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RESEARCH REPORT 157

Identification of the Water Quality Factors Which Prevent Fingernail Clams from Recolonizing the Illinois River, Phase II

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UNIVERSITY OF ILLINOIS
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DEPARTMENT OF THE INTERIOR • OFFICE OF WATER RESEARCH AND TECHNOLOGY

IDENTIFICATION OF THE WATER QUALITY FACTORS WHICH PREVENT FINGERNAIL CLAMS FROM RECOLONIZING THE ILLINOIS RIVER
PHASE II

by

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TECHNICAL COMPLETION REPORT

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March, 1981

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ABSTRACT

Water samples taken from the Illinois River on 5 October and 22 April 1977 inhibited the beating of the cilia on isolated clam gills, within two hours of exposure. The April sample was significantly more toxic than the October sample. Sediment taken on 14 December 1970 from Quiver Lake, a bottomland lake which receives sediment from the Illinois River and where fingernail clams were abundant prior to a die-off in 1955-58, was toxic to isolated clam gills. A sediment layer from the 2.6-5.1 cm depth showed the greatest toxicity, the 0-2.5 cm depth the next greatest toxicity, and deeper layers showed significantly less toxicity. From 3 April to 8 May 1980, intact fingernail clams were exposed to raw Illinois River water (containing suspended sediment), clean well water, and raw river water subjected to three treatments: (a) sand filtration (b) sand filtration + carbon filtration (c) sand filtration + clinoptilolite filtration. After two weeks of exposure, clams in raw river water suffered significantly greater mortality (42.5%) than other clams. After six weeks of exposure, 62.5% of the clams in raw river water had died, the next highest mortality (47.5%) occurred in sand-filtered water, and mortality in the other two treatments did not differ significantly from the well-water controls (24% mortality). The clams probably survived better in the treated water for two reasons: (1) clinoptilolite and carbon each removed ammonia, which is found in Illinois River water and which is toxic to fingernail clams (2) the additional physical filtration provided by the charcoal and clinoptilolite removed additional sediment, which contains unidentified toxic factors. Surviving clams grew better in river water and treated river water than in clean well water, probably because they fed upon fine organic matter which passed through the filters. The latter results indicate that the unidentified toxic factor acts directly on the clams, rather than indirectly by affecting their food supply. The rapid assay, using fingernail clam gills, and the deletion bioassay, where toxic components are selectively removed from raw water samples and the corresponding reduction in toxicity measured, are promising means of identifying effective treatments for complex wastes and polluted streams.

Sparks, Richard E., Michael J. Sandusky and Anthony A. Paparo

IDENTIFICATION OF THE WATER QUALITY FACTORS WHICH PREVENT FINGERNAIL CLAMS FROM RECOLONIZING THE ILLINOIS RIVER
Technical Completion Report to Office of Water Research and Technology, Department of the Interior, March 1981

KEYWORDS--water pollution effects/ bioassay/ bioindicators/ animal physiology/ fingernail clams/ Sphaerium transversum/ Musculium transversum/ Sphaeriidae/ silt/ ammonia/ suspended solids/ suspended sediment/ Keokuk Pool/ Mississippi River/ Illinois River/ pollutant identification/ toxicity/ clams/ mussels/ mollusks/ bivalves/ water pollution treatment/ water quality/ zeolites/ clinoptilolite/ activated carbon/ filtration/ pollution abatement/ sediment/ water pollution sources/ food webs/ limiting factors/ secondary productivity/ aquatic productivity/ assay

INTRODUCTION AND BACKGROUND

Richardson (1921, 1928) conducted surveys of the bottom fauna in the Illinois River and found that fingernail clams and snails were abundant or common in a 180-mile section of the river between the mouth and the upper end of Peoria Lake (Figure 1) during the years 1913-1915. The clam population declined after 1915 from river miles 80.1 to 180.5 (river miles are measured upstream from the confluence with the Mississippi River) as a result of the increased sewage pollution from the Chicago Sanitary and Ship Canal which opened January 1, 1900, and diverted Chicago sewage from Lake Michigan to the Illinois River. Degradation of the bottom progressed downstream at a rate of 8 to 16 miles per year (Richardson 1921). The bottom fauna exhibited a recovery pattern from 1920 to 1925 (Richardson 1921, 1928), probably in response to the installation of sewage and industrial waste treatment facilities in the Illinois Valley.

After 1925 there is a gap in bottom fauna data until a 1964 survey by Starrett and Paloumpis (1964). Starrett and Paloumpis found no fingernail clams in the Illinois River above Beardstown (river mile 86.9), so the clams had died out in a 100-mile section of the river sometime between 1925 and 1964. Anderson (1977) surveyed the bottom fauna in the Illinois River in 1975. Distribution of fingernail clams in the Illinois River in 1975 was essentially the same as it was in 1964. However, there appeared to have been a drastic decrease or loss of snails in the lower Illinois River. Although Starrett and Paloumpis (1964) found no snails above Beardstown, they reported an average of $34/m^2$ from Beardstown to Grafton; no snails were collected in the 1975 study.

There are three lines of evidence indicating that the die-off of fingernail clams occurred in the 1950's. Paloumpis and Starrett (1960) observed a die-off of fingernail clams in three Illinois River bottomland lakes in the 1950's. To use Quiver Lake as an example: in 1952, sphaeriid clams, mainly *Musculium transversum*, occurred in numbers exceeding $20,000/m^2$. During the next four years, 1953-1956, populations of fingernail clams and certain snails declined to zero. A slight recovery occurred in the following

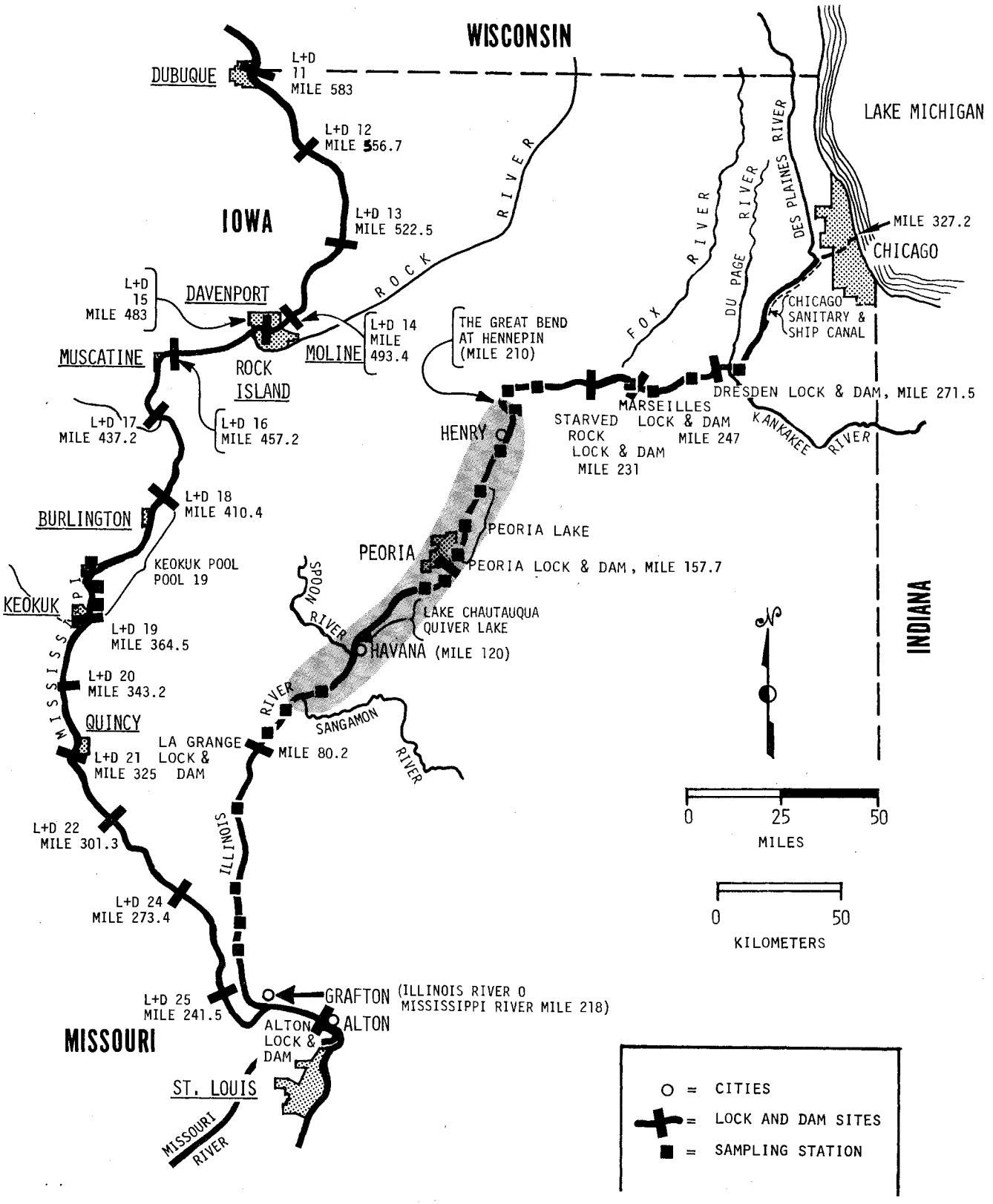

Figure 1. Map of Illinois and Mississippi Rivers (showing bottom sampling stations). Fingernail clams and other benthic organisms died out in the middle reach of the Illinois River in the mid-1950's (shaded area). Live clams for bioassays were obtained from Keokuk Pool, Mississippi River.

two years. However, in 1973, Sparks (unpublished data in the files of the Illinois Natural History Survey) found no sphaeriids, snails, or aquatic insects in Quiver Lake at the same stations.

A second line of evidence pointing to a 1950's die-off of benthic organisms is the abrupt decline in utilization of the Illinois Valley by lesser scaup ducks, which are one of the groups of diving ducks which feed on fingernail clams and snails (see Figure 2). Figure 2 shows that lesser scaup utilization of the Mississippi River, where fingernail clams are still common, showed a less severe decline in the 1950's than occurred in the Illinois Valley, followed by a recovery. Diving duck utilization of the Illinois River has never recovered to pre-1955 levels, indicating that fingernail clam populations have never recovered, since the diving ducks are quick to locate and utilize beds of clams.

The third line of evidence comes from an examination of fish stomachs by Paloumpis and Starrett in the 1960's. Fingernail clams are a favorite food of carp and other bottom-feeding fish, and the fish can often locate and feed upon beds of clams which go undetected by a biologist. Fingernail clams formed 50.2% by volume of the food items taken by carp collected below Beardstown, but no clams were found in carp collected above Beardstown (Starrett 1972). The clams had evidently died out in the Illinois River above Beardstown prior to the 1960's.

Although Anderson (1977) did not take any fingernail clams above mile 107 in 1975, other studies have shown that fingernail clams still occur in tributaries and in isolated pockets in the Illinois River. Biologists from the Metropolitan Sanitary District of Greater Chicago collected fingernail clams in the Calumet River and in the Chicago River and its south branch in 1975 (Metropolitan Sanitary District of Greater Chicago 1978). All locations were fairly close to inlets from Lake Michigan. Fingernail clams occur in the Des Plaines River above the entrance of the Chicago Sanitary and Ship Canal (personal communication, 1 June 1977. Mr. Thomas A. Butts, Illinois State Water Survey, Peoria, Illinois), and fingernail clams are regularly impinged on the intake screens at the R.S. Wallace Power Station located at river mile 162.5 on Lower Peoria Lake (personal communication, 1 June 1977, Mr. Guy R. McConnell, WAPORA, Inc.,

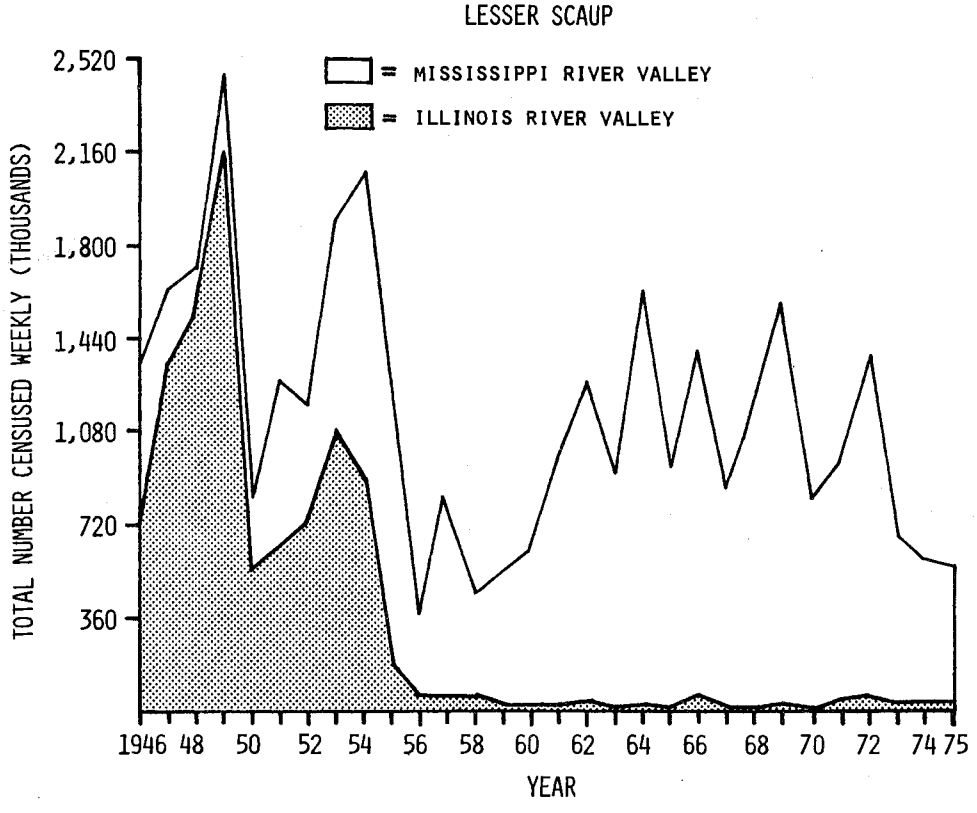

Figure 2. Utilization of the Illinois River by Lesser Scaup Ducks. The abrupt decline in the 1950's was probably due to a die-off of fingernail clams upon which the ducks feed. Lesser scaup continued to use the Mississippi River valley, where fingernail clams are still common. Source: Dr. Frank C. Bellrose, Wildlife Specialist, Illinois Natural History Survey.

