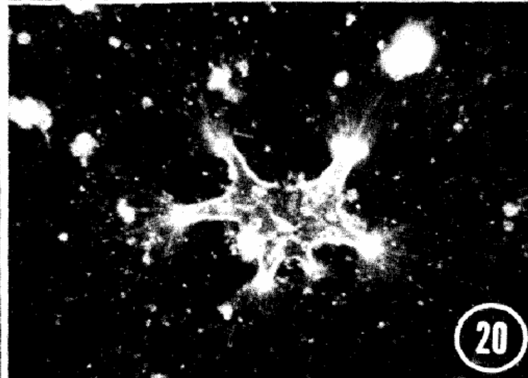
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yet its stickiness allowed it to re-attach easily. The (AS) variety was stuck firmly to the surface, being covered and surrounded by a mucoid mass. A very strong jet of water was required to dislodge it. Both varieties could be greatly distorted by the current flow, yet returned to their former shape.

A close study of these Lernaeophrya varieties showed that this suctorian is different from all others studied. It has many special adaptations for being a peritrich predator in environments like activated sludge where dissolved oxygen levels drop below 1 ppm. These suctoria can reach cross-sectional areas of up to 250 μm and are seen in activated sludge with cross-sectional areas of over 100 μm. Only a few oligochaete worms species can reach these cross-sectional thicknesses and they have circulatory systems and other special mechanisms for withstanding low oxygen levels. When a giant Lernaeophrya (PR) is placed under a glass coverslip and the light intensity raised to the highest for photomicrography, its responds to the heat and lowered dissolved oxygen by a mass surging and cyclosis of its cytoplasm. The macronucleus in the giants is composed of a labyrinth of thin strands. The food vacuoles being 5-10 μm in dm, are tiny compared to those of other suctoria. The contractile vacuole system is composed of a radiating net of tubules with tiny vesicles which empty into the larger contractile vacuole. These vacuoles can pulsate rapidly without completely collapsing. The pellicle is relatively thin ($\sim 0.3\mu$) compared to suctoria of comparable sizes.

We discovered another important response which led us to investigate further the physiology of Lernaeophrya. When a suctorian like Heliophrya is overcompressed, the tentacles swell and coil up. This damage can be repaired, yet it takes a long time. In Lernaeophrya, the tentacles were seen to quickly regain normal morphology after the compression was removed (by adding water under the coverslip). The tentacles would undergo a series of rapid contractions and relaxations. Also the contractile vacuoles increased their beat during tentacle repair. Since the inner tubule of the tentacle is composed of a bundle of microtubules, we used two microtubular binding chemicals, colchidine and vinblastine sulfate. Colchicine, at even 10^{-3} M, did not influence this recovery process compared to controls. Lernaeophrya treated with colchicine were able to feed normally. In contrast, vinblastine sulfate at 10^{-3} M was usually fatal, though sometimes not on the first compression and relaxation. Tentacles broke off, the mucoid layer over the suctorian was dissolved away and they became rounded up. An additional compression caused rupturing. Vinblastine sulfate at 10^{-6} M caused less severe responses, yet was fatal to large individuals, disruptive to medium-sized ones which nevertheless survived, and had slight effect on small Lernaeophrya. A 5% acetone solution was found to cause eventual rupture of tentacles as well as the body of suctoria.

From an ecological point of view, Lernaeophrya capitata plays an important role by controlling the population size of the peritrichs which are the most important bacteria feeders in activated sludge and in rivers that receive human organic wastes. Lernaeophrya can respond quickly to rapidly expanding peritrich populations, grow from a 31 μm individual with 5-10 tentacles, to a 800 μm individual with over 1000 tentacles, each essentially a separate mouth. Lernaeophrya can then synchronously produce enormous numbers of larvae while continuing to

feed on the swarming peritrich telotrochs. The *Lernaeophrya* larvae are rapid swimmers and quickly attach and are feeders themselves in minutes. Thus, *Lernaeophrya* is a specialized predator on peritrichs, forming a predator-prey complex which can respond quickly to increased numbers of bacteria. The predation of the *Lernaeophrya* controls the efficiency of the peritrich prey as well as the bacteria. *Lernaeophrya*, by removing the surplus peritrichs, reduce the demand on the limited dissolved oxygen required by inefficient and starving peritrichs that would die and cause over-bacterization and death of the community.

