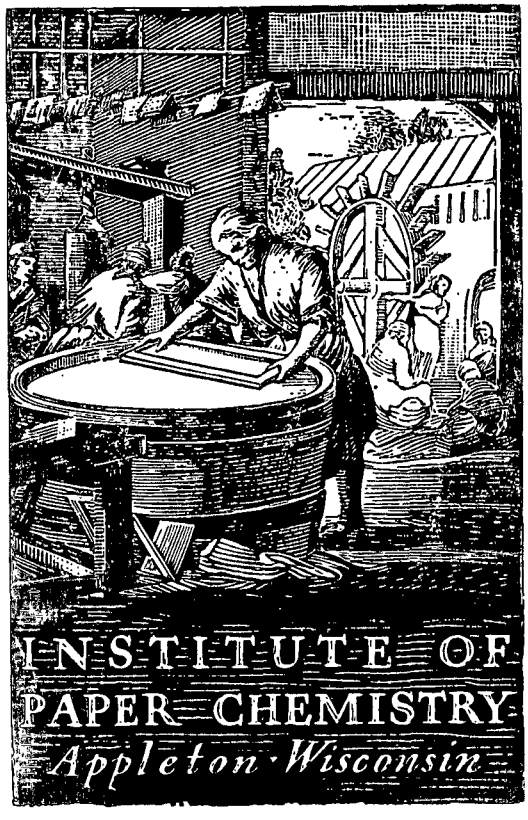

NONSETTLEABLE SOLIDS IN BIOLOGICALLY TREATED EFFLUENTS

PART A–Characterization of Nonsettleable Solids

PART B–Use of Solids as Food By Two Aquatic Food Chain Organisms

Project 3279

Report One

A Progress Report

to

MEMBERS OF THE INSTITUTE OF PAPER CHEMISTRY

March 28, 1980

THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

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NONSETTLEABLE SOLIDS IN BIOLOGICALLY TREATED EFFLUENTS

ABSTRACT

Nonsettleable solids discharged from biological secondary treatment systems were investigated for their value as food for two aquatic food chain organisms. Nonsettleable solids from oxygen activated sludge, air activated sludge and an aerated lagoon treatment system were characterized under summer and winter operating conditions. The activated sludge nonsettleable solids, because of their availability, were then used in a series of feeding experiments to determine whether the larvae of the filter feeding caddis fly, Hydropsyche sp., and the microcrustacean Daphnia sp. were able to utilize them as food.

Characterization of the collected materials revealed that 80-90% of the nonsettleable solids were very small particles with diameters of 1-2 microns. Of these particles, 40-65% were biological in nature with 22% alive at the time of collection. Solids collected during summer had higher viability than those collected during winter. Nonbiological components of the suspended solids could often be related to inorganic clay and coating materials used in the mill.

Feeding runs clearly demonstrated that both Hydropsyche as well as Daphnia can utilize the nonsettleable solids as food. Daphnia survived and reproduced on a diet of solids, and the larger (19.5 mg) caddis larvae also survived and grew. Smaller larvae consumed the solids, as confirmed by C^{14} tagging, but were unable to survive on a diet of 100% nonsettleable solids.

THE INSTITUTE OF PAPER CHEMISTRY

Appleton, Wisconsin

NONSETTLEABLE SOLIDS IN BIOLOGICALLY TREATED EFFLUENTS

PART A. CHARACTERIZATION OF NONSETTLEABLE SOLIDS

INTRODUCTION

Suspended solid materials discharged as a result of normal pulp and papermaking operations have been of environmental concern for a long period of time. These materials initially included bark, chips, knots, and fibers which were discharged directly into the receiving stream. Such direct discharges are rare in the industry today, as mills have primary treatment facilities which remove fibrous solids followed by a rigorous secondary treatment system which discharges a different kind of suspended solids. These solids are dispersed materials which do not settle during the normal retention times and are the subject of much current interest due to regulatory pressures which require ever increasing nonsettleable solids removal (1). Additional treatment equipment, therefore, may have to be installed to meet the proposed guidelines resulting in an increase of up to 74% in waste treatment expenditures (2).

A real question which must be considered is whether this money is best spent in the removal of post-biological treatment solids. Does the environmental impact of these materials justify the expense required to remove them? Traditional concerns about the environmental impact of suspended solids in general include their tendency to form sludge beds in receiving waters, their detrimental effects on fish gills and respiratory functions, and their interference with benthic organisms and habitats as well as increased turbidity and BOD_5 levels. These effects do not seem to be characteristic of nonsettleable solids.

Several recent investigations into the nature and effects of nonsettleable solids produced by pulp and paper industry treatment systems support this conclusion. The lack of solids deposition in areas receiving effluents from secondary treatment plants has been demonstrated by NCASI (3). Investigators at B. C. Research have found that only 20-47% of effluent nonsettleable solids could be precipitated after 24 hours of quiescent settling under laboratory conditions (4). In another study (5), nonsettleable solids were also found to have very little effect upon light penetration in river water and insignificant effect on the growth of an alga (Scenedesmus abundans).

A key question has to do with the composition and ultimate fate of these materials. Much of the earlier work on nonsettleable solids either focuses on some characteristic of the biological components or progresses from the assumption that all of the nonsettleable materials are biological in nature or origin. Environmental consequences of discharged solids may depend heavily upon the extent to which nonsettleable solids are biological materials produced in the secondary waste treatment system. Biological materials may be quite harmonious in the natural world and may possibly contribute to energy flow and productivity within natural communities.

OBJECTIVE

The objective of this part of the investigation was to characterize the nonsettleable solids collected from representative waste treatment systems under summer and winter operating conditions. Of primary interest were characteristics relating to nutritive value, energy content, and use as a food material.

PROCEDURES

SAMPLING SITES

Nonsettleable solid samples were collected from three sites employing three representative biological waste treatment systems. All mills providing samples were in Wisconsin and were within one hour charter air freight delivery time to the Institute.

Mill Site A is an oxygen activated sludge waste treatment system with a design capacity of 6.5 mgd. The mill has a chemimechanical pulp mill with an output of 147 tpd of hydrogen peroxide bleached hardwood pulp. This mill runs four paper machines with a daily production of 375 tpd of commercial coated grades of paper. This sample site provided a large number of the nonsettleable solids collections which were used as food for the feeding studies presented in Part B of this report.

Mill Site B has a two-stage air-activated sludge biological waste treatment system. This mill is a recycle paper operation producing pulp by deinking 100 tpd of waste paper. Water usage is 1.5 mgd with approximately 50% recycled from the waste treatment plant back into the mill. Mill B runs 2 paper machines which produce a line of tissue products.

Mill Site C uses an aerated lagoon biological treatment system which was the third waste treatment process used in this study. This plant produces 620 tpd of corrugating medium from hardwoods using a semichemical nonsulfur process. Water usage is 8 mgd which is treated in a series of aerated ponds with final settling which provides up to 10 days retention time.

SAMPLE COLLECTION AND HANDLING

Eighty liter grab samples were collected from final effluent streams at three different times during the winter months and three times during the summer months at each sample site. Samples were collected by either mill or Institute staff and transported by air or truck in polyethylene containers to the Institute laboratories.

Effluent was pumped through a continuous flow head in a centrifuge operated at 10,000 rpm (with a relative force of 8,500 xg) at 10°C. The collected solids were washed by a final flush of 2 liters of distilled water through the centrifuge head. Distilled water was used to avoid the addition of compounds which might interfere with the ATP and other assays. The use of moderate amounts of distilled water for suspending even pure cultures of bacteria has been demonstrated to be a useful diluent for routine microbiological application. The collected solids were removed from the centrifuge, resuspended and saved for analysis. Samples of all fractions including effluent, centrifuge supernatant as well as solids were used for analysis. Samples were either analyzed immediately as fresh materials or were preserved and stored in a manner compatible to the analytical test to be performed.

ANALYTICAL METHODS

Suspended solids determinations for most of the values were done by filtration through a 0.45 micron pore diameter Millipore filter. The maximum amount of material that would filter in an hour was used for weight determinations. Solids values were also obtained by using a second technique. This was done partly as a methods evaluation step but also to allow calculation of ash weights. Millipore filters were unable to produce ash weights due to combustion during drying. A second solids procedure involved the use of a standard Reeve Angel 934AH 5 micron pore glass

fiber filter according to standard methods (6). The glass fiber filter solids numbers were used only for ash determination; all other solids based numbers were derived from the Millipore technique.

Settleability was measured in an Imhoff Cone for initial samples only. During the first season of sample collecting it became apparent that settling did not occur even during a 24-hour settling period. For samples during the second season only spot checks were made for settleability.

Qualitative microscopic scans were made of all collected samples to characterize the general composition in terms of recognizable objects including fiber, bacteria, algae, zooplankton, grit, and detritus.

Turbidity was measured using a spectrophotometer, and results are presented as percent transmittance.

Analytical determinations of biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), organic carbon, inorganic carbon, and total nitrogen determined by Kjeldahl procedures were conducted according to standard methods (6).

Total phosphorus and elemental analysis determinations were provided by emission spectrographic analysis.

Carbohydrates were determined according to Tappi procedures (7). Heating values as Btu's were calculated according to Parr Manual 120.

Particle size distributions were made by means of a Coulter Counter Model B using operating procedures recommended by the manufacturer. An electrolyte solution of 1% NaCl and an aperture tube size of 50 microns were used.

Microbiological plate counts were made with selective agar and standard incubation and counting techniques.

Adenosine triphosphate (ATP) content was determined for all solids fractions to indicate living components (viability) of biological materials in the solids. The procedure was modified from Cheer (8). ATP was assayed by direct injection of 2 mL of sample into 8 mL of boiling tris buffer (pH 7.7) and continued boiling for 5 minutes. After the sample cooled, tris was added to restore the original volume. The samples were then frozen and stored at -20°C overnight. Analysis was conducted on the following day with 0.1 mL aliquot placed into an Aminco Chem-glow photometer and 0.1 mL of rehydrated firefly lantern extract (Calbiochem) injected into the sample. The light output of the consequent reaction is directly proportional to the ATP content of the sample and quantified by comparison to standards prepared with purified ATP.

RESULTS AND DISCUSSION

EFFLUENT CHARACTERISTICS

The characteristics of effluents sampled for nonsettleable solids determinations are presented in Table AI by season. Results for pH, temperature, turbidity, BOD_5, settleability, total suspended solids, organic carbon, inorganic carbon and ATP are presented in this table (more detailed information is included as Appendix AI). Temperature varied seasonally and between treatments. Mill A operated at a mean summer temperature of 40°C as compared to the lagoon of Mill C which averaged 27°C. Mill B operated at 34°C in the summer and 24°C in the winter. The lagoon understandably showed greatest summer-winter temperature changes.

Settleability proved to be negligible for all effluent samples collected during the winter period. Even after 24 hours of settling, solids failed to accumulate to a measurable degree. Except for spot checking to verify a lack of settling, no settleability determinations were made on summer collected samples. These results reaffirm the nonsettleable nature of the suspended solids in the effluents chosen for investigation.

Effluent solids proved to be fairly constant from season to season in both activated sludge treatment systems (Mills A and B). The aerated lagoon (Mill C) varied quite widely from summer to winter in solids production. The measurement of total suspended solids presented problems which required some exploration of alternative analytical procedures. This problem was not unique to this study. Other researchers also have looked at the effect of the measurement procedures on the final results for solids determination (9).

TABLE AI

CHARACTERISTICS OF EFFLUENTS USED FOR NONSETTLEABLE SOLIDS ANALYSES

	Mill B Air Activated Sludge		Mill C Lagoon		Mill A O_2 Activated Sludge	
	Summer $\bar{x}$	Winter $\bar{x}$	Summer $\bar{x}$	Winter $\bar{x}$	Summer $\bar{x}$	Winter $\bar{x}$
pH	7.4	7.3	6.9	7.1	7.1	6.6
Temperature, °C	34	24	27	12	40	31
Turbidity, %	86.3	92.3	28.3	30.3	49.3	47.0
BOD_5, mg/L	11.5	12	24.3	62.6	52.3	57.6
Settleability, 24 hours	--	0.96	--	0	--	1.1
Total suspended solids, mg/L	12.6	15.6	138	86	113	102
% Ash effluent solids	--	30	12.6	2.3	36.0	20.3
Organic carbon, mg/L	80.3	121	69.3	178.3	85.0	111.0
Inorganic carbon, mg/L	61	121	28	40	37	57
ATP, µg/mg/SS	0.62	0.13	0.31	0.09	0.35	0.13

This investigation required two suspended solids measurement approaches in order to provide the most comprehensive data. The Millipore filter technique with a 0.45 micron pore size was employed to entrain as many bacterial sized particles as possible. This technique, however, was not useful for ash determinations since the Millipore filters tended to combust with the subsequent loss of the sample. The glass fiber filter with a pore size of 5 microns was used to generate the second solids number and to provide ash determinations. All other solids based calculations were made with solids numbers from the Millipore filters. In Table AII solids values from both techniques are presented. (See Appendix AI for more details.)

For the determination of solids in effluents the Millipore technique was more efficient. From 7 to 26% more suspended solids were captured from the test effluents with this filter than with the standard glass filter. However, filter blinding occurred more rapidly with the Millipore, and smaller quantities of effluent could be filtered.

The difference in filtering efficiency for the collected solids fraction was not as evident. For two sample averages the glass filters had somewhat greater weights, while the remaining average weights did not show a difference greater than 8%. The difference in response was most likely due to the concentration of materials and the rate at which pore size changed at the higher concentration of solids.

SOLIDS CHARACTERISTICS

A complete set of characterization data was obtained for the collected nonsettleable solids. These solids were filtered from the effluent and washed to remove any contamination.

TABLE AII

COMPARISONS OF SUSPENDED SOLIDS COLLECTED FOR ANALYSIS

Effluent Solids	Mill B Air Activated Sludge	Mill C Lagoon	Mill A O_2 Activated Sludge
Millipore filter (0.45 micron) mg/L	14.1	112	108
Gooch glass fiber, mg/L	11.2	83.5	95.5
% Ash	30.0	7.5	28.2
Collected Solids			
Millipore filter mg/80 L	675	5524	5998
Glass fiber filters	738	5328	6222
% Ash	25.1	12.3	40

Each collection of solids was examined under the microscope for general composition. A summary of these qualitative observations is presented in Appendix AII. The greatest majority of recognizable materials appeared to be unicellular bacteria. Filamentous bacteria were also common along with a few rotifers and ciliated protozoans. Fibers and woody materials were scarce. Other components included algae, nematodes, fungi, flagellates and grit. Figure A1 shows 3 electron micrographs of a solids sample on a Millipore filter at 1000X, 3000X, and 10,000X magnifications. The sizes and shapes of some of the materials can be seen.

Analytical determinations for the collected nonsettleable solids are summarized in Table AIII (and in greater detail in Appendix AI). Thirteen parameters are summarized which reflect on the character and composition of the collected nonsettleable solids. In general it can be seen that the solids include biological and nonbiological components.

NONBIOLOGICAL SOLIDS CHARACTERISTICS

Ash content of the solids varied from 11.5% to 43% depending on the season and treatment system. For Mills A and B, ash content averages less than 25% but in Mill C ash averaged about 40%.

The elemental composition of these solids is summarized in Table AIV (and included in greater detail in Appendix AI). For Mill C it is apparent that aluminum, sodium and silicone are among the dominant elements revealed by analysis. These are also dominant materials in the solids from Mills A and B. This information would seem to indicate that clays and similar inorganic paper additives contributed to the nonbiological components of the solids to a significant degree.

800X 8,000X 2,400X

Figure A1. Scanning Electron Micrographs of Mill Nonsettleable Solids

TABLE ATII

CHARACTERISTICS OF NONSETTLEABLE SOLIDS BY SEASON AND WASTE TREATMENT SYSTEM

	Mill B Air Actived Sludge		Mill C Lagoon		Mill A O_2 Activated Sludge	
	Summer $\bar{x}$	Winter $\bar{x}$	Summer $\bar{x}$	Winter $\bar{x}$	Summer $\bar{x}$	Winter $\bar{x}$
Solids collected mg/80/L						
Millipore filter	580	770	4293	6756	6543	3453
Glass fiber filter	530	946	4036	6620	6440	6005
% Ash	--	25.1	11.5	13.0	36.6	43.0
Inorganic carbon, mg/g	80.0	116	6.2	5.3	3.2	6.4
Organic carbon, mg/g	410	514	203	373	190	369
TKN, mg/g	53.1	137.3	36.3	51	31.3	42.3
Phosphorous, mg/g	3.5	3.6	1.2	1.2	0.8	1.6
% Carbohydrates	4.8	6.3	3.8	8.9	6.1	8.5
Calories/mg	3.2	1.9	5.3	5.3	3.6	2.9
BOD_5, mg/g	213	286	131	169	168	305
COD, mg/g	1004	1380	1650	1594	928	931
ATP, µg/mg SS	0.54	0.14	0.27	0.04	0.15	0.12
% Analyzed elements	6.6	NA	7.7	NA	19.1	NA

TABLE AIV

ANALYZED ELEMENT CONTENT OF NONSETTLEABLE SOLIDS AS MEANS FOR SEASONAL SAMPLES

	Mill A O_2 Activated Sludge[a]	Mill B Air Activated Sludge	Mill C Lagoon
Element % Composition			
Aluminum	7.4	1.26	0.62
Barium	0.019	0.025	0.031
Boron	0.0055	0.010	0.0095
Chromium	0.0069	0.0	0.006
Copper	0.010	0.012	0.016
Calcium	1.00	2.2	1.86
Iron	0.27	0.10	1.3
Magnesium	0.47	0.96	0.62
Manganese	0.013	0.0057	0.061
Lead	0.005	0.009	0.017
Sodium[b]	2.05	31.6	1.19
Silicon	6.3	1.3	1.02
Titanium	0.66	0.54	0.25
Vanadium	0.0078	0.007	0.0035
Total %[c]	16.16	6.4	5.81

[a] Mill A data based on 17 analyses because of extra sampling for feed runs.
[b] Results as % total oven dry solids.
[b] This analytical method provides poor reliability for sodium. This number is questionable.
[c] Total excluding sodium.

