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Acid Mine Pollution: Effects on Survival, Reproduction and Aging of Steam Bottom Microinvertebrates

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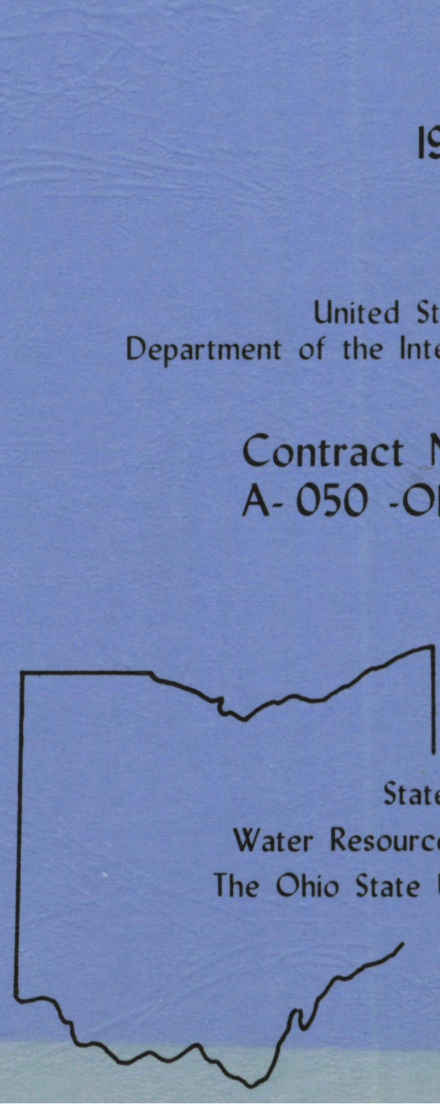

State of Ohio
Water Resources Center
The Ohio State University

ACID MINE POLLUTION: EFFECTS ON SURVIVAL, REPRODUCTION AND AGING OF STREAM BOTTOM MICROINVERTEBRATES

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Introduction

The project had three major objectives. The first was to study the relationship between acid mine pollution and the occurrence of benthic sand-dwelling microinvertebrates. Data were derived from 58 collections, all but ten of which came from streams in southeastern Ohio (Table 1). Sites, generally two per stream, were selected so as to provide a range of histories with respect to acid mine pollution. Some sites had no history of pollution; some are presently receiving drainage from mining activities and some have been the subject of reclamation projects. Collections were made from 14 stream sites during October-November of 1976 and a similar number, including 4 sites from 1976, during October-November of 1977. The 4 sites, 2 unpolluted and 2 polluted, which were studied both autumns were also studied at bimonthy intervals during the period of a year. Associated with the field work as well were the researches of two graduate students. One student has completed a Master's thesis on morphometry of a Tardigrada species occurring in a non-polluted stream and a taxonomic inventory of tardigrades inhabiting our study streams. The other student is working on a Doctoral dissertation, still in progress, on the population ecology of Rotifera species living in a gradient of physical-chemical conditions which occur as an unpolluted stream merges with a larger, acid mine polluted stream.

The second objective was the study of laboratory effects of acid mine pollution on life history parameters of selected organisms occuring in southeastern Ohio. A Gastrotricha species, on which preliminary information was already available, was studied with respect to effects on its intrinsic rate of increase of varied proportions of mine acid in a milieu of constant total ionic activity. A Rotifera species was studied as part of a non-thesis Master's research project designed to gain preliminary information with respect to its

Table I. Locations and collecting dates of streams and beaches surveyed under the Water Resources Grant.

	No. sites Collected	Year I 1976			Year I 1977			Year II 1977	
Non-polluted									
Mississippi River	2	July							
Strouds Run (Athens Co.)	2		Sept.	Nov.	March	April	June	July	Nov.
Dow Lake Beach	2			Oct. Nov.					Nov.
Margaret Cr. (Athens Co.)	2			Nov.					---
Oppossum Cr. (Athens Co.)	1			---					Oct.
Cherry Cr., Willow Cr. (Athens Co.,)	2			---					Oct.
Polluted and unreclaimed									
Sandy Run (Vinton Co.)	2		Sept.	Nov.	March	April	June	July	Nov.
Lake Hope Beach	2			Oct.					---
Long Run (Athens Co.)	2			Oct.					---
Raccoon Cr. (Hocking Co.)	2			Nov.					---
Sulphur Run (Athens Co.)	1			---					Oct.
Sunday Cr. (Athens Co.)	1			---					Nov.
Trace Run (Athens Co.)	3			---					Nov.
Snow Fork (Athens Co.)	1			---					Nov.
Polluted and reclaimed									
Minkers Run (Athens Co.)*	2			Oct.					---
Tick Ridge (Hocking Co.)**	2			Nov.					---
Long Hollow (Athens Co.)*	2			---					Nov.

* Mainly vegetative replanting, during the early 1950's.
** Total reclamation, during the mid 1970's, according to requirements of current law.

intrinsic rate of increase under non-polluted conditions. The subject of another completed Master's thesis was a study of several minute insect larvae to ascertain their tolerances to acid mine pollution and the effect of such pollutants on their basal metabolic rates through respirometry.

The third and last objective was the study of developmental and aging changes in the Gastrotricha test animal used for the second objective. This work is being carried out by a doctoral student, and is still in progress. Individuals have been raised in culture, and fixed for electron microscopy at specific developmental stages: post-hatch juvenile, pre-reproductive adult, reproductive adult, and aged adult. These materials have been examined and characterized to elucidate normal anatomy and fine structure, and to document changes occuring throughout the developmental sequence. In a later stage of the work, animals cultured in dilute acid mine drainage will be described and characterized in a similar manner.

A summary of student participation in the entire project is given in Table II. Nine different students were involved in each of two years, each involved in three to four aspects of the work.

Thus far the project has resulted in seven published abstracts, two published papers and a third in press, and three completed manuscripts which soon will be submitted for publication (Table III). In addition, data have largely been analysed for two other manuscripts, which will be completed during the spring of 1980, and data are on hand for a third, which should be completed during the summer of 1980. Thus the two year project will have resulted in at least seven published abstracts and nine published papers. Reprints of published abstracts and papers are included as separates; copies of the manuscript in press and of the three completed manuscripts are included as appendices.

Table II. Training of undergraduate and graduate students in various aspects of research, listed as Year I - Year II. Most individuals participated in more than one aspect of the study.

	Undergraduate Students	Graduate Students		Sub
	Senior Project Work	Master's Level	Doctoral Level	Total
Objective I - Field Study				
Collection and extraction of fauna	3 - 1	3 - 4	2 - 2	8 - 7
Enumeration of fauna	1 - 1	2 - 3	1 - 2	4 - 6
Taxonomic characterization of animals		1 - 2	1 - 1	2 - 3
Water analysis	0 - 1		2 - 2	2 - 3
Sediment analysis	0 - 1	0 - 1	0 - 1	0 - 3
Objective II - General Laboratory Studies				
Chemical characterization of water	1 - 1	1 - 1	0 - 1	2 - 3
Culture of animals	1 - 1	1 - 2	2 - 2	4 - 5
Determination of 24-hour LC_{50} levels		2 - 2	1 - 1	3 - 3
Objective III - Aging, Energetics and Sterility Studies				
Feeding		1 - 1	1 - 1	2 - 2
Osmoregulation and aging			1 - 1	1 - 1
Reproduction and aging			1 - 1	1 - 1
Number of different persons participating in the research:	3 - 2	4 - 4	2 - 3	9 - 9
Mean number of categories in which each person was trained:	2 - 3	2.8 - 4	6 - 5	3.2 - 4.1

Table III. List of published abstracts, publications and manuscripts thus far resulting from the two year study summarized herein.

Published Abstracts (7):

1977. Hummon, W.D. Meiobenthos of the Mississippi headwaters. Amer. Zool., 17:869.

1978. Hummon, M.R. Fine structural changes in Lepidodermella squammata (Gastrotricha: Chaetonotida) during maturation and aging. Ohio J. Sci., 78 Suppl: 11.

1978. Wainberg, R.H. A study of the Tardigrada from selected southeastern Ohio streams. Ohio J. Sci., 78 Suppl.: 11.

1978. Evans, W.A. The psammic Rotifera of an acid mine polluted stream. Ohio J. Sci., 78 Suppl.:87.

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Publications (3):

1978. Hummon, W.D., W.A. Evans, M.R. Hummon, F.G. Doherty, R.H. Wainberg and W.S. Stanley. Meiofaunal abundance in sandbars of acid mine polluted, reclaimed, and unpolluted streams in southeastern Ohio, pp 188-203. In: J.H. Thorp and J.W. Gibbons, eds. Energy and Environmental Stress in Aquatic Ecosystems. DOE Symposium Series (CONF-771114). Nat. Tech. Info. Service, Springfield, Virginia.

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(1980) Hummon, W.D. and D.P. Bevelhymer. Life table demography of the rotifer Lecane tenuiseta under culture conditions, and various age distributions. Hydrobiologia. (In Proof, 9 pp MS). [Appendix C]

Preliminary Manuscripts Completed (3):

Hummon, W.D. Influence of extraction-mesh size on apparent abundance and taxon structure of sandy streambed meiobenthos, with special reference aschelminth phyla. (11 pp MS). [Appendix A]

Wainberg, R.H. and W.D. Hummon. MOrphological variability of Isohypsibius saltursus and notes on other Tardigrada of southeastern Ohio streams. (29 pp MS). [Appendix B]

Doherty, F.G. and W.D. Hummon. Respiration of aquatic insect larvae (Ephemeroptera, Plecoptera) in acid mine water (10 pp MS). [Appendix D]

RESEARCH ACCOMPLISHMENTS

I. Field Research

Little quantitative inforamtion was available concerning the micrometazoa of North American streams prior to the onset of this project. Having no such data base for comparison, the first thing done was to establish a reliable and repeatable sampling scheme which would yield statistically valid quantitative information. This was accomplished during June and July 1976 at the University of Minnesota's Lake Itasca Biological Station, the sampling scheme being tested at two sites along the headwaters of the Mississippi River in July 1976 (Hummon, 1977 abstract; manuscript in preparation). Micrometazoans, dominated by rotifers, tardigrades, nematodes and gastrotrichs, were found to be as abundant as in many rich marine habitats. An important feature of the sampling scheme is that extraction is direct, without sieving, since it was found that sieving with even fine aperture sieves involves a serious underestimate of abundances due to the loss of tiny micrometazoans [Appendix A].

Southeastern Ohio sites sampled during the fall of 1976 involved streams with a variety of past histories with respect to acid mine pollution (Hummon, et al., 1978). The lower abundances of micrometazoa in unpolluted streams, compared to those of the headwaters of the Mississippi River, are probably due to two factors: most samples were taken in the fall rather than in the summer, and the streams occur in a carbonate-poor region of mainly sandstone parent material. Both abundance of total meiofauna and Diptera, and the number of taxa recorded, were negatively correlated with compensated noncarbonate conductivity (CNC) at the 14 sites studied. This index was derived empirically following the initial samplings in this series. CNC represents total conductivity (high under acid mine conditions), minus that which can be attributed to normal carbonate buffers, modified by both oxygenation status of stream and sediments and stream flow. Both of these modifying factors in the CNC formula are related biologically to the extent to which flowing stream water penetrates into the sediment habitat under study. A dendrogram of similarity relationships based on shared taxon diversity showed a central group of largely unpolluted sites with high taxon diversity and two lateral groups of polluted sites with lower diversities, one rotifer dominated and the other nematode dominated. It is suspected that the cause of rotifer-vs. nematode-domination of sites is related to sediment size and sorting.