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Radioisotope Confirmation of Ostracod Predation On the Heterotrich Ciliate *Spirostomum*

Spirostomum species often appear in the bottom sediments in aquaria established in the laboratory colonized with river floats, yet *Spirostomum* are almost never collected in the dishes in the floats. The *Spirostomum* and other benthic species probably live in spaces between the rubber stoppers of the float. Likewise, ostracods, *Paramecium* and *Euplotes* species are rare in the collected aufwuchs community but dominate in the aquarium sediment. When the benthic community is separated from the sediment using the vial-in-testtube method (Spoon, 1972) and placed in petri dishes, the ostracods, *Spirostomum*, *Paramecium*, and *Euplotes* community quickly degenerates with the *Spirostomum* disappearing first, then *Paramecium*, then *Euplotes* and finally the ostracods. Numerical studies by senior thesis student, Bob Johnson in 1973 indicated that the ostracod *Cypridopsis* were feeding directly on *Spirostomum intermedium* and *S. teres*. Direct observations of this feeding were not possible because the ostracods move so quickly and their mouth parts are hidden under a carapace. Senior thesis student Sam Locatelli worked with the senior investigator to attempt to resolve this problem. The ostracod was a species of *Cypridopsis* grown in an aquarium stocked originally from Piscataway Creek, Station 5 on the Potomac River. Two species of *Spirostomum* were used as food, the large *S. ambiguum* obtained from Carolina Biological Supply,and *S. intermedium* isolated from an aquarium stocked from Hains Point, Station 2 on the Potomac River. The *Spirostomum* were cultured in proteose-peptone Cerophyl wheat broth diluted 1:10 (Finley, 1965).

First, a study was made to determine which species of *Spirostomum* would be best for the radioisotope food chain study. In 1-inch plastic petri dishes with 2 ml media were placed 5 ostracods in four arrangements--10 *S. ambiguum* alone, 10 *S. intermedium* alone, or 10 of each *Spirostomum* species. Controls were set up without ostracods and with only the five ostracods. Each situation was repeated in four petri dishes. The numbers of *Spirostomum* and ostracods were counted after 1, 2, 3, 4, and 6 days. The whole experiment was then repeated. The data for one of these two replicates is graphed in Fig. 21. (Generally, the controls showed neither loss or division in their non-nutrient media over the six-day period for either *Cypridopsis* or *Spirostomum*.) The *S. intermedium* either with *S. ambiguum* or alone was fed on less heavily. (In this first replicate, the *S. ambiguum* disappeared more rapidly when together with *S. intermedium*. In the second replicate, *S. intermedium* disappeared more rapidly when together.) Possibly the slower movement and larger size of *S. ambiguum* made it a better prey of *Cypridopsis*. Maybe this naturally-occurring *S. intermedium* is better equipped to avoid its normal Potomac River predator, *Cypridopsis*. Nevertheless, these studies helped support the hypothesis that *Cypridopsis* feeds on *Spirostomum*, yet the ostracods could have been inadvertently rupturing the *Spirostomum*.

Tritiated thymidine was selected as the radioactive tracer because of the safety of tritium and the ability of thymidine to be incorporated rapidly by growing bacteria and subsequently passed from growing *Spirostomum* to the ostracods. *Spirostomum ambiguum* were allowed to