The presence of metals such as lead, chromium and copper was minimal in all samples analyzed. Thus, toxicity from heavy metals would not be anticipated and would not be expected to create objections to use of the nonsettleable solids sampled in this study as food by stream organisms.

Differences between waste treatment systems were large, with Mill A averaging three times the total inorganic elements of the other two. The largest components (Al and Si) probably originated from clays used in the manufacturing process and the waste treatment system. (Sodium values were eliminated from the total because of a lack of confidence in the analysis for determination of sodium.)

PARTICLE SIZE

The particle size is a large determinant in the settleability and removal efficiency of suspended solids. Analysis of particle size for every sample collected is summarized in Table AV (and Appendix AIII).

The range of particle sizes varied from the Coulter Counter's minimum size sensitivity limit of about 1.0 microns to approximately 20 microns. Few counts above 13 microns were recorded, and the majority of particles counted was below 2 microns in diameter. In 4 out of 6 average counts more than 90% of the particles counted were below 2 microns in diameter. No sample produced less than 73.6% of the particle count at less than 2 microns. In most cases 95% or better of the particles counted were less than 3.2 microns in diameter.

No consistent relationship between particle size and mill treatment system or season was observed. The particle size of the suspended solids was similar to the size of a "typical" bacterial cell, which is 1-2 μm with a volume of 1.5 μm^3 (10) as well as to some coating materials which have normal size ranges of 0.1-5 μm (11). The combined particle size distribution is plotted in Fig. A2.

TABLE AV

$\bar{x}$ PARTICLE SIZE DISTRIBUTION FOR EACH BIOLOGICAL TREATMENT SYSTEM BY DIAMETER

Diameter, μm	1.4	1.8	2.25	2.8	3.6	4.5	5.75	7.25	9.2	11.5
% Count										
Mill B O_2 Activated Sludge										
Winter	67	24	6.6	1.6	<1	<1	<1	<1	<1	<1
Summer	66.3	22.2	7.3	2.3	<1	<1	<1	<1	<1	<1
Mill A Air Activated Sludge										
Winter	78.5	14.0	4.5	1.5	<1	<1	<1	<1	<1	<1
Summer	70.6	20.3	5.3	1	<1	<1	<1	<1	<1	<1
Mill C Aerated Lagoon										
Winter	62.3	17.6	10.3	3.0	1.0	<1	<1	<1	<1	<1
Summer	72.3	19.3	6.3	1.6	<1	<1	<1	<1	<1	<1
Combined Average of 3 Systems and Both Seasons										
% Count	69.5	19.5	6.7	1.8	<1	<1	<1	<1	<1	<1
Winter average	69.2	18.5	7.1	2.0	<1	<1	<1	<1	<1	<1
Summer average	69.7	20.5	6.3	1.6	<1	<1	<1	<1	<1	<1

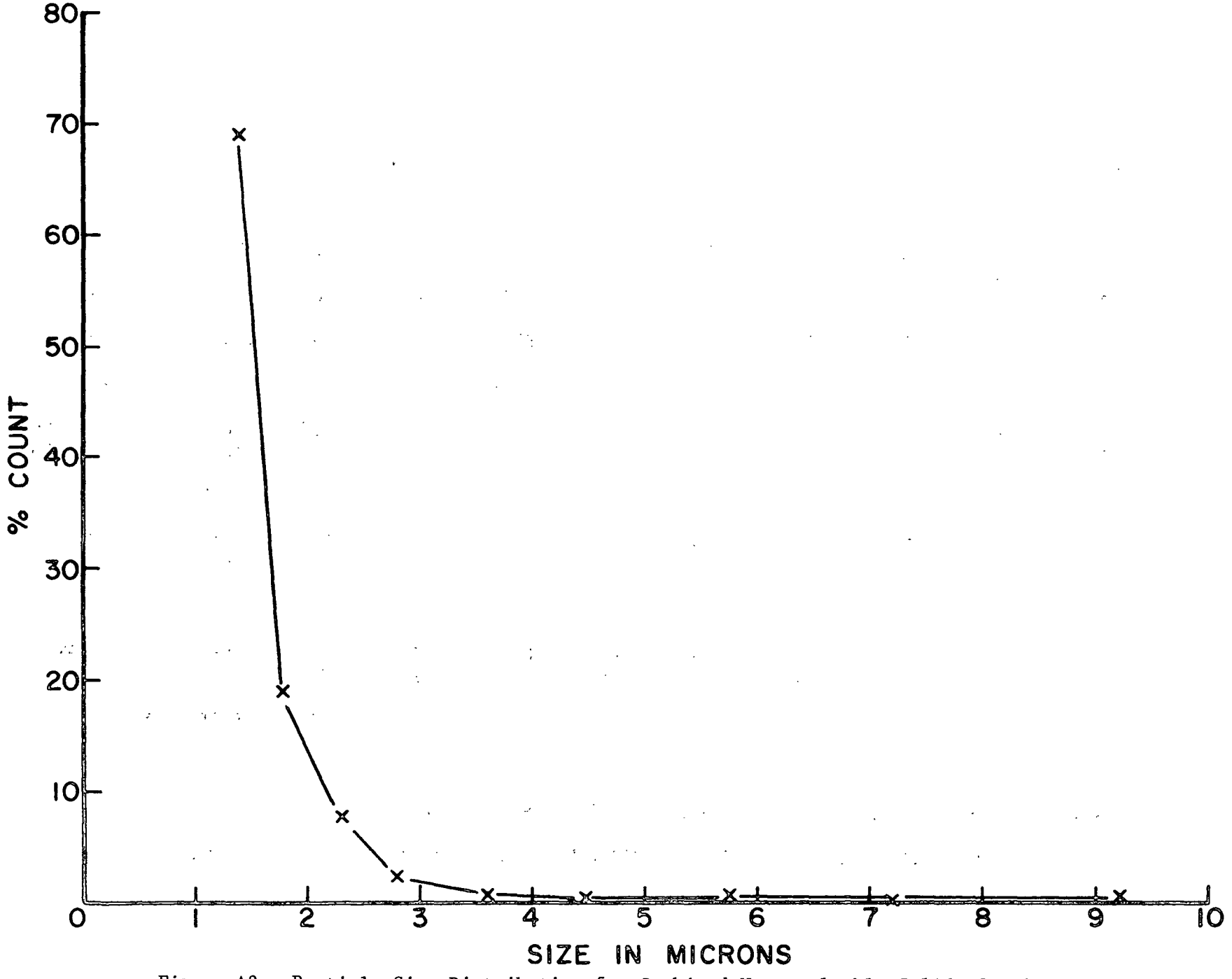

Figure A2. Particle Size Distribution for Combined Nonsettleable Solids Samples

The total population of solid particles is graphed as number per mg of solids and by volume as $\mu m^3/mg$ of solids for each treatment system for each season sampled. This information is found in Appendix AIII, Fig. 1-6.

The small particles exert an overwhelming influence on the total number of particles, but based on volume, the larger particles become more dominating. By count, less than 5% of the particles found were over 3 microns (Table AV). However, the average combined particle volume in the size range 3.2-12.8 was 38.7% for winter samples and 28.2% for summer samples. By either volume or count the very small and very difficult to remove particles dominate post biological effluent solids.

BIOLOGICAL CHARACTERISTICS

Data collected during this study indicate that a significant portion of the nonsettleable solids are biological materials. In Tables AVI and AVII data relevant to biological characteristics are summarized by season for the waste treatment systems at the three mills.

Total Kjeldahl nitrogen values were used in the cell mass calculation by converting organic nitrogen values to protein (conversion factor of 6.25X) and then using the assumption that 50% of the cell mass was protein (10). Although the percent of cell mass in collected solids was found to range from 39.1 to 66.3%, the winter cell mass fraction was smaller than the summer cell mass fraction.

ATP analysis showed that about 3.4 to 45.5% of the cell mass was alive. Samples collected during summer had higher viability than those collected during winter. The highest percent viability was recorded for summer air activated sludge systems while the lowest viability occurred for the winter-aerated lagoon system.

TABLE AVI

SUMMARY OF PLATE COUNTS FOR NONSETTLEABLE SOLIDS

	Mill A		Mill C		Mill A	
	Winter	Summer	Winter	Summer	Winter	Summer
Bacteria, No./mg	133×10^6	149×10^6	39.3×10^6	1.4×10^8	68.6×10^6	2.5×10^8
Counts, No./mg	5.7×10^6	74.9×10^6	20.8×10^6	0.87×10^8	26.0×10^6	0.5×10^8
Yeast + molds, No./mg	283 ± 46.1	169 ± 189.3	309 ± 172.2	211 ± 43.1	1170 ± 1075	137 ± 142.3
Fungi, No./mg	NA[b]	615[a]	NA	NA	NA	2175 ± 1984

[a] Number from one sample. Remaining numbers are means of all collections.
[b] NA = Not available.

TABLE AVII

BIOMASS COMPOSITION OF NONSETTLEABLE SOLIDS

	Mill A O_2 Activated Sludge		Mill B Air Activated Sludge		Mill C Lagoon	
	Summer	Winter	Summer	Winter	Summer	Winter
ATP µg/mg	0.15±0.07	0.12±0.05	0.54±0.11	0.138±0.09	0.27±0.10	0.044±0.016
% Viability of total nonsettleable solids from ATP	7.3±3.4	6.0±2.4	27±5.5	9.5±2.8	13.6±5.1	2.2±0.81
Viable cell mass, mg/g	72.3±50	9.1±3.7	62.2±32.1	33.7±35	41.3±17.2	11.1±7.2
Cell mass calculated from TKN, mg/g	391±169	529±100.2	663±106	1716±125	454±51.8	637±141
% of Biosolids which was cell mass	39.1±13.8	52.9±8.1	66.3±7.5	171.6±10.2	45.4±4.2	63.7±11.5
% Viability of cell mass from ATP	18.2±2.3	11.1±3.2	45.5±2.9	NA	29.9±10.1	3.4±0.62
% of Total solids alive by plate count	8.6±1.7	2.3±0.9	5.1±2.5	4.6±0.17	4.8±2.9	1.4±0.68

The proportion of the entire solids fraction (as opposed to the cell mass component) which was alive was understandably lower. Of the total solids, between 6.0 and 27.0% were alive at the time of collection. The pattern of lowest viability in winter is the same as previously indicated.

Microbiological plate counts were also used to calculate the percent of total solids which were viable. This method gave lower values than those obtained by the ATP method which measures material alive during collection rather than viability. Only 1.4 to 8.6% of the solids were found to be viable by plate counts. Summer samples remained the most viable, and the oxygen activated sludge system showed greatest solids viability using the plate count technique.

Cultured plate counts (Table AVI) also indicated that bacteria dominated the viable microfauna. This was true for all waste treatment systems and for both seasonal periods. This information corresponds with the particle size results which indicate dominance by particles in the size range of most bacteria.

Yeasts and molds were the next most profuse group of microbes, and these were present for all systems and for both seasons. Fungi were rare and were observed only for summer samples from two of the treatment systems.

Some reasons for this disagreement in viability results may be postulated. The plate counts actually measure organisms which are capable of reproducing. Specific culture media are required for this purpose and this may provide different results than the ATP test which measures all living organisms and not just those capable of colonizing a culture medium. It is also quite likely that the ATP method is more precise and quantitative for measuring the live organisms.

For purposes of this work the ATP figures will be used as most representative of living materials.

NUTRITIONAL CHARACTERISTICS

The nonsettleable solids have been identified as being composed of as much as 66% cell mass. The question then arises as to the nutritional value of this organic substance as food. As part of the solids characterization work, information was collected for caloric value, crude protein content, organic and inorganic carbon, ash and carbohydrate composition. The results of these determinations are summarized in Table AVIII.

Crude protein as percent of the solids varied from 19.5 to 69.7%. Protein levels were lower in summer solids for each mill treatment system with the protein in the oxygen activated sludge system being the lowest of all three summer averages.

Organic carbon levels showed similar distribution with levels lower in summer than winter and the lowest level for the Mill C treatment system. Inorganic carbon was not a large component of any of the samples.

Calories were calculated from Btu determinations and describe the energy value of the material. Calorie levels were fairly close for the averages of all mills for both seasons. Nonsettleable solids varied from 1.9 to 5.3 calories per mg of solids.

Carbohydrate content was rather low for all the types of solids. Levels, as percent composition, varied from 3.8 to 8.5% with considerable variations between the samples (standard deviations of 0.5 to 4.5). Seasonal variations were not pronounced, and Mills A and B and C were reasonably similar.

TABLE AVIII

SUMMARY OF NUTRITIONALLY RELATED INFORMATION COLLECTED FOR MILL EFFLUENT NONSETTLEABLE SOLIDS

	Mill B Air Activated Sludge		Mill C Lagoon		Mill A O_2 Activated Sludge	
	Winter $\bar{x}$	Summer $\bar{x}$	Winter $\bar{x}$	Summer $\bar{x}$	Winter $\bar{x}$	Summer $\bar{x}$
Calories/mg	1.9	3.2	5.3	5.3	2.9	3.5
% Crude protein calculated from TKN	69.7	33.0	31.8	22.4	26.4	19.5
% Phosphorous[a]	NA	0.56	NA	0.7	NA	0.59
% Inorganic carbon	11.6	7.9	0.53	0.62	0.63	0.32
% Organic carbon	51.2	41.0	37.5	20.2	36.8	18.9
% Ash	25.1	NA	13.0	11.5	43	36.6
Carbohydrates % Composition						
Rhamnan	0.64	0.50	0.48	0.27	0.55	0.48
Araban	0.04	0.02	0.39	0.23	0.20	0.02
Xylan	0.20	0.16	0.52	0.34	0.39	0.33
Mannan	0.49	0.47	0.48	0.39	0.55	0.51
Galactan	0.40	0.39	0.85	0.51	0.60	0.77
Glucan	4.5	3.2	1.93	1.96	6.21	3.97
Total	6.38	5.02	4.5	3.84	8.5	6.11

[a]Phosphorous from emission spec. as %.

The dominant sugar was predictably glucan which reached levels as high as 6.2%. All other identified sugars were present at levels of less than 1%; these included rhamnan, araban, xylan, mannan, and galactan.

The nutritional information for the solids indicates that there is potential for use of these solids as a food or as a supplement. The utilization and availability of nonsettleable solids as food may depend on the size of the particles and their accessibility to organisms.

COMPARISON OF WASTE TREATMENT SYSTEMS

To better investigate the relationships among the nonsettleable solids parameters for the three systems, we conducted a two way computer analysis of variance for 26 characteristics of the solids. The similarity between treatment systems as well as seasons for each parameter was compared (see Appendix AIV).

Comparing the three treatment systems, we found that the solids characteristics whose mean values were significantly different at the 0.05% confidence limit or better were: phosphorus, calories/mg, effluent temperature, effluent BOD_5, solids COD in mg/g, percent ash in the collected solids, inorganic carbon and organic carbon. The parameters were those most influenced by the type of mill and waste treatment system. It is reasonable to expect different mills to have different solids loadings as well as different temperatures. Because of the relationship among temperature, solids and carbon, it is not surprising to see significantly different BOD_5's for the effluent.

Of equal interest are parameters which did not change for mill systems. These include: pH, effluent ash, % carbohydrate, solids BOD_5, ATP of the effluent and the solids, and the particle size of the solids.

There were some significant differences in solids characteristics due to seasonal influences of combined mill samples. Effluent temperature is a characteristic which would differ logically due to the influence of the aerated lagoon which operates at close to ambient temperatures. The activated sludge systems varied much less in operating temperatures. Likewise the influence of Mill A summer plate counts contributed to differences in plate counts by season. ATP content of the effluent was different seasonally but effluent solids were not. This means that solids viability changes seasonally and is predictably lower in the winter. Particle counts for both effluent and collected solids were seasonably different as were the caloric content and the BOD_5 of the solids.

This information supports the observations of most treatment plant operators that there is a seasonal difference in the operational efficiency of waste treatment plants. The widely noted observation that solids loss is greater in the winter than in the summer from biological treatment systems was also observed in this study. Moreover, the data indicates that winter solids have a greater percentage of cellmass or biological materials than summer solids but that this cell mass contains less living material than during summer collection periods. It has been observed during this study that there are also differences in the effluents and solids produced under different seasonal operating conditions.

Further comparisons of the nonsettleable solids characteristics were made using an analysis of correlation for coefficient "r." This calculation provides additional information on the relationship between the characteristics of the solids and solids plus effluents. Summarized correlation coefficient information is included with this report as Appendix AV.

Highest correlations existed between effluent solid levels and collected solid levels which only indicated that collection and solids analysis techniques were accurate. A high correlation between solids and BOD_5 of the effluent also existed. This indicates that a significant amount of the effluent BOD_5 which remains can be attributed to the nonsettleable solids. This indication was also supported by the BOD_5 data collected for centrifuge supernatant which showed a drop in BOD_5 when nonsettleable solids were removed.

Other high correlations occurred between the ATP of the effluent and the ATP of the solids.

Some parameters were correlated which did not logically relate to each other and these provided little expansion of an understanding of the nonsettleable solids. These included correlations such as temperature and calcium, organic carbon with heavy metals, and BOD_5 with aluminum.