The fall 1977 series included 14 sites, four repeated from fall 1976 for continuity and ten others (manuscript in preparation). Based on experimental data from the previous year, the compensated noncarbonate conductivity (CNC) formula has been modified so as to be calculated independently of any faunistic information: it had originally been dependent only on proportionate depths of fauna in the sediments, not on taxon or abundance information. Again the same series of faunistic parameters was found to be negatively correlated with CNC, namely abundances of total meiofauna and of Diptera, and the number of taxa recorded. These correlation coefficients were significant for data from fall 1976, from fall 1977 and for data from both years combined, despite the fact that abundances for 1977 exceeded by two-fold those for 1976 at all four sites which were sampled in both years. By scaling all fall 1977 abundances down to 1976 levels based on the repetitive data from these four sites (two polluted, two non-polluted), abundances of total meiofauna and of Diptera became even more negatively correlated with CNC and the Nematoda taxon was added to the list showing significant negative correlation.

As a result of this two year study we are in no doubt that, for areas such as southeastern Ohio where carbonate buffers are naturally limiting, general meiofaunal abundances are inversely related to the total amount of acid mine pollution present in stream waters and sediments. We also have evidence from one stream (two sites), where flowing water, fish and insect larvae all have been declared to be recovered from the effects of past pollution, that the residual effects are much in evidence in both sediments and their micrometazoa. We do not know how fast recovery occurs after the abatement or cessation of acid input takes place. However, we now have basic information on the organisms, understanding of the habitat, and the technology by which to monitor such recovery.

The four sites (two polluted on Sandy Run and two unpolluted on Strouds Run) which were sampled during the falls of 1976 and 1977 were also sampled at bimonthly intervals during the intervening year. Though the data are largely unanalysed as yet, some conclusions are apparent. Maximum abundances are usually present in June, minimum abundances between January and March. Maximum abundances of the two unpolluted sites were only one-third those recorded from the headwaters of the Mississippi River, and those of the two polluted sites only one-half those of the unpolluted sites. Fall abundances at all four sites averaged only one-third of maximum abundances. Differences are even more striking when individual taxa are considered, with varying patterns of abundance occurring from one taxon to another. Unfortunately, because of a lack of basic taxonomic work on the individual taxa, it has not yet been possible to deal with the data at the species level.

A year-round morphometric study was made with respect to one species of Tardigrada from one of the non-polluted sites (Appendix B), information necessary as background to a detailed study of such a taxon. Species were also indentified from most of our study sites, though tardigrades are not abundant in polluted streams. This represents at present our best known taxon and indicates the amount of work yet to be done on other, perhaps more difficult taxa. Work is also under way with respect to the Rotifera, but is as yet only partially analysed.

II. Laboratory Research

Progress was made with respect to an analysis of factors present in mine acid which affect life history parameters of one test organism, the common freshwater gastrotrich Lepidodermella squammata (Hummon and Hummon, 1979). We found for various mixtures of lake and acid mine water having a total conductivity of 350 - 380 µmho/cm, that mean life expectancy was greatly reduced and reproduction ceased if the proportion attributable to carbonates was less than 5%. As the carbonate fraction was increased, mean life expectancy quickly normalized and reproductive effort was proportionately increased to near normal. The variance of reproductive effort in intermediate carbonate fractions, however, was increased, indicating that many animals were either fully reproductive or non-reproductive. These results indicate a large amount of latent heterozygosity in animals from a cloned population which were in theory genetically identical.

Work was begun on a second test animal (Appendix C) in order to set a base line of information for future studies with acid mine pollution. Lecane tenuiseta, a rotifer, was found to be longer lived and to produce more eggs more slowly and over a longer period of time than the gastrotrich. The resulting rate of increase for the rotifer, however, differed little from that of the gastrotrich, which compensated for its fewer eggs by quicker development.

A second line of approach to laboratory studies was inaugurated, using small aquatic insect larvae to determine whether the toxic effects of acid mine pollution were mediated through respiratory metabolism (Appendix D). Contrary to expectations, the results indicated no difference in oxygen uptake per unit dry weight under control or acid mine conditions.

III. Development and aging studies.

The fine structure of the primary test animal, Lepidodermella squammata, has been studied in specimens from a clone maintained in culture in the laboratory. This work has been carried out by a second doctoral student involved throughout the project. Gross changes observed in overall shape among normally aging animals, and among animals cultured in acid mine water, imply some disruption in osmoregulation and possible change in muscle tone. A fixation method that does not distort the animals is especially important. Best results have been obtained by direct but gradual introduction of a fine stream of fixative into a spot plate with a swimming animal. For electron microscopy, alternating groups of thick and thin sections of animals oriented for both longitudinal and cross sectioning have been prepared and studied. Identification of tissue types and arrangements is best accomplished in electron micrographs at 2000 to 20,000 X: these results can then be used to interpret adjacent thick sections with light microscopy, at 200 to 1,000 X.

Aspects of fine structure of importance to the study include the integument, the osmoregulatory system of protonephridia, the feeding apparatus, and the reproductive system. The integument consists of a series of overlapping scales, whose bases adhere closely to the epidermal syncytial layer of the normal adults. On the ventral side, the scalar pattern is interrupted by two longitudinal rows of cilia, which provide the locomotor system. Interference with normal ciliary activity is characteristic of animals in distress, either because of advancing age or adverse conditions.

The protonephridia can be observed in living animals using the light microscope at 400 to 1000 X, as very fine moving lines along the lateral portions of the abdomen: this represents the continual beating of the pair of flagella that comprise each of the two protonephridia. Flagellar beating continues in animals in distress, and has been detected in animals considered by other criteria to be dead or nearly dead. In serial electron micrographs of cross sections, the origin of the protonephridia was found to occur at the pharyngo-intestinal junction. A tangled system of canals is present in proximity to each protonephridial channal, and is concentrated ventrolaterally to each channal, with components extending along the ventral ciliary rows. No fine structural changes have been observed in the protonephridial system of aging animals.

The feeding apparatus of L. squammata includes a complex arrangement of tubular structures and filamentous projections at the EM level. In living animals, the mouth appears to be held against the substratum as the animal swims forward, and mouth and pharynx open as a result of quick contractions of the pharyngeal muscles. Gastrotrichs are generally considered to be bacteriophagous, but little is known of their selectivity in feeding. Both the culture work and fine structural evidence indicate that they both ingest and digest yeast cells. The structure of the mouth does not appear to alter with aging: its fine structure appears crystalline and is probably quite stable.

Basic information on the fine structure of the reproductive system in adults cultured in lake water correlates with the light microscopic evidence. In actively reproducing individuals, a large central germinal vesicle surrounded by lipid-rich storage material is prominent, and in electron micrographs, a series of several smaller eggs can be identified, posterior and lateral to the largest developing egg. In post-hatch juveniles, six putative egg nuclei can be identified in each of two posterior lateral locations. Cell division does not generally occur in these animals after completion of embryological development and hatching, and it appears that the number of egg nuclei is also determined prior to hatching. These findings indicate that the maximum reproductive effort in cultured animals of about six eggs is not directly limited by the availability of egg nuclei in the adult. The observed reduction in reproductive output in animals cultured in acid mine water thus may be attributed to altered rates of development of the egg nuclei already present at time of hatching.

Lepidodermella squammata has been considered to be an obligate parthenogen, based on the ability of a single isolated egg to produce viable eggs and offspring, and the absence of recognizable male structures. During the course of this study, dense elongate structures were noted in electron micrographs of post-reproductive animals, and it was suggested (Hummon, 1978) that these might represent sperm. This suggestion has since been corroborated in a study at another laboratory, using different techniques (Science 205:302-303. 1979). It has not been determined in either laboratory whether and under what conditions the sperm are functional.

Aging changes have been detected in several tissues: egg cytoplasm, epidermal tissue layer, pharyngeal muscle, and gut. The changes in egg nucleus and cytoplasm which occur during their maturation are not considered as an aging change, and are repeated up to six times in each animal. The most mature egg is clearly recognizable both by its more anterior position and by its altered appearance and size; the characteristics are similar in a post-hatch juvenile and in a post-reproductive adult whose most mature egg is certain never to be laid. Among the remaining eggs, however, changes occur with age, and in the aged animal, these reserve eggs become packed with large vesicles, presumably lipid, and are distended.

There is no apparent change in the nature of the cuticle with age, although it must increase in extent during growth of the animal. The subcuticular epidermis is a syncytium, and is densely packed with organelles in the post-hatch juvenile, with conspicuous rough endoplasmic reticulum and golgi. In the aged adult, the syncytium appears attenuated, and cell organelles are poorly represented and sparse, although the layer remains intact.

The pharyngeal muscle of all animals is arranged around a triangular lumen, and is described as tri-radiate in cross section. In all animals of all ages, there are areas of dorsal pharyngeal cells that appear filled with dense deposits of finely granular material and with little or no muscle fibrils. Their nature has not been determined. Muscle cells are conspicuous in the rest of the pharynx, with thick and thin filaments arranged in approximately hexagonal array in appropriate section. In young animals, these cells are densely filled with muscle filaments, mitochondria and nuclei. In aged animals, large vaculoles are present, and the general integrity of the fibers appears distorted. No changes were noted in the pharyngeal cuticle.

The gut contents of young animals are finely divided, with microvilli surrounding a triradiate lumen. There is evidence of intracellular digestion, with putative food particles enclosed in vacuoles. Little lipid is present. In the aged animal, the same cell organelles are present, but vacuoles are 3-10 times larger, often appear empty, and there are conspicuous lipid deposits present.

Some of the aging changes noted are to be expected from the known life history patterns of this animal. A metabolically active epidermis in the actively growing animal is indicated by the electron microscope evidence, and a quiescent tissue is demonstrated in the aged animal. Similarly, the loss of integrity in the aged pharyngeal muscle may be an indication of general decline and failure of cells to carry out repair at the necessary level. The accumulation of lipid in the gut and in the reserve egg nuclei in the aged animals is less understandable, and it implies the sequestering of considerable food resources in a way that apparently is not available to the animal.

This portion of the project is not yet completed, but will be pursued by the concerned graduate student with other support. As shown in the results of section II, it has been demonstrated that culture of *Lepidodermella squammata* in the presence of dilute acid mine water results in an increased variance in reproductive characteristics so that it has proved difficult to obtain individuals truly representative of the conditions occurring in the population as a whole. It is hoped that these unexpected problems can be resolved. Information now available on aging changes will then be used as a tool in the analysis of development and aging of the test animal when exposed to dilute acid mine drainage.

APPLICATION OF RESEARCH RESULTS.

Specifically interested in the results of this study are the following: Wayne National Forest and Soil Conservation Service, US Department of Agriculture; Ohio Environmental Protection Agency and Ohio Department of Natural Resources. The general results of the project have been communicated to representatives of the above agencies, and to industry and the public, through public meetings concerning acid mine pollution held on the Ohio University campus, and through hearings by Ohio EPA on the Hocking River Basin portion of the State Water Quality Management Plan.

The general collection format has recently been modified so as to be useful as a Meiobenthic Bioassay. Information obtained using the bioassay will be compatible with the vast array of data derived from the field portion of the project. Moreover, the general results of the bioassay can be expected to be at least 90% as accurate as the research results with expenditure of only 25% of the time per site. We will become involved with the Ohio EPA and Ohio DNR during summer 1980, and following, to assess the effects of reclamation efforts on sites heavily damaged by acid mine pollution.

Appendix A

Influence of extraction - mesh size on apparent abundance and taxon structure of sandy streamed meiobenthos, with special reference to aschelminth phyla.

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Abstract

Analysis of preserved material, passed through sieves of 500, 250, 125, 62 and 37 μm openings, was made for 16 higher taxa of freshwater meiofauna (animals < 2 mm in length). Only 38% of total meiofauna was cumulatively retained by the 62 μm sieve and 89% by the 37 μm sieve. Animals passing through these sieves were mostly gastrotrichs, rotifers and nematodes. H' taxon diversity was used to characterize structure, and $S_{H'}$ similarity, to compare the results for fauna retained cumulatively on various sieves with those for unsieved material. Only the 37μm sieve yielded both a comparable H' value and a highly similar structure of ordered taxa. The time-inefficiency of using decreased sieve openings is not compensated for by increased accuracy of results, especially with respect to aschelminth phyla. Freshwater meiobenthos are better extracted from small unsieved sand samples by multiple decantation, followed by multiple Sedgwick-Rafter cell counts.