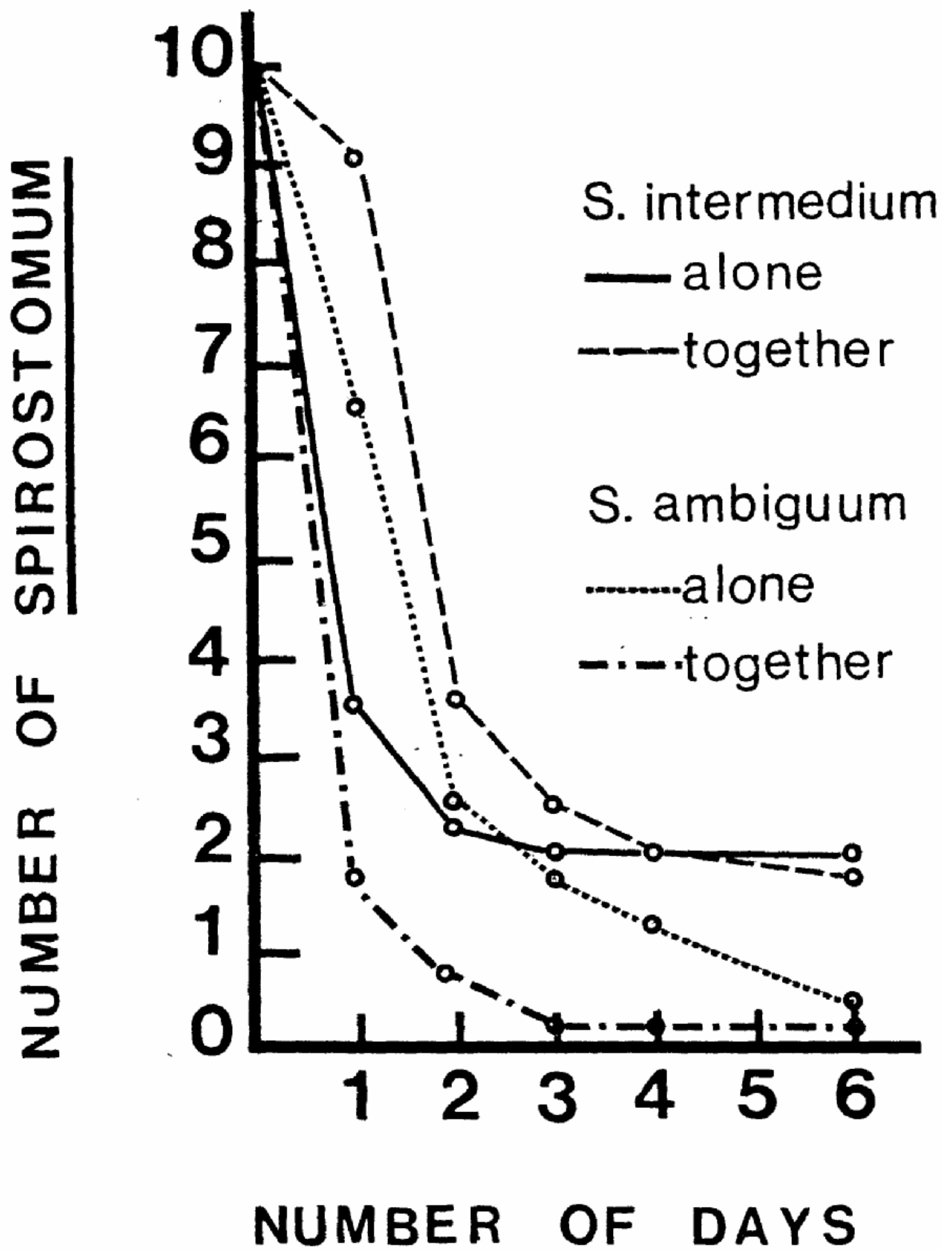

Fig.21. Graph showing numbers of Spirostomum ambiguum and S. intermedium prey remaining after 1, 2, 3, 4, and 6 days exposure to five ostracods (Cypridopsis). (Each point is the average in four dish replicates.)

grow for 24 hours in a culture flask of PPCW media containing the tritiated thymidine. Then approximately 250 *Spirostomum* in equal volumes (20 ml) of the mixed culture media were placed in 3" plastic petri dishes. Then 25 washed ostracods were added to each appropriate dish. In the first experiment, there were three replicates of the following groups: *Spirostomum* not fed on for 6 days, *Spirostomum* fed on for 6 days (reduced to about 125 individuals), and ostracods fed *Spirostomum* for 3, 4, 5, and 6 days. The ostracods were washed free of *Spirostomum*, digested in a scintillation vial containing 0.8 ml of NCS Tissue solubilizer, held at 47°C for 24 hours before receiving 10 ml acidified scintillation fluid. After 48 hours refrigeration, the vials were brought to room temperature and analyzed by a Beckman Model LS-100C liquid scintillation counter (Dr. Sam Pancake assisted in the design of this radioisotope procedure). The results of this study are shown in Fig. 22.

It can be seen from the two separate counts of the scintillations that the curves indicate uptake of the tritium label followed a logarithmic path. This further supported the belief that the *Spirostomum* were actually ingested and assimilated.

In a second experiment, a control was added to determine if the ostracods could absorb the label from the media. All the *Spirostomum* were removed from three dishes after feeding for three days in the labeled media with bacteria. To these dishes ostracods were added. Table 9 shows the results of this experiment. There was a 92% increase in label in the ostracod group with *Spirostomum*. The twofold increase in label in *Spirostomum* preyed upon than not exposed could be due to the predation increasing the feeding efficiency of the *Spirostomum*. These preliminary studies are being expanded with better controls to describe this predator-prey system. These experiments lend strong support to the hypothesis that the ostracods do capture, ingest, and assimilate *Spirostomum*. The ostracods feeding may involve both active capture and rupture and filter-feeding on the cytoplasmic particles.