Charleston, Illinois). The fingernail clams taken at the power plant may have washed downstream from populations which managed to survive in areas of Peoria Lake where spring water enters through the river bottom. There are several areas in Peoria Lake, such as the vicinity of Spring Bay (miles 173.0-180.0), where spring water is known to enter the lake. Starrett (1971) found, during a mussel survey in 1966, that in this region of Peoria Lake there was an increase in the dissolved oxygen (2.0-6.0 mg/l dissolved oxygen) and a corresponding increase in the number of mussels collected. Between June and November, 1973, biologists from the environment consulting firm, WAPORA, took 1 fingernail clam in 21 Ponar dredge samples near Hennepin (mile 212.0) and 9 fingernail clams in 21 Ponar dredge samples near Havana (mile 118.6) (WAPORA 1974). The Illinois Natural History Survey has also collected fingernail clams in Quiver Creek, a tributary of the Illinois River near Havana. In the reach of the Illinois River from mile 89.0 to mile 145.3, which includes the Havana region, a bedrock valley overlain with sand deposits lies to the east of the Illinois River. Groundwater flows through the sand into the Illinois River at the rate of about 8.75 m^3/s (309 cfs) during low-flow conditions (Singh and Stall 1973). The good quality groundwater flowing into the river along the sandy eastern bluffs may make some areas marginally suitable for fingernail clams. However, the clams apparently are still not abundant enough here or in Peoria Lake to attract and hold large flocks of diving ducks (personal communication, 1 June 1977, Mr. Robert Crompton, Wildlife Field Assistant, Illinois Natural History Survey).

Since fingernail clams go through an entire life cycle in 33 days (Gale 1969), they are capable of quickly repopulating an area from which they have been eliminated. There is some factor in the Illinois River which currently prevents recolonization of the river from the residual populations remaining in Peoria Lake and the tributary streams.

The same unknown factor which is affecting fingernail clams in the Illinois River, may also be affecting clams in other Midwestern rivers. For example, the Illinois Natural History Survey documented a decline in the population and growth rate of fingernail clams in the Keokuk Pool, Mississippi River in 1976 and 1977. The reduction in the population of fingernail clams apparently had an impact on diving duck use of Keokuk Pool, since there was a 49% decrease in the peak population

of canvasback ducks on Pool 19 between 1975 and 1976--a decline which cannot be attributed to a decline in the duck population produced on the northern breeding grounds, which amounted to only a 3% decrease. The canvasback usage of Pool 19 increased in the fall of 1977, but the ducks were evidently substituting snails and aquatic plants for fingernail clams (Paveglio and Steffeck 1978). Both aquatic plants and snails were much more abundant in Pool 19 in 1977 than in previous years. The plants increased because the upper Mississippi was much clearer than in previous years and water levels were low and stable as a result of the 1976-77 drought in the upper Midwest. Some of the species of snails whose populations increased in 1977, such as *Helisoma trivolvis*, use aquatic plants as habitat. Although the decrease in one type of food for fish and waterfowl in Pool 19 was compensated for by an increase in other types of food in 1976-77, the experience on the Illinois River in the mid-1950's shows that we cannot be assured that one declining food resource will naturally be replaced by another. Managers of the fish and wildlife resources are helpless if the causes of the decline are not known, because it is impossible to know what corrective action can be taken, or indeed, whether it is even feasible to take corrective action.

The purpose of the research described in this report and in an earlier one (Anderson et al. 1978) is: (1) to determine why fingernail clams died out in a 100-mile reach of the Illinois River in the 1950's and have since failed to recolonize, (2) to develop and use the information to prevent a similar die-off in other Midwestern rivers, such as the upper Mississippi. Phase I of this research (Anderson et al. 1978) developed both a rapid bioassay technique using isolated gills from fingernail clams and a more time-consuming chronic bioassay technique using intact fingernail clams. Thirteen water quality factors and a sample of Illinois River water were tested on fingernail clam gills, and two of the same factors, potassium and un-ionized ammonia, were tested on intact clams. Although normal gill function was impaired by Illinois River water, suspended particles, cyanide, heavy metals, low dissolved oxygen levels, and temperature changes, the results were not considered conclusive because the factors were not tested on intact clams. The intact clams were not killed, nor their growth impaired, by potassium concentrations which occur in the Illinois River, but their growth was slowed at un-ionized ammonia concentrations

of .34 mg/l (as un-ionized ammonia nitrogen, NH_3-N), which do occur occasionally in the upper reaches of the Illinois River.

The research described in this report is a follow-up to the earlier work. We completed the following tasks: (1) development of improved methods for maintaining fingernail clams in the laboratory; (2) determination of toxicity to clam gills of different layers of sediment from the Illinois River; (3) determination of effects of raw Illinois River water and sediment on intact clams; and (4) determination of effects on intact clams of raw Illinois River water treated to remove certain components, including ammonia and sediment. The following project objectives were <u>not</u> achieved: (1) analysis of shells and tissues of clams exposed to treated and untreated river water by microprobe techniques; (2) analysis of treated and untreated river water by x-ray diffraction; (3) testing of the effects of treated river water on clam gills; and (4) identification of the toxic factor in Illinois River water and sediment, which prevents fingernail clams from recolonizing portions of the river where they were formerly abundant.

METHODS

COLLECTION OF FINGERNAIL CLAMS

Fingernail clams were collected from the Keokuk Pool of the Mississippi River using an eighteen-foot boat equipped with a crane and a Ponar grab sampler. Fingernail clams were separated from the mud by pressure-sieving the Ponar samples through a 30-mesh screen with a 12-volt battery-operated water pump. The clams were carried to the laboratory in 37-liter plastic coolers equipped with aerators and half-filled with Mississippi River water.

Clams were separated from the mud in the laboratory at Havana and shipped via parcel post in plastic bags or jars to the laboratory at Southern Illinois University, where the effects of sediments on clam gills were determined. Styrofoam insulation minimized temperature changes in the shipping containers. The clams were delivered within three to five days of capture.

The clams retained at Havana were used in bioassays and in tests of different culture techniques. All clams collected on a particular date were regarded as a single "stock", and were maintained separately from stocks obtained on other dates. Each stock was assigned an identification number, in order according to date.

CULTURE TECHNIQUES

Mud, water and organisms from the Keokuk Pool, Mississippi River were transferred from the plastic cooler to a 37.8-liter glass aquarium. The stock aquarium was in a water bath to maintain temperature at 20C and received unchlorinated well water from a small modified proportional diluter (Mount and Brungs 1967) built for this project (Figure 3). Clams collected during the winter were kept in the plastic cooler overnight with an aerator, so the temperature warmed gradually from 1C to 20C in 24 hours.

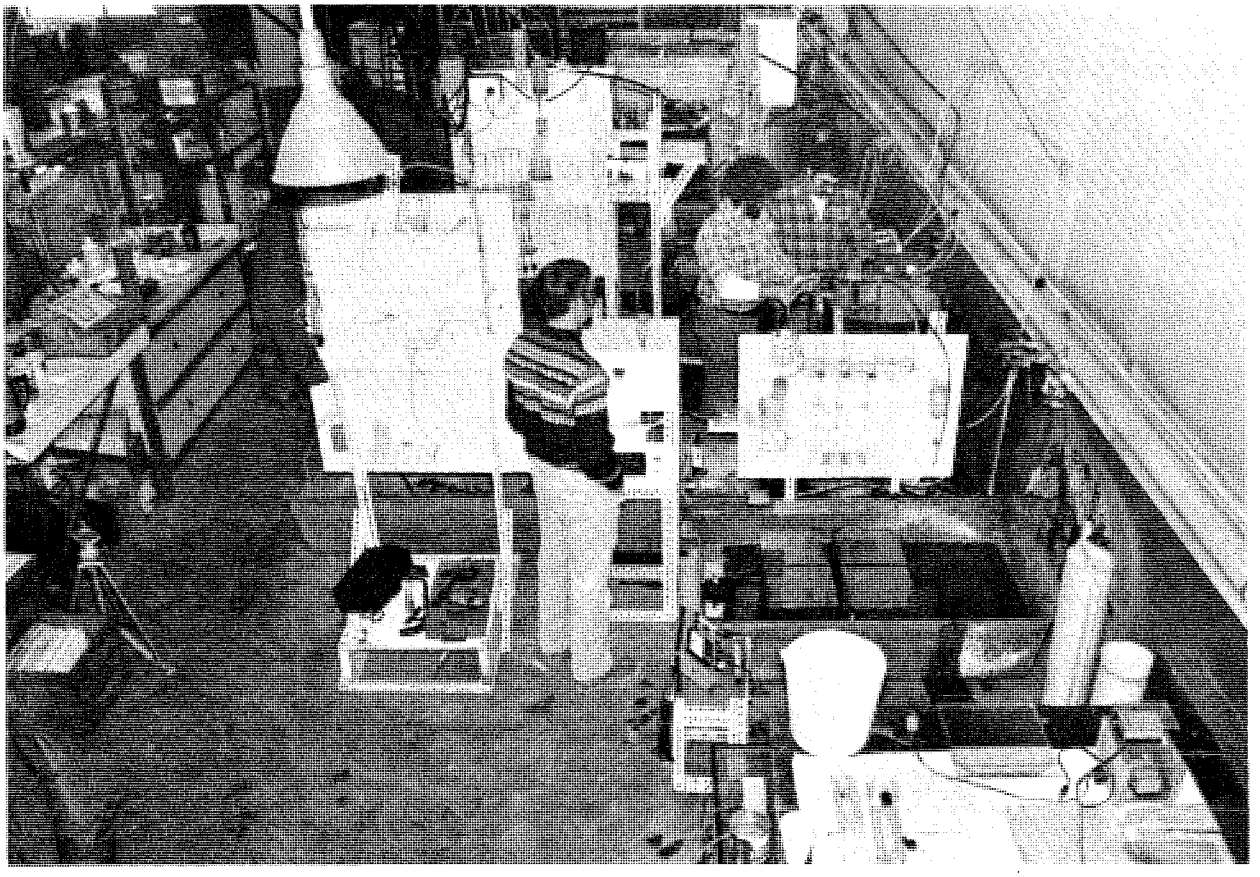

Figure 3. Bioassay Laboratory. The proportional diluter (right) supplied well water and algae to the stock aquaria.

A small sample of mud from the aquarium was passed through a 30-mesh sieve to separate organisms. Twenty small clams (*Musculium transversum*, 2-3 mm in shell length) were separated from detritus and other organisms under a microscope and placed in a 100-mm petri dish. Two petri dishes, containing 20 clams each, were placed on top of the mud in the stock tank, and removed at 2-week intervals to measure the shells and count the number of live clams.

The clams were examined under a microscope and shell lengths measured with an ocular micrometer. If the clams in the petri dishes did not survive well (>20% mortality), the entire stock was discarded and not used for bioassays. An exception was made for stock 8, where heavy mortality between the 2-week and 4-week check was attributable to a power failure which interrupted water flow and aeration to the stock aquaria. These clams were used in deletion bioassay number 1 because no other clams were available.

A small amount of the sieved sediment was added to each petri dish, and the remainder stored at room temperature in unchlorinated well water in a 3.8-liter glass jar. Silt was added to the petri dishes because we found that only 7 out of 20 clams survived a 4-week test in bare petri dishes, whereas 18 out of 20 survived in silt from the Mississippi River. The beneficial effect of the sediment seemed to last only 2 weeks, so the old sediment was discarded and stored sediment added to the dishes every 2 weeks when the clams were examined and counted.

Fingernail clams are very active and climb the sides of glass containers, so the petri dishes were covered with a plastic snap-on lid in which a 50-mm hole was cut to allow circulation of water. The hole was covered with 30-mesh nylon screen. Because the clams were small, active, fragile-shelled, and nearly transparent, some were unavoidably lost when the contents of the petri dishes were being sieved at 2-week intervals. If any clams were missing at each 2-week check, the numbers missing were recorded and are reported in the tables of results. Of course, missing clams were not counted as survivors or dead clams. Dead clams were easily identified under the microscope, because the shells gaped and were usually empty. When a clam dies, the elastic hinge ligament forces the shell open and the soft body parts decay and disappear within a few hours.