Acknowledgements

Support by a grant from the Office of Water Research and Technology, U.S. Department of Interior, through the Ohio Water Resources Center, Columbus, is gratefully appreciated. I also wish to thank Dr. David Fry, University of Indiana, Bloomington, for numerous helpful discussions on the fresh-water habitat and fauna, Dr. David Parmalee, Director of the Lake Itasca Biological Laboratory, University of Minnesota, for facilitating the research, and my wife Margaret Raper Hummon, Ohio University, Athens, for help in making the collections and discussing the data.

One of the most persistent problems in dealing with meiobenthos (animals < 2 mm in length, Swedmark, 1964) is in effectively extracting them from the substratum and gaining reliable quantitative counts. The problem is greater with muddy than with sandy substrata and greater with freshwater than with marine meiobenthos. In the first instance it is because much of the sediment is extracted along with the animals, in the second because of the smaller mean size of the freshwater animals themselves; in either case efficient extraction tends to be nullified by observational inefficiencies.

Sieving is probably the most common method of extracting preserved, quantitative material, though other methods have been used, such as elutriation (usually though not always combined with sieving), floatation, centrifugation and direct counts. Sieves with openings up to 200 - 250 μm have been popular for freshwater benthos (Edmundson & Winberg, 1971, and references), though sieves with openings down to 50 μm have recently been advocated (Mundie, 1971; Shiozawa & Barnes, 1977).

For freshwater meiobenthos (microbenthos, interstitial fauna), both Kajak and Ruttner-Kolisko (in Edmundson & Winberg, 1971, pp. 63, 124) consider that small subsamples are best treated in an unsieved condition, the animals presumably being unumerated by direct counts. In the present paper, data are presented which reinforce this conclusion with respect to sieving, and the Sedgwick-Rafter counting procedure is utilized to overcome many of the problems encountered in the enumeration of unsieved freshwater (or marine) meiobenthos.

MATERIALS AND METHODS

Material was collected from the headwaters of the Mississippi River near Lake Itasca, Minnesota in early July 1976 for another study (ms in preparation). Ninety-six 2.7 cm^3 samples representing various stations and depths from two sand bars were narcotized with 1% $MgCl_2$ for 10-15 minutes, extracted by multiple decantation and fixed in 10% formalin with Rose Bengal, as described elsewhere

(Hummon, et al., 1978). Following enumeration by multiple Sedgwick-Rafter cell counts, the material, containing flocculent debris, silt-clay, fecal pellets and micrometazoans, was pooled into a single flask. This pooled material later was washed through 500, 250, 125, 62, and 37 μm opening sieves, with sieved and non-retained fractions held for subsequent analysis.

Sieved fractions were washed into jars and made up to 50 ml (500, 250, 125 μm sieves) or 100 ml (62, 37 μm sieves). Non-retained residues were allowed 24 hrs to settle before most of the supernatant was carefully siphoned from each of several flasks. Their contents were combined and the process repeated until concentrated to 150 ml in a jar. Fractions from the 500 and 250 μm sieves were enumerated to major taxa by direct counts, all animals being tallied. Fractions from the remaining sieves and the unsieved residues were subsampled into 10 Sedgwick-Rafter (S-R) cells each and enumerated to major taxa. In all, 2,531 animals were tallied, comprising some 13.1% of the estimated total of 19,307 animals in 16 taxa. S-R replicate data were subjected to variance/mean and chi square analyses (Elliott, 1977), for total meiofauna and for each taxon, when represented by 3 or more individuals per replicate set.

To determine the effect of sieving on apparent taxon structure, H' taxon diversity (Pielou, 1975) was calculated for the cumulative fraction that would have been retained by each sieve size, from coarest to finest, or for the unsieved material. Similarity between fractions was then calculated using the $S_{H'}$ formula for shared taxon diversity (Hummon, 1974), and the results arranged into a hierarchical dendrogram.

RESULTS

Results on the fraction-residue enumeration analysis are given in Table I. Tricopterans and coleopterans were all retained on the 500 μm sieve and acarines on the 250 μm sieve, though relatively fewer from other taxa were retained. By the 125 μm sieve all members of 6 taxa and over half of 5 others were retained,

Table III. List of published abstracts, publications and manuscripts thus far resulting from the two year study summarized herein.

Published Abstracts (7):

1977. Hummon, W.D. Meiobenthos of the Mississippi headwaters. Amer. Zool., 17:869.

1978. Hummon, M.R. Fine structural changes in Lepidodermella squammata (Gastrotricha: Chaetonotida) during maturation and aging. Ohio J. Sci., 78 Suppl: 11.

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Preliminary Manuscripts Completed (3):

Hummon, W.D. Influence of extraction-mesh size on apparent abundance and taxon structure of sandy streambed meiobenthos, with special reference aschelminth phyla. (11 pp MS). [Appendix A]

Wainberg, R.H. and W.D. Hummon. MOrphological variability of Isohypsibius saltursus and notes on other Tardigrada of southeastern Ohio streams. (29 pp MS). [Appendix B]

Doherty, F.G. and W.D. Hummon. Respiration of aquatic insect larvae (Ephemeroptera, Plecoptera) in acid mine water (10 pp MS). [Appendix D]

RESEARCH ACCOMPLISHMENTS

I. Field Research

Little quantitative inforamtion was available concerning the micrometazoa of North American streams prior to the onset of this project. Having no such data base for comparison, the first thing done was to establish a reliable and repeatable sampling scheme which would yield statistically valid quantitative information. This was accomplished during June and July 1976 at the University of Minnesota's Lake Itasca Biological Station, the sampling scheme being tested at two sites along the headwaters of the Mississippi River in July 1976 (Hummon, 1977 abstract; manuscript in preparation). Micrometazoans, dominated by rotifers, tardigrades, nematodes and gastrotrichs, were found to be as abundant as in many rich marine habitats. An important feature of the sampling scheme is that extraction is direct, without sieving, since it was found that sieving with even fine aperture sieves involves a serious underestimate of abundances due to the loss of tiny micrometazoans [Appendix A].

Southeastern Ohio sites sampled during the fall of 1976 involved streams with a variety of past histories with respect to acid mine pollution (Hummon, et al., 1978). The lower abundances of micrometazoa in unpolluted streams, compared to those of the headwaters of the Mississippi River, are probably due to two factors: most samples were taken in the fall rather than in the summer, and the streams occur in a carbonate-poor region of mainly sandstone parent material. Both abundance of total meiofauna and Diptera, and the number of taxa recorded, were negatively correlated with compensated noncarbonate conductivity (CNC) at the 14 sites studied. This index was derived empirically following the initial samplings in this series. CNC represents total conductivity (high under acid mine conditions), minus that which can be attributed to normal carbonate buffers, modified by both oxygenation status of stream and sediments and stream flow. Both of these modifying factors in the CNC formula are related biologically to the extent to which flowing stream water penetrates into the sediment habitat under study. A dendrogram of similarity relationships based on shared taxon diversity showed a central group of largely unpolluted sites with high taxon diversity and two lateral groups of polluted sites with lower diversities, one rotifer dominated and the other nematode dominated. It is suspected that the cause of rotifer-vs. nematode-domination of sites is related to sediment size and sorting.

The fall 1977 series included 14 sites, four repeated from fall 1976 for continuity and ten others (manuscript in preparation). Based on experimental data from the previous year, the compensated noncarbonate conductivity (CNC) formula has been modified so as to be calculated independently of any faunistic information: it had originally been dependent only on proportionate depths of fauna in the sediments, not on taxon or abundance information. Again the same series of faunistic parameters was found to be negatively correlated with CNC, namely abundances of total meiofauna and of Diptera, and the number of taxa recorded. These correlation coefficients were significant for data from fall 1976, from fall 1977 and for data from both years combined, despite the fact that abundances for 1977 exceeded by two-fold those for 1976 at all four sites which were sampled in both years. By scaling all fall 1977 abundances down to 1976 levels based on the repetitive data from these four sites (two polluted, two non-polluted), abundances of total meiofauna and of Diptera became even more negatively correlated with CNC and the Nematoda taxon was added to the list showing significant negative correlation.

As a result of this two year study we are in no doubt that, for areas such as southeastern Ohio where carbonate buffers are naturally limiting, general meiofaunal abundances are inversely related to the total amount of acid mine pollution present in stream waters and sediments. We also have evidence from one stream (two sites), where flowing water, fish and insect larvae all have been declared to be recovered from the effects of past pollution, that the residual effects are much in evidence in both sediments and their micrometazoa. We do not know how fast recovery occurs after the abatement or cessation of acid input takes place. However, we now have basic information on the organisms, understanding of the habitat, and the technology by which to monitor such recovery.

The four sites (two polluted on Sandy Run and two unpolluted on Strouds Run) which were sampled during the falls of 1976 and 1977 were also sampled at bimonthly intervals during the intervening year. Though the data are largely unanalysed as yet, some conclusions are apparent. Maximum abundances are usually present in June, minimum abundances between January and March. Maximum abundances of the two unpolluted sites were only one-third those recorded from the headwaters of the Mississippi River, and those of the two polluted sites only one-half those of the unpolluted sites. Fall abundances at all four sites averaged only one-third of maximum abundances. Differences are even more striking when individual taxa are considered, with varying patterns of abundance occurring from one taxon to another. Unfortunately, because of a lack of basic taxonomic work on the individual taxa, it has not yet been possible to deal with the data at the species level.

A year-round morphometric study was made with respect to one species of Tardigrada from one of the non-polluted sites (Appendix B), information necessary as background to a detailed study of such a taxon. Species were also indentified from most of our study sites, though tardigrades are not abundant in polluted streams. This represents at present our best known taxon and indicates the amount of work yet to be done on other, perhaps more difficult taxa. Work is also under way with respect to the Rotifera, but is as yet only partially analysed.

II. Laboratory Research

Progress was made with respect to an analysis of factors present in mine acid which affect life history parameters of one test organism, the common freshwater gastrotrich Lepidodermella squammata (Hummon and Hummon, 1979). We found for various mixtures of lake and acid mine water having a total conductivity of 350 - 380 μmho/cm, that mean life expectancy was greatly reduced and reproduction ceased if the proportion attributable to carbonates was less than 5%. As the carbonate fraction was increased, mean life expectancy quickly normalized and reproductive effort was proportionately increased to near normal. The variance of reproductive effort in intermediate carbonate fractions, however, was increased, indicating that many animals were either fully reproductive or non-reproductive. These results indicate a large amount of latent heterozygosity in animals from a cloned population which were in theory genetically identical.

Work was begun on a second test animal (Appendix C) in order to set a base line of information for future studies with acid mine pollution. Lecane tenuiseta, a rotifer, was found to be longer lived and to produce more eggs more slowly and over a longer period of time than the gastrotrich. The resulting rate of increase for the rotifer, however, differed little from that of the gastrotrich, which compensated for its fewer eggs by quicker development.

A second line of approach to laboratory studies was inaugurated, using small aquatic insect larvae to determine whether the toxic effects of acid mine pollution were mediated through respiratory metabolism (Appendix D). Contrary to expectations, the results indicated no difference in oxygen uptake per unit dry weight under control or acid mine conditions.

III. Development and aging studies.

The fine structure of the primary test animal, *Lepidodermella squammata*, has been studied in specimens from a clone maintained in culture in the laboratory. This work has been carried out by a second doctoral student involved throughout the project. Gross changes observed in overall shape among normally aging animals, and among animals cultured in acid mine water, imply some disruption in osmoregulation and possible change in muscle tone. A fixation method that does not distort the animals is especially important. Best results have been obtained by direct but gradual introduction of a fine stream of fixative into a spot plate with a swimming animal. For electron microscopy, alternating groups of thick and thin sections of animals oriented for both longitudinal and cross sectioning have been prepared and studied. Identification of tissue types and arrangements is best accomplished in electron micrographs at 2000 to 20,000 X: these results can then be used to interpret adjacent thick sections with light microscopy, at 200 to 1,000 X.