LITERATURE CITED — PART A

1. EPA. Development document for interim final and prepared effluent limitation guidelines and new source performance standards for the bleached kraft, groundwood, sulfite, soda, deink, and nonintegrated paper mills segment of the pulp, paper and paperboard mills point source category. Vol. I and II. U.S. EPA 440/76/047a, 1976.

2. Gelb, B. A. Measuring the cost of industrial water pollution control. Case Study I, Pulp Paper, and Paperboard Mills. Supplement to Conference Board Report No. 700, 1976. 113 p.

3. Costa, H. S., D. L. Borton, and McKeown, J. J. Impact of residual biosolids on receiving water quality. 1978 Tappi Environmental Conference Proceedings, pp. 57-63.

4. Lee, E. G. H., Wolden, C., and Mueller, J. C. Analysis and characterization of suspended solids in pulp and paper mill effluent. Pulp Paper Can. 78 (9):205-9(Sept., 1977).

5. Academy of Natural Sciences of Philadelphia. Fate and effects of suspended solids for secondary biological treatment of waste water. Academy of Natural Sciences of Philadelphia, Div. of Limnology and Ecology No. 77-31, Oct., 1977. 145 p.

6. American Public Health Association, American Water Works Association and Water Pollution Control Federation. Standard methods for the examination of water and waste water. 14th ed. APHA, New York, 1975. 1193 p.

7. Borchardt, L. G. and C. V. Piper. A gas chromatographic method for carbohydrates as alditol-acetates. Tappi 53(2):257-61(Feb., 1970).

8. Cheer, S., Gentile, J. and Hegre, C. Improved methods for ATP analysis. Anal. Biochem. 60:102(1974).

9. Mueller, J. C. Analysis of suspended solids by centrifugation — an alternative to the standard procedure. Project report for COFI Environmental Committee. B. C. Research, Vancouver, B.C. 17 p.

10. Gunsalus, E. C. and Stanier, R. Y., eds. The bacteria; a treatise on structure and function. Vol. 1, Academic Press, 1960. 513 p.

11. Parham, R. A., Kaustinen, H. M. Papermaking materials, an atlas of electron micrographs. The Institute of Paper Chemistry, Appleton, Wisconsin. 55 p.

PART B. USE OF SOLIDS AS FOOD BY TWO AQUATIC FOOD CHAIN ORGANISMS

INTRODUCTION

In Part A of this report the characteristics and composition of non-settleable solids from three types of biological waste treatment systems were described. The composition of these solids indicate that they may have value as food for organisms which normally feed on microorganisms dispersed throughout their normal environment. The objective of this part of the investigation was to determine whether the nonsettleable solids characterized in Part A could be used successfully as food by two aquatic organisms.

In order to be considered as a successful food, organic substances must be conducive to three biological functions. The first function is ingestion. A material has to be in a shape, form and location where it can be ingested. Once injested the food must be able to be digested by the organism using it for food. Confirmation of ingestion alone is insufficient to prove digestion. The third necessary function is assimilation. If the digested material can be assimilated and can contribute to the growth and survival of the organism it is a successful food.

The current work investigates these food functions for two organisms. Organisms which would be able to feed on these types of materials include a variety of aquatic invertebrates. Included in this group is a family of caddis fly larvae called Hydropsychidae. The Hydropsychidae are a family of net-spinning insects which are diverse and widespread over North America. The immature stages of these insects are aquatic and feed only in the aquatic larval stage of development. These larvae collect food from the aquatic environment by filtering it through a finely woven net and rely for food upon materials which remain suspended in the water column.

Hydropsyche are known to eat small algae, fungi, bacteria and detritus (1-2), and are found in nearly all productive good quality streams in North America.

A second test animal was also investigated which makes up a major part of the zooplankton in many lakes and rivers. This organism is Daphnia sp. which is also a filter feeder and normally diets on algae and bacteria (3-5). This organism is easy to culture under laboratory conditions and is one for which a large amount of life history data is available.

PROCEDURES

ARTIFICIAL STREAMS AND TEST ORGANISMS

Hydropsyche was studied in the laboratory under riverine conditions. A laboratory scale apparatus was designed to keep water volumes and food requirements at manageable levels in a simulated flowing water habitat. This was done by constructing 6 doughnut-shaped circular tanks out of 0.95 cm (see Fig. B1) Plexiglas. The tanks were 33.5 cm in diameter for the outside ring and 9 cm in diameter for the inside ring. The capacity of the outside ring was 7.5 liters. An artificial substrate consisting of unlapped porcelain balls, 5 cm in diameter was used to simulate rock river bottoms. A total of 30 balls was placed in each habitat. Water movement was provided by means of a 4-bladed Plexiglas paddle with blades 75 mm wide by 83 mm long. Six paddles were attached in series to a 13 mm steel shaft attached to a gear reduction drive. The drive was powered by a 3/4 horsepower continuous duty electric motor. The paddles turned at 20 revolutions per minute which produced a water velocity (over substrate balls) of 22.9 cm/sec (measured with a timed float). Each of 6 habitat tanks were placed in line with the shaft which produced duplicate flow conditions in each tank. The different tanks were used for different food rations.

The habitats were filled with city water dechlorinated by means of activated carbon. The chambers were cleaned periodically of accumulations of settled food or waste materials by means of a siphon tube without disturbing the populations.

The Hydropsyche used as test organisms were obtained from the Fox River in Appleton, Wisconsin from an area free from the influence of known point source waste discharges. Organisms were transported to the lab in river water, hand sorted to collect correct species and transferred to shallow aerated containers for

measuring. Each individual of each test population was blotted on dried pulp sheets and weighed on a sartorius analytical balance. Each organism's head capsule length was measured on a compound microscope with an ocular micrometer. The Hydropsyche larvae were placed into the test chamber and allowed to acclimate for three days prior to initiation of a feeding experiment. Feeding experiments continued until the onset of pupation at which time the surviving larvae were collected, weighed and measured as before.

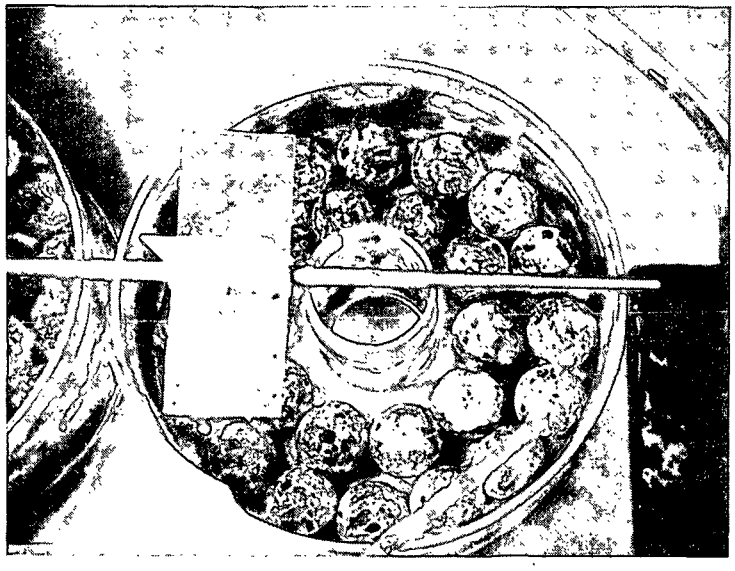

Figure B1. View of Flowing Water Habitats Used for Solids Feeding Studies

FOOD AND FEEDING

Test organisms were fed varying diets of control food and experimental food. Control food was a commercial tropical fish food HiProMin made by Hartz Mountain. This flake food was finely ground with a mortar and pestle and then sieved through a fine mesh plankton net. The food was weighed dry and added slowly to the surface of the water rotating in the habitats. Analytical and nutritional information for this food is included in Appendix BI.

Experimental food consisted of nonsettleable solids collected from Mill C on a weekly basis and recovered by centrifugation as described in Part A of this report. Solids were fed fresh on day 1 of the three day feeding program and then frozen for use on the last 2 days of the program. Characterization information for the special collection of Mill A solids used as food are included in Appendix BI, Table BII.

Food rations were established based on an estimated maximum use rate of 25% of body weight per week for the first experiment. This was later reduced to 20% because of underutilization. A separate food quantity was calculated for each tank population. The total population of 50-100 individuals was weighed and an average weight per insect was obtained. Twenty five, and later 20, percent of the average insect weight times the total number of individuals yielded the weekly food ration. This amount was divided into thirds and was fed on each of the three weekly feeding days. After each count period the weekly food rate was adjusted based on the number of survivors.

HABITAT MONITORING

Conditions in each habitat were monitored daily to protect against sudden changes. The following parameters were measured:

Temperature

pH

Dissolved oxygen

Conductivity

NH_4

Nitrite-nitrate

Suspended solids

Total solids

Meters were used to measure temperature, pH, dissolved oxygen and conductivity. Nitrate was measured using a Hach procedure (6). This procedure was checked against the Standard Methods colorimetric method (7) and found to give similar results. Solids determinations were done with glass fiber filter techniques as described in Part A of this report.

NET SIZING AND SOLIDS UPTAKE EXPERIMENTS

The nets of the Hydropsyche larvae were collected from the porcelain substrate by submersing a glass slide under a well formed net. The net attachments were then severed with a needle and the net was floated onto the slide. Care was required to prevent distortion, collapse or folding of the web. A cover slip was used to protect the specimen while net meshes were measured by means of an ocular micrometer in a microscope.

We investigated solids uptake by caddis nets and substrate by adding fresh biosolids stained with Rose Bengal to a new unfed larval population. Solids were stained with 0.1 g/L Rose Bengal (determined experimentally) added at 25% of the body weight of an acclimated but unfed population. Suspended solids determinations were made over time and larvae were sacrificed and disected to check for ingested solids.

DAPHNIA SP. FEEDING

Daphnia sp. were cultured in the laboratory from a single individual obtained from a collection of Fox River fauna. Daphnia habitats were 3-liter glass aquaria with 10 individuals in each jar. A food density of 0.01 mg/mL was determined to be above minimum food density required (5, 8-9) and this was used as the initial food rate. Supplemental food was added three times a week based on an assumed consumption of 1-7% body weight per day. A value of 10%

of body weight was used to ensure adequate minimums. Populations were fed HiProMin control food, biosolids from Mill B, a ration consisting of half control food and half solids (50/50), solids generated under laboratory conditions, and zero food. At the end of the test, counts were made of harvested Daphnia and each individual was measured for body carapace length to determine adults from progeny.

Biosolids generated in the lab were also fed to Daphnia. Solids were produced in lab scale batch type activated sludge units to provide comparisons with mill effluent solids. The units used were two-liter reaction vessels with a five-day sludge age, aeration with compressed air and mixing by means of a mechanical stirrer. A nutrient medium was used rather than an effluent and included the following:

Glucose	1.2 g/L
Yeast extract	0.2 g/L
$MgSO_4$	0.1 g/L
$FeSO_4$	0.1 g/L
$CaCl_2$	0.1 g/L

This medium was autoclaved at 120°C, 15 lb pressure at 20 minutes. The generated biosolids were characterized for:

ATP
Total suspended solids
Ash
Total Kjeldahl nitrogen
Phosphorous
BOD_5
Organic carbon
COD
Particle size

Test procedures were described in Part A.

C^{14} TAGGING STUDY

C^{14}-tagged solids were fed to the test organisms which were then measured for radioactivity. A schematic design of tests and procedures is included in Fig. B2. Solids were generated in the lab scale reaction vessel using C^{14}-labeled glucose in a sterile ethanol-water solution. The glucose had an SA = 237 mc/mM equivalent to 500 microcuries. Radioactive glucose was used in the biogenerator to produce tagged solids.

Mill solids were tagged by 24-hour exposure in the reaction vessel to the C^{14}-tagged glucose which was added as a nutrient source. The tagged solids were removed by centrifugation, washed and resuspended. The solids were then filtered and the filter with solids was dissolved in NCS tissue solubilizer[1] overnight at 50°C. Test organisms were also washed and solubilized in tissue solubilizer. Solubilized samples were added in 0.1 mL aliquots to 10 mL of Cocktail D and analyzed by means of a liquid scintillation counter.

Fifty Daphnia were fed at previously described rates for 24 hours. Ten were then removed to filtered clean water for 24 hours to ensure a cleared gut tract. Another group of 10 was killed immediately in 4% formalin and tested. The third batch was transferred to a separate container and fed control food until a reasonable number of progeny had been produced. The progeny were separated out (by size), solubilized and counted to determine whether or not tagged glucose had been passed along to offsprings.

Caddis fly larvae were treated somewhat differently. Populations of large and small larvae were set up and fed for 4 days. Following that period, subsamples of 10 individuals of each size larvae were killed and solubilized and counted. Another subsample was killed and the gut tract was dissected out.

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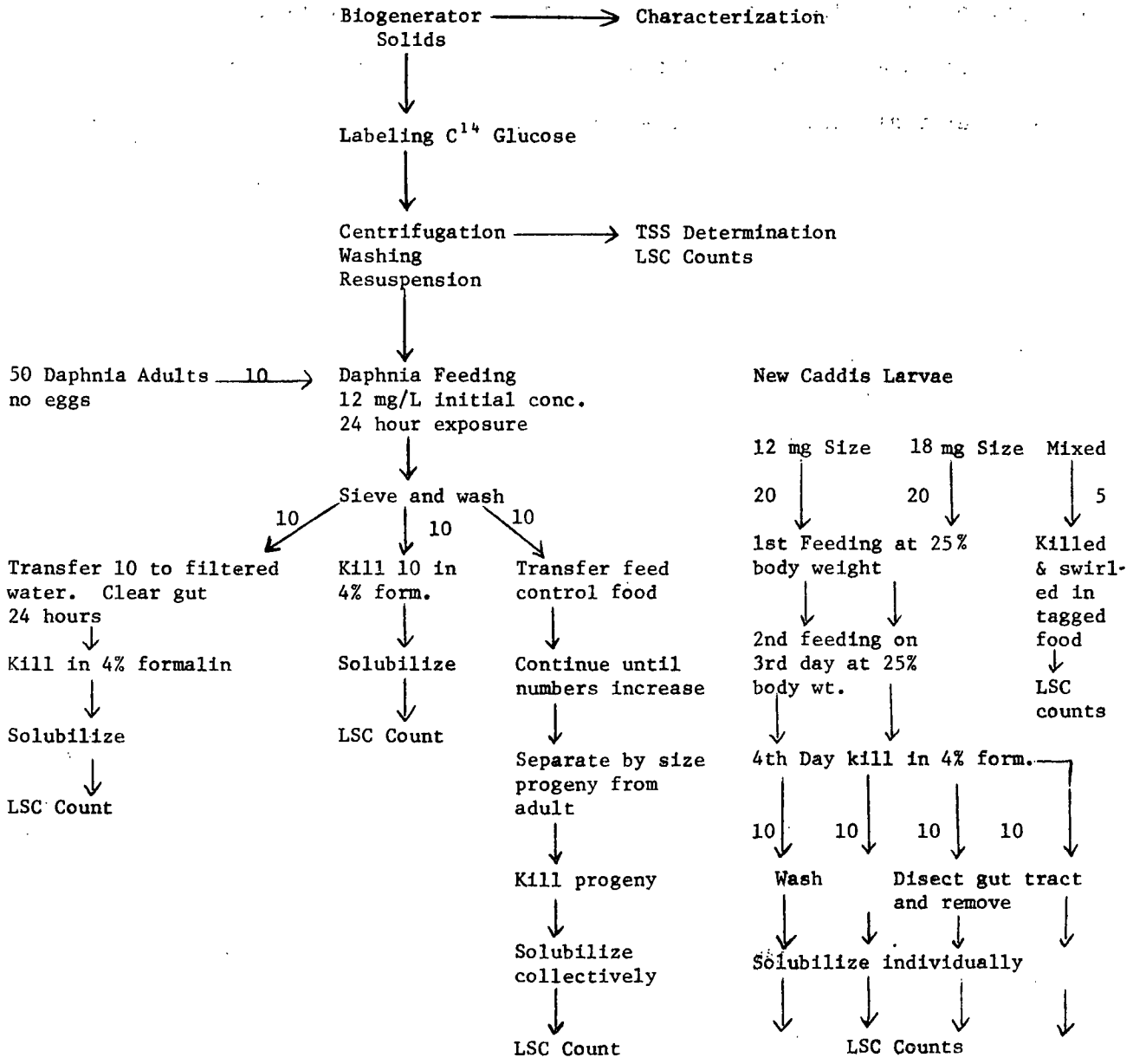
[1]Amersheim Corporation.



Note: LSC = liquid scintillation count.

Figure 2. Schematic Layout of C^{14}-Radio Tagged Solids Study

The remaining organisms were solubilized and counted. An additional subsample of 5 unexposed individuals was killed and merely swirled in tagged food. Then these were washed and solubilized and counted to determine a background count for tagged material which might be attached to the organism rather than actually injested.

RESULTS AND DISCUSSION

HYDROPSYCHE FEEDING EXPERIMENTS

During the course of Hydropsyche sp. feeding experiments daily monitoring of the ring tank habitats was maintained. Parameters to be monitored were chosen to reflect conditions which would affect the organisms' health such as temperature, pH, dissolved oxygen and conductivity. Also monitored were parameters which would be affected by the organisms such as ammonia and nitrate levels and suspended as well as dissolved solids. When ammonia or nitrate levels began to show increases, the water in the tanks was changed and any solids accumulations removed by siphon. This information is summarized in Appendix BII with mean values. The means do not adequately show daily variations which led to cleaning procedures but do show comparisons between habitats receiving different foods.