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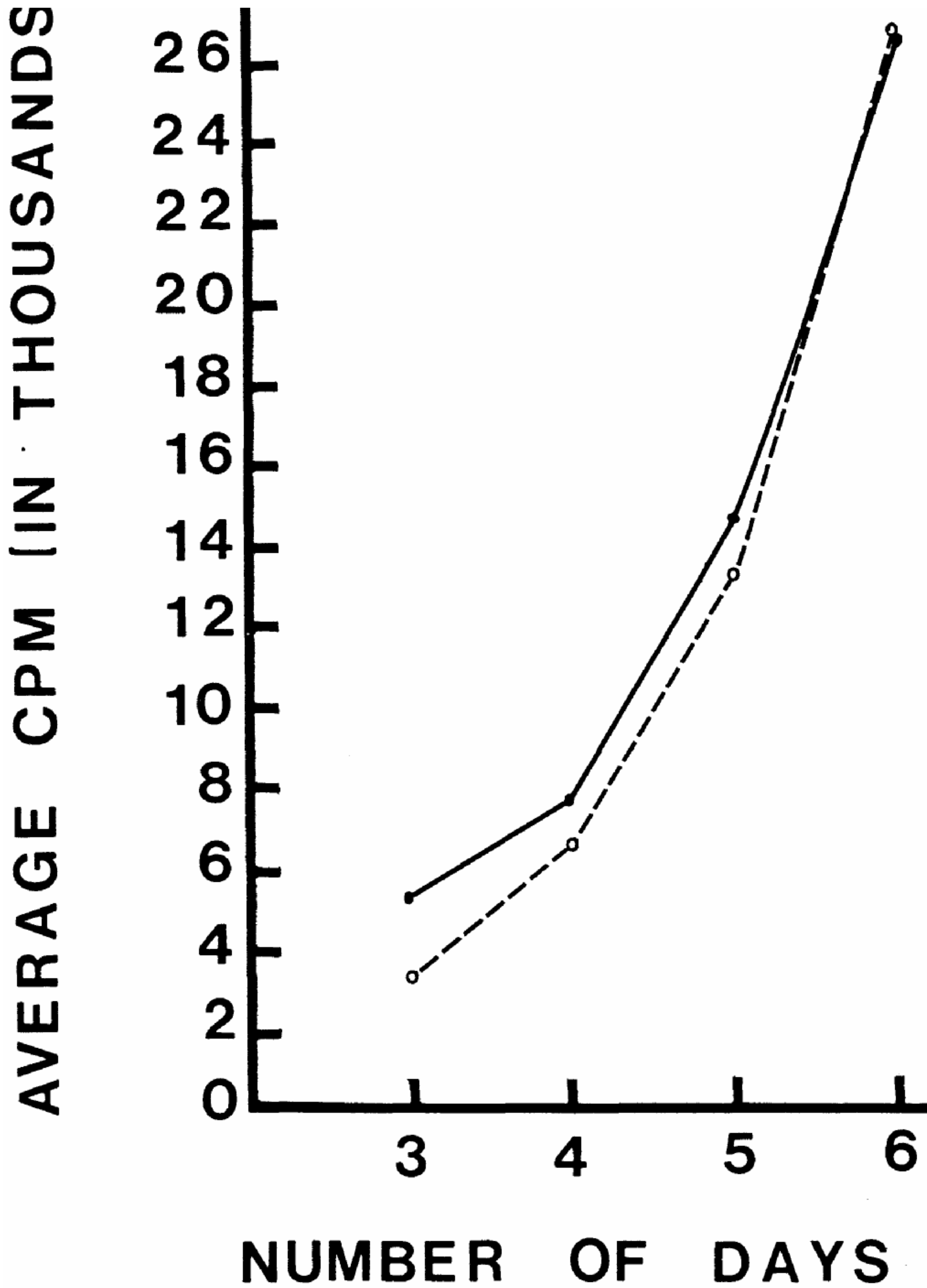

-g. 22. Average counts per minute for count Cycle 1(straight line) and Cycle 2 (broken line) obtained by liquid scintillation of the washed ostracods after 3, 4, 5, and 6 days predation on <u>Spirostomum ambiguum</u>.

Table 9. Numbers of scintillations for tritiated thymidine labeled Ostracod--*Spirostomum*--Bacteria system after three-day exposure.