Aspects of fine structure of importance to the study include the integument, the osmoregulatory system of protonephridia, the feeding apparatus, and the reproductive system. The integument consists of a series of overlapping scales, whose bases adhere closely to the epidermal syncytial layer of the normal adults. On the ventral side, the scalar pattern is interrupted by two longitudinal rows of cilia, which provide the locomotor system. Interference with normal ciliary activity is characteristic of animals in distress, either because of advancing age or adverse conditions.

The protonephridia can be observed in living animals using the light microscope at 400 to 1000 X, as very fine moving lines along the lateral portions of the abdomen: this represents the continual beating of the pair of flagella that comprise each of the two protonephridia. Flagellar beating continues in animals in distress, and has been detected in animals considered by other criteria to be dead or nearly dead. In serial electron micrographs of cross sections, the origin of the protonephridia was found to occur at the pharyngo-intestinal junction. A tangled system of canals is present in proximity to each protonephridial channal, and is concentrated ventrolaterally to each channal, with components extending along the ventral ciliary rows. No fine structural changes have been observed in the protonephridial system of aging animals.

The feeding apparatus of L. squammata includes a complex arrangement of tubular structures and filamentous projections at the EM level. In living animals, the mouth appears to be held against the substratum as the animal swims forward, and mouth and pharynx open as a result of quick contractions of the pharyngeal muscles. Gastrotrichs are generally considered to be bacteriophagous, but little is known of their selectivity in feeding. Both the culture work and fine structural evidence indicate that they both ingest and digest yeast cells. The structure of the mouth does not appear to alter with aging: its fine structure appears crystalline and is probably quite stable.

Basic information on the fine structure of the reproductive system in adults cultured in lake water correlates with the light microscopic evidence. In actively reproducing individuals, a large central germinal vesicle surrounded by lipid-rich storage material is prominent, and in electron micrographs, a series of several smaller eggs can be identified, posterior and lateral to the largest developing egg. In post-hatch juveniles, six putative egg nuclei can be identified in each of two posterior lateral locations. Cell division does not generally occur in these animals after completion of embryological development and hatching, and it appears that the number of egg nuclei is also determined prior to hatching. These findings indicate that the maximum reproductive effort in cultured animals of about six eggs is not directly limited by the availability of egg nuclei in the adult. The observed reduction in reproductive output in animals cultured in acid mine water thus may be attributed to altered rates of development of the egg nuclei already present at time of hatching.

Lepidodermella squammata has been considered to be an obligate parthenogen, based on the ability of a single isolated egg to produce viable eggs and offspring, and the absence of recognizable male structures. During the course of this study, dense elongate structures were noted in electron micrographs of post-reproductive animals, and it was suggested (Hummon, 1978) that these might represent sperm. This suggestion has since been corroborated in a study at another laboratory, using different techniques (Science 205:302-303. 1979). It has not been determined in either laboratory whether and under what conditions the sperm are functional.

Aging changes have been detected in several tissues: egg cytoplasm, epidermal tissue layer, pharyngeal muscle, and gut. The changes in egg nucleus and cytoplasm which occur during their maturation are not considered as an aging change, and are repeated up to six times in each animal. The most mature egg is clearly recognizable both by its more anterior position and by its altered appearance and size; the characteristics are similar in a post-hatch juvenile and in a post-reproductive adult whose most mature egg is certain never to be laid. Among the remaining eggs, however, changes occur with age, and in the aged animal, these reserve eggs become packed with large vesicles, presumably lipid, and are distended.

There is no apparent change in the nature of the cuticle with age, although it must increase in extent during growth of the animal. The subcuticular epidermis is a syncytium, and is densely packed with organelles in the post-hatch juvenile, with conspicuous rough endoplasmic reticulum and golgi. In the aged adult, the syncytium appears attenuated, and cell organelles are poorly represented and sparse, although the layer remains intact.

The pharyngeal muscle of all animals is arranged around a triangular lumen, and is described as tri-radiate in cross section. In all animals of all ages, there are areas of dorsal pharyngeal cells that appear filled with dense deposits of finely granular material and with little or no muscle fibrils. Their nature has not been determined. Muscle cells are conspicuous in the rest of the pharynx, with thick and thin filaments arranged in approximately hexagonal array in appropriate section. In young animals, these cells are densely filled with muscle filaments, mitochondria and nuclei. In aged animals, large vaculoles are present, and the general integrity of the fibers appears distorted. No changes were noted in the pharyngeal cuticle.

The gut contents of young animals are finely divided, with microvilli surrounding a triradiate lumen. There is evidence of intracellular digestion, with putative food particles enclosed in vacuoles. Little lipid is present. In the aged animal, the same cell organelles are present, but vacuoles are 3-10 times larger, often appear empty, and there are conspicuous lipid deposits present.

Some of the aging changes noted are to be expected from the known life history patterns of this animal. A metabolically active epidermis in the actively growing animal is indicated by the electron microscope evidence, and a quiescent tissue is demonstrated in the aged animal. Similarly, the loss of integrity in the aged pharyngeal muscle may be an indication of general decline and failure of cells to carry out repair at the necessary level. The accumulation of lipid in the gut and in the reserve egg nuclei in the aged animals is less understandable, and it implies the sequestering of considerable food resources in a way that apparently is not available to the animal.

This portion of the project is not yet completed, but will be pursued by the concerned graduate student with other support. As shown in the results of section II, it has been demonstrated that culture of *Lepidodermella squammata* in the presence of dilute acid mine water results in an increased variance in reproductive characteristics so that it has proved difficult to obtain individuals truly representative of the conditions occurring in the population as a whole. It is hoped that these unexpected problems can be resolved. Information now available on aging changes will then be used as a tool in the analysis of development and aging of the test animal when exposed to dilute acid mine drainage.

APPLICATION OF RESEARCH RESULTS.

Specifically interested in the results of this study are the following: Wayne National Forest and Soil Conservation Service, US Department of Agriculture; Ohio Environmental Protection Agency and Ohio Department of Natural Resources. The general results of the project have been communicated to representatives of the above agencies, and to industry and the public, through public meetings concerning acid mine pollution held on the Ohio University campus, and through hearings by Ohio EPA on the Hocking River Basin portion of the State Water Quality Management Plan.

The general collection format has recently been modified so as to be useful as a Meiobenthic Bioassay. Information obtained using the bioassay will be compatible with the vast array of data derived from the field portion of the project. Moreover, the general results of the bioassay can be expected to be at least 90% as accurate as the research results with expenditure of only 25% of the time per site. We will become involved with the Ohio EPA and Ohio DNR during summer 1980, and following, to assess the effects of reclamation efforts on sites heavily damaged by acid mine pollution.

Appendix A

Influence of extraction - mesh size on apparent abundance and taxon structure of sandy streamed meiobenthos, with special reference to aschelminth phyla.

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Abstract

Analysis of preserved material, passed through sieves of 500, 250, 125, 62 and 37 μm openings, was made for 16 higher taxa of freshwater meiofauna (animals < 2 mm in length). Only 38% of total meiofauna was cumulatively retained by the 62 μm sieve and 89% by the 37 μm sieve. Animals passing through these sieves were mostly gastrotrichs, rotifers and nematodes. H' taxon diversity was used to characterize structure, and $S_{H'}$ similarity, to compare the results for fauna retained cumulatively on various sieves with those for unsieved material. Only the 37μm sieve yielded both a comparable H' value and a highly similar structure of ordered taxa. The time-inefficiency of using decreased sieve openings is not compensated for by increased accuracy of results, especially with respect to aschelminth phyla. Freshwater meiobenthos are better extracted from small unsieved sand samples by multiple decantation, followed by multiple Sedgwick-Rafter cell counts.

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One of the most persistent problems in dealing with meiobenthos (animals < 2 mm in length, Swedmark, 1964) is in effectively extracting them from the substratum and gaining reliable quantitative counts. The problem is greater with muddy than with sandy substrata and greater with freshwater than with marine meiobenthos. In the first instance it is because much of the sediment is extracted along with the animals, in the second because of the smaller mean size of the freshwater animals themselves; in either case efficient extraction tends to be nullified by observational inefficiencies.

Sieving is probably the most common method of extracting preserved, quantitative material, though other methods have been used, such as elutriation (usually though not always combined with sieving), floatation, centrifugation and direct counts. Sieves with openings up to 200 - 250 μm have been popular for freshwater benthos (Edmundson & Winberg, 1971, and references), though sieves with openings down to 50 μm have recently been advocated (Mundie, 1971; Shiozawa & Barnes, 1977).

For freshwater meiobenthos (microbenthos, interstitial fauna), both Kajak and Ruttner-Kolisko (in Edmundson & Winberg, 1971, pp. 63, 124) consider that small subsamples are best treated in an unsieved condition, the animals presumably being unumerated by direct counts. In the present paper, data are presented which reinforce this conclusion with respect to sieving, and the Sedgwick-Rafter counting procedure is utilized to overcome many of the problems encountered in the enumeration of unsieved freshwater (or marine) meiobenthos.

MATERIALS AND METHODS

Material was collected from the headwaters of the Mississippi River near Lake Itasca, Minnesota in early July 1976 for another study (ms in preparation). Ninety-six 2.7 cm^3 samples representing various stations and depths from two sand bars were narcotized with 1% $MgCl_2$ for 10-15 minutes, extracted by multiple decantation and fixed in 10% formalin with Rose Bengal, as described elsewhere

(Hummon, et al., 1978). Following enumeration by multiple Sedgwick-Rafter cell counts, the material, containing flocculent debris, silt-clay, fecal pellets and micrometazoans, was pooled into a single flask. This pooled material later was washed through 500, 250, 125, 62, and 37 μm opening sieves, with sieved and non-retained fractions held for subsequent analysis.

Sieved fractions were washed into jars and made up to 50 ml (500, 250, 125 μm sieves) or 100 ml (62, 37 μm sieves). Non-retained residues were allowed 24 hrs to settle before most of the supernatant was carefully siphoned from each of several flasks. Their contents were combined and the process repeated until concentrated to 150 ml in a jar. Fractions from the 500 and 250 μm sieves were enumerated to major taxa by direct counts, all animals being tallied. Fractions from the remaining sieves and the unsieved residues were subsampled into 10 Sedgwick-Rafter (S-R) cells each and enumerated to major taxa. In all, 2,531 animals were tallied, comprising some 13.1% of the estimated total of 19,307 animals in 16 taxa. S-R replicate data were subjected to variance/mean and chi square analyses (Elliott, 1977), for total meiofauna and for each taxon, when represented by 3 or more individuals per replicate set.

To determine the effect of sieving on apparent taxon structure, H' taxon diversity (Pielou, 1975) was calculated for the cumulative fraction that would have been retained by each sieve size, from coarest to finest, or for the unsieved material. Similarity between fractions was then calculated using the $S_{H'}$ formula for shared taxon diversity (Hummon, 1974), and the results arranged into a hierarchical dendrogram.

RESULTS

Results on the fraction-residue enumeration analysis are given in Table I. Tricopterans and coleopterans were all retained on the 500 μm sieve and acarines on the 250 μm sieve, though relatively fewer from other taxa were retained. By the 125 μm sieve all members of 6 taxa and over half of 5 others were retained, yielding a misleading 62.7% mean of all taxa which represented only 15.3% of total meiofauna. By the 62 μm sieve all members of most taxa were retained and the majority of all except 2 taxa, yielding an 85.5% mean of all taxa, but only 37.8% of total meiofauna. Even with the 37 μm sieve, less than 90% of total meiofauna were retained and only 30.6% of Gastrotricha, accounting for the relative paucity of records for this taxon from the freshwater meiobenthos literature.

Chi square tests (9 df) of 33 S-R taxon-replicate sets indicated that all fell between P = .90 and P = .10 of randomness, with a mean barely exceeding P = .50. Rotifer replicates showed both the greatest aggregation and the greatest uniformity of any taxon, including total meiofauna. More extensive information is given in Table II with regard to replicate S-R counts, taken this time from the sandy streambed meiobenthos of southeastern Ohio (Hummon, et al., 1978, and unpublished data). Fauna were narcotized, fixed, and extracted as above. Subsample sets of 8 or 12 S-R replicates were taken from 1,152 samples over a period of two years by 9 different workers, each of whom had received at least minimal training prior to beginning work. Except for tardigrades and ostracods, fewer than expected chi square values exceeded the .05 level of significant aggregation. There was no clear correlation between aggregated replicates and either the source of the material being analysed or the density of the taxon (or total meiofauna) represented.