Of most interest is the solids data. Total dissolved solids did not vary between feeding runs for any of the food rations including starved populations. Suspended solids levels showed greater variability and also failed to show any relationship to whether food was added to the tanks or not. This is largely due to the fact that suspended solids were sparsely distributed and large enough samples to produce reliability in the data were not available.

In order to investigate the influence of the caddis larvae on solids removal two removal experiments were conducted. Solids were added to habitats with and without caddis larvae and monitored in the water for 24 hours thereafter. The results are listed in Appendix BIII and summarized in Fig. B3. In the first test a greater solids loading was added initially than in the second. In both cases there was a decline in suspended solids in all habitats including those without the larval nets within the first hour. Impingement between porcelain

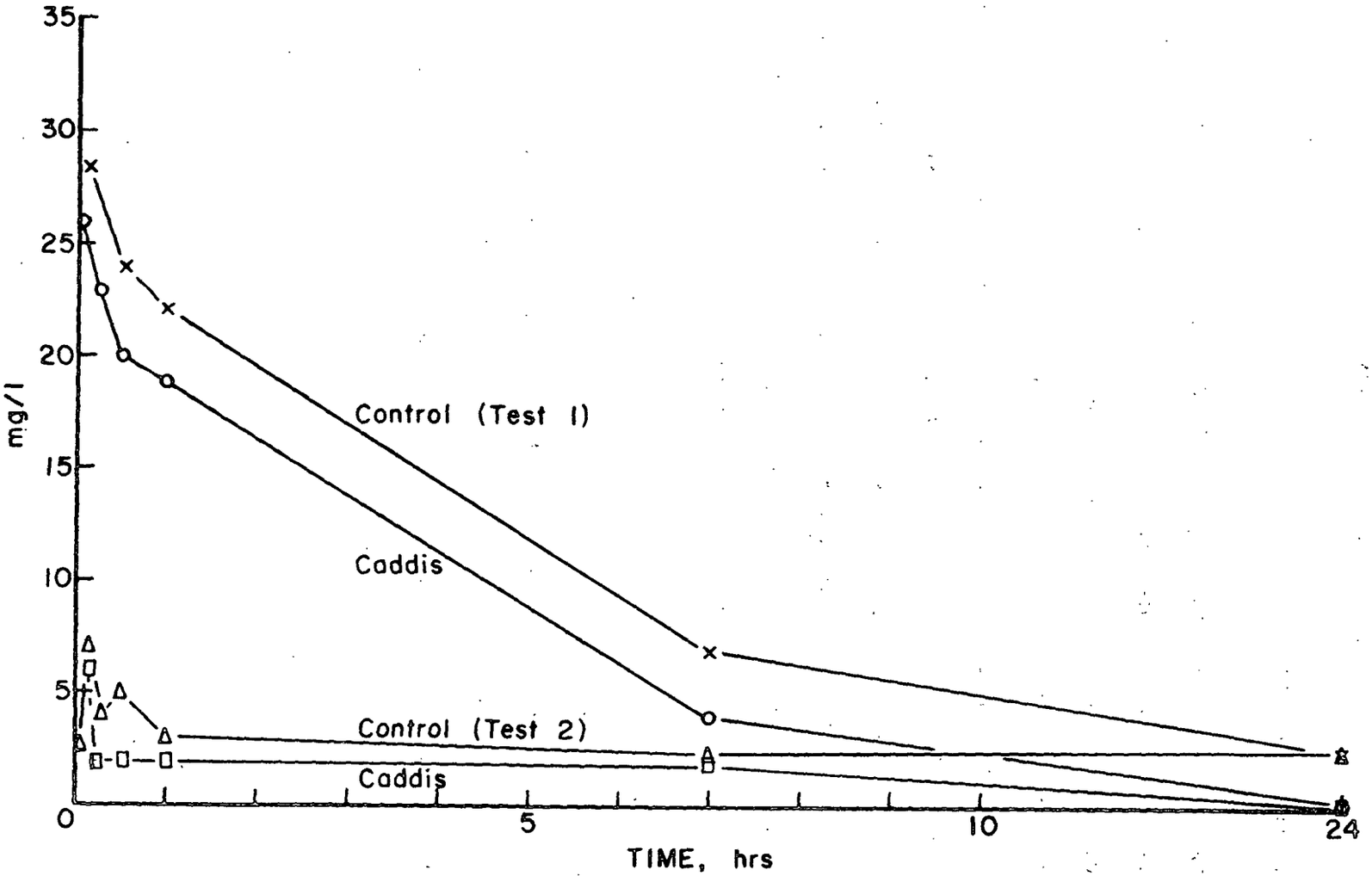


Figure B3. Solids Uptake by Caddis Larval Nets

spheres and settling in quiescent current shadows probably affected this initial removal in bugless habitats. By the end of 24 hours it was observed that nearly all suspended solids had been removed in tanks containing larvae while in the control tanks suspended solids levels evened out and remained at moderate levels. On the average for the two tests 4 times as much suspended solids remained in the chambers without caddis larvae than in those with.

The results of this solids uptake study implies that the nets are capable of removing the small nonsettleable solids from the water flowing past. Thus, these materials should be available as food for the organisms.

CADDIS NET SIZE INVESTIGATIONS

It is generally accepted that net-spinning filter-feeding caddis fly larvae feed on particles that fall within preferred size ranges (2, 10). The size of the net meshes is believed to select for the preferred food size which changes as the animal grows. The question remains as to whether the caddis mesh size or net characteristics are adequate for the removal of nonsettleable suspended solids. Information on net size and shape was collected during several of the feed runs. This data is summarized in Table BI.

While a very rough pattern in the data indicates that smaller larvae have a smaller mesh size there is no correlation in this data between larval size (weight) and mesh size ($r = 0.28$).

It was also observed qualitatively that mesh size varied within a net and that the mesh size rather than number of meshes changed as the configuration of the net changed. When nets were spun between balls this resulted in an inverse pyramid shape with small meshes at the peak and large meshes at the base. Regardless of the size range no meshes were observed that would apparently be small enough to

filter one- to five-micron particles such as those found in nonsettleable solids. However the nets readily captured these particles and became quite encumbered with them. It seems that the net fiber characteristics allow the particles to adhere to the strands rather than to be trapped between them. In Fig. B4, photomicrographs of caddis nets show particles adhered to the net strands. It would appear that the nets are able to "filter" particles that are much smaller than the openings in the net itself. As the particles accumulate they contribute to the effective surface area of the net and assist in capturing more materials.

TABLE BI

NET MESH SIZE RELATIONSHIPS FOR HYDROPSYCHE LARVAE
IN NONSETTLEABLE SOLIDS FEEDING HABITATS

Run	Food Ration	$\bar{x}$ Initial Caddis Weight, µg	$\bar{x}$ Initial Caddis Head Capsule, µ	$\bar{x}$ Net Mesh, microns	S. Dev. σ	N Nets	N Meshes
1	Zero food	18.6	1.17	325 x 396	(131 x 140)	3	8
2a	Control food	3.75	0.56	320 x 426	(103 x 108)	4[a]	20
a	Zero food	5.90	0.56	161 x 233	(110 x 161)	5	29
b	Control food	3.75	0.56	328 x 539	(113 x 167)	4	19
b	Nonsettleable solids	5.39	0.56	275 x 325	(50 x 65)	1[a]	5
b	50/50	6.53	0.56	273 x 419	(101 x 98)	4[a]	19
b	Zero food	5.90	0.56	142 x 197	(43 x 86)	2[a]	10
4	Control	7.12	--	242 x 345	(70 x 146)	2[a]	10
	Nonsettleable solids	5.1	--	325 x 424	(67 x 78)	3[a]	18
	50/50	6.47	--	309 x 419	(39 x 54)	3[a]	14
	Nonsettleable solids	9.0	--	327 x 433	(61 x 60)	3[a]	18

[a] Remaining nets had no measurable meshes.

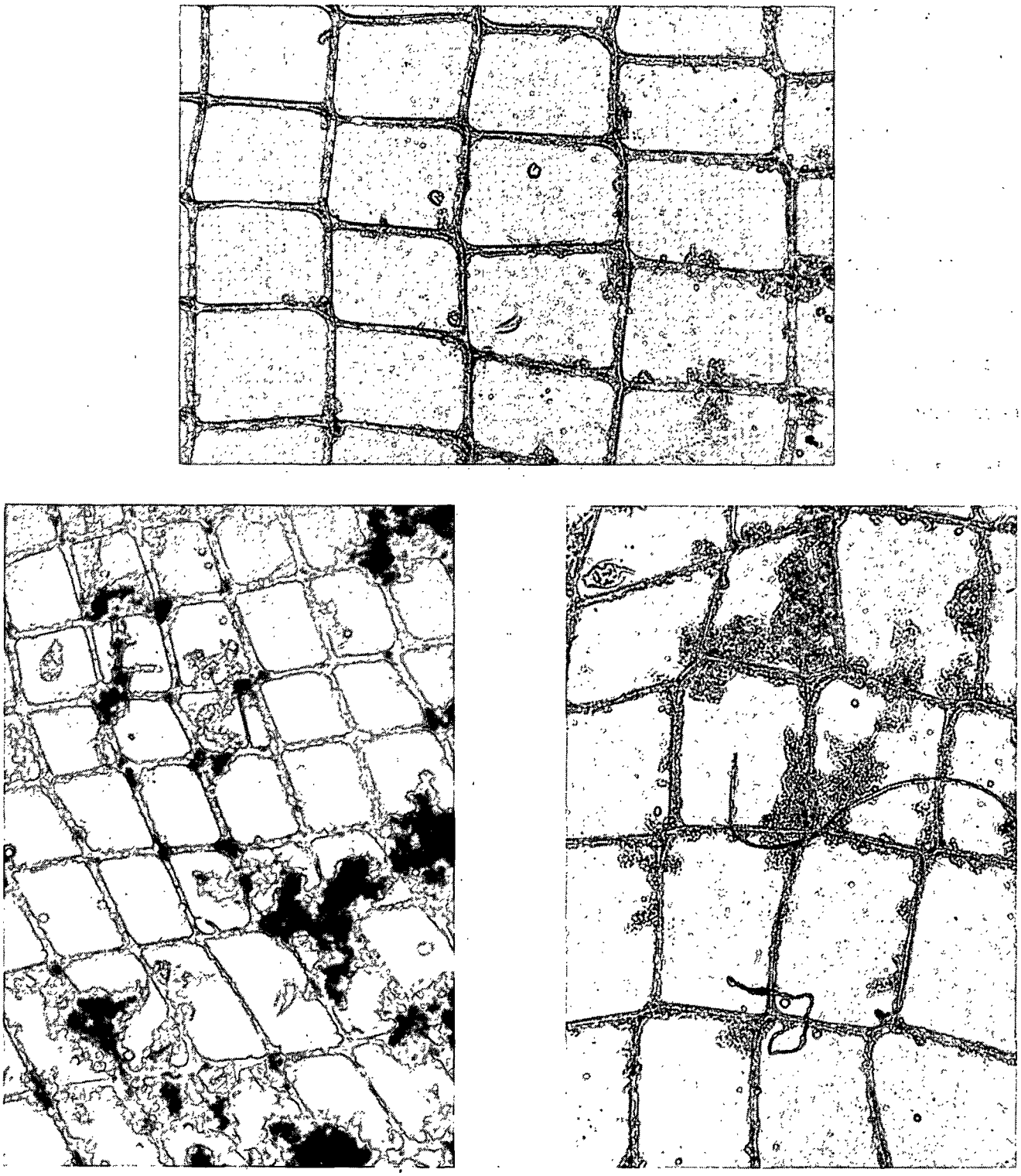


Figure B4. Hydropsyche Larval Nets at 40X Magnification Showing Net Structure With Captured Food and Detritus Particles

It was apparent from these observations and other data that the nets did allow access by the caddis larvae to the nonsettleable solids.

GROWTH AND SURVIVAL OF HYDROPSYCHE

To measure the use and value of postbiological solids to the caddis fly larvae extensive growth and survival experiments were conducted with both large larvae (18-25 mg) as well as small larvae (3-9 mg). The caddis larvae were captured from wild populations and acclimated in the laboratory without feeding for each experiment. The experiments terminated either with the onset of pupation (the next stage in the caddis life cycle preparatory to becoming an adult) or with the loss of a significant number of individuals from any of the food rations.

The growth and survival data are summarized in Table BII and Fig. B5 (for details see Appendix BIV). From these data it can be seen that the larvae which were not fed showed an average weight loss of 1.6 mg with a survival rate of only 57.0%. Larger caddis fly larvae on a diet of control food increased in weight by 4.02 mg and had a survival rate of 72.5%. Larvae which were fed a mixture of suspended solids plus control food showed a weight gain similar to that of the control at 4.7 mg with a better survival (84%). Large Hydropsyche fed a diet of pure suspended solids showed an average gain of 3.17 mg with a survival rate of 67.5%. Statistical tests of the data by analysis of variance methods showed no significant difference (at 95% confidence level) between growth or survival for the different food rations. The postbiological solids were as good a food source as was the control.

However from data in Table BII and Fig. B4 it is apparent that the small caddis were not as able to use the suspended solids as were the large larvae. While the control food caddis grew by 5.5 mg with a survival rate of 66% the suspended solids-fed larvae had a survival rate of only 15%. This was worse than

the survival rate of 19% for those larvae which were not fed at all. The best survival (72.3%) was achieved by the larvae fed a mixture of control food plus solids. This is meaningful as it indicates that the solids were not harmful or inhibitory to the small larvae. The differences between the survival for solids fed larvae and control food larvae (including control food plus solids) were significant at the 95% confidence level.

TABLE BII

GROWTH AND SURVIVAL OF CADDIS FLY LARVAE FOR EXPERIMENTAL FOOD STUDIES

	Food	$\bar{x}$ Initial Weight, mg	$\bar{x}$ Weight Change, mg	$\bar{x}$ % Survival
Large caddis	100% control	20.8	+ 4.02	72.5
	50% control[a]	21.6	+ 0.9	80.0
	100% biosolids	21.5	+ 3.17	67.5
	50/50[b]	20.6	+ 4.7	84.0
	Zero food	20.8	- 1.6	57.0
Small caddis	100% control	6.79	+ 5.5	66.3
	100% biosolids	7.18	+ 0.43	15.1
	50/50[b]	7.0	+ 2.16	72.3
	Zero food	6.9	+ 1.34	19.0

[a]One experiment fed half the amount of the "100% control" ration.
[b]Ration of half control food and half biosolids.

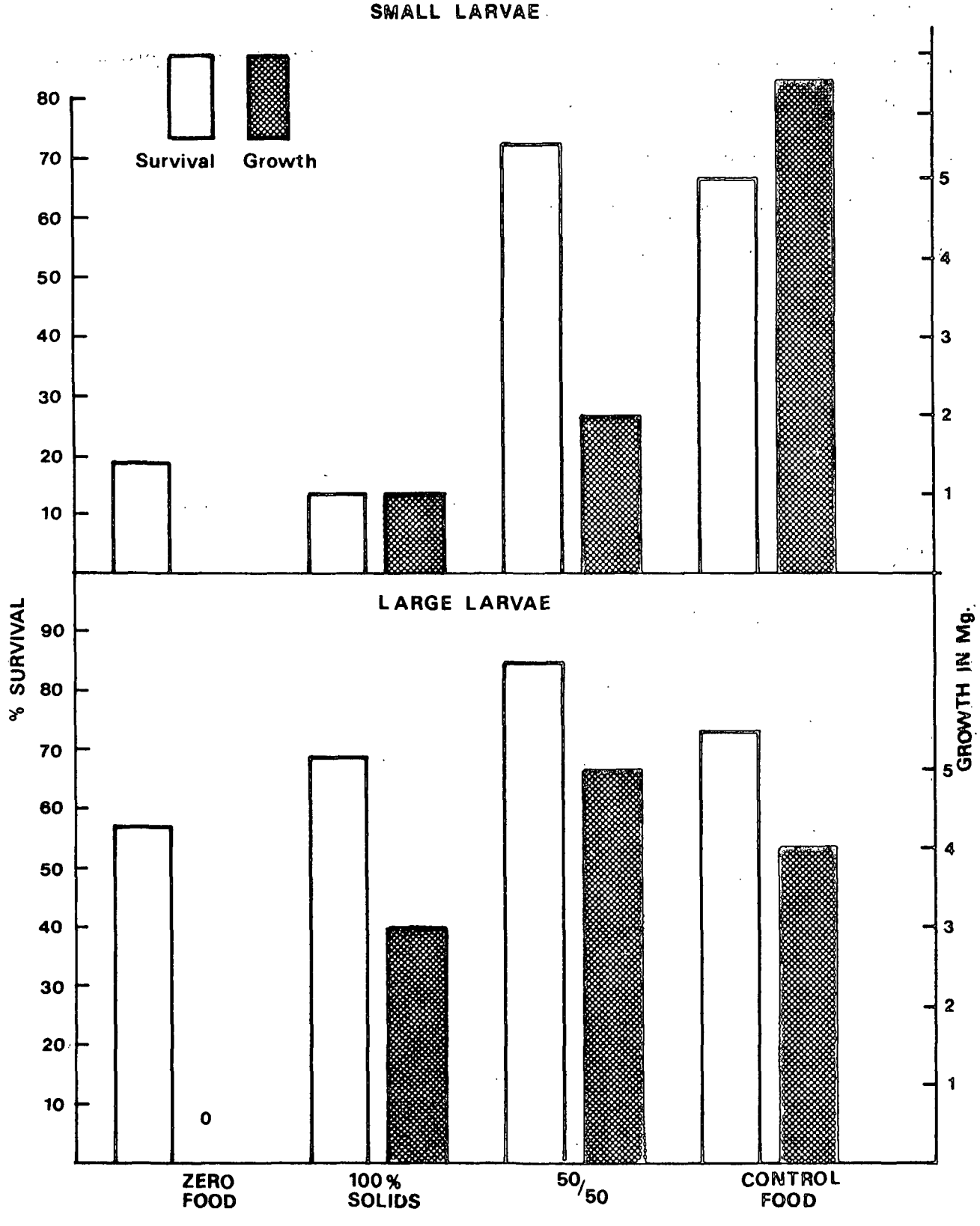


Figure B5. Survival and Growth of Hydropsyche During Feeding Experiments with Non-settleable Solids

The response by the small larvae to the solids diet was unexpected because the larger larvae have demonstrated during two separate experiments that they can do well on this diet. Since quantities were present to excess, availability was probably not limiting. One possible explanation is that the dietary requirements of the larvae such as protein requirements, trace minerals or vitamins change with age and growth. The diet of pure solids did not provide that nutritional requirement to the small larvae. Since no information is available on the nutritional or dietary requirements of these organisms the deficiencies cannot be documented at this time.