Number of Organisms	CPM	Average CPM	CPM	Average CPM
Experimental				
125 Eaten *S. ambiguum*	31267.8		32065.4	
125 Eaten *S. ambiguum*	20609.7	25081.9	20490.2	25376.8
125 Eaten *S. ambiguum*	23368.1		23574.9	
Experimental				
25 Ostracods Fed	74225.1		78006.2	
25 Ostracods Fed	68145.6	75405.1	70985.4	79025.5
25 Ostracods Fed	83844.7		88085.0	
Control				
250 Uneaten *S. ambiguum*	34514.6		36262.9	
250 Uneaten *S. ambiguum*	72636.0	57470.0	75614.2	59799.3
250 Uneaten *S. ambiguum*	65259.4		67520.7	
Control				
25 Ostracods Alone	30267.8		30695.0	
25 Ostracods Alone	39444.4	39049.4	35270.5	37785.4
25 Ostracods Alone	47435.9		47390.8	

Literature Searches

In order to conduct this research program, several extensive literature searches have been completed this last year. All literature on the suctorian ciliates published over the past fifty years has been cataloged by the senior investigator. In collaboration with the late Harold Finley of Howard University and his graduate student Colliston Rose, and the senior thesis student Michael Phelan, an attempt was made to compile the complete literature on the peritrich ciliates. Over 1,200 references have been cross-cataloged on four separate file systems; that is, by author, date, subject, and taxonomy. A manuscript entitled, "The Literature on the Peritrich Ciliates" is being completed for publication. The graduate student, Miss Janine Ramsey, compiled a complete file of copies of papers on cannibalism among protozoa. The senior thesis student Mr. Pat Ryan attempted to research the complete literature on cannibalism compiling over 350 references. Mr. Ryan also made an extensive study of three strains of the ciliate predator *Didinium*, attempting to determine if its giant formation occurred through cannibalism. His results were inconclusive. The senior thesis student Mr. Philip Schmidt compiled all the literature on the genus *Didinium*. The senior thesis student Mr. Peter Eisenhardt made a literature search of the effects of the pesticide methoxychlor on fish and invertebrates. All of these literature searches bear directly on the Potomac River studies and especially on the main research interests of the principal investigator.

Publications Generated from This Research Project

On June 5th 1975, the principal investigator participated in the symposium at Alexandria, Virginia entitled "The Potomac Estuary, Biological Resources, Trends and Options" sponsored by the Interstate Commission on the Potomac River Basin and the Maryland Power Plant Siting program. His presentation entitled, "Microbial Communities of the Upper Potomac Estuary: The Aufwuchs" was published with the Symposium on April 1976 with William T. Mason and Kevin C. Flynn as the editors. This seven-page article was essentially a summary of the 117-page grant report submitted to O.W.R.R. in August 1975. This work, especially that dealing with ciliate protozoa, was also presented at the annual meeting of the Society of Protozoologists in New Orleans, June 5th at the closing session for which the senior investigator served as chairman.

The paper entitled, "Poly(ethylene oxide), a New Protozoan Slowing Agent, Compared to Methyl Cellulose and Protoslo" with Charles Feise as junior author was accepted for publication by the Journal of Protozoology. Scores of colleagues have tried this new non-toxic substance and found it to be superior to any other slowing agent. Also, unlike methyl cellulose, it is readily dissolved in salt water.

The senior author is contributing articles to the two-volume tribute to Dr. Harold Finley, who passed away in the spring of 1975. The senior investigator authored the technique communication entitled, "Use of Thin, Flexible Plastic Coverslips for Microscopy, Microcompression and Counting of Aerobic Microorganisms." It was this new microscopic method that permitted the extensive light microscopic study on *Heliophrya* feeding on *Paramecium*. This twenty-page article entitled "Observation on the Behavior and Feeding Mechanism of the Suctorian *Heliophrya erhardi* (Rieder) Matthes preying on *Paramecium*" by D. M. Spoon, G. B. Chapman, R. S. Cheng, and S. F. Zane. The following page illustrates the stages in this feeding process. These two papers will appear in the first Finley memorial volume of the Transactions of the American Microscopical Society.

The senior investigator on several occasions over the past four years has obtained cyst-like corpses of paramecia in the suctorian cultures. Dr. Joe L. Griffin studied these three-month old corpses with transmission and scanning electron microscopy and demonstrated a remarkable state of preservation even though bacteria were present. Dr. Griffin and the principal investigator are publishing a paper entitled " Natural Preservation of Cyst-like Corpses of *Paramecium aurelia* in cultures of a suctorian" in the second Finley memorial volume to appear this fall.