Fig. 1 shows the dendrogram of joined similarity of values, based on shared diversity of ordered taxa, as well as the respective H' values. Only material from the 37 μm sieve showed both a comparable H' value and a highly similar taxon structure to that from unsieved material. Materials from the 125 and 62 μm sieves, while comparable and similar to one another, tended to overestimate taxon diversity and showed lower similarity with materials from the 37 μm sieve and unsieved materials. Materials from the 500 and 250 μm sieves had even less comparability and similarity, either between themselves or with other materials.

DISCUSSION AND CONCLUSION

Whether or not to use sieving as a portion of the taxon enumeration procedure for freshwater meiobenthos depends upon the purpose and design of the study. If one were primarily interested in aquatic insect larvae, curstacea or oligochaetes, these data indicate that use of a 62 μm sieve would be adequate; use of coarser sieves would not be advised. While it is true that material analysed here was of limited scope, the large proportion of tiny dipterans allow the conclusions to be generalized to temporary meiobenthos of other locations and seasons. On the other hand, if one were interested in the entire meiobenthos, and particularly in members of the aschelminth phyla, the alternatives lie between use of a 37 μm sieve or treating unsieved material in some other enumerative manner. Use of a 37 μm sieve is time consuming, inefficient with respect to retention of juvenile nematodes and smaller species of rotifers and gastrotrichs, and even in many sandy sediments does not solve the problem of observational inefficiencies. A preferred method is to subsample small samples and enumerate taxa using multiple Sedgwick-Rafter cell counts. In the S-R cell, observational efficiency is favored by shallow depth (1mm) and broad area (10 sq. cm).

Single S-R cell counts were used for meiobenthos by Neel (1948) and Urban (1971), though as part of a more complex collection-assessment procedure than mine (detailed in Hummon, et al., 1978). Edmondson (in Edmondson & Winberg, 1971 p. 134) advocates use of counts from two cells for normally acceptable results and of counts from three or more cells for increased accuracy. Kutkuhn (1958) concludes that use of five cell counts will provide a 90% probability of being within the 95% confidence limits of random subsampling. Data included herein indicate that use of eight-twelve cell counts provides at least a 95% probability of being within these confidence limits for total meiofauna and a wide variety of taxa. Such cell counts are reliable and repeatable, and require no greater expenditure of effort than that generally needed to sieve materials through a 37 μm sieve. And, unlike sieving, cell counts include enumeration to taxon in the process.

Taxon similarity is an important means of assessing changes in community structure over time or space. Replicate samples from the same habitat taken temporally tend to have mean $S_{H'}$ values of 50 to 70; those from spatially different portions of the same general habitat tend to have mean $S_{H'}$ values of 25 to 45 (Hummon, 1974; 1976; Hummon & Hummon, 1977; Hummon, et al., 1978). Sieving with anything coarser than 37 μm openings tends to result in sizable alterations of apparent meiobenthic taxon structure. Data given above indicate that these alterations yield similarities which approach or fall below those found between samples taken over a temporal or spatial series. Such discrepancies can best be avoided by multiple decantation of small unsieved samples, followed by subsampled enumeration of taxa using multiple Sedgwick-Rafter cell counts.

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FIGURE LEGEND

FIG. I. Dendrogram showing similarity of ordered taxa for meiofauna that would be retained cumulatively on various sized sieves or in unsieved material, along with H' values in bits for the respective fractions. Data are based on $S_{H'}$ values of shared taxon diversity. SS = Sieve size, openings in μm; U = unsieved.

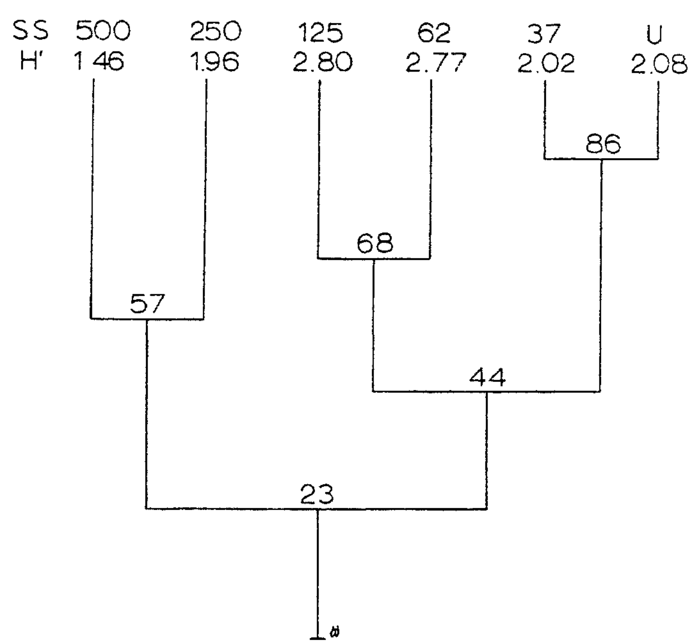

TABLE I. Cumulative percent of total meiofauna and of individual taxa that would be retained on sieves of decreasing pore sizes or that would be present in unsieved material.

Taxon	Number	Sieve Size 500 μm	250 μm	125 μm	62 μm	37 μm	Unsieved
Total Meiofauna	19,307	0.6	2.4	15.3	37.8	88.6	100.0
Gastrotricha	1,145	--	--	--	2.6	30.6	100.0
Nematoda	1,673	0.5	3.5	20.5	60.5	80.3	100.0
Rotifera	11,415	--	--	0.7	12.1	90.7	100.0
Tardigrada	2,550	--	--	31.6	95.1	99.4	100.0
Turbellaria	117	--	6.0	23.1	82.9	100.0	100.0
Ostracoda	62	--	11.3	51.6	100.0	100.0	100.0
Harpacticoida	529	--	1.5	52.2	100.0	100.0	100.0
Cyclopoida	119	--	4.2	60.5	100.0	100.0	100.0
Diptera	1.074	7.6	22.7	68.3	100.0	100.0	100.0
Oligochaeta	520	5.0	24.0	94.2	100.0	100.0	100.0
Cladocera	65	--	--	100.0	100.0	100.0	100.0
Plecoptera	11	9.1	9.1	100.0	100.0	100.0	100.0
Ephemeroptera	21	9.5	52.4	100.0	100.0	100.0	100.0
Acarina	2	--	100.0	100.0	100.0	100.0	100.0
Tricoptera	3	100.0	100.0	100.0	100.0	100.0	100.0
Coleoptera	1	100.0	100.0	100.0	100.0	100.0	100.0
Mean of Taxa		14.5	27.2	62.7	84.6	93.8	100.0
SD		33.6	38.6	38.0	31.9	17.7	--

TABLE II. Proportion of taxon - replicate sets, analysed by means of whole Sedgwids - Rafter cell counts, for which aggregated dispersion of subsamples was indicated. Data were based on 1,152 faunal samples from the sandy streambed meiobenthos of southeastern Ohio.

Taxon	No. S-R Replicate Sets Assessed	Percent Exceeding the 95% Confidence Limits of Randomness
Tardigrada	74	8.1
Ostracoda	14	7.1
Total Meiofauna	951	4.8
Rotifera	555	4.7
Nematoda	506	4.5
Diptera	229	3.9
Oligochaeta	188	3.7
Cyclopoida	70	2.9
Harpacticoida	74	2.7
Gastrotricha	242	2.1
Turbellaria	118	1.7
Cladocera	20	0.0
Ephemeroptera	2	0.0
Collembola	1	0.0
Summary	3,044	4.2

Appendix B

Morphological variability of *Isohypsibius saltursus* and notes on other Tardigrada of southeastern Ohio streams.

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Abstract: The eutardigrade *Isohypsibius saltursus* has a life cycle incorporating seven instars, under non-polluted stream conditions at Strouds Run west. Information was derived from frequency plots of body, buccal apparatus and buccal tube lengths, correlated with diagnostic characters such as buccal tube width and hind-claw branch lengths. Results were applied to data from shed cuticles, simplex stages and gravid females. Sexual maturity began at the end of the second instar, body length ca 240 μm. Buccal apparatus and buccal tube lengths regressed against body length produced slopes of 0.15 and 0.09 respectively, values apparently diagnostic for local populations. Variability in body length was accounted for almost entirely by morphometric instar growth, with ecological parameters, aside from sediment size and sorting, having little effect. Measurements of mounted specimens may be adversely affected by orientation, mounting

medium, coverslip pressure, or the optical system used; caution is advised when describing or identifying animals in the mounted condition. Tardigrade species-numbers and abundances were reduced under acid-mine polluted and reclaimed conditions; specimens obtained from these sites were usually difficult to identify because of poor body condition.

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The phylum Tardigrada is comprised of the "water bears", common micrometazoans in aquatic and semi-aquatic habitats within marine and freshwater ecosystems. Ranging in size from 50 to 1200 μm, tardigrades possess a short cylindrical body from which project four pairs of legs tipped with claws or adhesive pads. Claws are used for clinging to vegetation, debris and sediment granules, and the legs for the distinctive sluggish-crawling or creeping mode of locomotion. The digestive tract, modified to facilitate ingestion of liquids, contains a triradiate pump-sucking pharynx, which is utilized after a long pair of stylets pierce a food source releasing its contents, and a simple intestine. Respiratory or circulatory organs are lacking, as is correlated with the minute size of tardigrades.

To date, the only study of Ohio tardigrades was by Fleeger & Hummon (1975) on soil species. No freshwater tardigrade species have been reported previously. The main purpose of the present study was to analyze morphometric variability within a single species, obtained from an unpolluted stream site over the period of a year. This variability was partitioned between intrinsic factors, such as allometric growth or molt cycles, and extrinsic factors, such as one or more ecological parameters or artifacts of the measuring-mounting system.

METHODS AND MATERIALS

Field work was done in July and September 1977, and in February and April 1978, at a site on the freshwater stream Strouds Run west, Athens County, Ohio (NE 1/4, NE 1/4, Sec. 35, T5N, R13W, Ohio River Survey). On each of these four days, a series of sediment samples was collected using a 20cc plastic coring syringe holding sediments of 8.5cm depth and 1.85 cm diameter. Samples gathered at substratum depths of 0-1, 1-2, 3-4, and 6-7cm, beneath surface elevations of +2, -3 and -10 cm (relative to waterline), have been considered in this study.

Fifteen replicates for each elevation and depth were divided among three replicate jars so that each jar contained five core segments, or 13.5cm^3 of sediment.

Live tardigrades and shed cuticles were extracted in the laboratory from two jars per set, using a separatory funnel elutriator, through which tap water was passed from bottom to top for five minutes each at slow, medium and high speed. Materials retained on a 37 μm sieve were washed into Petri-dishes and carefully examined for tardigrades using a Wild M8 binocular microscope. Contents of the third jar per set were utilized for sediment analysis, modified from the sieving and pipette analysis of Folk (1968).

Animals were removed from Petri-dishes, using a mouth pipette, narcotized with 50% ethanol, and transferred by Irwin loop to a drop of tap water on a microscope slide, without cover-slip, to allow for further relaxation and slow recovery. A series of 10 measurements was taken on each animal (Fig. 1) using a Leitz HM-LUX compound microscope, equipped with phase contrast optics and a calibrated occular micrometer. Magnification of 600X was used for all measurements except body length, which was assessed at 150X; estimated error factors were ±1 and ±5 μm, respectively.

Growth instars were interpreted separately for measurements obtained from live tardigrades and shed cuticles. A total of 424 live animals (396 possessing a buccal apparatus) and 77 complete or nearly complete and 18 incomplete cuticles (with or without eggs) was used in the study of instars.

Animals were identified using the taxonomic keys of Cuénot (1932), Morgan & King (1976), Ramazzotti (1972) and Schuster et al. (1977). Identifications were made on narcotized animals, to prevent errors due to specimen orientation or mounting. In the July and September collections, every fifth tardigrade was permanently mounted while narcotized using one of three Turtox media: aniline-blue staining CMCP-9AB, acid-fuchsin-red staining CMCP-9AF, or non-staining CMCP-10.