SOLIDS RECOVERY FROM HABITATS

During the course of the Hydropsyche feeding experiments profuse growths of attached, periphytic organisms were observed on the porcelain balls being used as a substrate for the caddis larvae. Because some qualitative diffusion occurred in the tanks an attempt was made to evaluate the materials remaining after the feeding runs.

Solids were scraped and brushed off of the balls and weighed. Chlorophyll components were collected to document algal activity. Results of this preliminary data collection are summarized in Table BIII.

From this information it was observed that where similar counts of nonsettleable solids were introduced a greater amount of periphytic solids was recovered from habitats receiving mill solids than from habitats receiving control food or no food. (Tanks 5 and 6 for Run 5 were fed at a lower rate.) This was consistent for both runs and was also true for the 50/50 food ratios. This may be due to the introduction of living biomass with the mill solids which were then available to colonize the porcelain balls. The control food on the other hand was all nonliving and may have lacked an innoculum of living cells.

TABLE BIII

PERIPHYTIC SOLIDS PRODUCED ON HABITAT SUBSTRATES
DURING FEEDING STUDIES

	Total S. Solids, mg		Chlorophyll		Pheophytin, mg/m^2	
	Run 1	Run 5	Run 3	Run 5	Run 3	Run 5
100% control food	1481.3	983.4	4.78	10.5	2.67	1.05
100% Biosol.	2826.8	1308.7	15.5	15.4	2.23	0.95
50/50 Ration	2254.9	1010.8	1.88	8.2	0.78	0.8
No food	602.3	42.4	0	3.8	2.0	-0.3
100% Biosol. with no larvae	1749.7	--	7.34	--	0.72	--
100% Biosol. with small larvae	--	559.2	--	5.1	--	1.1
50% Control food	--	517.0	--	0.6	--	1.05

Run 3 — data collected from one ball.
Run 5 — data is average of two balls.

The chlorophyll levels were also much higher in the 100% mill solids food ration than in the control. However in the 50/50 ration a significant difference was not apparent. This result is less likely to be dependent upon introduced material as living algae was a rare component of the mill solids used. The alga growth present was likely to be due to contaminants introduced with the water or with the caddis collected from the Fox River. The quantities of algae found in the 100% solids tanks may indicate some stimulation of the algae by the solids added as food. The release of nutrients in the introduced solids may be sufficient to stimulate some increased algal production under the conditions of this experiment. Further work would be necessary in order to document or further understand this apparent response to mill solids.

DAPHNIA SP. FEEDING RESULTS

Another organism which feeds on small plant and animal particles is the daphnid crustacean. Several feed run trials lasting from 18-25 days (which encompasses the normal life span of a Daphnia) were established. A summary of the Daphnia growth and survival information is presented in Table BIV and graphically displayed in Fig. B6 (for details see Appendix BV). Since Daphnia reproduce parthenogenetically the "growth" variable is population growth. The size of the individual does not change once the adult stage is reached. From the described data it can be seen that the nonsettleable solids provide a very adequate food source for the Daphnia. In the first test 5 individuals resulted in an average of 89 after 18 days for a population increase factor of 18 times. The control food produced 36 times the starting number but the starved populations all died.

TABLE BIV

SUMMARIZED DAPHNIA SP. FEEDING EXPERIMENTS

Test	Food	Initial Number of Organisms	$\bar{x}$ Final Number	Population Increase
1	Control food	5	181.5	36.3X
	Nonsettleable solids	5	89.5	17.9X
	Zero food	5	0	0
2	Control food	10	40.5	4X
	Mill-nonsettleable solids, low density	10	60	6X
	Mill-nonsettleable solids, high density	10	45.5	4.5X
	Laboratory generated solids	10	148.3	14.8X
	Zero food	10	13	0.3X

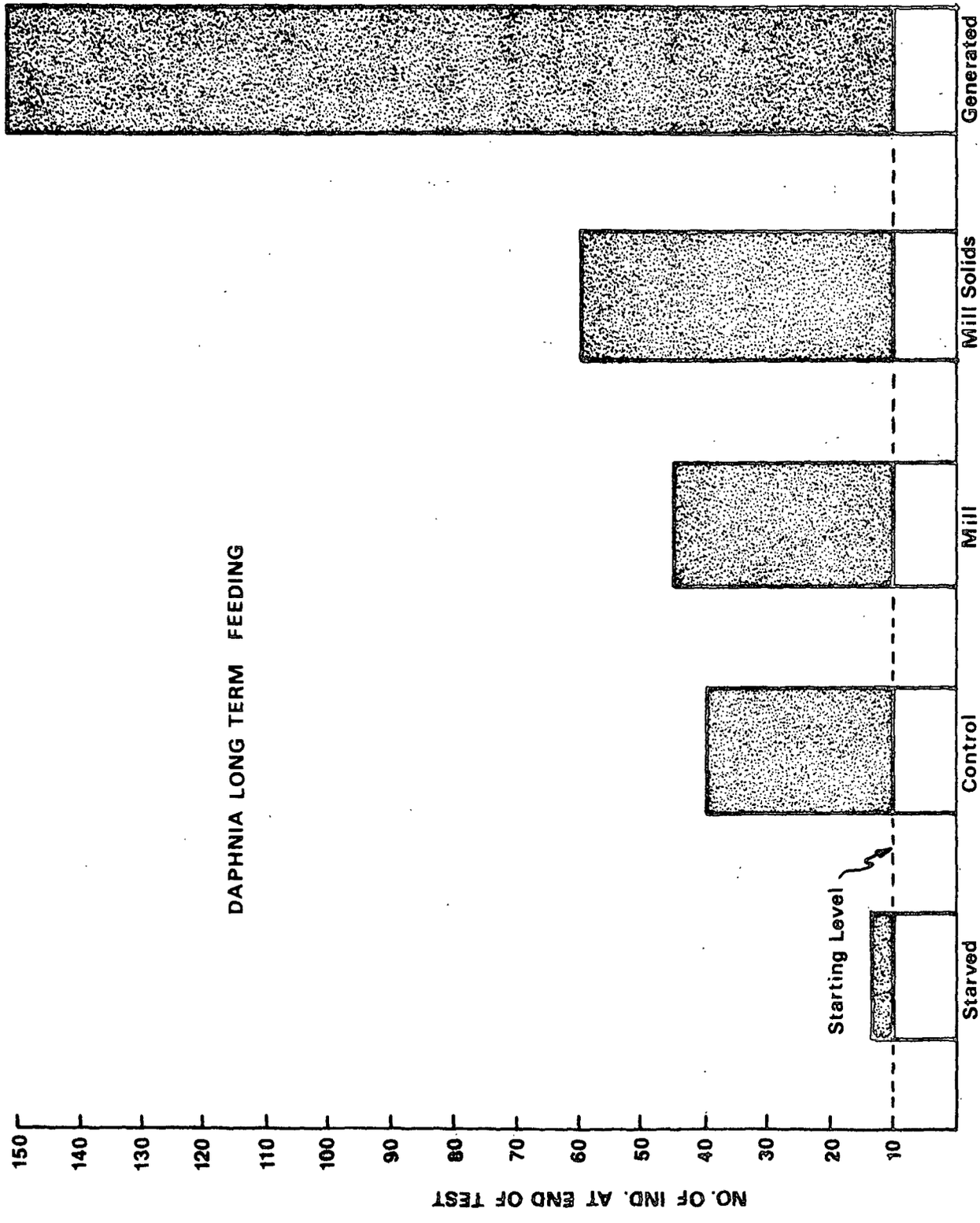


Figure B6. Production of Daphnia During Long Term Feeding Experiments with Nonsettleable Solids

In the following tests smaller population increases were seen but there was little difference between control food and nonsettleable solids. Control food population increased by 4 times while solids-fed populations increased by 4.5 times and 6 times the original count. Solids generated in the laboratory and used as food showed increases of nearly 15 times original counts and even starved populations managed to survive (possibly due to bacterial contamination though they did not increase appreciably). The great success for laboratory-generated solids may have been due to the availability of this material to the individual Daphnia. Laboratory-generated solids were more viable (up to 100% viability measured by ATP content) than the mill-produced solids and the bacteria may have reproduced and provided more food than in other diets. This was not tested but this response to laboratory generated solids further supports the conclusion that these materials provide an adequate food source for Daphnia and could be used as food by them in natural environments receiving treated mill effluents.

C^{14} LABELING STUDIES

The C^{14} radioactive tagging studies were done in order to look further at the response of small caddis larvae and to verify assimilation of postbiological solids. These studies produced information on uptake and assimilation rates for both caddis sizes as well as for Daphnia.

Data for the C^{14} uptake studies are summarized in Table BV (details in Appendix BVI). The schematic design in Fig. B2 illustrates the tests conducted on the test organisms. Precautions were taken to eliminate all external counts. Background counts were tested for in the tissue solvent, untagged test animals and suspension media. In addition dead test organisms were swirled in tagged solids to

TABLE BV

SUMMARY OF SOLIDS ASSIMILATION BY HYDROPSYCHE AND DAPHNIA
DETERMINED BY C^{14} LABELING

Food	Newly Fed Daphnia	Gut Cleared Daphnia	Large Whole Hydropsyche	Large Hydropsyche Gut Removed	Small Whole Hydropsyche	Small Hydropsyche Gut Removed
Mill Solids						
$\bar{x}$ Corrected cpm[a]	313	150	6905	10,673	1764	835
$\bar{x}$ cpm per mg body wt.	3130	1500	513	509	287	192
Food assimilated — micrograms	30	14	600	900	200	70
Food assimilated — as % body wt.	30	14	3.9	4.2	3.2	1.5
Food assimilated — as % food fed	0.1	0.02	1.0	1.5	0.35	0.12
Lab Generated Solids						
$\bar{x}$ Corrected cpm	102	150	465	270	164	64
$\bar{x}$ cpm per mg body wt.	1020	1500	28	23	39	23
Food assimilated — micrograms	20	30	100	50	30	10
Food assimilated — as % body wt.	20	30	0.6	0.4	0.8	0.3
Food assimilated — as % food feed	0.03	0.1	0.2	0.1	0.1	0.01

[a] cpm = counts per minute.

account for any radioactivity that might be due to particles attached rather than ingested. Corrected counts in Table BV have these background counts subtracted.

One question which remained from the caddis larval food studies was answered by the C^{14} tagging studies. The small Hydropsyche larvae ingested both mill- and laboratory-generated solids. Counts per minute (cpm) recorded for small caddis averaged 1764 and for large caddis 6905. For whole small caddis larvae this represents 200 micrograms and for larger whole larvae 600 micrograms of food. When the gut tracts were removed to eliminate counts due to ingested materials which may not have been assimilated the small larvae had retained only the equivalent of 70 micrograms of food. The larger larvae without gut tracts retained counts equivalent to 900 micrograms. Thus it appears that the smaller larvae are not as efficient at converting the solids to body tissue as are the larger larvae. The smaller organisms were nearly as successful at ingesting the materials as their large counterparts were but they were not as efficient at assimilating them. The data does clearly support the conclusion that Hydropsyche larvae can ingest and assimilate the nonsettleable particles found in biologically treated effluents.

Tagging studies done on Daphnia supported the conclusion made from the long-term feeding work that biological solids are very successfully used by Daphnia. In Fig. B7 a comparison between Daphnia and caddis fly larvae and between the two solids sources is made with a bar graph. Daphnia were very efficient at converting the solids averaging a conversion of food equivalent to 30% of their body weight. In fact the Daphnia used as much of the solids for food as did the large caddis fly larvae in spite of the very great differences in body size between them.

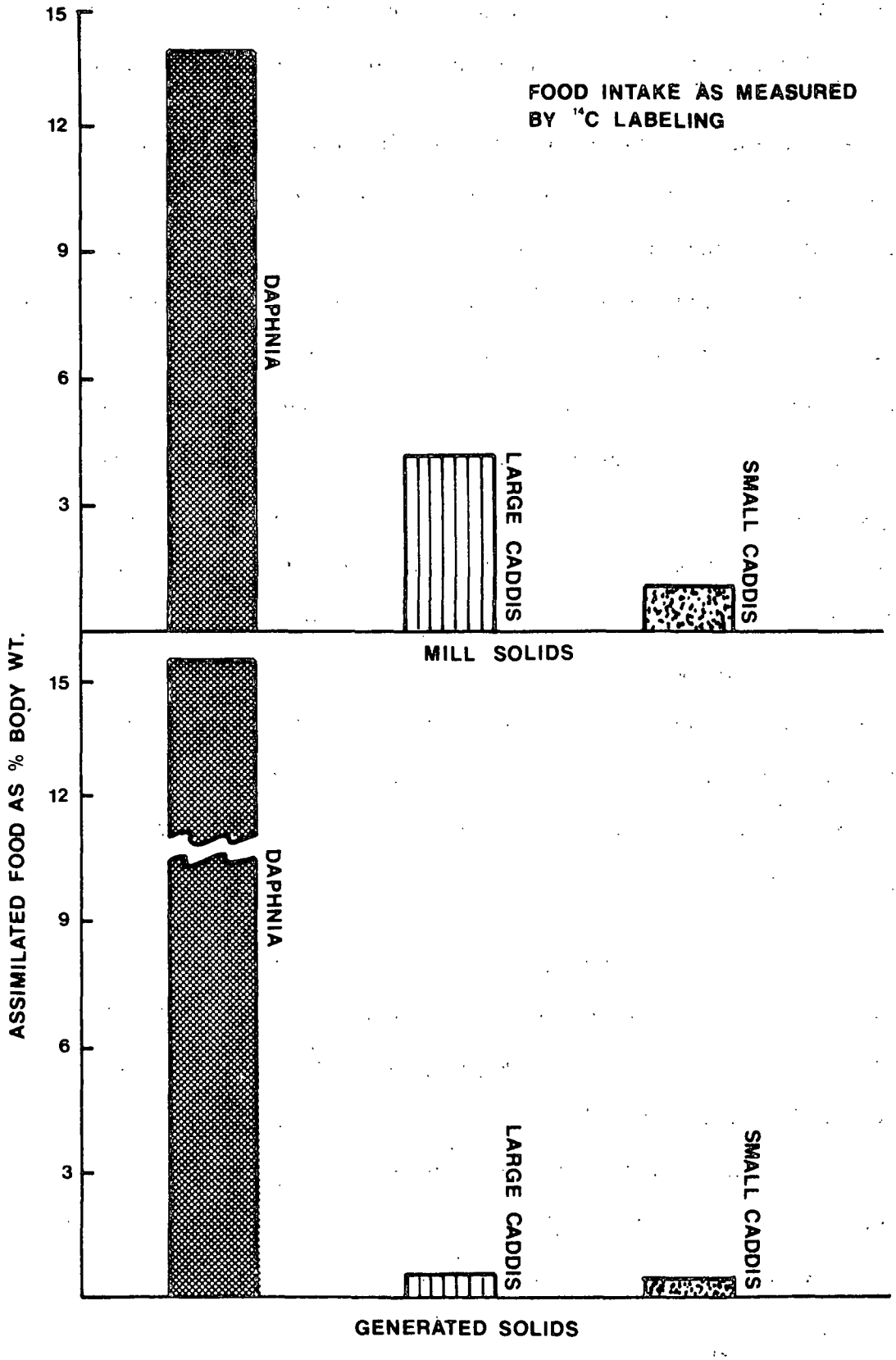


Figure B7. Assimilation of C^{14} Labeled Solids by Daphnia and Caddis Larvae

To examine the persistence and significance of assimilated solids the radiotagged adult Daphnia were allowed to reproduce in the absence of tagged foods and the daughters were analyzed. Nine adult Daphnia contained a count of 45 cpm. Smaller organisms in the next size category, and which were offsprings of the C^{14}-fed originals, exhibited a count (with possible background counts removed) of 2 cpm. The smallest organisms which appeared to be offsprings of the daughters did not exhibit any count at all.

CONCLUSIONS — PART A AND PART B

In the three mill systems studied, the vast majority of solids discharged from the waste treatment plants were small in size. Particle sizes of less than 2 μm in diameter found in this study would hardly settle under normal river conditions. These nonsettleable solids were 40-65% biological in nature of which 5 to 35% were alive. Viability was greater in summer samples than in winter samples, with the greatest difference evident for the aerated lagoon treatment system. Nonbiological materials included a great amount of inorganic clay and coating materials contributed by the papermaking process.

The nonsettleable solids were used as food by both the invertebrate as well as the crustacean filter feeder. The large Hydropsyche sp. survived and grew on a diet of 100% nonsettleable solids. The smaller larvae consumed the solids without ill effect, but as a solitary food the solids were nutritionally inadequate for them. Daphnia sp. survived and reproduced normally on a diet of 100% nonsettleable solids and actually produced more young than did the controls.