Stock clams were fed a concentrated suspension of the algae *Scenedesmus quadricauda* (Chlorophyta), delivered to the stock aquaria with a 50-ml pipette twice a day.

The following changes were made in our culture techniques during the course of this research: (1) Starting with stock 9 (collected 23 October 1979) an automatic feeding system was added to the stock tanks, which delivered 3.2 ml of concentrated algal suspension to each stock tank every 5 minutes. (2) Starting with stock 11 (collected 20 March 1980) petri

dishes containing clams and sieved sediment were placed in bare aquaria containing no other clams or sediment, following a suggestion by Gale (1972:22) that the decomposing remains of dead clams may induce a resting state (no growth) in live clams kept in the same container.

Water temperatures and dissolved oxygen concentrations in the stock aquaria were measured 5 times a week, pH 2 or 3 times per week, and alkalinity once a week. Results are reported in Table 1.

ADDITION BIOASSAYS, USING CLAM GILLS

During Phase I of this project, water samples from the Illinois River and sediment samples from an adjacent backwater lake connected to the river were collected and tested on fingernail clam gills. Not all the results had been analyzed and graphed at the time the Phase I Report was prepared (Anderson et al. 1978), so the methods and results are presented in this report. Since sediment or river water were added to clean water, these tests are called *addition bioassays*.

Sediment

On 14 December 1976 a sediment core was taken from Quiver Lake, which opens into the Illinois River at Havana. Quiver Lake was the location where Paloumpis and Starrett (1960) documented a die-off of fingernail clams in 1955-58, and where Sparks failed to find any live clams in 1973. The core was extruded from the 10-cm diameter steel corer onto a plastic tray, and divided into the following segments: (1) surface to 2.5 cm depth (2) 2.6-5.1 cm depth (3) 5.2-7.6 cm depth (4) 7.7-10.2 cm depth. Each segment of the core was placed in a separate jar and shipped to Southern Illinois University for testing. At Southern Illinois University, the core segments were stored overnight in a refrigerator, warmed to room temperature the next day, and used immediately. Equal volumes of wet mud from each core segment were placed in 1 liter of invertebrate physiological solution, making four test solutions in all. After the effects on the clam gills were determined, a measured volume of test solution was passed through a membrane filter, air dried, and weighed. The sediment concentration (in mg per liter) was then calculated. The average particle size was measured under

a microscope with an ocular micrometer and particle counts made with a hemocytometer.

The clams had been acclimated at least one week in invertebrate physiological solution in Instant Ocean aquaria at a temperature of 17C and a pH ranging from 7.8 to 8.2. Gills were excised from the clams and placed in petri dishes, where a continuous flow of standard physiological solution or solution to which the sediments had been added was maintained by means of metering pumps. Temperatures and dissolved oxygen concentrations in the petri dishes were monitored by thermistor meters and membrane electrodes. The clam gills were observed under a microscope, and the movement of particles across a known distance in the microscope field was timed so that the particle transport rate (in μm per second) could be calculated. The average rate in a microscope field showing approximately 50 gill filaments was determined. Gills from 5-7 clams were observed, and the means and standard deviations for the transport rates are reported in the results.

River Water

Water samples were dipped from one foot below the surface of the Illinois River at Havana on 5 October 1977 and 22 April 1978. The samples of river water and a sample of well water from the laboratory at Havana were shipped to the laboratory at Southern Illinois University for testing on clam gills. At Southern Illinois University, the water samples were refrigerated overnight, warmed to room temperature the next day, and used immediately. Apparatus and methods specially developed for this research (Anderson et al. 1978) were used to determine the ciliary beating rate of lateral cilia (in beats per second) on isolated clam gills from 5-7 clams as they were exposed to the 3 samples of water, and to samples of river water diluted in various proportions with well water.

DELETION BIOASSAYS, USING INTACT CLAMS

A submersible sewage pump delivered raw Illinois River water and sediment to a 208-liter polyethylene drum containing a float switch. Whenever water in the reservoir fell below a set point, the submersible pump switched on. When the reservoir was full, the pump switched off, and the back flow of water in the pipe purged debris from the impeller and intake

screen of the pump. The pump was capable of grinding up and passing 3.8-cm solids, but the protective intake screen was approximately 1-cm mesh. A small pump circulated the water in the reservoir, to keep the sediments from settling out, and supplied a head box which delivered water by gravity flow to the test chambers and treatment systems described below. All parts of the delivery water system were made out of nontoxic materials, including glass, polyethylene, teflon, nylon and silicone rubber. The only exception was the metal impeller of the pump, but analyses by atomic absorption and flame emission spectroscopy showed no differences in metal concentrations between water samples taken simultaneously at the intake and in the reservoir.

Figure 4 shows how the raw river water was treated to *remove* certain components, hence these tests are called *deletion bioassays*.

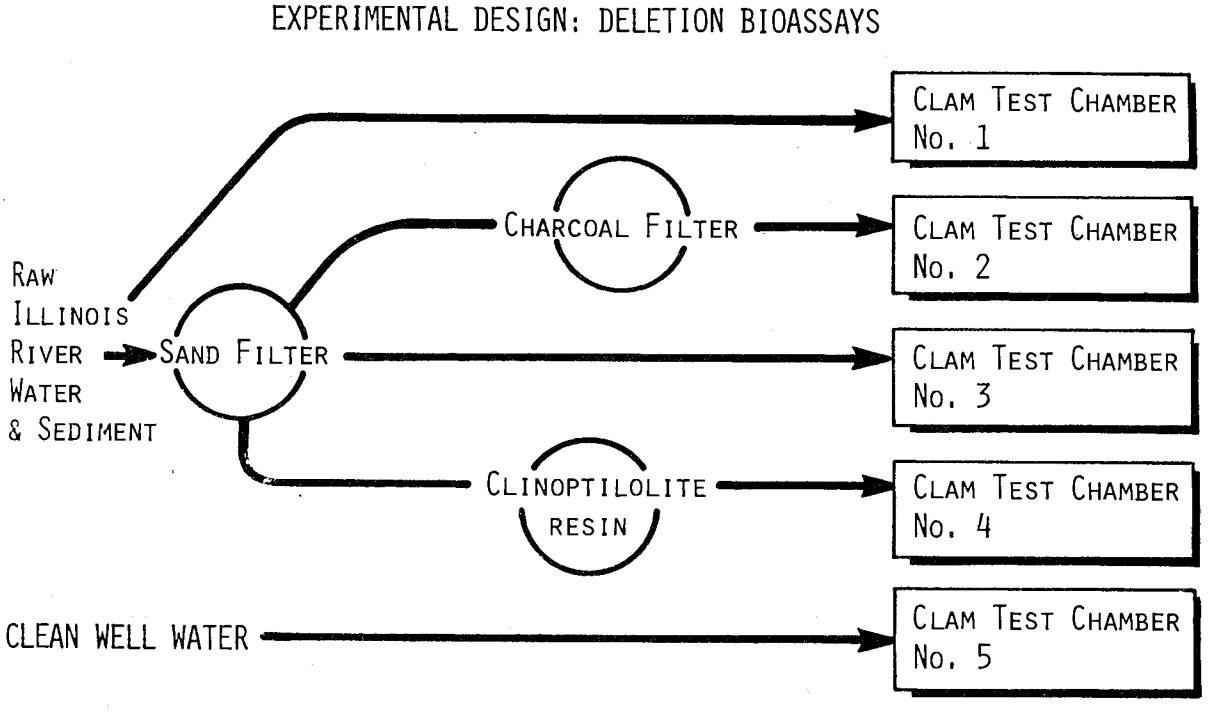

Figure 4. Schematic Diagram of Deletion Bioassay.

Treatment consisted of filtration through various media. Sand was used to remove suspended material, charcoal to remove a broad class of organic chemicals, and clinoptilolite to remove ammonia. Sand-filtered water was delivered to the charcoal filter and the clino filter to prevent rapid clogging of these expensive media with sediment. Raw Illinois River water and unchlorinated well water were also delivered to individual test chambers, so that the following 5 types of water were tested: (1) unchlorinated well water, (2) Illinois River water filtered through sand and clinoptilolite, (3) Illinois River water filtered through sand and charcoal, (4) Illinois River water filtered through sand, and (5) raw Illinois River water containing suspended sediment.

The filters were made from plastic garbage cans (see Figure 5.)

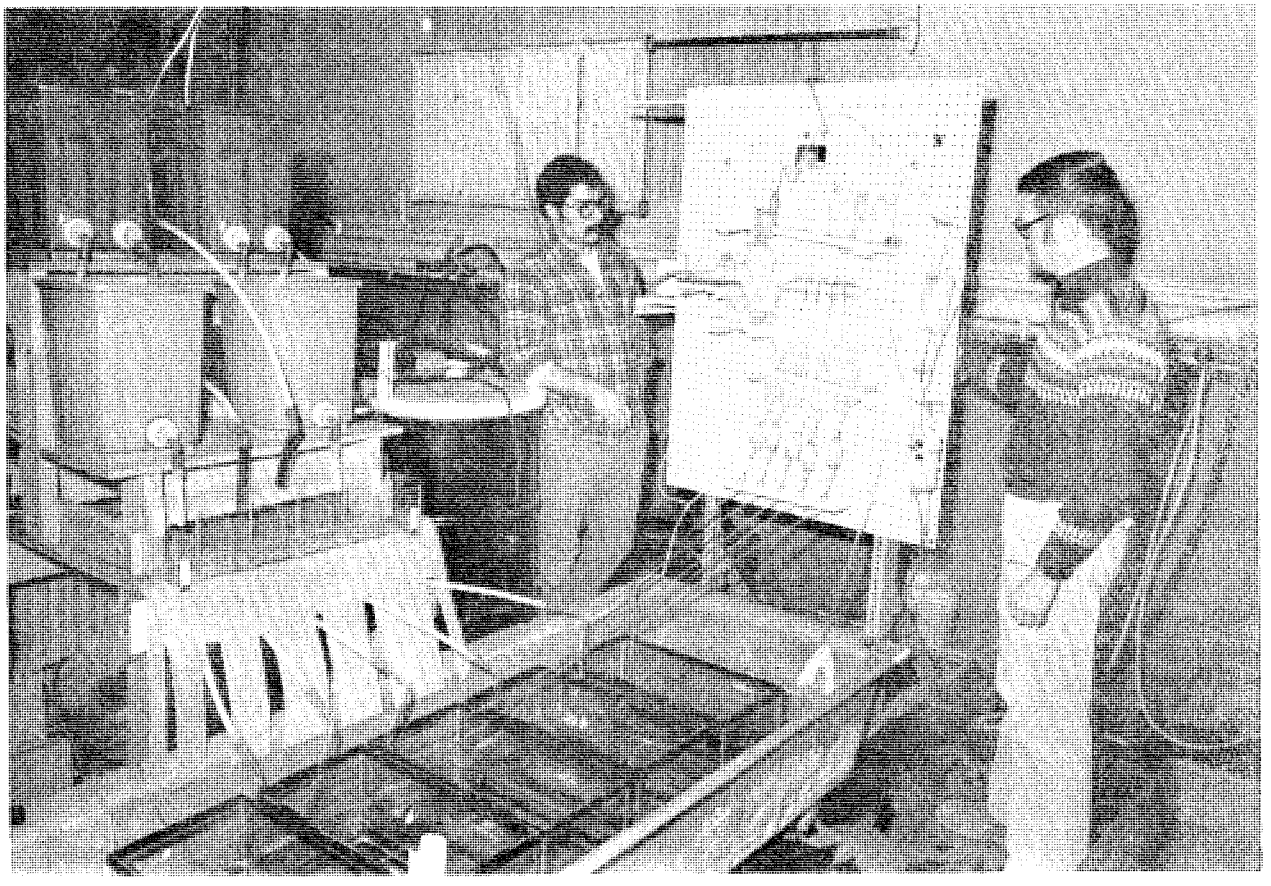

Figure 5. Deletion Bioassay Apparatus. The automatic algae feeder is mounted on the white pegboard. The black 55-gallon drum is the reservoir for river water. The household garbage cans contain the filtration media. Test chambers are in a water bath in the foreground.

The outlet at the bottom of each garbage can was covered with plastic screen and a pad of fiberglass in the bottom of the can kept the media from plugging the outlet. Sand, charcoal, or clino was added next, and covered with another pad of fiberglass which could be removed easily and washed. Water flowed by gravity from the constant head box to the sand filter, then to the other filters or test chambers.

The test chambers were 37.8-liter glass aquaria with outlets to maintain the volume at 23 liters. The test chambers were immersed in a water bath to control water temperature. Water flow from the deletion apparatus into the test chambers was approximately 100 ml per minute and was checked 3 times a day. Sediment occasionally clogged the delivery tubes overnight, but because of the large volume of the test chambers in relation to the small size and oxygen requirements of the clams, oxygen levels in the test chambers did not decline. The maximum length of time the flows could have stopped was 12 hours, and we do not feel that these infrequent stoppages had any effects on the results of our deletion bioassays, which lasted 3-6 weeks.