Animals in permanent mounts were remeasured, so that paired results for 40 animals mounted in each medium could be analyzed.

RESULTS

Two eutardigrade species were found at Strouds Run west. The more abundant was Isohypsibius saltursus Schuster, Toftner and Grigarick, 1978 , a species only recently described from Lake Tahoe, California, and confirmed by D.R. Nelson (East Tennessee State University, Johnson City) and R.O. Schuster (University of California, Davis). Using Ramazzotti (1972), this species keyed out to I. augusti (Murray, 1907), although the dissimilar Isohypsibius-type claws were much thicker and had distinct accessory points in I. saltursus (Fig. 1b). Further, distance from the stylet supports to the posterior end of the buccal tube was 2X the buccal tube width in I. saltursus (Fig. 1c), but 3X the width in I. augusti (Schuster et al. 1977). Three pharyngeal macroplacoids were present, the first and last usually equal in length and the middle somewhat variable (Fig. 1c). This species has also been identified from the James River, Virginia (Munson, 1980).

Plotting buccal apparatus and the tube length against frequency for I. saltursus yielded seven instars (Fig 2a, b), whereas plotting length against frequency did not clearly define instars (Fig. 2c). Using buccal tube widths and placoid and claw IV branch lengths (Table I), it was possible to define ranges for body lengths corresponding to the seven instars indicated by buccal apparatus and the tube data. This series was further generalized (Table II) to shed cuticles, gravid females, and simplex stages, which lacked the buccal apparatus. Sexual maturity began at the end of the second instar, ca 240 µm body length. Egg lengths were 50-72 µm, with up to eight eggs being noted in some larger specimens of instar VI. No shed cuticles, gravid females, or animals in the simplex stage were found representing the final instar (VII).

Scattergram plots, indicating direct relationships between body length and buccal apparatus and tube lengths, were confirmed by linear regression (Table III) for both individual collection days and pooled results, each being significant at the .001 level. Slopes for combined data, of 0.15 and 0.09 respectively, remained constant between instars. These slope and intercept relationships appear to be diagnostic for I. saltursus from the Strouds Run west site.

Body lengths in all collections measured 140-750 µm. February and April collections showed a reduction in number of tardigrades and a general decrease in size of measured characters, when compared to July and September collections (Table IV). Multiple stepwise regression was performed on body length results to determine how they varied with extrinsic ecological parameters (date of collection, season and time since last precipitation; elevation with respect to waterline; sediment depth and mean sand grain size; and sample replication), as opposed to the intrinsic factor of morphometric growth expressed as instars (Table V). More than 88% of body length variability was accounted for by morphometric changes attributable to instar growth. Effects of extrinsic ecological parameters on body length were negligible, increasing both the multiple R and R^2 results only slightly, and accounting for less than 1% of the body length variability when combined. Simple R results (Table V) showed .94 correlation between body length and instar growth. Correlation between body length and extrinsic parameters was highest with elevation relative to waterline, followed in descending order by collection date, sediment depth, and sample replicate, although only the first two factors were significant.

Numbers of tardigrades (Table IV) were drastically reduced in February and April, a trend found in other meiofaunal groups as well, particularly oligochaetes, turbellarians, rotifers and gastrotrichs. When figures for the abundance of I. saltursus on these four collection dates were combined with those of Hummon (unpubl. data), they yielded the annual growth cycle shown in Fig 3. Peak abundances occurred in early to mid-summer and early fall, and ebbs in winter.

The decline observed during the summer of 1977 may be explained by a period of extended drought. During this time, the sample site was often above water, placing meiofauna under severe stress and probably accounting for the reduction in numbers.

Numerous eidostic (Hummon, 1971) and metric changes were noted when *I. saltursus* specimens were mounted in Turtox media and under coverslip pressure. Body length was often reduced and the cuticle wrinkled, probably resulting from fluid loss to the hyperosmotic mounting medium. The first two placoids were often pushed together by tissue shrinkage, appearing as a single placoid with a median constriction; sometimes all three placoids appeared as a broadened, continuous bar. The pharynx was often flattened by coverslip pressure, changing its shape from an oval (length>width) to a circle or even a rotated oval (length<width). Claw branches, while unchanged in size, were often oriented so poorly that critical taxonomic features could not be clearly distinguished. In some cases incorrect identifications might have been made had not the genus and species of the live, unmounted animal been determined earlier. Changes in body length and ratio of pharynx length to width, both important in species descriptions, were also noted.

Body, buccal apparatus, and buccal tube lengths of animals measured live and after mounting in the Turtox media are given in Table VI, along with results of paired t-tests. Body and buccal tube differences were significant (.001>P) for each set of data analyzed, while buccal apparatus differences were of lesser significance for CMCP-9AF and CMCP-10 and not significant for CMCP-AB. Results generally substantiate eidostic changes in the pharynx, noted above, the pharynx being the only part of the buccal apparatus which is not wholly part of the buccal tube. Increased pharynx dimensions, under coverslip pressure, appeared variably to counteract shrinkage found in the buccal tube under the mounted condition.

Data for mean particle sizes and mean body lengths of *I. saltursus* and their respective sorting and standard deviation values were subjected to intercorrelation analyses. Results indicate significant relationships between both mean values and their respective deviation values, and between mean body lengths and mean particle sizes, regardless of how partical size is measured (Fig. 4). This indicates that coarser grains laid down by rapidly running waters, directly following periods of measurable precipitation, were better sorted (i.e. more uniform) and contained a narrower range of interstices, in which lived smaller but less variable sized tardigrades. Likewise, finer grains laid down by waters of fluctuating speeds, following trace precipitation or a longer time period following a previous precipitation, were more poorly sorted (i.e. less uniform) and contained a broader range of interstices, in which lived larger but more variable tardigrades. Caution is urged at two points: the amount of material necessary for an accurate determination of sedimentary parameters is vastly greater than that needed to provide living space for one or more tardigrades; and for each set, the sample analyzed for sediments was assumed to be representative of other samples from which live tardigrades were extracted.

NOTES ON OTHER TARDIGRADA

Macrobiotus pullari (Murray, 1907), the second species found at Strouds Run west, was collected during the months of November, February and April, and was more abundant in the latter two months when the population of I. saltursus at this site was scarce. Live specimens were obtained from February and April samples, the former yielding eight animals 150-330 μm in length and the latter containing four animals and one shed cuticle, lacking eggs, with lengths of 170-360 μm (Table VII). November specimens, obtained from the preserved material of Hummon (unpubl. data), were used for identification purposes only. The legs of this species possessed pairs of similar claws of the V-shaped echinogenitus-type (Fig. 5a), with strong accessory points and crescent-shaped basal lunules. Two macroplacoids were present, the first being 2X the length of the second and containing a noticeable median constriction, which divided it into two nearly equal structures (Fig. 5b).

Tardigrades from studies conducted over the two years 1976-78 (Hummon et al. 1978 and unpubl. data) are shown in Table VIII, along with the site conditions (unpolluted, acid-mine polluted, or reclaimed) under which they occurred. In general, animals were more numerous at unpolluted than at other sites, although there is some overlap between unfavorable unpolluted sites and favorable polluted sites. Specimens from unpolluted sites were also in better condition and hence were more easily identified, than those from other sites.

Unpolluted sites - Sample sites at Strouds Run north, Margaret Creek west, and Oppossum Creek yielded numerous specimens identified as Macrobiotus dispar (Murray, 1907). This species possessed massive macronyx-type claws in which the principal arms of each double claw were long and had noticeable accessory points (Fig. 5c). Under oil immersion, a distinct chitinous bar was seen to connect the doubleclaws of each leg. Two macroplacoids were present, the first being about 2X the length of the second and having a median constriction, which appeared to divide this placoid in half when viewed in the mounted condition (Fig. 5d).

Macrobiotus hastatus (Murray, 1907), was found in small numbers at Cherry Creek. This small tardigrade had doubleclaws of the V-shaped echinogenitus-type (Fig. 5e), and possessed a small lunule at the base of each leg. The pharynx contained three macroplacoids, with the first two very close together and of about equal length; a smaller third macroplacoid followed the first two.

Two previously discussed species, I. saltursus and M. pullari, were found at the Margaret Creek south and west sites respectively, bringing the species total to five from all unpolluted sites and no more than two from a single site. Though no tardigrades were found at the upper, unpolluted site on Trace Run, they were found at all seven of the other non-polluted sites for a spatial presence of 88%.

In the two year-round study sites at Strouds Run, tardigrades were found 13 of 14 times for a temporal presence of 93%.

Acid-mine polluted sites - Both the lower and upper sites at Sandy Run contained tardigrade species. The former contained a specimen identified by its distinctive C-clamp shaped macronyx-type claws as Macrobiotus macronyx (Dujardin, 1851). This specimen was unique in that it also contained three epizootic protozoans on its body. The specimen may have been dead before it was collected, having been transported to the sampled area from somewhere upstream. The latter site at Sandy Run yielded a poorly preserved Hypsibius species, whose poor condition made further identification impossible. The few tardigrade specimens from Long Run and Snow Fork were not seen. Material collected from the majority of polluted sites, 9 of 14, lacked tardigrades, with a spatial presence of but 36% and a temporal presence in the two year-round study sites at Sandy Run of but 29%.

Reclaimed sites - The lower and upper sites at the reclaimed stream near Tick Ridge yielded one poorly preserved specimen each, believed to be Hypsibius arcticus (Murray, 1907).

DISCUSSION

Tardigrades possess a state of eutely (Marcus, 1928), in which the number of cells is not increased after birth. Growth occurs by expansion of cells, animals shedding their old cuticle and any related hardparts to accommodate the increase in size. Preceding ecdysis, cuticular components of the buccal apparatus are discharged, resulting in the simplex stage. Just prior to the shedding of the cuticle itself, the tardigrade enters a period of retrograde growth that ceases shortly after it exits through the split anterior end of the old skin. A new buccal apparatus, proportionate to the new body length, is secreted by salivary glands shortly after the molt is completed. Hypodermal cells secrete a new cuticle and, since the claws remain attached to the molted cuticle, new claws are formed by secretions of glands located at the tip of each leg.

Higgins (1959) demonstrated that annual growth of the eutardigrade Macrobiotus islandicus Richters produced body- and buccal apparatus-length - frequency curves which contained a series of peaks and valleys, representing instars and ecdyses. He also showed a high positive correlation between body and buccal apparatus length. We found seven such growth instars for Isohypsibius saltursus based on correlation between body, buccal apparatus, and buccal tube lengths, and on buccal tube width and claw IV branch lengths. Use of body length itself did not indicate distinct instars, because of overlapping body lengths at the end of one instar and beginning of the next. Occasionally, as Hallas (1972) had previously indicated, smaller specimens possessed larger buccal apparatus and tube lengths than larger ones. We attribute this to allometric growth within an instar. Buccal apparatus and claws, being comprised of dense chitinous material (Jeuniaux, 1975), cannot expand as can the more flexible cuticle; hence, their maximum sizes for a particular instar would be established at the onset of the instar. Viewed throughout the life of an individual, buccal apparatus length shows sharply stepwise growth relative to the more undulating clinal growth of total body length. Whether males and females possess identical growth patterns is difficult to determine, due to the lack of sexual dimorphism.

For I. saltursus from Strouds Run west regression slope and intercept values, based on ratios of body length to buccal apparatus and tube lengths respectively, appear fairly constant over time and instar. Whether this is true for geographically separated populations of this species is not yet known. The holotype from Lake Tahoe, California, belonging to instar VII, showed body and buccal tube lengths of 561 and 33 μm. Beginning from our intercept the regression slope of this animal would be 0.02, not 0.09.