Cultures of Protozoa and Micrometazoa

The senior investigator has spent an average of eight hours per week maintaining the isolated and aquarium cultures. Table 10 shows a list of those species in culture in late summer of 1975. Since then the senior investigator has acquired subcultures of the peritrich collection of the late Harold Finley of Howard University. *Rhabdostyla* should be added to the list of peritrichs in isolated culture. It was discovered that most of the species of peritrichs from the activated sludge at Blue Plains could be grown on 1:10 proteose peptone Cerophyll wheat (PPCW) media developed by Dr. Finley. An *Epistylis* species that readily encysts was isolated from the sewage treatment plant. A new suctorian species of *Heliophrya* has been isolated which resembles *H. rotunda*. A second strain of the suctorian *Lernaeophrya capitata* was isolated from the E.P.A. activated sludge experimental tanks. It differs markedly from the Potomac River strain. A key difference is its tenacious attachment to the culture dish, while the Potomac River strain has essentially no adhesion. The senior investigator, after years of attempts, discovered a highly successful means of growing the ciliate *Spirostomum*. They are grown in testtubes with a smaller vial inside with 1:10 PPCW media. The outer diameter of the vial is about 1 mm smaller than the inner diameter of the 15 cc testtube. This restricts gaseous exchange favoring growth of the microaerophilic *Spirostomum*. Dense populations are maintained with subculturing needed only every 40 days, signaled when the *Spirostomum* go to the bottom of the tubes. A large mayorellid amoeba that eats both suctoria and peritrichs has been isolated from the activated sludge.

Zooflagellates
- Anthophysa vegetans
- Anisonema prosgeobium
- Bodo sp.
- Chilomonas sp.
- Codonocladium umbellatum
- •Codosiga botrytis
- Collodictyon triciliatum
- Cryptomonas sp.
- Heteronema acus
- Mastigella simplex
- Mastigamoeba reptans
- Monas sp.
- •Monosiga varians
- •Monosiga sp. 2
- •Notosolenus apocamptus
- •Paranema trichophorum
- •Peranemopsis inflexum

Sarcodines
- •Actinophrys sol
- •Actinosphaerium eichorni
- Amoeba discoides
- Amoeba verrucosa
- Amoeba vespertilio
- Arcella coronata
- Arcella dentata
- •Arcella discoides
- Arcella vulgaris
- Biomyxa vagans
- Centropyxis aculeata
- Centropyxis ecornis
- Centropyxis hemisphaerica
- Clathruline elegans
- ★Conchiliopodium bilimbosom
- Difflugia corona
- Difflugia globosa
- Difflugia urceolata
- Diplophrys archeri
- Flabellula sp.
- Gromia sp.
- Hartmanella sp.
- Hyalodiscus sp.
- ★Mayorella vespertilio
- Microgromia sp.
- Naegleria sp.
- Penardia mutabilis
- Thecamoeba verrucosa
- Vahlkampfia limax
- Vanella sp.

Ciliates

Holotrichs
- Amphileptus sp.
- Chilodonella cucullulus
- Chilodonella uncinata
- •Chilodonella nana
- •Cinetochilum margaritaceum
- •Coleps hirtus
- •Colpidium colpoda
- Colpoda cucullus
- •Cyclidium sp.
- Cyrtolophosis mucicola
- Didinium armatum
- Didinium balbiani
- ★Didinium nasutum
- Dileptus anser
- Enchelyodon sp.
- Frontonia leucas
- Hemiophrys fusidens
- Homolozoon vermiculare
- Lacrymaria olor
- Lacrymaria sp. 2
- Lechriopyla sp.
- Lembadion sp.
- Lembus sp.
- Litonotus fasciola
- Litonotus sp. 2
- Loxodes sp.
- Mesodium sp.
- •Metapus es
- Metapus spiralis
- Nassula sp.
- ★Paramecium aurelia
- Paramecium calkinsi
- ★Paramecium caudatum
- ★Paramecium multimicronucleatum
- Paramecium putrinum
- •Paramecium trichium
- Peritromus sp.
- Phascolodon vorticella
- Prorodon teres
- Prorodon sp. 2
- Pseudoprorodon sp.
- Stokesia vernalis
- ★Tetrahymena sp.
- Trachelius ovum
- Tracheophyllum sp. 1
- Tracheophyllum sp. 2
- Trichopelma sp.
- Urocentrum turbo
- Urozoma sp.