C^{14} tagging verified that solids were ingested and assimilated and in the case of the Daphnia sp. the assimilated materials could be passed along to offsprings.

ACKNOWLEDGMENTS

Work for this project was done at The Institute of Paper Chemistry with the financial support of its member companies. Special credit needs to be given to participating staff. Jud Conkey produced microbiological solids analysis results, ATP, and plate count information as well as being involved with experimental design. Sally Berben assisted in data collection and was critical to the Daphnia feed study work. Michael Tesmer assisted in biological data analysis. Hardev Dugal reviewed and criticized the manuscript as well as contributed to designing the solids characterization work. Special recognition of numerous support staff needs also to be made. The efforts of all these are greatly appreciated.

LITERATURE CITED — PART B

1. Slack, H. D. The food of caddis fly (Trichoptera) larvae. J. Animal Ecology (5):105-15(1936).

2. Williams, N. E. and H. B. N. Hynes. Microdistribution and feeding of the net-spinning caddis flies (Trichoptera) of a Canadian stream. Oikes 24(1):73-84(1973).

3. Hayward, R. S. and D. N. Fallup. Feeding, filtering and assimilation in Daphnia schoedleri as affected by environmental conditions. Arch. Hydrobiologie 77(2):139-63(1976).

4. Schindler, J. E. Food quality and zooplankton nutrition. J. Animal Ecology 40(3):589-95(1971).

5. Romanenko, V. I. Experimental study of bacterial production in water and consumption by Daphnia. Microbiology 39(4):617-20(1970).

6. Hach Chemical Company. Wastewater Analysis Handbook, Hach Chemical Company, Loveland Co., 1978. 630 pp.

7. American Public Health Association, American Water Works Association, and Water Pollution Control Federation. Standard methods for the examination of water and wastewater. 14th ed., APHA, New York, 1975. 1193 pp.

8. Tzuka, Yasihiko. Feeding of Daphnia on planktonic bacteria. Japanese Journal of Ecology 21(3 and 4):127-33(1971).

9. Baudoin, M. F. Weight, size and chemical composition of some fresh water zooplankton: Daphnia hyalina. Limnology + Oceanography 17(4):645-9(1972).

10. Wallace, J. B. Biology, microdistribution and food of filter feeding Trichoptera in a southern Appalachian stream. Presented at North American Benthological Society Meeting, LaCross, Wisconsin. 1976.

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APPENDIX AI

CHARACTERISTICS BY SEASON FOR NONSETTLEABLE SOLIDS
AS MEANS AND STANDARD DEVIATIONS

Effluent Characteristics

Mill		pH	Temp., °C	Turbidity, % t	BOD_5, mg/L	Settle-ability, 24 hr	Total Suspended Solids, mg/L	% Ash Eff. Solids	Organic Carbon, mg/L	Inorganic Carbon, mg/L	ATP, µg/mg SS
A	O_2 Activated Sludge										
	Summer	7.1 ± 0.2	40 ± 2.6	49.3 ± 19.3	52.3 ± 27.0	--	113.3 ± 52.6	36.0 ± 15.1	85 ± 36.0	36.6 ± 8.9	0.35 ± 0.17
	Winter	6.6 ± 0.1	30.6 ± 2.5	47 ± 15	57.6 ± 16.6	1.07 ± 0.66	102 ± 37.4	20.3 ± 4.8	111 ± 19.7	57. ± 14.1	0.13 ± 0.03
B	Air Activated Sludge										
	Summer	7.4 ± 0.6	33.7 ± 2.6	86.3 ± 12.3	11.5 ± 10.6	--	12.6 ± 5.3	--	80.3 ± 34.2	61 ± 3.0	0.62 ± 0.32
	Winter	7.3 ± 0.2	24 ± 3.2	92.3 ± 0.5	12 ± 5.6	0.96 ± 0.29	15.6 ± 5.0	3.0 ± 18.7	121[a]	121[a]	0.128±0.122
C	Aerated Lagoon										
	Summer	6.9 ± 0.6	27 ± 2.2	28.3 ± 3.4	24.3 ± 7.6	--	138 ± 17.5	12.6 ± 15.4	69.3 ± 24.2	28 ± 12.1	6440 ± 1902
	Winter	7.1 ± 0.2	12 ± 2.2	30.3 ± 5.5	62.6 ± 22.0	0	86 ± 14.4	2.3 ± 3.3	178.3 ± 27.9	40.3 ± 8.3	6005 ± 1605

[a]One sample.

Collected Solids Characteristics

Mill		Suspended Solids, mg/80 L (Glass Filter)	Ash, %	Inorganic Carbon, mg/g	Organic Carbon, mg/g	TKN, mg/g	Phosphorous, mg/g	Carbohydrates, %	Calories, cal/mg
A	O_2 Activated Sludge								
	Summer	530 ± 117	--	80.0 ± 16.1	410 ± 117	53.1 ± 8.6	3.5 ± 0.68	4.8 ± 0.9	3.2 ± 1.5
	Winter	946 ± 278	25.1 ± 17.7	116.6 ± 55.8	514 ± 32.8	137.3 ± 10	3.6 ± 2.2	6.3 ± 4.5	1.9 ± 0.52
B	Air Activated Sludge								
	Summer	4036 ± 387	11.5 ± 4.9	6.2 ± 1.2	202 ± 27	36.3 ± 4.3	1.2 ± 0.15	3.8 ± 0.82	5.3 ± 0.04
	Winter	6620 ± 1603	13 ± 1.4	5.3 ± 1.2	373 ± 128	51 ± 11.2	1.2 ± 0.5	8.9 ± 2.3	5.3 ± 0
C	Aerated Lagoon								
	Summer	6440 ± 1902	36.6 ± 5.5	3.2 ± 1.1	190 ± 42	31.3 ± 13.5	0.8 ± 0.34	6.1 ± 0.71	3.6 ± 0.35
	Winter	6005 ± 1605	43 ± 9.3	6.4 ± 1.7	368 ± 117	42.3 ± 8.0	1.6 ± 0.8	8.5 ± 3.8	2.9 ± 0.61

APPENDIX AI (Continued)

CHARACTERISTICS BY SEASON FOR NONSETTLEABLE SOLIDS
AS MEANS AND STANDARD DEVIATIONS

Mill		BOD_5, mg/g	COD, mg/g	ATP, µg/mg SS	Analyzed Elements, %	Crude Protein, %	Rhamnose, %	Araban, %	Xylan, %	Mannan, %	Galactan, %	Glucan, %
A	O_2 Activated Sludge											
	Summer	213 ± 72	1004 ± 532	0.54 ± 0.11	6.6 ± 1.1	19.5 ± 8.4	0.48 ± 0.13	0.02 ± 0.01	0.33 ± 0.16	0.51 ± 0.12	0.77 ± 0.18	3.97 ± 0.24
	Winter	286 ± 66	1380 ± 168	0.138 ± 0.09	NA	26.4 ± 4.9	0.55 ± 0.16	0.2 ± 0.05	0.39 ± 0.21	0.55 ± 0.29	0.60 ± 0.29	6.21 ± 2.8
B	Air Activated Sludge											
	Summer	130.6 ± 44	1650 ± 127	0.27 ± 0.1	7.7 ± 0.99	33.0 ± 5.3	0.50 ± 0.10	0.02 ± 0.02	0.16 ± 0.01	0.47 ± 0.02	0.39 ± 0.005	3.2 ± 0.81
	Winter	169 ± 18	1594 ± 543	0.044 ± 0.016	NA	69.7 ± 5.9	0.64 ± 0.23	0.04 ± 0.02	0.20 ± 0.19	0.49 ± 0.27	0.40 ± 0.21	4.5 ± 3.6
C	Aerated Lagoon											
	Summer	168.3 ± 35.8	928 ± 269	0.149 ± 0.072	19.1 ± 5.4	22.4 ± 2.8	0.27 ± 0.03	0.23 ± 0.06	0.34 ± 0.13	0.39 ± 0.08	0.51 ± 0.15	1.96 ± 0.24
	Winter	305 ± 148	931 ± 216	0.121 ± 0.05	NA	31.8 ± 7.0	0.48 ± 0.06	0.39 ± 0.01	0.52 ± 0.10	0.48 ± 0.02	0.85 ± 0.10	1.93 ± 0.96

APPENDIX AII

QUALITATIVE MICROSCOPIC CHARACTERISTICS OF MILL NONSETTLEABLE SOLIDS

		Unicellular Bacteria[a]	Filamentous Bacteria	Fungi	Algae	Amoebae	Flagellates	Free Ciliates	Stalked Ciliates	Rotifers	Nematodes	Fiber	Grit
Mill A	Winter	3	2	0	+	0	1	+	+	1	0	1	1
	Summer	3	2	0	+	0	+	0	+	+	0	+	+
Mill B	Winter	3	+	+	+	0	0	0	0	0	0	+	2
	Summer	3	+	+	1	0	0	0	0	0	0	+	1
Mill C	Winter	3	2	+	0	0	0	+	2	1	0	+	2
	Summer	3	1	0	1	0	0	+	+	0	+	+	2

[a] All values are means for all observations collected during the period.