Calculations of regression slope and intercept values were made from data on Murray's preserved type series of I. augusti: (n=21; measurements from Van der Land, 1966). Body length vs. buccal apparatus length gave a slope of 0.14, intercept 41.29; body length vs. buccal tube length gave a slope of 0.07, intercept 26.63. The slopes, if representative of live as well as preserved specimens, lie within the ranges we calculated for I. saltursus (Table III), but the intercepts are considerably greater than those we calculated. Our population thus appears to possess an allometrically larger buccal apparatus, relative to body length, than either I. saltursus from Lake Tahoe or I. augusti from Scotland.

Schuster et al. (1977) assumed that I. saltursus specimens with body length of 200-245 µm were juveniles. Similarly, we found no shed cuticles with eggs for animals below 240 µm. A nine month reproductive cycle was noted for animals from Lake Tahoe, with greatest abundance in August-September and the first noticeable population depletion occurring in mid-October. Just under 50% of shed cuticles in that population contained eggs, of 67-82 µm in diameter. These trends were noted at Strouds Run west, although egg diameters were 50-72 µm in slightly more than half of the cuticles which bore eggs.

Swedmark (1964) suggested that micrometazoan body size may vary with sediment size, accounting for discrepancies in length reported for a given species from different sediments. Seasonal variation in body length was noted by Hallas (1972) within a population of soil eutardigrades, reflecting the limitation set by pore spaces in the soil, migration between substratum layers, and the relatively small number of samples taken. Multiple regression results indicated that for I. saltursus instar growth accounted for the majority of body length variability recorded. Effects of extrinsic factors must have cancelled those of intrinsic instar growth to some extent, since both elevation of sediment surface and collection data had greater impact in simple than in multiple regressions. On the other hand, we did find evidence supporting an inverse relationship between body length and sediment size.

Although metric changes alone have been considered for eutardigrades, indications of morphological changes due to growth may also involve meristic and eidostic characters. This was demonstrated for several heterotardigrades by McGinty & Higgins (1968), Renaud-Mornant & Anselme-Moizan (1969), Grigarick et al. (1975) and Lattes (1975), working with Batillipes mirus Richters, Stygarctus bradypus Schultz, Echiniscus trisetosus Cuénot and E. oihonnae Richters, and E. quadrispinosus Richters, respectively. We found no meristic changes in Isohypsibius saltursus with growth, and eidostic changes were mostly those introduced by coverslip pressure in mounted specimens.

Results of variability due to mounting and measuring systems have not often been quantified. McGinty & Higgins (1968) stated that variation may reflect inefficiencies in an optical system, in preservation techniques, in methods of observation, and in orientation of a specimen. Pilato (1975) held further that some structures, such as buccal tube and pharngeal bulb, whose dimensions are used in the identification of tardigrade species, "may have their measurements altered through technical manipulation of the specimens, such as the distortion of the body dimensions because of coverslip pressure". If the Turtox media used in this study are indicative of other mountants, such as Hoyer's, which are used for tardigrades, then key measurements may be significantly altered due to osmotic fluid loss to the medium. Extreme care should be used when attempting to describe or identify specimens which have been so mounted, ideally, the description of Tardigradia should be performed on unsqueezed narcotized specimens. Information on changes (particularly metric ones) occurring after permanent mounts have been completed being used to supplement these results.

In conclusion, we have shown that variability in body length was accounted for almost entirely by morphometric instar growth, with ecological parameters, other than sediment size and sorting, having relatively little effect. Caution is urged in working with mounted specimens because of possible adverse effects resulting from improper orientation, certain types of mounting media, coverslip pressure or inferior optical systems. Six species in three genera were identified from southeastern Ohio streams, four species in unpolluted streams and two in those having a past history of acid-mine pollution. Tardigrade species numbers and abundances were reduced under polluted conditions, with specimens being difficult to identify because of generally poor body condition.

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FIGURE LEGENDS

FIG. 1 - Diagrammatic sketches of Isohypsibius saltursus showing measurements made and discussed in this study: (a) BL, body length, dorsal midline from mouth to hind end of body in an extended specimen; (b) hind claw branches from insertion of claw to tip of claw branch for both the primary and secondary branches; (c) buccal mechanism showing buccal tube width (BTW), buccal apparatus length (BAL) from mouth to end of pharynx, buccal tube length (BTL) from mouth to end of tube itself, pharyngeal part of the buccal tube (PP) from posterior end of buccal tube to stylet support (SS), macroplacoid lengths (PL) and pharynx length (PHL). Pharynx width (PHW) not shown.

FIG. 2 - Frequency plots of buccal apparatus length (2a) and buccal tube length (2b) in Isohypsibius saltursus, indicating the presence of seven instars and of body length (2c), lacking distinct indication of instars.

Fig. 3 - Plot of adjusted log-transformed abundance values for Isohypsibius saltursus, expressed as animals per 10 sq. cm. Geometric means are shown adjacent to corresponding points on the graph. Triangles, this study; circles, Hummon et al. (1978 and unpubl. data).

Fig. 4 - Diagrammatic representation of correlation values (R) between animal body lengths and sediment sizes based on all samples containing 2 or more specimens of I. saltursus (N = 22). Abbreviations: mean body length (Mean BL); standard deviation of body length (S.D. BL); mean phi size (MN ϕ); mean micrometer particle size (MN μm); sorting coefficient of particle size (SO ϕ). Symbols for significance levels are as in Table V.

Fig. 5 - Claws (a) and buccal apparatus (b) of Macrobiotus pullari; claws (c) and buccal apparatus (d) of M. dispar; and claws (e) of M. hastatus from southeastern Ohio sites.

TABLE I - Instar number and body length (BL) ranges for Isohypsibius saltursius based on measurements of buccal tube width (BTW), three macroplacoid lengths (PL) listed in order from first to third, and paired branches of the hindclaws (CLAW IV). Overlaps in body lengths appear to be transitional in respect to the other measurements between the previous and succeeding instars. All measurements are in μm.

Instar	BL range	BTW	PL	CLAW IV
I	<170	2-2½	2½,2,2½	10,8 and 8,7
II	170-270	3	3,2½,3 or 3,3,3; rarely 3,2,4	12,8 and 9,7 or 12,8 and 10,8
III	240-345	4	4,3,3, or 4,3,4; 4,3,5, at 300; rarely 5,3,5, at 340	12,9 and 10,8 or 12,10 and 10,9; 15,10 and 12,10 at 280
IV	320-430	4 until 375 5	5,3,5 or 5,4,4,	15,12 and 12,10; 18,12 and 14,10-12 at 350
V	400-460	5 until 420 6	6,4,5 or 6,4,6; rarely 7,4,6 or 7,5,7	18,12 and 14-15,12-14
VI	450-560	6 until 500 7	7,5,7; rarely 7,5,6 or 7,5,8	20,12 and 15,10 or 20,15 and 14-15,12-14
VII	>540	7-8	7,5,7 until 570; becomes 8,6,8	22,15 and 15-18, 12
	(largest was 750)	(14 at 750)	(14,12,10 at 750)	(32,22 and 22,20 at 750)

TABLE II - Body, claw IV and placoid (where applicable) lengths of shed cuticles, gravid females and simplex animals of Isohypsibius saltursus, associated with various growth instars. Abbreviations are as in Table I.

	Instar	BL	CLAW IV	PL
SHED CUTICLES WITH EGGS:				
	end of II	240 (2-3 eggs)	12, 8 and 10, 8	-
	end of III	320-350 (2-7 eggs)	15, 10 and 12, 10	-
	end of IV	400-420 (4-7 eggs)	18, 12 and 15, 12; or 20, 15 and 14, 12	-
	end of V	445-450 (2-5 eggs)	18, 12 and 15, 14; or 22, 14 and 15, 12	-
	end of VI	500-530 (2-8 eggs)	20, 15 and 15, 14	-
SHED CUTICLES WITHOUT EGGS:				
	end of III	310-330	15, 12 and 12, 10; or 18, 12 and 15, 10	-
	end of III	360	18, 12 and 14, 10	-
	end of IV	380-390	21, 12 and 18, 11; or 21, 16 and 18, 15	-
	end of IV	410-435	18, 15 and 14, 12; or 20, 15 and 15, 12	-
	end of V	440-470	18, 12 and 15, 14; or 20, 15 and 18, 12; or 21, 15 and 18, 14	-
	end of VI	500	20, 12 and 15, 10	-
GRAVID FEMALES:				
	end of III	340 (5 eggs)	15, 12 and 12, 10	5,3,5
	end of VI	530 (7 eggs)	20, 15 and 15, 12	7,5,7
SIMPLEX ANIMALS:				
	start of I (?)	140	10, 8 and 8, 7	-
	start of III	220	12, 10 and 10, 8	-
	start of IV	270-280	18, 12 and 12, 10	-
	start of IV	310-320	18, 12 and 14, 12	-
	start of IV	350	18, 12 and 14, 12	-
	start of V	390	18, 12 and 14, 12	-
	start of VI	420	20, 15 and 14, 12	-

TABLE III - Linear regression results for body length (BL) versus buccal apparatus length (BAL) and buccal tube length (BTL) of *Isohypsibius saltursus*. All results are significant at the .001 level.

BL vessus BAL

Date	Slope	Intercept	Correlation (R)
July 27, 1977	0.15	21.61	0.87
Sept. 30, 1977	0.15	24.97	0.81
Feb. 4, 1978	0.13	31.32	0.99
Apr. 2, 1978	0.12	25.96	0.93
Combined data:	0.15	22.86	0.85

BL versus BTL

Date	Slope	Intercept	Correlation (R)
July 27, 1977	0.08	19.22	0.74
Sept. 30, 1977	0.09	20.19	0.72
Feb. 4, 1978	0.09	21.54	0.97
Apr. 2, 1978	0.07	21.00	0.80
Combined data:	0.09	18.80	0.75

TABLE IV - Body length (BL), buccal apparatus length (BAL) and buccal tube length (BTL) of Isohypsibius saltursus shown with their respective standard deviation values (S.D.) for each collection day and the pooled results.

Date	Variable	Mean (S.D.) μm	Number of animals
July 27, 1977	BL	341.81 (101.28)	116
	BAL	72.01 (17.14)	
	BTL	47.68 (11.47)	
Sept. 30, 1977	BL	378.65 (90.14)	267
	BAL	82.32 (16.66)	
	BTL	55.10 (11.48)	
Feb. 4, 1978	BL	359.17 (206.80)	6
	BAL	79.50 (27.98)	
	BTL	52.67 (18.47)	
Apr. 2, 1978	BL	298.57 (116.54)	7
	BAL	62.86 (15.54)	
	BTL	40.86 (9.63)	
Combined data:	BL	366.15 (97.81)	396
	BAL	78.91 (17.68)	
	BTL	52.64 (12.10)	

TABLE V - Summary table for multiple stepwise regression and simple regression, considering the sources of variability for body length results presented in Table IV for Isohypsibius saltursus (n = 396). Key symbols: NS P < .05; * .05 < P; ** .01 < P; *** .001 < P.

VARIABLE	MULTIPLE R VALUE	R^2 VALUE	CHANGE IN R^2	CUMULATIVE % ACCOUNTABLE VARIABILITY	SIMPLE R VALUE
Instar growth	0.93943	0.88252	0.88252	88.25	0.93942***
Collection date	0.93944	0.88254	0.00002	88.25	0.10633*
Elevation of sediment surface	0.94061	0.88474	0.00220	88.45	0.22225**
Sediment depth	0.94088	0.88526	0.00052	88.53	0.06097^{ns}
Sample replicate	0.94091	0.88532	0.00006	88.53	-0.04605^{ns}

TABLE VI - Compared results for mean (and standard deviation) of body length (BL), buccal apparatus length (BAL), and buccal tube length (BTL) in <u>Isohysibius saltursus</u>. Individuals were measured, mounted in one of the media and remeasured. Two-tailed t-test results for paired differences are given at the right. Symbols for significance levels are as in Table 5.