Suctoria
- •Acineta tuberosa
- Acineta limnetis
- Acineta grandis
- Acineta foetida
- Anarma sp.
- Astrophrya arenaria
- Dendrosoma radians
- ★Heliophrya "burbancki"
- ★Heliophrya erhardi
- •Heliophrya riederi
- ★Heliophrya rotunda
- ★Lernaeophrya capitata
- •Metacineta mystacina
- Metacineta sp. 2
- Metacineta sp. 3
- Metacineta sp. 4
- Paracineta crenata
- Paracineta sp. 2
- •Podophrya fixa
- Solenophrya sp.
- Sphaerophrya magna
- Sphaerophrya pusilla
- Sphaerophrya sp. 3
- Squalorophrya macrostyla
- Trichophrya epistylidis
- Trichophrya sp. 2
- Tokophrya infusionum
- Tokophrya lemnarum
- Tokophrya quadripartita

Peritrichs
- ★Campanella umbellaria
- ★Carchesium polypinum
- ★Carchesium granulata
- Cothurnia sp. 1
- Cothurnia sp. 2
- Epistylis cambari
- Epistylis hentscheli
- Epistylis niagarae
- ★Epistylis plicatilis (3 varieties)
- Epistylis urceolata
- Hastatella radians
- Laegenophrya vaginicola
- Omphrydium eichhorni
- ★Opercularia coarctata
- Opercularia coronata
- Opercularia minima
- Opercularia phryganeae
- •Platycola longicollis (3 varieties)
- Scyphidia sp.
- Systylis hoffi
- ★Telotrochidium hennequyi
- Vaginicola yurvula
- Vaginicola grandis
- Vaginicola ingenita
- Vaginicola striata
- Vorticella aequilata
- ★Vorticella campanula (4 varieties)
- Vorticella convallaria
- Vorticella "finleyi"
- Vorticella mayeri
- ★Vorticella microstoma (2 varieties)
- ★Vorticella minuta
- Vorticella monilata
- Vorticella octava
- ★Vorticella striata
- Zoothamnium adamsi
- Zoothamnium pygmaeum
- Zoothamnium simplex

Spirotrichs

Heterotrichs
- Condylostoma sp.
- Spirostomum ambiguum
- •Spirostomum intermedium
- Spirostomum minus
- Spirostomum teres
- •Stentor coeruleus
- •Stentor "finleyi"
- Stentor roeseli

Oligotrichs
- Codonella cratera
- Halteria grandinella
- Strombidium turbo
- Strombidium sp. 2

Hypotrichs
- Actinotricha sp.
- •Aspidisca costata
- Aspidisca lynceus
- •Aspidisca sulcata
- Euplotes aediculatus
- ★Euplotes eurystomus
- Euplotes moebiusi
- Euplotes patella
- •Chaetospira mülleri
- Chaetospira sp. 2
- Holosticha sp. 1
- Holosticha sp. 2
- Holosticha sp. 3
- Hypotrichidium conicum
- Oxytricha fallax
- Oxytricha ludibunda
- ★Oxytricha sp. 3
- •Stylonychia mytilis
- Stylonychia pustulata
- Uroleptus sp. 1
- Uroleptus sp. 2
- Urostyla sp.

Coelenterates
- Cordylophora lacustris
- •Hydra americana
- Protohydra sp.

Parazoa (sponges)
- Spongilla lacustris
- Spongilla sp. 2

Rhabdocoels
- •Stenostomum sp. 1
- Stenostomum sp. 2
- Stenostomum sp. 3
- •Microstomum sp.
- •Macrostomum sp.

Triclads
- Dugesia trigrina

Bryozoa
- •Plumatella repens
- Pectinatella magnifica
- Hyalinella punctata

Gastrotrichs
- •Chaetonotus sp. 1
- •Chaetonotus sp. 2

Rotifers
- Asplanchna priodonta
- Branchionus bidentata
- Branchionus plerodinoides
- Cephalodella gibba
- Cephalodella auriculata
- Cephalodella sp. 3
- Cephalodella sp. 4
- Conochilus sp.
- Coolotheca sp.
- Erignatha sp.
- Euclanis dilatata
- Keratella americana
- Lacinularia flosculosa
- Lecane ohioensis
- •Lepadella patella
- Limnais melicerta
- •Monostyla sp.
- Notommata sp.
- •Philodina roseola
- Polyarthra sp.
- Ptygura sp.
- Rotaria sp.
- Synchaeta sp. 1
- Synchaeta sp. 2
- Testudinella sp.
- Trichocerca sp.

Cladocerans
- Bosmina sp.
- •Ceriodaphnia sp.
- Daphnia pulex

Calanoid copepods
- •Diaptomus sp.
- Limnocalanus sp.

Cyclopoid copepods
- •Cyclops sp.
- •Megacyclops sp.
- Numerous other species (20+)

Harpacticoid copepods
- 2 species

Ostracods
- •Cypris sp.
- •Cypridopsis sp.