Key

4 — Very numerous

3 — Numerous

2 — Moderate

1 — Slight

+ — Trace

0 — None

APPENDIX AIII

PARTICLE SIZE AND VOLUME DISTRIBUTIONS FOR MILL NONSETTLEABLE SOLIDS

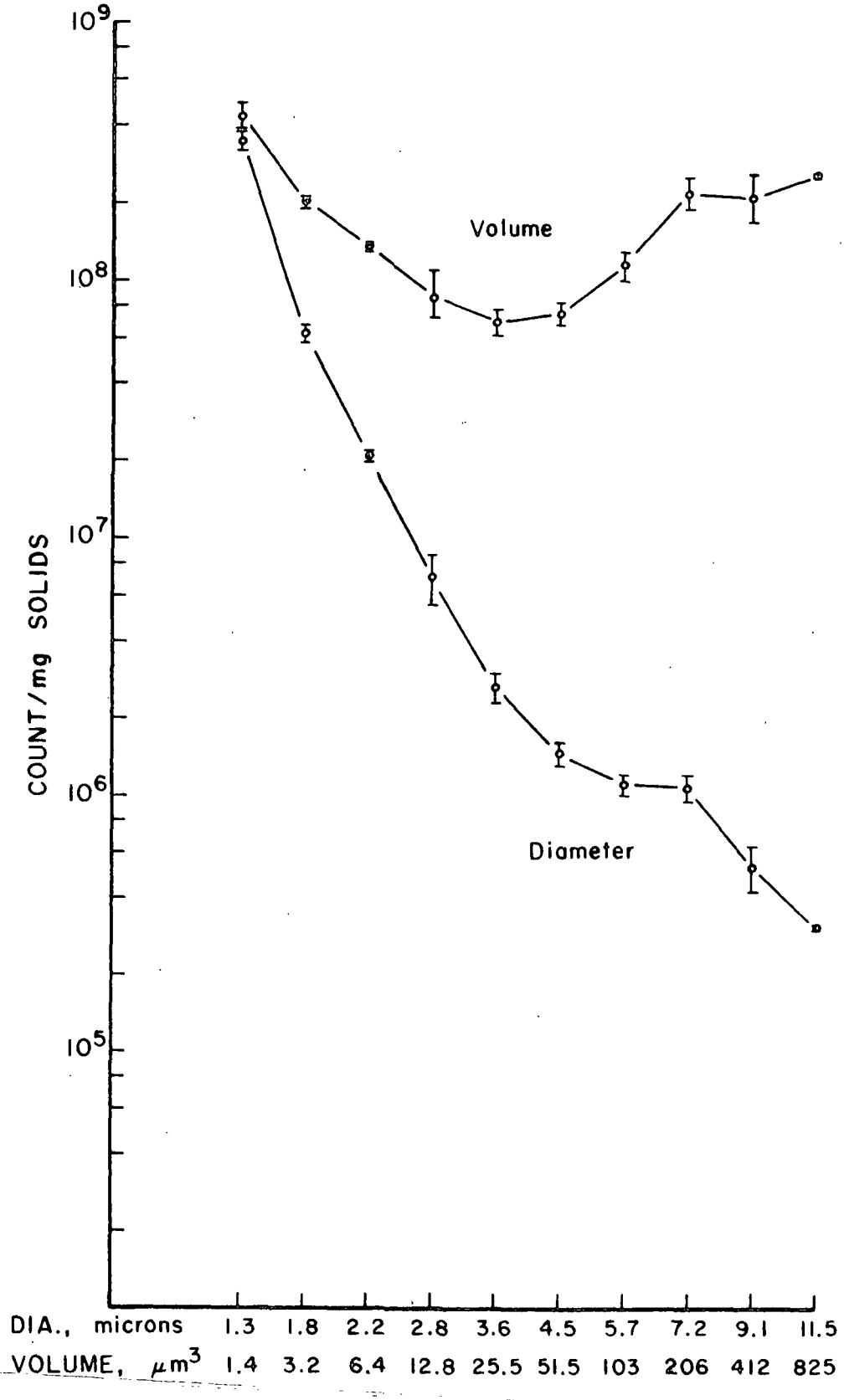

Figure 1. Mill A Winter Collection

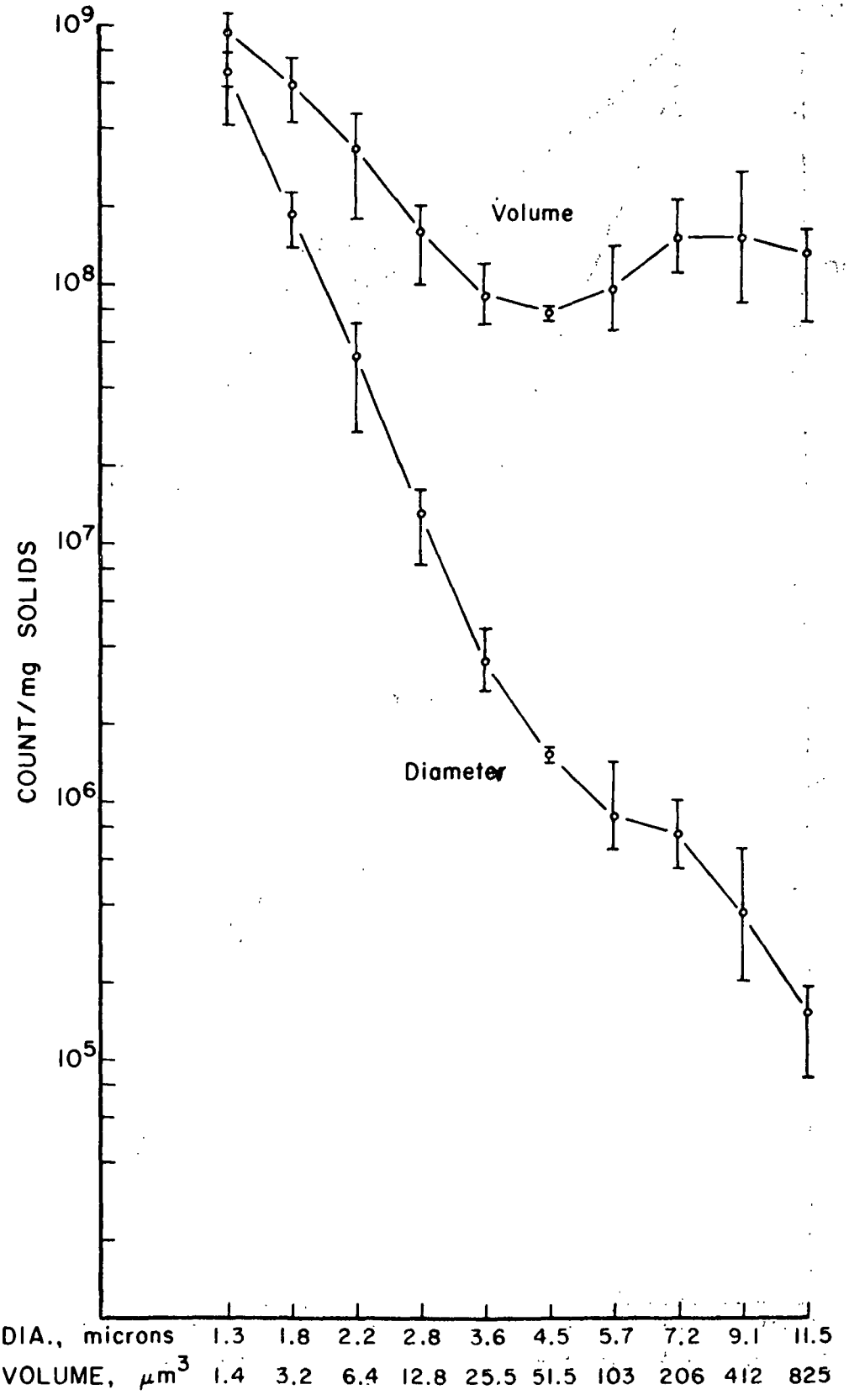

Figure 2. Mill A Summer Collection

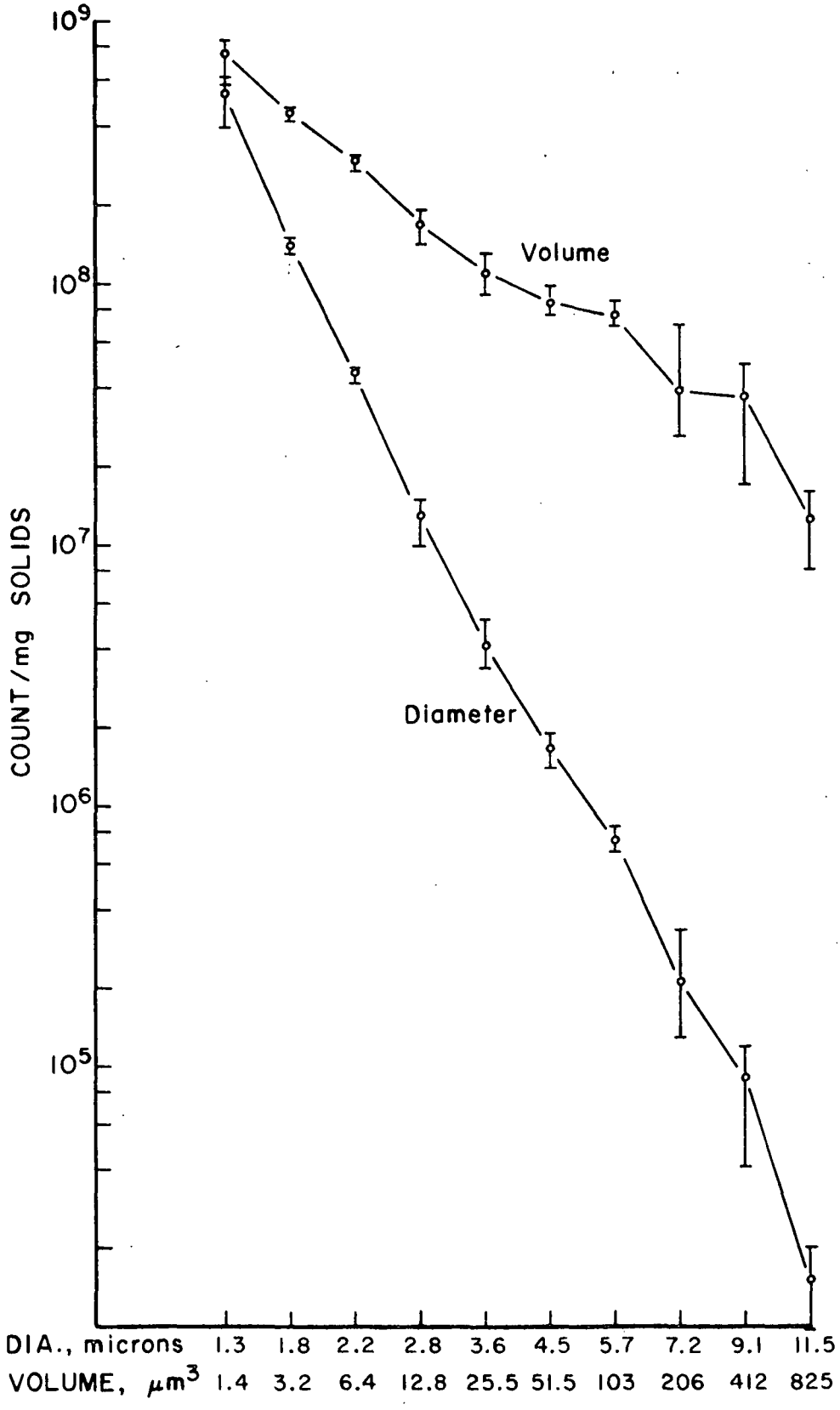

Figure 3. Mill B Summer Collection

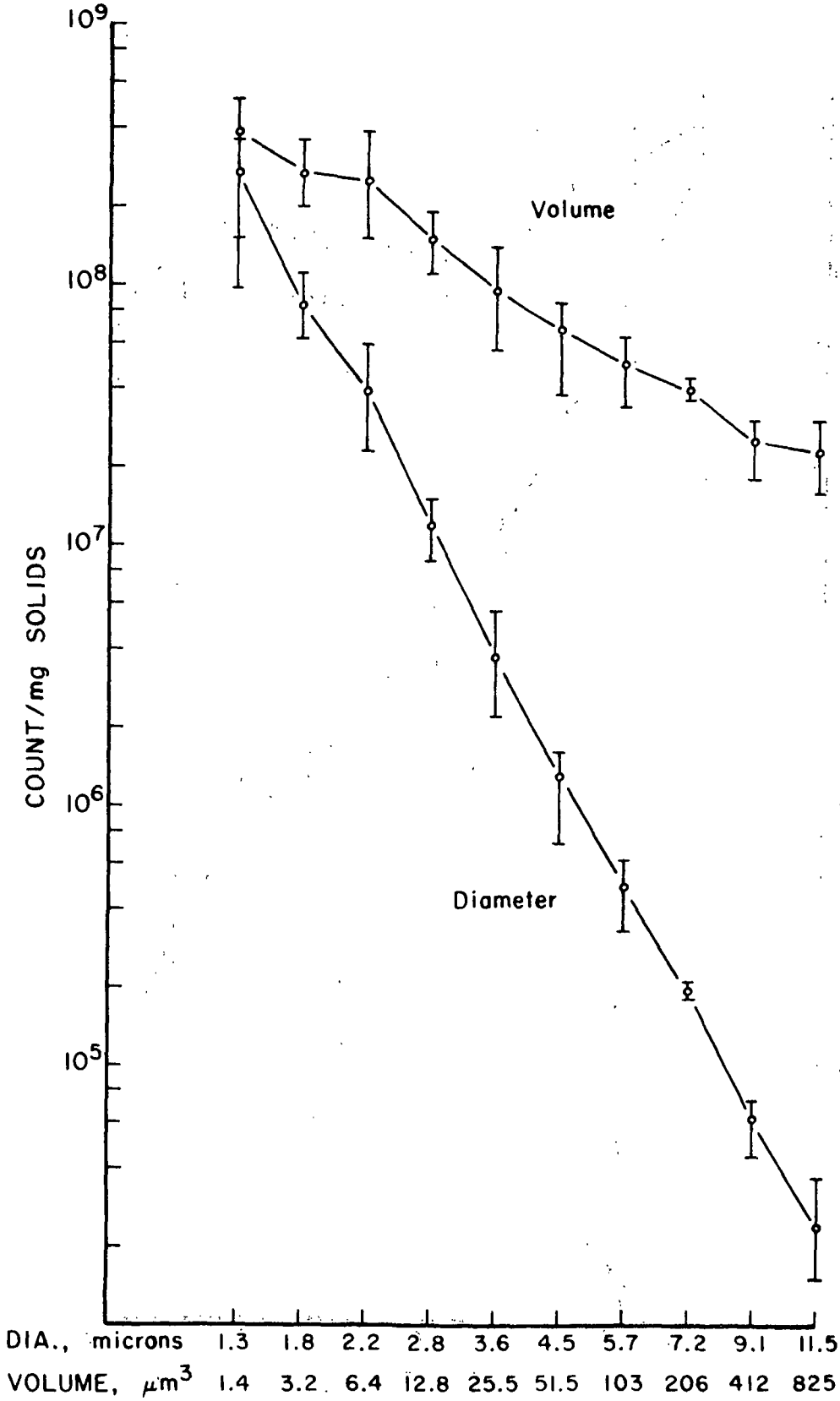

Figure 4. Mill B Winter Collection

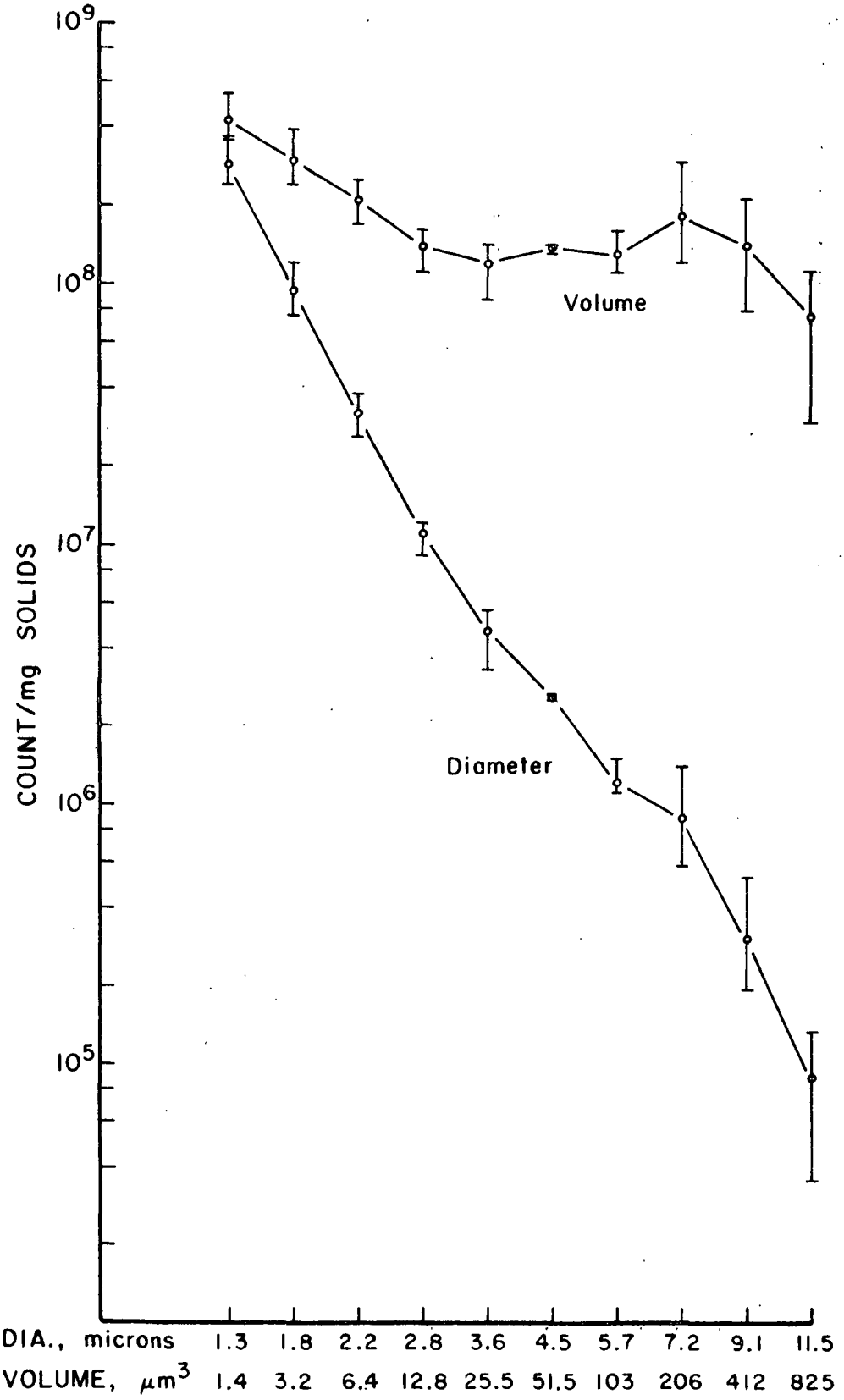

Figure 5. Mill C Summer Collection

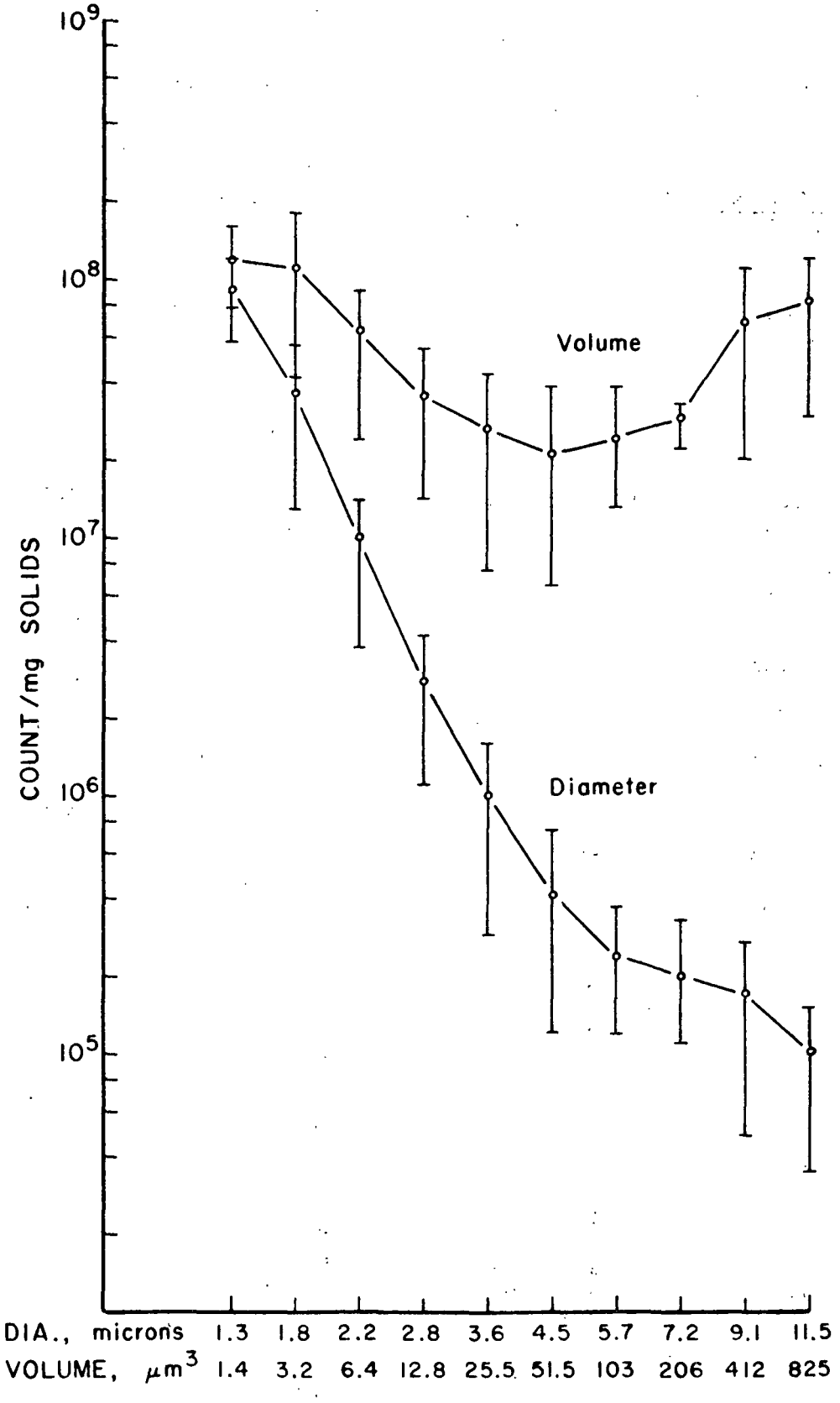

Figure 6. Mill C Winter Collection

APPENDIX AIV

SUMMARIZED ANALYSIS OF VARIANCE CALCULATIONS FOR NONSETTLEABLE SOLIDS

Parameter	F from Table 0.05	F from Table 0.01	F Calculated	Standard Deviation, σ	Level of Significance
pH					
Mills	3.84	6.93	2.2	0.65	N.S.[a]
Seasons	4.75	9.33	0.57	0.32	
Temp.					
Mills	3.88	6.93	40.24	19.4	0.01
Seasons	4.75	9.33	61.20	24.0	0.01
Effluent Solids					
Mills	3.88	6.93	23.31	135.7	0.01
Seasons	4.75	9.33	1.26	31.5	N.S.
Collected Solids					
Mills	3.88	6.93	26.4	72.6	0.01
Seasons	4.75	9.33	0.67	1.05	N.S.
% Ash-Effluent Solids					
Mills	3.80	6.93	2.11	26.1	N.S.
Seasons	4.75	9.33	0.19	22.04	N.S.
% Ash-Collected Solids					
Mills	3.88	6.93	6.42	35.2	0.05
Seasons	4.75	9.33	1.05	14.2	N.S.
Bacterial Plate Counts-Effluents					
Mills	3.88	6.93	24.25	157	0.01
Seasons	4.75	9.33	24.38	157	0.05
Bacterial Plate Counts-Solids					
Mills	3.88	6.93	2.19	0.86	N.S.
Seasons	4.75	9.33	8.69	1.7	0.05
Inorganic Carbon-Solids					
Mills	3.88	6.93	30.6	131.5	0.05
Seasons	4.75	9.33	1.34	27.4	N.S.
Organic Carbon					
Mills	3.88	6.93	8.02	252	0.05
Seasons	4.75	9.33	12.93	320	0.05
TKN					
Mills	3.95	7.2	74.1	84.5	0.01
Seasons	4.84	9.65	64.3	78.7	0.01
Phosphorous					
Mills	3.88	6.93	10.4	33.2	0.01
Seasons	4.75	9.33	0.46	0.7	N.S.
% Carbohydrate					
Mills	3.98	7.2	0.63	2.2	N.S.
Seasons	4.84	9.6	4.5	5.8	N.S.

APPENDIX AIV (Continued)

SUMMARIZED ANALYSIS OF VARIANCE CALCULATIONS FOR NONSETTLEABLE SOLIDS

Parameter	F from Table 0.05	F from Table 0.01	F Calculated	Standard Deviation, σ	Level of Significance
Calories/mg solids					
Mills	3.98	7.2	33.18	3.5	0.01
Season	4.84	9.6	6.69	1.5	0.05
BOD_5 (Solids)					
Mills	3.98	7.2	3.63	126.2	N.S.
Seasons	4.84	9.6	5.64	157	0.05
BOD_5 (Effluent)					
Mills	4.1	7.5	6.94	47.1	0.05
Seasons	4.9	10.0	3.25	32.2	N.S.
COD (Solids)					
Mills	3.88	6.93	5.93	270	0.05
Seasons	4.75	9.33	0.42	228	N.S.
ATP (Effluent)					
Mills	3.98	7.2	3.44	0.30	N.S.
Seasons	4.84	9.65	13.62	0.60	0.01
ATP (Solids)					
Mills	3.98	7.2	1.64	0.26	N.S.
Seasons	4.84	9.65	3.03	0.36	N.S.
Coulter Counts (Effluent)					
Mills	4.1	7.56	0.036	1.39	N.S.
Seasons	4.96	10.04	52.16	16.8	0.01
Coulter Counts (Solids)					
Mills	3.98	7.2	2.3	2.86	0.01
Seasons	4.84	9.65	35.8	4.77	0.05

[a] N.S. = not significant.