During deletion bioassay number 1, a 50-ml pipette was used to deliver the concentrated algal suspension to each test chamber twice a day. However, high concentrations of algae could not be maintained in the test chambers with pipette feeding. An automatic feeding system (separate from the one used on the stock aquaria) delivered 100 ml of algal suspension to each test chamber every 5 minutes during deletion bioassay number 2. Algal concentrations were measured by procedures described in Standard Methods (American Public Health Association 1976:1024-26).

Clams from stocks number 8 (collected 21 June 1979) and 11 (collected 20 March 1980) were used in deletion bioassays 1 and 2, respectively. The procedures were the same as in the culture tests: (1) each test chamber held 2 petri dishes containing 20 clams each (for a total of 40 clams exposed to each test solution), (2) the clams were 2.4 to 3.0 mm in shell length, (3) clam survival and growth were checked at 2-week intervals, and (4) sieved sediment collected from the Mississippi River on the same date the clams were collected was added to the petri dishes at the beginning of the bioassay and at 2-week intervals thereafter.

Water temperatures and dissolved oxygen concentrations in the test chambers were measured 5 times a week, pH 2 or 3 times per week, and alkalinity

once a week. Results are reported in Tables 3 and 5. Water levels on a gage at Havana were recorded during deletion bioassays 1 and 2 and are plotted in Figure 10.

Variance tests (Snedecor and Cochran 1967) were used to determine whether there were significant differences in clam mortality between treatments. Mortalities in duplicate petri dishes within each test chamber were pooled. An analysis of variance, ANOVA (Steel and Torrie 1960), was used to determine whether there were significant differences in shell lengths of clams exposed to the different test solutions and the clean well water. If the mortality in a test solution was significantly different from mortality in the well water control, the length data were not analyzed, because of the possiblity that mortality was size-dependent. If, for example, the rate of uptake and effect of a toxic substance in the river water depended on the body volume or gill surface area of a clam, mortality would be size-dependent, and size differences between clams exposed to the various treatments and the well water would reflect differential mortality rather than differential growth. All differences were considered significant at a probability, $P \leq .05$.

RESULTS AND DISCUSSION

RESULTS OF DIFFERENT CULTURE TECHNIQUES

Water chemistry and temperature in the stock aquaria are given in Table 1.

Clam Mortality

Clam stocks 7-11 maintained during this phase of the research had lower mortalities after 2 and 4 weeks in the laboratory than stocks 1-6 maintained during Phase I (Figures 6 and 7). The confidence limits

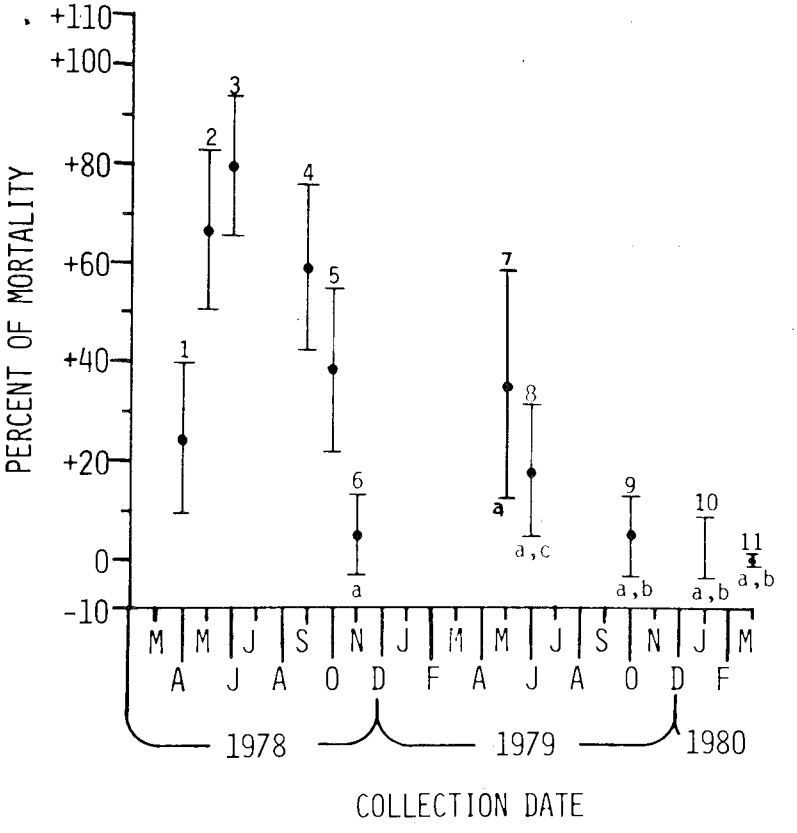

Figure 6. Stock Clam Mortality at Two Weeks. Brackets indicate 95% confidence limits. [a]Mississippi River silt added. [b]Algae added automatically every five minutes. [c]Algae added by pipette twice a day.

TABLE 1

Water Chemistry and Temperature in Stock Tanks

Stock Number	Collection Date	Temperature (C) Mean	Temperature (C) Range	pH Mean	pH Range	Dissolved Oxygen (Mg/l) Mean	Dissolved Oxygen (Mg/l) Range	Total Alkalinity (Mg/l as $CaCO_3$) Mean	Total Alkalinity (Mg/l as $CaCO_3$) Range
1	4/21/78	16.2	14.6-17.7	8.00	7.94-8.08	8.50	8.32-9.45	220.0	220
2	5/17/78	18.2	17.1-19.8	7.98	7.92-8.01	8.17	5.10-9.00	220.9	213-230
3	6/23/78	20.8	18.8-23.1	8.06	7.87-8.18	7.94	5.78-8.62	214.0	204-226
4	9/6/78	21.4	19.9-22.0	8.06	7.74-8.17	7.64	3.99-8.76	171.4	152-196
5	10/17/78	21.5	20.5-22.0	7.89	7.69-8.00	7.39	6.40-8.10	150.0	148-152
6	11/9/78	21.1	18.3-22.5	8.05	8.01-8.14	7.98	7.20-8.98	147.0	146-148
7	6/4/79	20.6	18.1-22.7	7.85	7.35-8.01	6.88	1.20-8.60	161.0	152-170
8	6/21/79	21.1	18.8-23.1	8.16	8.11-8.18	7.90	7.51-8.31	167.3	156-176
9	10/23/79	19.6	16.1-21.2	7.98	7.78-8.08	8.15	7.42-9.08	139.2	136-144
10	1/3/80	20.8	18.3-22.1	7.99	7.89-8.07	8.87	8.28-9.49	154.2	152-158
11	3/20/80	21.7	20.0-24.1	8.26	8.05-8.48	8.47	8.05-8.91	156.0	146-164

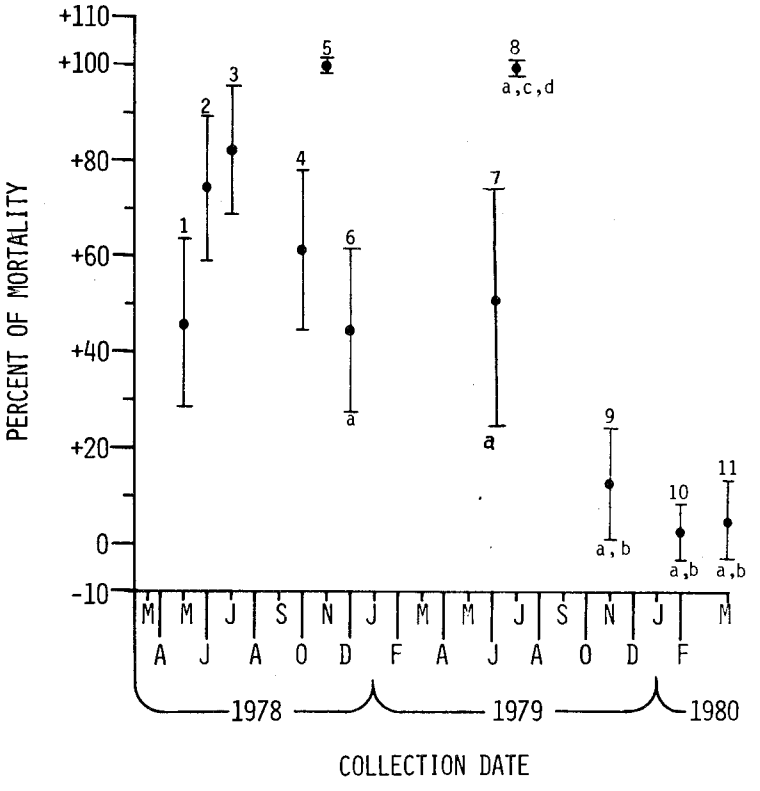

Figure 7. Stock Clam Mortality at Four Weeks. [a]Mississippi River silt added. [b]Algae added automatically every five minutes. [c]Algae added by pipette twice a day. [d]Clams affected by stoppage of water flow and aeration during a power failure which occurred between the 2-week and 4-week check.

for stock 7 were much wider than for the other stocks, because of the small initial sample size (20 clams in stock 7, 40 in the others), necessitated by the paucity of small clams in the bottom samples obtained from the Mississippi River on 4 June 1979.

Addition of Mississippi River silt to the petri dishes, starting with stock 6, improved clam survival, and automatic feeding of algal suspensions, starting with stock 9, further improved survival (Figures 6

and 7). Clams kept in bare petri dishes generally developed a cottony growth of fouling organisms, probably fungi and bacteria, on their shells, which may have interfered with siphoning. Clams which burrowed in silt kept their shells clean. Benjamin and Burky (1978:60) indicate that deposit feeding may be an important supplemental feeding strategy in fingernail clams, hence the clams may have fed on organic detritus and bacteria in the Mississippi silt. *Musculium transversum* from the Mississippi River ingest a wide variety of algae and diatoms (Gale and Lowe 1971:309 - 310). While most of the algae ingested by *Musculium transversum* pass through the digestive tract intact (Gale and Lowe 1971: 511), clams may derive nourishment from the bacterial growth coating the algae. Stock 8, which exhibited slightly poorer survival at 2 weeks than stocks 9-11, was fed algae twice a day with a 50-ml pipette. In contrast, stocks 9-11 were fed algae continuously, resulting in a greater concentration of algae in the stock aquaria. After 4 weeks, the mortalities observed for stocks 9-11 were significantly less than for all previous stocks (Figure 7).

Clam Growth

Although stocks 7-11 all grew during the first two weeks in the laboratory, they failed to grow during the next two weeks after the original silt was replaced (Table 2). Gale (1972:22) has suggested that the tactile stimulation associated with the observation and measurement of *Musculium transversum* in the lab may elicit a resting state (no growth) in the clams. Thomas (1959) reported that another sphaeriid clam (*Sphaerium partumieum*) frequently exhibited a resting state lasting up to 6 weeks when reared in the laboratory. Gale (1972:22) also felt that the presence of the decomposing remains of parental stock in clam growth chambers may have been a factor inducing a resting state. However, Imlay and Paige (1972:211, 213, 214) reported that the unidentified species of *Musculium* they used grew satisfactorily in lab tests where the clams were removed periodically for measuring. In addition, Imlay and Paige felt that the clams were feeding on bacteria from the decomposing food they placed in the test chambers. Clam stocks 1-10 were maintained in petri dishes that were placed in stock aquaria which contained the

TABLE 2

Growth and Survival of Stocks of Fingernail Clams*

	No. Alive	Mean Shell Length (mm)	Variance	Standard Deviation	Range (mm)	Mean Growth Increment (mm)
Stock 7[1] Collected 06/04/79						
Initial	20	2.5	0.04	0.20	2.2-2.9	--
2 Weeks	13	2.7	0.06	0.24	2.2-3.0	0.2
4 Weeks	10	2.7	0.05	0.22	2.2-3.0	--
Stock 8[1,3] Collected 06/21/79						
Initial	40	2.7	0.02	0.14	2.4-2.9	--
2 Weeks	32	2.8	0.01	0.07	2.6-2.9	0.1
4 Weeks	--	--	--	--	--	--
Stock 9[2] Collected 10/23/79						
Initial	40	2.5	0.01	0.11	2.3-2.7	--
2 Weeks	38	2.6	0.01	0.11	2.4-2.8	0.1
4 Weeks	34	2.6	0.02	0.15	2.4-2.9	--
Stock 10[2] Collected 01/03/80						
Initial	40	2.6	0.0246	0.1568	2.4-3.1	--
2 Weeks	39	2.8	0.0584	0.2416	2.5-3.6	0.2
4 Weeks	39	2.8	0.0685	0.2618	2.5-3.6	--
Stock 11[2] Collected 03/20/80						
Initial	40	2.4	0.0067	0.0821	2.3-2.6	--
2 Weeks	40	2.7	0.0270	0.1642	2.4-3.0	0.3
4 Weeks	38	2.7	0.0268	0.1638	2.4-3.0	--

*all stocks were maintained in silt
[1]algae delivered by pipette
[2]algae delivered automatically every 5 minutes
[3]electrical failure terminated test after 2 weeks

remainder of that particular stock collection. These clams were, therefore, exposed to the decomposing remains of clams in that stock which had perished. Clams from stock 11, placed in an aquarium not containing other clams, responded with the fastest growth seen for any stock group for the first 2 weeks (Table 2). These clams, however, failed to grow after the 2-week check when the original silt was replaced.