Other Crustacea
- •Asellus sp.
- •Gammarus sp. 1
- Gammarus sp. 2

Hirudinea
- Clossophonia complanata
- Helopdella sp.
- Illinobdella richardsoni

Oligochaetes
- •Aelosoma hemprichi
- Aelosoma sp. 2
- •Aulophorus furcatus
- •Chaetogaster disphanus
- •Chaetogaster diastrophus
- •Dero sp.
- •Nais communis
- Ophidonais sp.
- •Pristina sp.
- •Stylaria proboscidea

Insect larvae
- Chironomids (15+ species)
- Rheotanytarsus sp.
- •Psychoda sp.
- Stonefly (10+ species)
- Mayfly larvae (8+ species)
- Sialis sp. 1
- Sialis sp. 2

Mollusca
- •Bithynia sp.
- Bulimid species
- •Goniobasis virginica
- •Gyraulus sp.
- Lanx sp.
- •Lymnaea sp.
- •Physa sp.
- •Pisidium dibium
- Planorbid species

Organisms maintained in laboratory
- ★In Isolated Culture
- •In Aquarium Culture

TABLE 10. Tentative List of Species of the Aufwuchs Microfaunal Community of the Upper Potomac River from Chain Bridge to Mt. Vernon (July, 1975)

Survey of Potomac River Sites

Upon receipt in February 1976 of the official letter authorizing funds for this project, the expensive and time-consuming river survey portion of the work was begun. On February 20th, the floats were set out. On March 20th, none of these floats were recovered; two floats were set out at each site. On April 24th, only one area 4 float was recovered. On June 25 and July 2, floats were recovered from areas 2, 4, and 5. Both floats plus their ropes on buoys at site 3 were lost. New floats were set out with a notice tied to the ropes saying, "Do not remove, Coast Guard Permit, etc " Subsequently, no floats have been removed. The floats for sites 2, 4, and 5 collected June 25 and July 2 were thoroughly studied and counts made. (This data will be included in the annual report for 1976-1977.) The following table shows all the physical-chemical data collected for these four collection dates. Using either our own boat or the Chesapeake Lightship range boat, we made a total of eight trips on the river during this survey period. Five different students have assisted in this work.

Preparation of Illustrative Keys to the Aufwuchs Members

The illustrative keys for the suctoria of the Potomac River are nearly complete with permanent slides, measurements of dimensions, drawings and photographs. Much progress has been made on the key to the peritrich ciliates of the Potomac River. Senior thesis student Mr. Michael Phelan added substantially to this work. A separate key on the ciliates of the activated sludge is also nearing completion. The graduate student Miss Janine Ramsey worked for a total of 174 hours preparing Protargol slides of the laboratory ciliates. Emphasis was placed on the suctoria, especially *Lernaeophrya capitata*. Permanent slides were made of about twenty species of ciliates.

Table 11.

Physical-Chemical Data Collected at the Potomac River Sites by D. M. Spoon,

Site 2-Hains Point
Site 3-Blue Plains Sewage Plant
Site 4-Broad Creek
Site 5-Piscataway Creek

A. March 20
B. April 24
C. May 21 & 29
D. June 23 & 25

SITE		2	3	4	5
Air °C Temperature YSI Thermister	A	20.0	20.0	19.0	15.0
	B	----	----	21.0	21.5
	C	20.0	18.0	18.0	19.0
	D	28.0	26.0	29.0	28.0
Water Temperature °C	A	8.5	8.0	9.0	7.0
	B	---	---	22.0	21.5
	C	19.0	21.0	21.0	21.0
	D	26.5	22.5	27.5	27.0
pH (Electrode)	A	7.3	7.2	7.2	7.2
	B	---	---	6.6	6.7
	C	6.7	6.4	6.4	6.5
	D	6.8	7.0	7.0	7.0
D.O.(ppm) (H)-Hach Kit otherwise YSI electrode	A	12(H)	8(H)	13(H)	16(H)
	B	----	---	9(H)	7(H)
	C	8.9	4.1	4.8	6.0
	D	8.1	7.2	6.3	6.2
Secchi (Inches)	A	--	--	--	--
	B	--	--	22	22
	C	24	20	20	20
	D	20	20	20	20
NO_3 (ppm)	A	3.5	2.8	1.8	1.4
	B	---	---	1.2	2.4
	C	1.1	2.2	3.1	3.5
	D	3.6	3.9	4.4	5.3
PO_4 (ppm) (ortho & meta)	A	0.03	0.75	0.82	0.4
	B	----	----	0.4	0.22
	C	0.32	1.9	0.65	0.4
	D	0.22	0.26	0.95	0.38