APPENDIX AV

CORRELATION COEFFICIENTS r FOR NONSETTLEABLE SOLIDS COMPARISONS

r	Level of Correlation, > 0.90	> 0.80	> 0.70
0.97	Effluent solids with collected solids		
0.90	Effluent solids with effluent BOD_5		
0.90	Effluent plate counts with % aluminum (solids)		
0.90	Effluent plate counts with % calcium (solids)		
0.92	Effluent plate counts with % silicon (solids)		
0.95	Organic carbon with % sodium (solids)		
0.94	Phosphorous with % sodium		
0.91	Effluent ATP with solids ATP		
0.91	Heavy metals with % sodium		
0.82		Effluent temperature with % calcium (solids)	
0.81		Effluent temperature with silicone	
0.86		Collected solids with effluent BOD_5	
0.83		Collected solids with % calcium	
0.83		Collected solids with % sodium	
0.86		Inorganic carbon with total Kjeldahl nitrogen	
0.89		Inorganic carbon with % sodium	
0.88		Organic carbon with heavy metals	
0.83		Phosphorous with inorganic carbon	
0.85		BOD_5 with % aluminum	
0.85		BOD_5 with % calcium	
0.84		BOD_5 with % silicone	
0.88		Particle counts effluent with particle counts solids	
0.85		Aluminum with % calcium	
0.84		Calcium with % silicone	
0.78			Effluent solids with inorganic carbon
0.73			Effluent solids with phosphorous (solids)
0.70			Effluent solids with % aluminum
0.76			Effluent solids with % calcium
0.79			Effluent solids with % sodium
0.70			Effluent solids with % silicone
0.78			Collected solids with inorganic carbon
0.74			Collected solids with phosphorous
0.77			Collected solids with % aluminum
0.78			Collected solids with % silicone
0.72			% ash (effluent solids) with % ash (collected solids)
0.70			% ash (collected solids) with % aluminum
0.78			% ash (collected solids) with % calcium
0.72			Total Kjeldahl nitrogen with % sodium
0.79			Phosphorous with % analyzed elements
0.76			% analyzed elements with sodium
0.79			% aluminum with inorganic carbon
0.70			Inorganic carbon with % sodium

APPENDIX BI

QUALITATIVE MACROSCOPIC CHARACTERISTICS OF MILL NONSETTLEABLE SOLIDS

TABLE I

MANUFACTURERS GUARANTEED ANALYSIS OF HiProMin TROPICAL FISH FLAKE FOOD USED AS A CONTROL FOOD

Crude protein	Not less than 53.0%
Crude fat	Not less than 3.5%
Crude fiber	Less than 3.3%
Moisture	Less than 5.7%
Calcium	5.3-6.4%
Phosphorous	Not less than 2.2%
Vitamin A	5,000 USP units
Vitamin D_3	300 I.U.
Vitamin E	2.6 I.U.
Thiamine	0.4 mg
Pyridoxine	0.7 mg
Riboflavin	3.0 mg
Vitamin B_{12} Activity	18.5 mc mg
d-Pantothenic acid	1.0 mg
Niacin	3.2 mg
Choline	231.0 mg

APPENDIX BI (Continued)

TABLE BII

CHARACTERISTICS OF NONSETTLEABLE SOLIDS USED AS FOOD FOR HYDROPSYCHE WALKERI AND DAPHNIA SP.

	Mill A Special Collections			Lab-Generated Solids
	$\bar{x}$	S.D.	n	
Effluent pH	7.01	0.45	22	--
Effluent temperature	35.6°C	5.9	22	--
Effluent suspended solids	137.6 mg/L	57.5	21	360 mg/L
Collected nonsettleable solids	4919 mg	1797	22	--
Ash, %	36.5	10.8	22	12.8
Aluminum, %	7.2	3.1	14	--
Chromium, %	0.006	0.002	14	--
Copper, %	0.010	0.002	14	--
Calcium, %	1.04	0.45	14	--
Iron, %	0.27	0.19	14	--
Magnesium, %	0.48	0.19	14	--
Phosphorus, %	0.55	0.11	13	--
Sodium, %	2.25	0.98	14	--
Titanium, %	0.64	0.30	14	--
Inorganic carbon, mg/L	25.5	12.1	16	--
Organic carbon, mg/L	998.8	535.2	15	--
Carbohydrates, %	8.48	1.72	10	--
Calories, mg solids	3.44	0.62	14	--

APPENDIX BII

SUMMARY OF MONITORED PARAMETERS IN RING TANKS DURING HYDROPSYCHE SP. FEEDING EXPERIMENTS

Run		Tank No.[a] (Food Ration)	Test Duration, weeks	Temp., °C	pH	D.O., mg/L	NO_3, mg/L	NH_3, mg/L	Sus. Solids, mg/L	Total Solids, mg/L	Food Rate Feeds	Food Rate Week
1	a.	Control, 100%	10	16.4 (1.6)	8.0 (0.15)	9.1 (1.0)	2.02 (0.7)	0.58 (1.4)	0.8 (1.3)	253 (61.5)	156 mg	468 mg/wk.
	b.	Biosolids, 100%	10	16.3 (1.6)	8.0 (0.14)	9.3 (0.68)	1.31 (0.7)	0.19(0.27)	2.8 (3.9)	264 (49.7)	156 mg	468
	c.	Biosolids & control 50/50	10	16.5 (1.6)	8.0 (0.16)	9.2 (0.68)	2.10 (1.3)	<0.3 (0.67)	10.0(13.8)	273 (54.2)	156 mg	468
	d.	Zero food	10	16.6 (1.6)	8.1 (0.18)	9.2 (0.69)	0.86 (0.5)	<0.1 (0.09)	13.1(16.3)	255 (39.5)	0	0
	e.	100% Biosolids, 0 bugs	8	16.7 (1.6)	7.9 (0.17)	9.2 (0.72)	0.72(0.37)	<0.1	6.5(10.2)	184 (39.0)	156.0	468
2	a.	100% Control food	7	21.0 (1.1)	7.7 (0.17)	8.6 (0.3)	2.13 (1.1)	0.34(0.45)	0.71(1.0)	217 (33.9)	25→33[a]	75→99
	b.	Biosolids, 100%	7	20.8(0.85)	7.7 (0.14)	8.6 (0.32)	1.61(0.42)	0.34(0.42)	0.71(1.1)	212 (33.0)	36→9[a]	108→27
	c.	50/50	7	21.0 (1.0)	7.7 (0.14)	8.6 (0.32)	2.0 (0.81)	1.09(1.06)	2.4 (7.7)	225 (42)	44→22[a]	132→66
	d.	Zero food	7	21.8 (1.0)	7.7 (0.14)	8.6 (0.3)	1.4 (0.51)	0.28(0.33)	0.63(0.9)	219 (34)	0	0
	e.	100% Biosolids, 0 bugs	7	21.1 (1.1)	7.7 (0.16)	8.7 (0.3)	1.21(0.35)	0.33(0.31)	0.88(1.1)	211 (33.4)	36→9[a]	108→27
3	a.	100% Control food	4	20.8 (1.2)	7.2 (0.3)	8.7 (0.24)	2.8 (1.3)	0.25(0.12)	0.52(0.79)	214 (33.3)	63.4	190.2
	b.	100% Biosolids	4	20.5 (1.3)	7.3 (0.2)	8.8 (0.18)	1.4 (0.31)	0.20 (0.1)	0.61(0.78)	197 (24.0)	55.5	166.5
	c.	50/50	4	20.7 (1.2)	7.4 (0.2)	8.7 (0.19)	1.5 (0.40)	0.21(0.10)	0.34(0.44)	178 (17.4)	53.2	159.6
	d.	Zero food	4	21.0 (1.4)	7.4(0.23)	8.7 (0.20)	1.6 (0.3)	0.20(0.10)	0.52(0.7)	205 (30.7)	0	0
	e.	100% Biosolids, 0 bugs	4	21.2 (1.1)	7.5(0.22)	8.7 (0.20)	1.54(0.52)	0.25(0.06)	0.84(0.9)	202 (26.7)	55.5	166.5
4	a.	100% Control	4	19.8 (1.5)	7.7(0.21)	8.9 (0.25)	2.2 (0.88)	6.4 (3.8)	0.29(0.36)	224 (30.0)	48.0	144
	b.	100% Biosolids	4	19.4 (1.9)	7.5(0.74)	9.0 (0.25)	1.6 (0.49)	0.32(0.16)	0.3 (0.2)	197 (22.7)	34.1	102
	c.	50/50	4	19.6 (1.3)	7.7(0.15)	9.0 (0.25)	1.8 (0.42)	0.30(0.14)	0.2 (0.34)	205 (19.7)	43.0	129
	d.	Zero food	4	19.9 (1.4)	7.7(0.15)	8.9 (0.26)	1.7 (0.36)	0.30(0.17)	0.27(0.39)	229 (36.5)	0	0
	f.	100% Biosolids	4	21.0 (1.2)	7.7(0.17)	8.7 (0.25)	2.35(1.05)	0.48(0.37)	0.50(0.51)	245 (16.1)	60.4	181.2
5	a.	100% Control food	10	15.8 (2.0)	7.8(0.10)	9.5 (0.20)	0.80(1.79)	3.2 (1.4)	0.39(0.61)	229 (45.3)	76.4	229.2
	b.	100% Biosolids	10	15.5 (2.1)	7.9(0.11)	9.6 (0.48)	2.47(1.2)	0.27(0.14)	1.3 (1.1)	118 (117)	74.3	222.9
	c.	50/50	10	15.8 (2.0)	7.9(0.08)	9.6 (0.42)	3.21(1.68)	0.42(0.49)	0.90(0.72)	233 (48.5)	72.8	218.4
	d.	Zero food	10	16.4 (1.8)	7.9(0.16)	9.4 (0.34)	0.72(0.53)	0.16(0.12)	0.32(0.29)	179 (43)	0	0
	e.	100% Biosolids, small bugs	10	15.7 (2.1)	7.9(0.09)	9.7 (0.49)	1.59(0.64)	0.27(0.34)	0.8 (0.08)	208 (37.9)	22.5→12[a]	67.5→36
	f.	50% Control food	10	15.9 (2.2)	7.8(0.16)	9.6 (0.50)	1.71(1.0)	0.19(0.15)	0.97(1.4)	186 (49.4)	36.9→31[a]	111→93

[a] Food rations include 100% biosolids, 100% control food, half control food and half biosolids (50/50) or 100% control food fed at half the rate (50% control food).
[b] Food rates revised during experiment based on interim weights and counts.
(σ) = standard deviation.

APPENDIX BIII

SOLIDS UPTAKE RATES FOR ARTIFICIAL STREAMS WITH AND WITHOUT LARVAE

	Suspended Solids Level, mg/L		
	Control Aquaria	Caddis Aquaria	Gut Contents
Test 1			
Prior to feeding	0	0	None
Immediately following feeding	28.4	26.0	Gut full, stained material in 1st third of tract
10 Minutes	25.0	23.0	--
30 Minutes	24.0	20.0	Gut partially filled, no stained material
60 Minutes	22.0	19.0	--
7 Hours	7.0	4.0	All gut contents pink
24 Hours	2.4	0.4	--
Test 2			
Prior to feeding	2.8	0	No gut tract analysis for Test 2
Immediately following feeding	7.0	6.0	
6 Minutes	4.0	2.0	
30 Minutes	5.0	2.0	
60 Minutes	3.0	2.0	
7 Hours	2.4	2.0	
24 Hours	2.0	0.8	

APPENDIX BIV

GROWTH AND SURVIVAL FOR HYDROPSYCHE SP. DURING FEEDING RUNS

Run	Food Ration	No. Larvae	Initial $\bar{x}$ wt. per Larva, mg	Initial $\bar{x}$ head capsule, µs	No. Larvae alive	Final $\bar{x}$ wt. per Larva	$\bar{x}$ Head Capsule	Survival, %	$\bar{x}$ weight change at end of feeding	$\bar{x}$ Head capsule length change	Test Duration, weeks
1	1. 100% Control	50	18.75	1.19	30	25.8	1.18	65	+7.05	-0.01	10
	2. 100% Biosolids	50	18.71	1.15	22	25.4	1.15	47	+6.7	0.00	10
	3. 50/50	50	19.57	1.17	32	27.1	1.38	86	+7.6	+0.21	10
	4. Zero food	50	18.60	1.17	28	19.0	1.03	58	+0.4	-0.14	10
2	1. 100% Control	100	3.75	0.56 (.14)[a]	62	7.23	--	62	+3.48	--	7
	2. 100% Biosolids	100	5.39	0.56 (.14)	4	3.85	--	4	-1.54	--	7
	3. 50/50	100	6.53	0.56 (.14)	74	6.53	--	74	0.0	--	7
	4. Zero food	100	5.90	0.65 (.14)	27	3.52	--	27	2.38	--	7
3	1. 100% Control	100	9.52	--	72	19.74	--	72	+10.22	--	4
	2. 100% Biosolids	100	8.33	--	13	8.93	--	13	+0.6	--	4
	3. 50/50	100	8.0	--	78	11.68	--	78	+3.68	--	4
	4. Zero food	100	8.30	--	12	10.4	--	12	+1.74	--	4
	5. 100% Biosolids	100	8.56	--	14	12.19	--	14	+3.63	--	4
4	1. 100% Control	100	7.12	--	65	12.4	--	65	+5.3	--	4
	2. 100% Biosolids	100	5.1	--	22	6.6	--	22	+1.5	--	4
	3. 50/50	100	6.47	--	65	9.2	--	65	+2.8	--	4
	4. Zero food	100	6.5	--	18	5.4	--	18	-1.1	--	4
	5. 100% Biosolids	100	9.0	--	12	5.4	--	12	-3.6	--	4
5	1. 100% Control	50	22.9	0.63	40	23.9	0.62	80	+1.0	+0.01	10
	2. 100% Biosolids	50	24.2	0.63	44	23.5	0.65	88	-0.7	+0.02	10
	3. 50/50	50	21.8	0.63	41	23.6	0.66	82	+1.8	+0.03	10
	4. Zero food	50	23.0	0.63	28	19.4	0.66	56	-3.6	+0.03	10
	5. 100% biosolids	50	6.7	--	13	6.9	0.49	26	+0.2	--	10
	6. 50% Control food	50	21.6	0.63	40	22.5	0.67	80	+0.9	+0.04	10

[a] Head capsules measured on a separate population of 100 individuals. Same initial $\bar{x}$ starting length.

APPENDIX BV

DAPHNIA GROWTH AND SURVIVAL DURING SOLIDS FEEDING STUDIES

Test	Food Rations	Experiment Duration, days	Initial No.	Final No.	$\bar{x}$ Length, microns	Distribution by Length			
						0-0.75 mm	0.76-1.25	1.5-1.75	>2
1	Control food	18	5	212	1.01	178	34	--	--
	Control food	18	5	151	1.2	107	42	2	--
	100% Biosolids	18	5	105	0.98	81	23	1	--
	100% Biosolids	18	5	74	1.2	56	17	1	--
	Zero food	18	5	0	--	--	--	--	--
2	Control food	25	10	39	1.1	14	2	22	1
	Control food	25	10	80	0.8	60	--	12	8
	Control food	25	10	5	1.1	1	3	--	1
	Control food	25	10	38	0.9	23	1	14	--
	Biosolids low density	25	10	45	0.9	4	12	18	1
	Biosolids low	25	10	75	1.2	24	17	20	14
	Biosolids high	25	10	16	0.76	11	--	3	2
	Biosolids high	25	10	75	1.0	39	5	24	7
	Generated solids	25	10	119	1.0	65	6	29	19
	Generated solids	25	10	211	1.1	127	17	60	28
	Generated solids	25	10	198	0.9	124	7	31	36
	Generated solids	25	10	65	1.3	22	3	19	21
	Starved	25	10	4	1.3	0	0	2	2
	Starved	25	10	31	1.4	4	0	24	3
	Starved	25	10	0	--	0	0	0	0
	Starved	25	10	18	1.4	4	0	9	5

APPENDIX BVI

C^{14} TAGGING DATA FOR NONSETTLEABLE SOLIDS FEEDING STUDIES IN COUNTS PER MINUTE

	Biogenerated Solids	No. Test Organisms	Mill Solids	No. Test Organisms
Tagged solids, mg	4,703		11,938	
Large caddis				
Untagged controls	7	--	7	--
Swirled controls	71	3	71	3
$\bar{x}$ Whole fed individuals	425	6	6,905	4
$\bar{x}$ Gut tracts removed	392	6	10,673	5
Small caddis				
Untagged controls	7	--	7	--
Swirled controls	13	5	13	5
$\bar{x}$ Whole fed individuals	192	5	1,764	5
$\bar{x}$ Gut tracts removed	115	4	835	5
Daphnia				
Untagged controls	--	--	--	--
Fed and killed	103	12	312	9
Fed and clear gut tract	172	9	150	10
Offspring Adults	45	9	--	--
Md	2	40	--	--
Small	0	146	--	--
Swirled Daphnia	7.2	--	7.2	--
Solubilizer	8	--	8	--
Glucose feed	72,000 cpm	--	72,000	--