Storage of the silt at room temperatures may result in prolonged bacterial activity which depletes the silt of nutrients the clams may use,or the microbial flora may shift from species the clams can utilize to less nutritious or less assimilable species (personal communication, 22 May 1980, Dr. Robert Gorden, Aquatic Microbiologist, Illinois Natural History Survey, Champaign, Illinois). Silt used in future testing will be collected fresh from the Mississippi River every two weeks or stored in a refrigerator.

ADDITION BIOASSAYS, USING CLAM GILLS

Sediment

The deeper layers of sediment from Quiver Lake were less toxic to fingernail clam gills (depressed the particle transport rate less) than the two shallower layers (Figure 8). Although we did not attempt to date the sediment layers, these findings are consistent with the fact that fingernail clams thrived in Quiver Lake until the die-off in 1955-58 (Paloumpis and Starrett 1960). If a toxic material were introduced to the river, starting in 1955, it might be bound to the sediments deposited at that time (perhaps represented by the second layer). The deeper and older sediments (third and fourth layers) would not contain the toxicant. The decline in toxicity from the second layer to the surficial layer might reflect a reduction in toxicant input to the river or dilution by a greater volume of sediment eroded from farmlands and banks of tributary streams.

The size (μm), weight (mg/l) and particle concentration (particles/l x 10^6) for each sediment layer, following dilution to make up a test solution, are given below (mean ± standard deviation):

Layer	Size	Weight	Particle Concentration
0.0-2.5 cm	5.3 ± 1.2	53.8 ± 8.2	2.8 ± 0.9
2.6-5.1 cm	2.8 ± 0.9	44.1 ± 6.7	5.1 ± 1.9
5.2-7.6 cm	4.5 ± 1.1	61.2 ± 11.2	3.8 ± 1.2
7.7-10.2 cm	3.8 ± 0.9	78.1 ± 9.8	4.5 ± 0.6

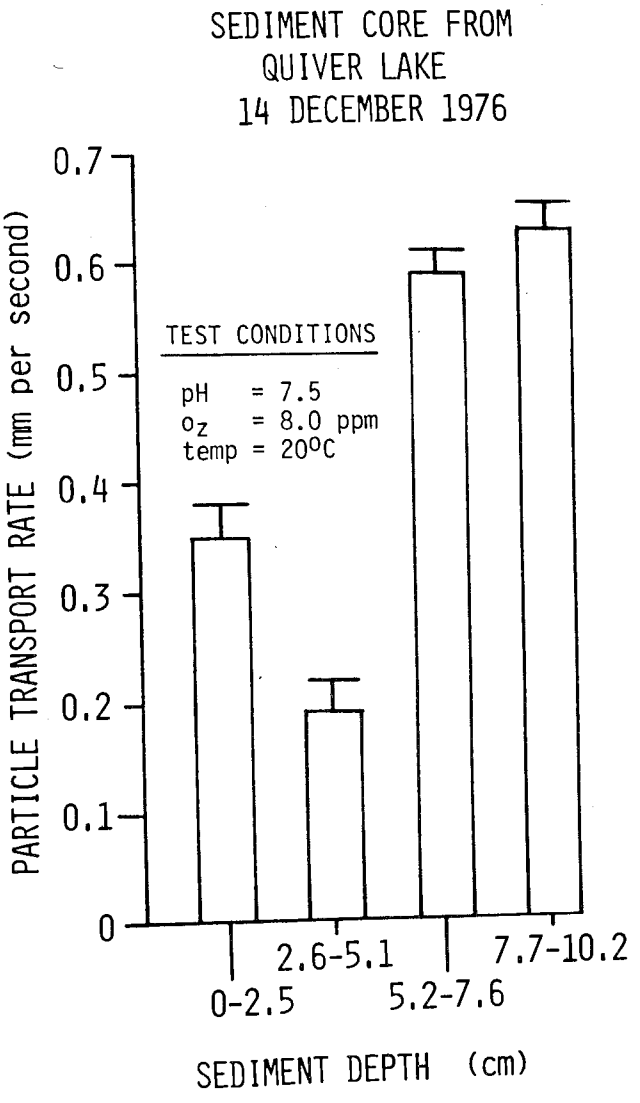

Figure 8. Response of Clam Gills to Four Layers of Sediment from Quiver Lake, Illinois River.

River Water

Anderson et al. (1978) demonstrated that the cilia on isolated clam gills beat normally for at least 6 hours in well water from our laboratory at Havana, but the ciliary beating rate dropped to practically zero when the gills were exposed to Illinois River water for 2 hours. The present results confirm the earlier ones: samples of water taken from the Illinois River on two dates severely inhibited the cilia on clam gills, and reduction in inhibition was proportional to the dilution of the river water with well water (Figure 9). It is not surprising

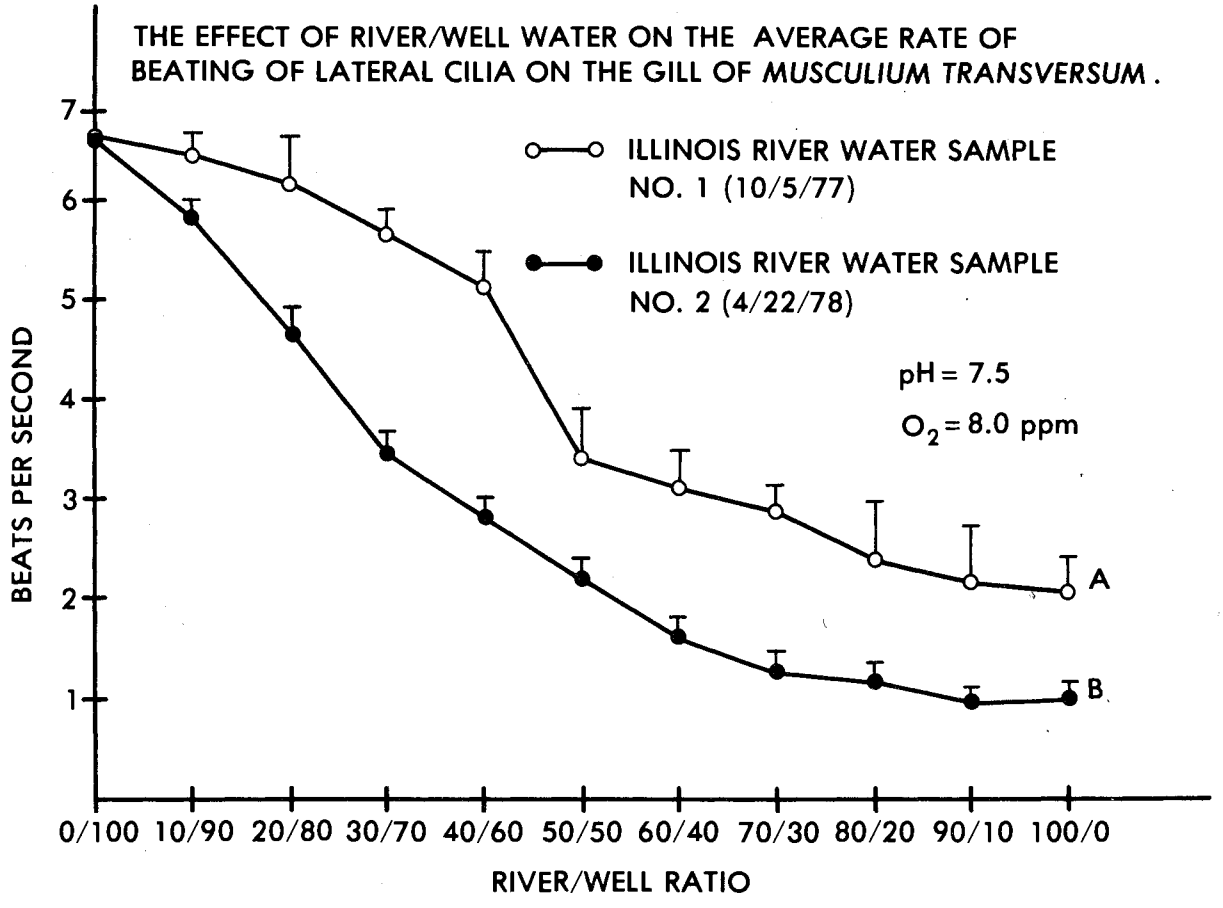

Figure 9. Response of Clam Gills to Illinois River Water.

that the degree of inhibition differed significantly between water samples taken on different dates (Figure 9), because the input of toxicant to the river could vary; the volume of flow in the river (hence the dilution) varies; and physical/chemical factors, such as toxicant absorption or desorption from sediment, may vary.

DELETION BIOASSAYS, USING INTACT CLAMS

Deletion Bioassay Number 1

Deletion bioassay number 1 started on 29 June 1979. Water levels in the Illinois River during the bioassay are shown in Figure 10 and the water chemistry and temperature in Table 3. The test terminated

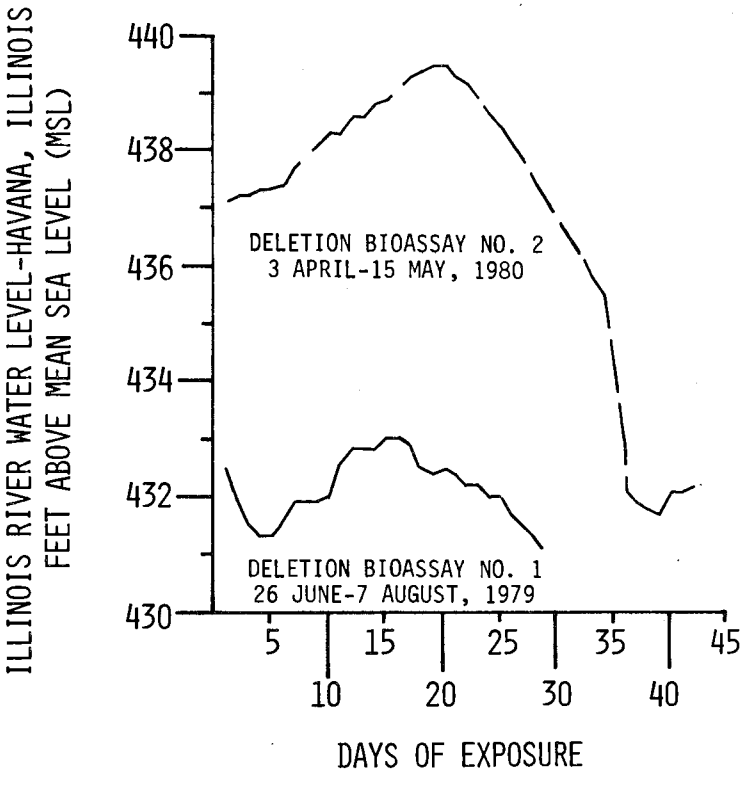

Figure 10. Water Levels in the Illinois River During Deletion Bioassays 1 and 2.

TABLE 3

Water Chemistry and Temperature During Deletion Bioassay No. 1

Test Conditions	Temperature (C) Mean	Range	pH Mean	Range	Dissolved Oxygen (Mg/l) Mean	Range	Total Alkalinity (Mg/l as $CaCO_3$) Mean	Range
Control (well water)	22.7	20.8-25.7	8.05	7.99-8.12	8.35	7.82-8.62	168.1	148-194
Sand + Clinoptilolite Filtered	23.6	21.7-26.0	8.27	8.17-8.36	7.31	6.97-7.69	158.8	126-176
Sand + Charcoal Filtered	23.5	21.7-26.0	8.31	8.18-8.49	7.60	7.20-8.01	155.6	132-170
Sand Filtered	23.6	21.7-26.0	8.25	8.16-8.32	7.13	6.30-7.70	159.6	130-176
Raw River Water	23.7	21.9-26.0	8.25	8.09-8.35	6.52	5.61-7.62	160.0	128-176
Illinois River Conditions	25.5	22.8-28.8	8.12	7.75-8.27	6.08	3.59-7.58	156.6	122-176

prematurely on 7 August 1979 when a pump delivering well water to the control chamber failed and could not be repaired or replaced immediately.

Clam Mortality. There were no significant differences in mortality between clams maintained in well water and in raw river water or treated river water (Figures 11 and 12, Table 4).

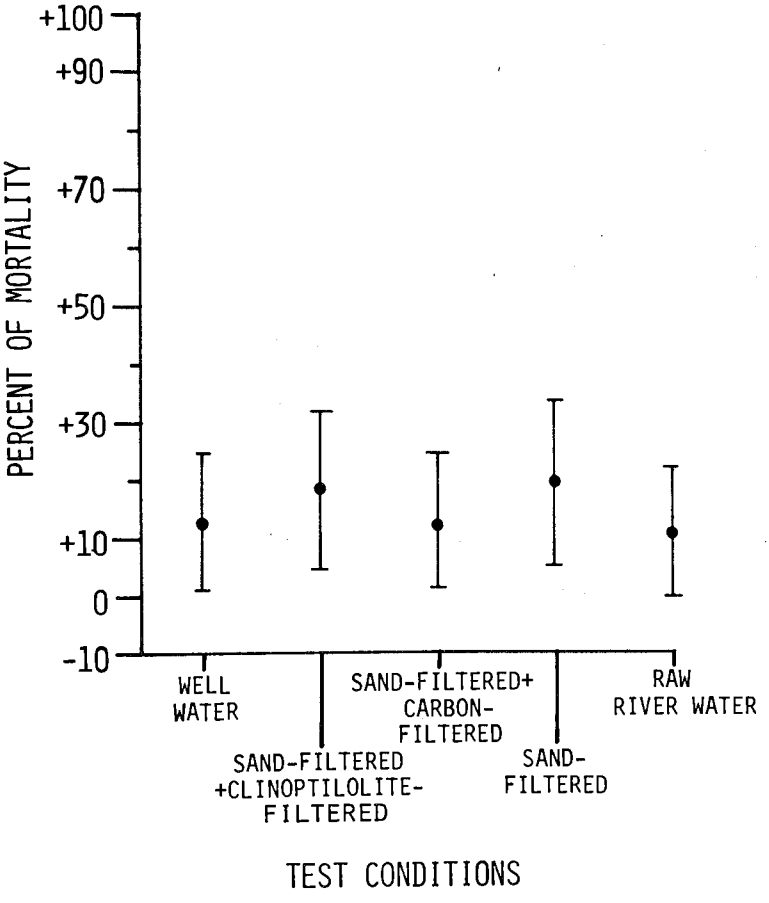

Figure 11. Mortality of Clams Exposed to Well Water, Raw Illinois River Water and Treated River Water for Two Weeks in Deletion Bioassay Number 1. Brackets show 95% confidence limits.

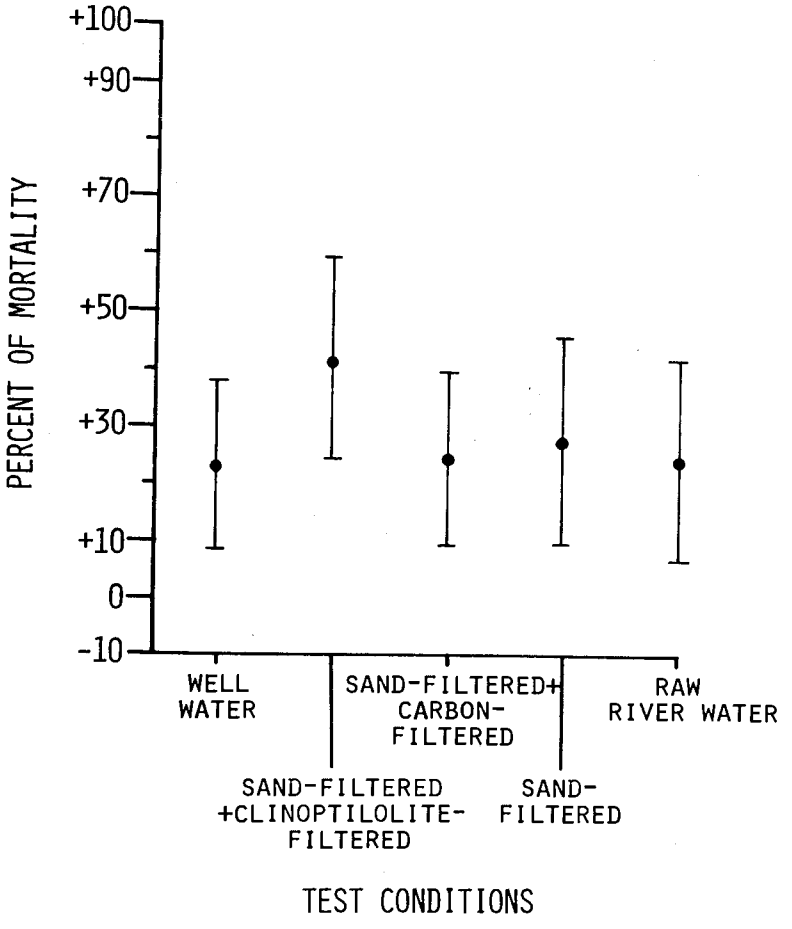

Figure 12. Mortality of Clams Exposed to Test Conditions for Four Weeks in Deletion Bioassay Number 1.

Clam Growth. Clams maintained in raw river water and sand-filtered water grew significantly more than clams exposed to other treatments (Figures 13 and 14, Table 4).

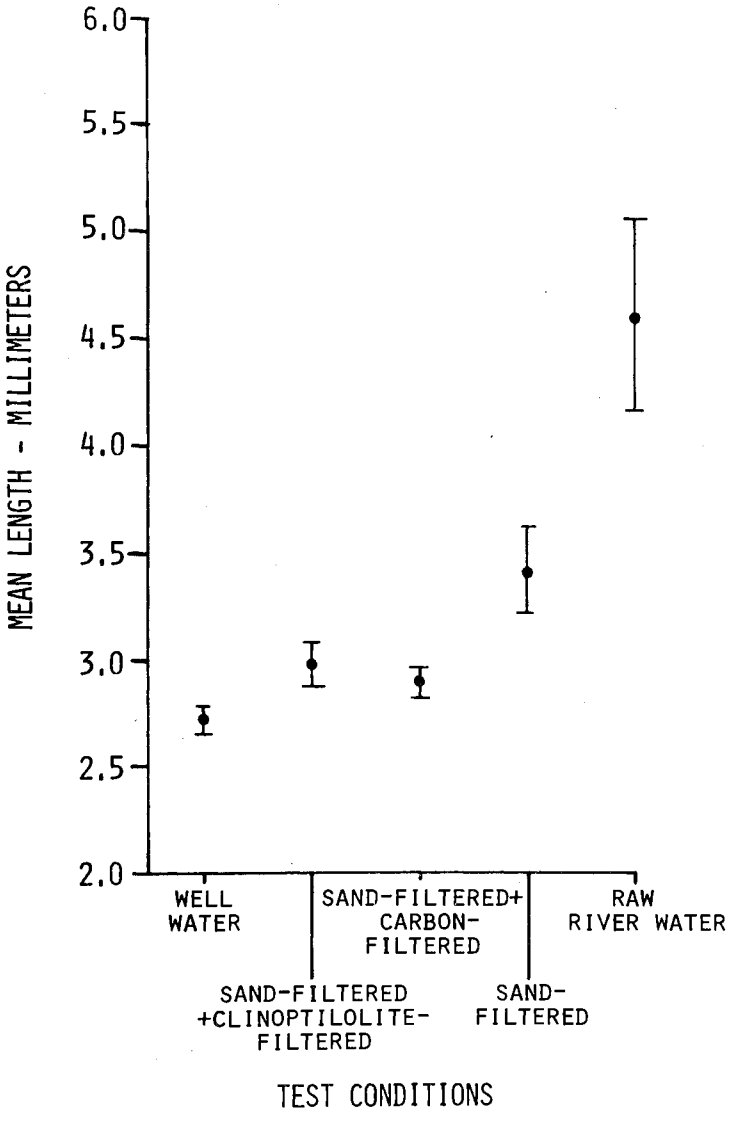

Figure 13. Shell Length of Clams Exposed to Well Water, Raw Illinois River Water and Treated River Water for Two Weeks in Deletion Bioassay Number 1. Brackets show 95% confidence limits.

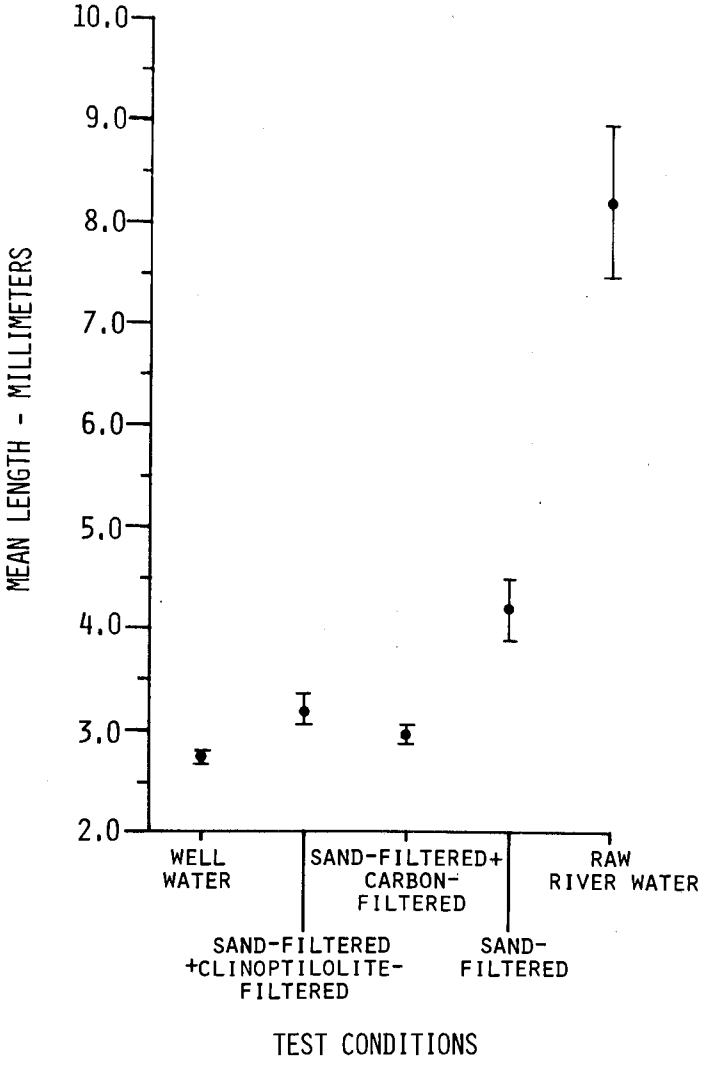

Figure 14. Shell Length of Clams Exposed to Test Conditions for Four Weeks in Deletion Bioassay Number 1.

TABLE 4

Results of Deletion Bioassay No. 1

	No. Alive	No. Missing	Mean Shell Length (mm)	Vari-ance	Standard Deviation	Range (mm)	Mean Growth Increment (mm)
Initial							
Control[1]	40	--	2.7	0.03	0.16	2.4-3.0	--
Sand-Clino[2]	40	--	2.7	0.02	0.15	2.4-2.9	--
Sand-Char[3]	40	--	2.7	0.03	0.16	2.4-3.0	--
Sand[4]	40	--	2.7	0.02	0.15	2.4-3.0	--
Raw[5]	40	--	2.7	0.02	0.15	2.4-3.0	--
2 Weeks							
Control	34	1	2.7	0.03	0.18	2.4-3.0	--
Sand-Clino	31	2	3.0	0.09	0.29	2.4-4.0	0.3
Sand-Char	34	1	2.9	0.04	0.21	2.5-3.2	0.2
Sand	29	4	3.4	0.29	0.54	2.8-5.0	0.7
Raw	33	3	4.6	1.58	1.26	2.5-6.5	1.9
4 Weeks							
Control	31	0	2.7	0.03	0.17	2.4-3.0	--
Sand-Clino	24	2	3.2	0.15	0.38	2.7-4.2	0.2
Sand-Char	28	2	3.0	0.06	0.25	2.5-3.6	0.1
Sand	21	7	4.2	0.52	0.72	2.8-5.6	0.8
Raw	24	2	3.2	0.15	0.38	2.7-4.2	0.2

[1]well water
[2]river water, filtered through sand and clinoptilolite
[3]river water, filtered through sand and charcoal
[4]river water, filtered through sand
[5]raw Illinois River water and silt

Clams maintained in well water did not grow at all (Table 4)! Laughlin et al. (1981) report a phenomenon called hormesis, where animals exposed to sublethal stress grow more than unstressed animals. The stress evidently triggers hormonal and neuronal responses which activate defensive mechanisms against the stress. The increased hormonal/neuronal activity increases growth as a side effect. A more likely explanation for the lack of growth in clams in well water during our experiments, in contrast to the rapid growth in raw river water, is a lack of food. The clams were kept in Mississippi River silt which had been stored at room temperature and which had probably lost its nutritive value for clams, as explained above. In addition, there were probably not enough algae added to the test chambers (50 ml of algal suspension added by pipette twice each day) for the clams to feed upon. The clams in raw river water received a constant influx of silt, organic detritus, and microorganisms, which they undoubtedly fed upon. Toxic materials were either not present in the river water and sediment from 26 June to 7 August 1979, or toxic effects could not be detected because the clams in the clean well water and treated well water were starving, while clams in river water were receiving food. Another indication that lack of food limited the growth of the clams during bioassay number 1 is the fact that clam growth was inversely proportional to the amount of filtration of the river water. We observed that some fine sediment passed through the sand filter into the test chambers. Less sediment entered test chambers which received water passed through two filters (sand + clino or sand + charcoal). The more filtration, the less food the clams received from the river water.

Deletion Bioassay Number 2

The automatic feeding system was installed prior to the start of deletion bioassay number 2 on 3 April 1980. The mean numbers of algal colonies (a colony was defined as two or more algal cells) per ml of

suspension delivered to the test chambers during bioassay number 2 were:

4 April	3032
16 April	3666
18 April	9197
24 April	324
7 May	2294
14 May	2909

One hundred ml of algal suspension was delivered to each test chamber every 5 minutes. The concentrations of algae delivered to the test aquaria during deletion bioassay number 2 are of the same order of magnitude as the total green algae (Chloroplyta) numbers found in Keokuk Pool, Mississippi River, by Gale and Lowe (1971:508).

Water chemistry and temperature in the test chambers during bioassay 2 are reported in Table 5.

Clam Mortality. Clams exposed to raw Illinois River water during deletion bioassay number 2 suffered significantly greater mortality than all other groups after 2 weeks and 4 weeks of exposure (Table 6, Figures 15 and 16). After 6 weeks, clams exposed to Illinois River water had significantly greater mortality than all groups except those clams exposed to sand-filtered Illinois River water (Figure 17). The control group of clams, exposed to unchlorinated well water, had the lowest mortality after 4 weeks and 6 weeks (Figures 16 and 17).

Clam Growth. Although mortality of fingernail clams exposed to raw river water was high, growth of the survivors after 2 weeks and 4 weeks of exposure was only slightly worse than clams in treated water (Table 6, Figures 18 and 19) and after six weeks, comparable to the clams in treated water (Table 6 and Figure 20). Clams in clean well water showed the poorest growth of all, so lack of food may still have been affecting the control clams despite automatic feeding of algae. Sediments continuously accumulated in test chambers receiving raw river water as well as in chambers receiving filtered river water, but no additional sediment accumulated in the well water control. As mentioned above, the

TABLE 5

Water Chemistry and Temperature During Deletion Bioassay No. 2

Test Conditions	Temperature (C) Mean	Temperature (C) Range	pH Mean	pH Range	Dissolved Oxygen (Mg/1) Mean	Dissolved Oxygen (Mg/1) Range	Total Alkalinity (Mg/1 as $CaCO_3$) Mean	Total Alkalinity (Mg/1 as $CaCO_3$) Range
Control (well water)	22.1	20.5-23.2	8.09	7.73-8.22	8.62	7.77-9.22	176.7	154-216
Sand + Clinoptilolite Filtered	22.3	20.8-23.3	8.27	8.11-8.59	8.23	7.70-8.90	173.1	160-190
Sand + Charcoal Filtered	22.3	20.9-23.2	8.25	8.11-8.41	8.33	8.0-8.72	172.0	148-194
Sand Filtered	22.5	21.0-23.5	8.22	8.02-8.40	8.23	7.48-8.95	174.9	156-198
Raw River Water	21.5	20.8-22.9	8.27	8.05-8.48	8.11	7.11-9.00	174.9	154-194
Illinois River Conditions	18.5	8.4-20.2	8.39	8.14-8.81	9.85	6.8-14.1	171.4	158-186

TABLE 6

Results of Deletion Bioassay No. 2

	No. Alive	No. Missing	Mean Shell Length (mm)	Vari-ance	Standard Deviation	Range (mm)	Mean Growth Increment (mm)
Initial							
Control[1]	40	--	2.6	0.02	0.15	2.4-2.9	--
Sand-Clino[2]	40	--	2.6	0.02	0.14	2.4-2.9	--
Sand-Char[3]	40	--	2.6	0.02	0.13	2.4-3.0	--
Sand[4]	40	--	2.6	0.02	0.13	2.4-2.9	--
Raw[5]	40	--	2.7	0.02	0.13	2.4-2.9	--
2 Weeks							
Control	37	1	2.7	0.02	0.15	2.5-3.0	0.1
Sand-Clino	35	0	3.6	0.24	0.49	2.5-4.3	1.0
Sand-Char	38	0	3.6	0.20	0.45	2.7-4.3	1.0
Sand	36	0	3.7	0.23	0.48	2.6-4.4	1.1
Raw	23	0	3.2	0.15	0.38	2.7-4.0	0.5
4 Weeks							
Control	34	1	2.8	0.02	0.15	2.5-3.0	0.1
Sand-Clino	33	0	4.0	0.35	0.59	2.7-4.8	0.4
Sand-Char	31	0	4.0	0.44	0.67	2.5-5.0	0.4
Sand	34	0	4.1	0.43	0.66	2.7-4.9	0.4
Raw	17	0	3.7	0.37	0.60	2.8-4.6	0.5
6 Weeks							
Control	29	0	2.8	0.02	0.14	2.5-3.0	--
Sand-Clino	30	0	4.4	0.32	0.56	3.6-5.4	0.4
Sand-Char	29	0	4.1	0.30	0.55	3.1-5.1	0.1
Sand	21	0	4.8	0.63	0.79	2.9-6.0	0.7
Raw	15	0	4.3	0.62	0.79	2.9-5.6	0.6

[1]well water
[2]river water, filtered through sand and clinoptilolite
[3]river water, filtered through sand and charcoal
[4]river water, filtered through sand
[5]raw Illinois River water and silt

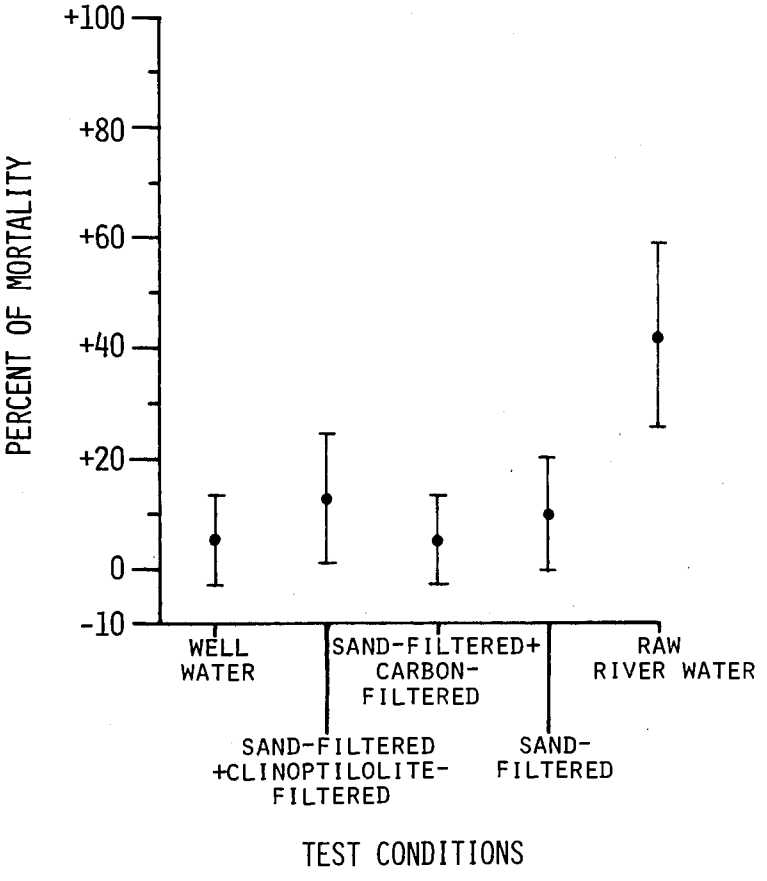

Figure 15. Mortality of Clams Exposed to Well Water, Raw Illinois River Water and Treated River Water for Two Weeks in Deletion Bioassay Number 2. Brackets show 95% confidence limits.

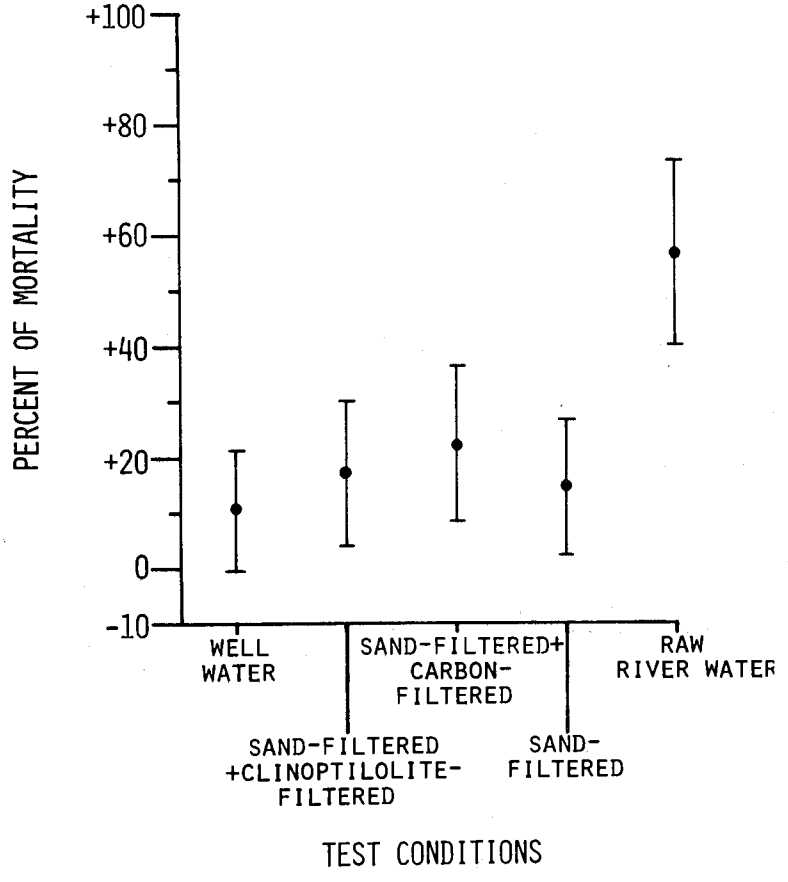

Figure 16. Mortality of Clams Exposed to Test Conditions for Four Weeks in Deletion Bioassay Number 2.

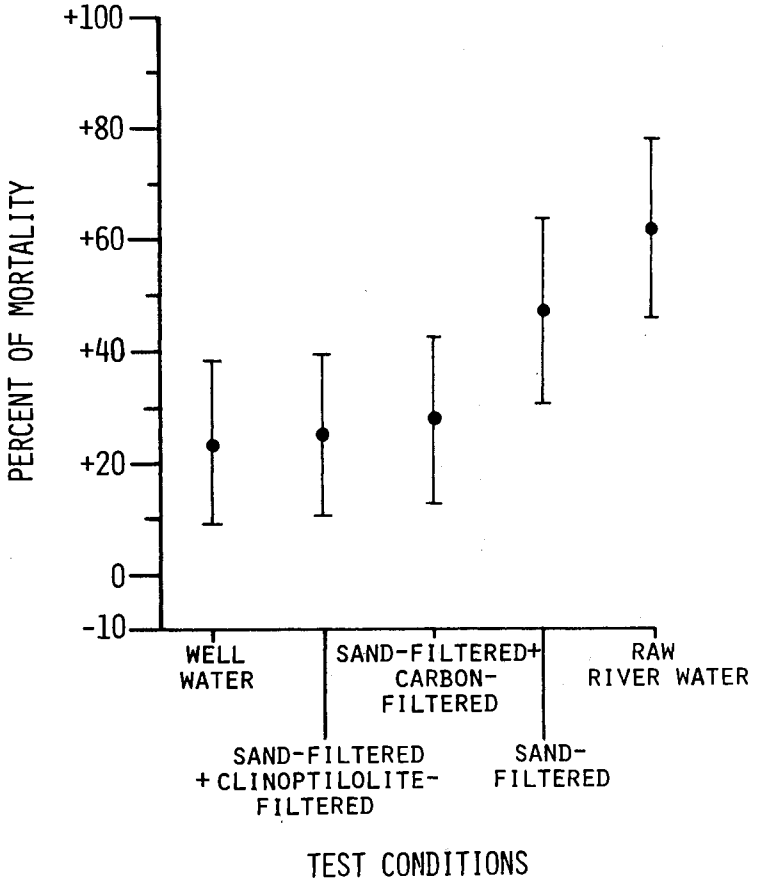

Figure 17. Mortality of Clams Exposed to Test Conditions for Six Weeks in Deletion Bioassay Number 2.

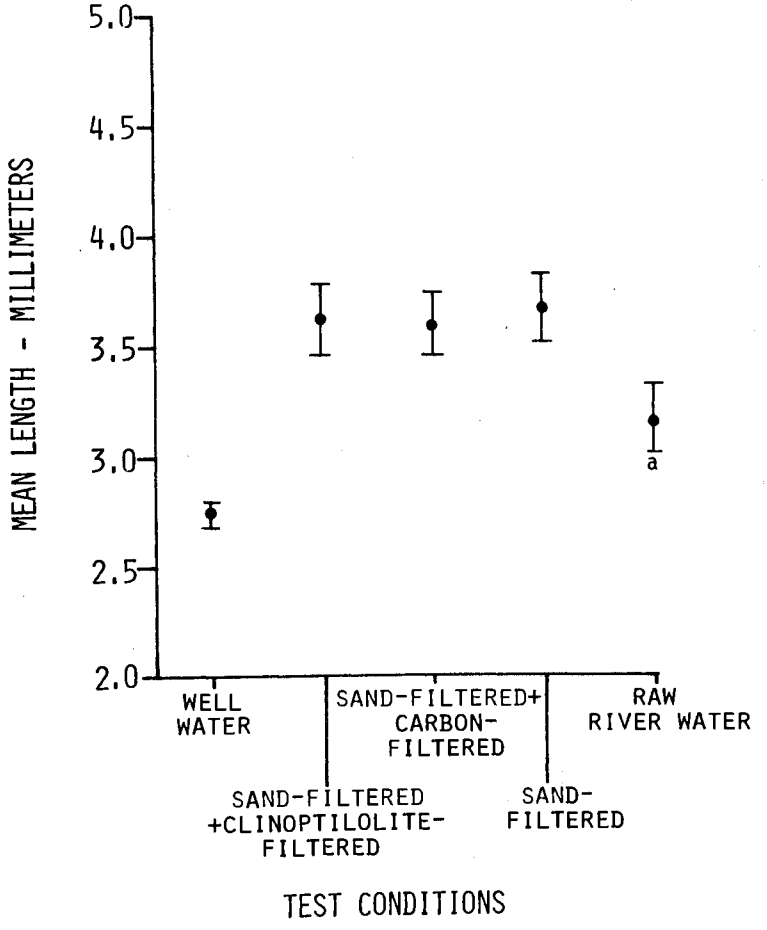

Figure 18. Growth of Clams Exposed to Test Conditions for Two Weeks in Deletion Bioassay Number 2. [a]See note in text (METHODS, page 17) regarding possibility of size-selective mortality.

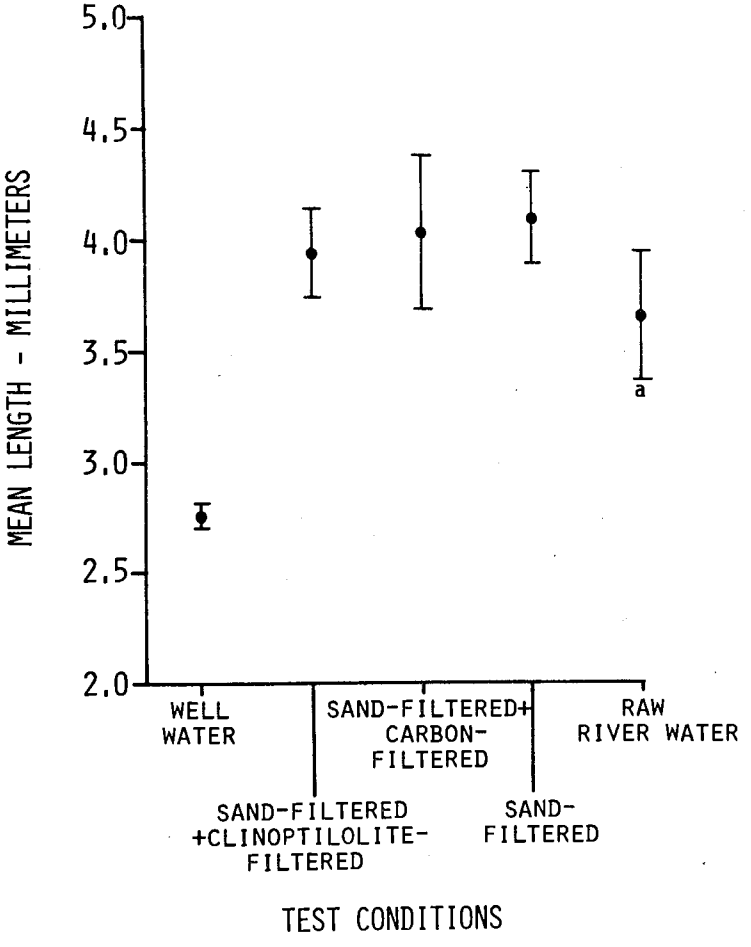

Figure 19. Growth of Clams Exposed to Test Conditions for Four Weeks in Deletion Bioassay Number 2.

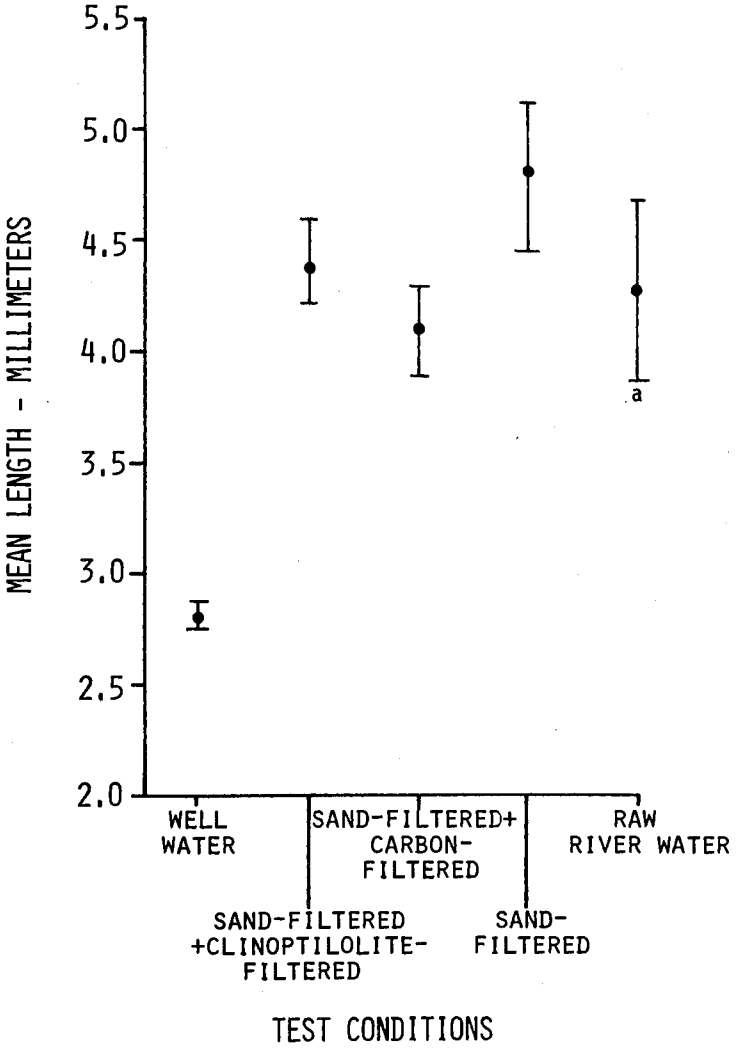

Figure 20. Growth of Clams Exposed to Test Conditions for Six Weeks in Deletion Bioassay Number 2.

food value of the Mississippi silt which was added at 2-week intervals may have been depleted during storage.

Comparison of growth of clams exposed to raw Illinois River water for 4 weeks in deletion bioassays 1 and 2 (Figures 14 and 19), shows that clams in bioassay 1 attained twice the growth of clams in bioassay 2, despite the improved feeding method employed in bioassay 2. The mortality of clams in bioassay 2, however, was almost three times that in bioassay 1 (Figures 12 and 16). These results indicate that whatever is affecting the fingernail clams in the Illinois River, and preventing recolonization of the river by clam populations in tributary streams, is acting directly on the clams and not indirectly, by affecting their food supply. Moreover, the as yet unidentified factor apparently fluctuates in intensity, since only 20% of the clams exposed to raw river water died between 26 June and 24 July 1979 whereas almost 60% died between 3 April and 1 May 1980. Water levels in the Illinois River were much higher in April 1980 than in June-July 1979 (Figure 10), so the intensity of the factor may vary with discharge.

Another factor which may have affected the clams indirectly during bioassay number 2 was an episode of elevated pH levels in the river. The pH levels in the river are normally less than 8.0, but both our laboratory and the laboratories of the Illinois Power Company stations at Havana and Hennepin (see map, Figure 1) recorded pH values above 8.0 for approximately 10 days, reaching highs of 8.8 on the 21st day of the bioassay (24 April 1980) and 8.5 on the 35th day (8 May 1980). While these pH levels probably did not have a direct effect on the clams, they might well have had an indirect effect, by increasing the percentage of ammonia which exists in the toxic un-ionized form (Anderson et al. 1977:61-81).

SUMMARY

1. Illinois River water is toxic to fingernail clam gills. Water samples taken from the river on 5 October 1977 and 22 April 1978 inhibited the beating of the cilia on isolated clam gills, and the April sample was significantly more toxic than the October sample.

2. Illinois River sediment is toxic to fingernail clam gills. A sediment core was taken from Quiver Lake, a bottomland lake which receives sediment input from the Illinois River and where fingernail clams were abundant prior to a die-off in 1955-58. A sediment layer from the 2.6-5.1 cm depth showed the greatest toxicity, the 0-2.5 cm depth the next greatest toxicity, and deeper layers showed significantly less toxicity. Although the sediment layers were not dated, this pattern of toxicity is consistent with the observed die-off. Deeper, hence older layers of sediment, do not show the toxicity of the shallower, younger layers. The decline in toxicity in the surficial layer could result from a reduction in the input of toxicant, or greater dilution of a steady toxicant input by an increased sediment input.

3. Raw water from the Illinois River, containing suspended sediment, is toxic to intact fingernail clams. After six weeks of exposure, 62.5% of the clams in raw river water died. The next highest mortality (47.5%) occurred in sand-filtered water, while mortality in the other two treatments (charcoal and clinoptilolite filtration) did not differ significantly from the mortality of 24% in the well-water controls. The clams probably survived better in the treated water for two reasons: (1) clinoptilolite and charcoal each removed ammonia, which is found in Illinois River water and which is toxic to fingernail clams (2) the additional physical filtration provided by the charcoal and clinoptilolite removed additional sediment, which contains unidentified toxic factors.

4. The toxicant is fairly fast acting, since clams in raw river water suffered 42.5% mortality within two weeks, and ciliary inhibition on isolated clam gills occurred within two hours of exposure to raw river water.

5. The results of both the gill assay and the deletion assay show that the toxicity of the river varies with time, with April 1978 and April

1980 samples showing greater toxicity than samples taken in October 1977 and June-July 1979.

6. The unidentified toxic factor in the river water apparently affects clams directly, rather than indirectly by affecting their food supply. Growth of the surviving clams in raw river water and treated river water did not differ significantly at the end of 6 weeks, but was significantly better than in the controls, indicating that the survivors were deriving some nutritional benefit from the detritus and microorganisms which managed to pass through the filtration system.

7. In conclusion, Illinois River water and sediment contain an unidentified factor which is toxic to fingernail clams. Major detritus-based food webs in the Illinois River, leading from microorganisms and organic matter to diving ducks and bottom-feeding fish valued by man, are not likely to be restored unless the toxic factor is controlled.

RECOMMENDATIONS

This research has shown that Illinois River water and sediment are toxic to fingernail clams, but the research has not identified the specific toxicant or toxicants. The toxicant could be identified, using the gill bioassay developed in this research in conjunction with physical-chemical methods of partitioning water and sediment samples. We recommend the following approach: (1) take sediment cores from a series of lakes and pools along the entire Illinois River, where sediments are known to be accumulating, (2) use the gill bioassay (which provides results rapidly--within hours) to determine where the "hot spots" of toxicity are along the river, and which layer of sediment within each "hot spot" contains the greatest toxicity, (3) take a large volume sample of the most toxic sediment layer from the lake with the most toxic sediment and chemically extract and partition the components of the sediment, (4) test the extracts and partitioned components on fingernail clam gills to determine which component has the greatest toxicity, (5) analyze the most toxic component to provide specific chemical identification of the toxicant or toxicants, (6) verify the identification by measuring the toxicity of the pure chemical (obtained from a chemical supply house) to fingernail clam gills and intact clams, and (7) make recommendations to the state and federal environmental protection agencies regarding control of the toxic material and the prognosis for restoration of detritus-based food webs in the Illinois River.

RELATION OF THIS RESEARCH TO WATER RESOURCES PROBLEMS

There were four major biological communities existing in the Illinois River, prior to the severe degradation of the river which occurred in the 1950's: (1) the plankton communities (phytoplankton and zooplankton), (2) communities occurring on hard substrates, such as rocks, downed trees, and shells of large mussels, (3) the periphyton, insects, and snails associated with beds of aquatic plants in shallow water, and (4) the mud-bottom communities in deeper water, which were dominated by fingernail clams (*Musculium transversum*) and burrowing mayflies of the genus *Hexagenia*. These communities were responsible for fixing or processing energy and organic matter--making it available for higher level consumers such as fish and ducks, which are valued by man. The communities also provided "tertiary" treatment of man's organic waste, converting it from an oxygen-demand problem into biomass usable by the higher level consumers. The mud-bottom community was eliminated from a 100-mile reach of the Illinois River and its connecting lakes in 1955-58. The aquatic plant beds had disappeared by 1958.

The research described in this report has shown that Illinois River water and sediment is still toxic to the fingernail clam, a representative of the mud-bottom community. Hence, the mud-bottom community will not recover until the toxicity is reduced. The research has also developed methods for: (1) culturing fingernail clams in the laboratory, (2) rapidly measuring toxicity using isolated fingernail clam gills, and (3) determining the efficacy of pollution treatment using intact fingernail clams in deletion bioassays. These methods could be coupled with chemical partitioning and analysis of Illinois River water and sediment to identify the specific toxicant or toxicants affecting the benthic community. If the toxicant is not identified, there is a possibility that it may be controlled incidentally, as the result of pollution control measures now being undertaken. However, there is also a possibility that a great deal of money and effort will be spent inefficiently on solutions before the problems (in this case, the toxic substances) have been specifically and precisely identified.

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