MEDIA	CONDITION	VARIABLE	MEAN	(S.D.) μm	N	t-value	
CMCP-9AB	Live	BL	444.62	(60.52)	39		
		BAL	90.45	(9.76)	38		
		BTL	59.66	(7.34)	38	BL	17.31***
						BAL	0.61 n.s.
	Mounted	BL	281.28	(69.86)	39	BTL	6.23***
		BAL	88.50	(9.59)	38		
		BTL	50.18	(4.64)	38		
CMCP-9AF	Live	BL	380.71	(86.52)	35		
		BAL	80.03	(14.41)	34		
		BTL	51.65	(9.27)	34	BL	9.48***
						BAL	-3.53**
	Mounted	BL	285.43	(83.12)	35	BTL	4.47***
		BAL	83.74	(16.16)	34		
		BTL	48.03	(8.34)	34		
CMCP-10	Live	BL	417.03	(80.13)	37		
		BAL	87.24	(14.41)	34		
		BTL	57.82	(11.19)	34	BL	12.20***
						BAL	2.11*
	Mounted	BL	274.87	(65.76)	37	BTL	5.49***
		BAL	81.35	(12.73)	34		
		BTL	50.18	(6.97)	34		

TABLE VII - Measurements from live *Macrobiotus pullari* specimens found at Strouds Run west. Abbreviations are as in Fig. 1. All measurements are in μm.

DATE	BL	BAL	BTL	PP	BTW	PH.L	PH.W	PL	CLAW IV	(N)
1978 Feb. 4	150-160	55-58	38	10	3	25-28	20-22	5,3	12, 8 and 12, 8	(5)
	250	72	48	10	4	35	30	8,5	18, 12 and 18, 12	(1)
	330	70-75	45-50	10	4	38-42	35	8,5	18, 12 and 18, 12 or 19, 14 and 18, 14	(2)
1978 Apr. 3	170	60	38	8	2½	25	20	4,3	12, 10 and 12, 9	(1)
	280	82	42	10	4	38	32	8,5	20, 12 and 18, 12	(1)
	300	78	55	10	4	42	35	8,6	20, 15 and 20, 15	(1)
	330	(shed cuticle lacking eggs)				--	--	--	22, 15 and 20, 15	(1)
	360	100	65	10	5	45	38	10,6	21, 15 and 21, 15	(1)

TABLE VIII - Tardigrade species found at various unpolluted, acid-mine polluted, and reclaimed streams in southeastern Ohio.

STREAM	SITE	DATE	Number/10 sq. cm surf. $G_{\bar{x}}$	(95% C.L.)	SPECIES PRESENT
Unpolluted streams					
Strouds Run	West	Sept. '76	45.8	(1.3 to 967.9)	
		Nov. '76	7.6	(0.4 to 50.8)	
		Mar. '77	0.7	(0.0 to 7.4)	
		Apr. '77	9.0	(0.0 to 131.0)	*I. saltursus*; *M. pullari*
		June '77	102.1	(22.0 to 461.8)	
		July '77	23.2	(3.4 to 133.7)	
		Nov. '77	20.3	(2.1 to 144.0)	
	North	Sept. '76	0.5	(0.0 to 5.1)	
		Nov. '77	5.5	(2.1 to 12.6)	
		Mar. '77	6.0	(0.0 to 55.4)	*M. dispar*
		Apr. '77	3.1	(0.0 to 18.6)	
		June '77	0.5	(0.0 to 4.5)	
		Nov. '77	3.3	(0.0 to 64.6)	
Margaret Creek	West	Nov. '76	25.5	(2.3 to 213.9)	*M. dispar*; *M. pullari*
Oppossum Creek		Oct. '77	4.6	(0.0 to 19.7)	*M. dispar*; *Isohysibius* sp.
Margaret Creek	South	Nov. '76	2.6	(0.0 to 38.1)	*I. saltursus*
Willow Creek		Oct. '77	0.7	(0.0 to 9.1)	not seen
Cherry Creek		Oct. '77	0.5	(0.0 to 4.5)	*M. hastatus*
Polluted Streams					
Sandy Run	Lower	Nov. '76	4.3	(0.0 to 38.6)	*M. macronyx*
		Apr. '77	0.4	(0.0 to 2.7)	
	Upper	Nov. '76	0.4	(0.0 to 3.4)	*Hypsibius* sp.
		Mar. '77	1.2	(0.0 to 8.2)	
Long Run	Lower	Oct. '76	1.6	(0.0 to 15.4)	not seen
	Upper	Oct. '76	1.3	(0.0 to 10.1)	not seen
Snow Fork		Nov. '77	0.5	(0.0 to 4.7)	not seen
Reclaimed Streams					
Tick Ridge	Lower	Nov. '76	0.3	(0.0 to 2.4)	*H. arcticus* (?)
	Upper	Nov. '76	0.3	(0.0 to 2.1)	*H. arcticus* (?)

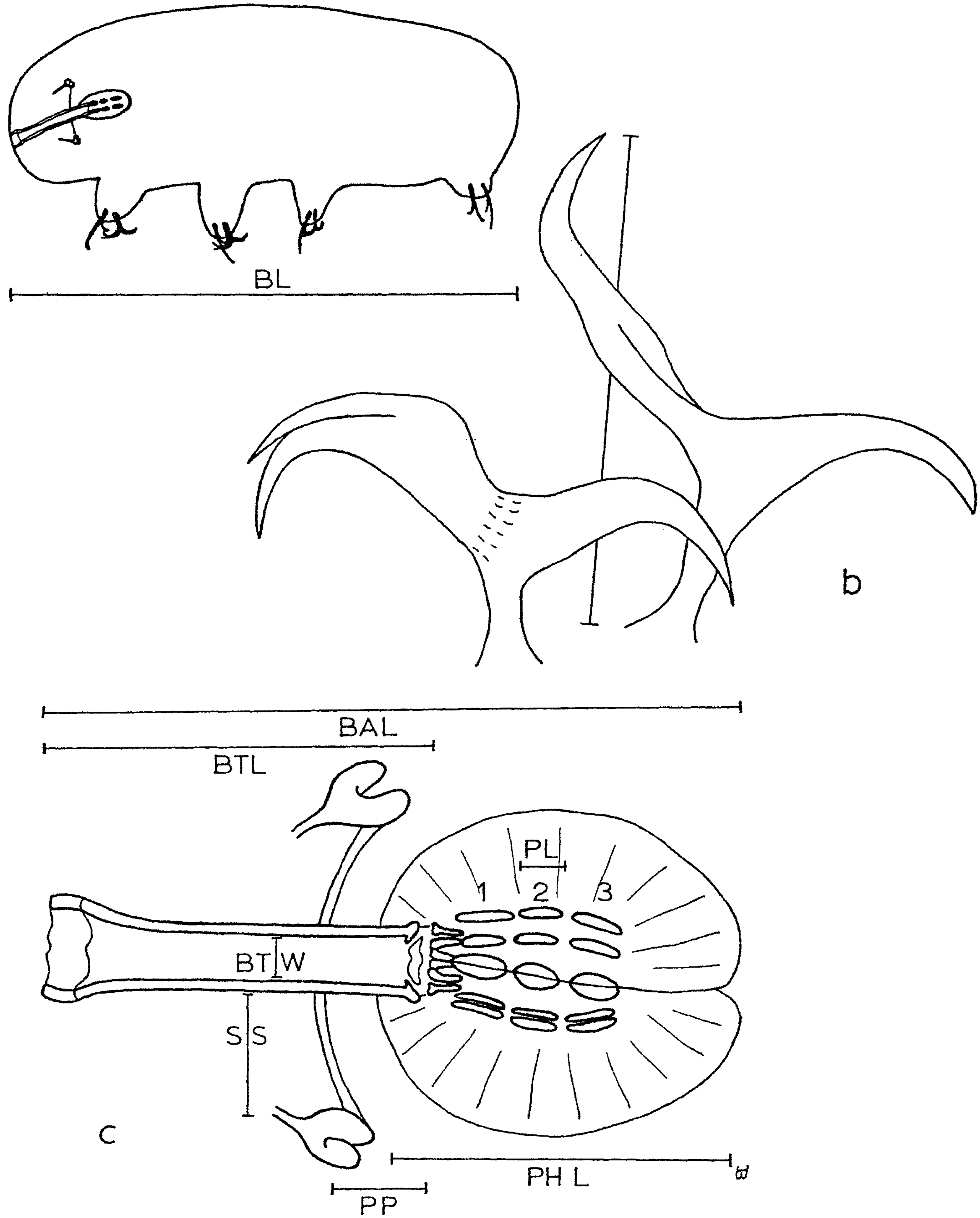
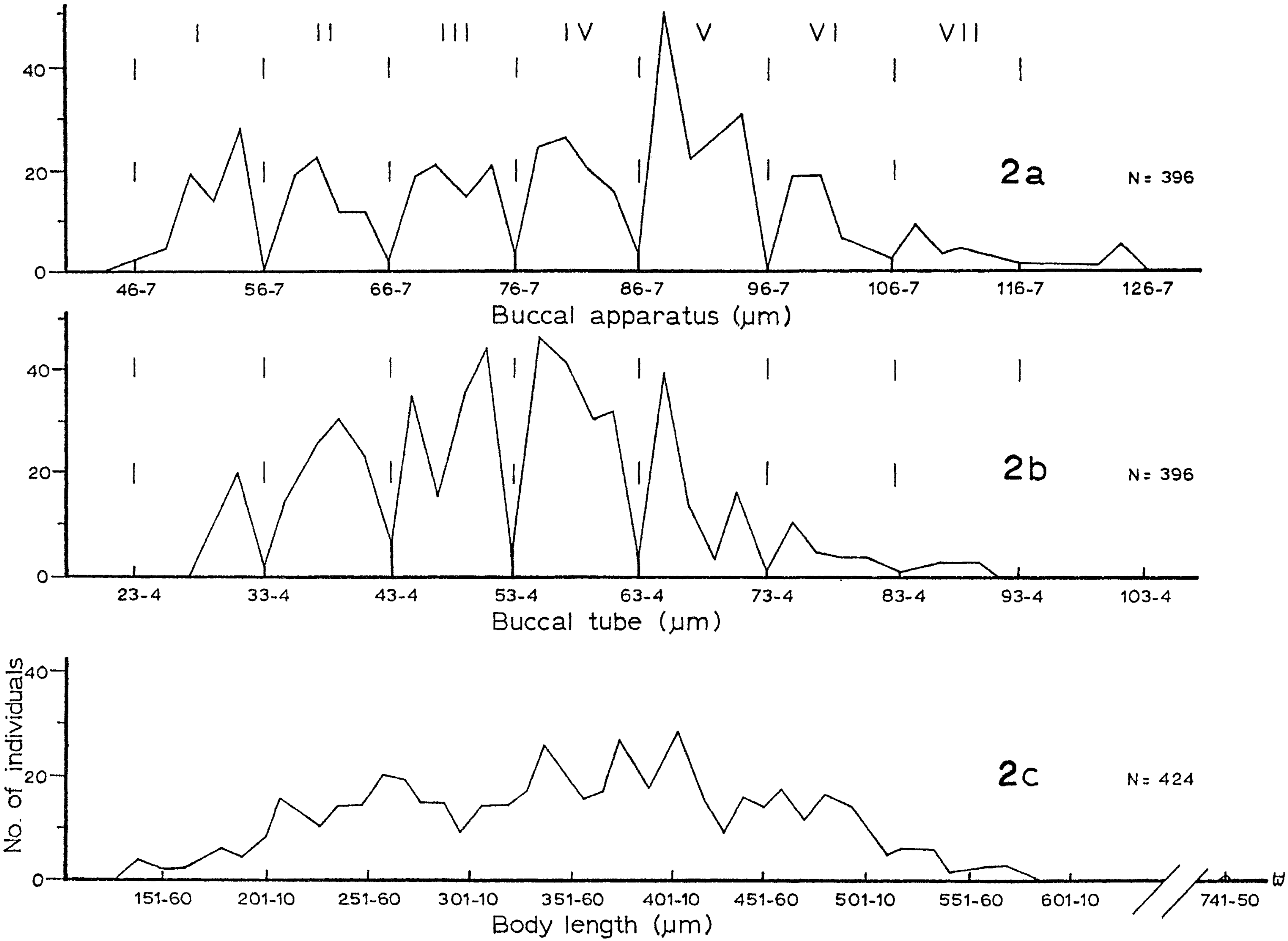
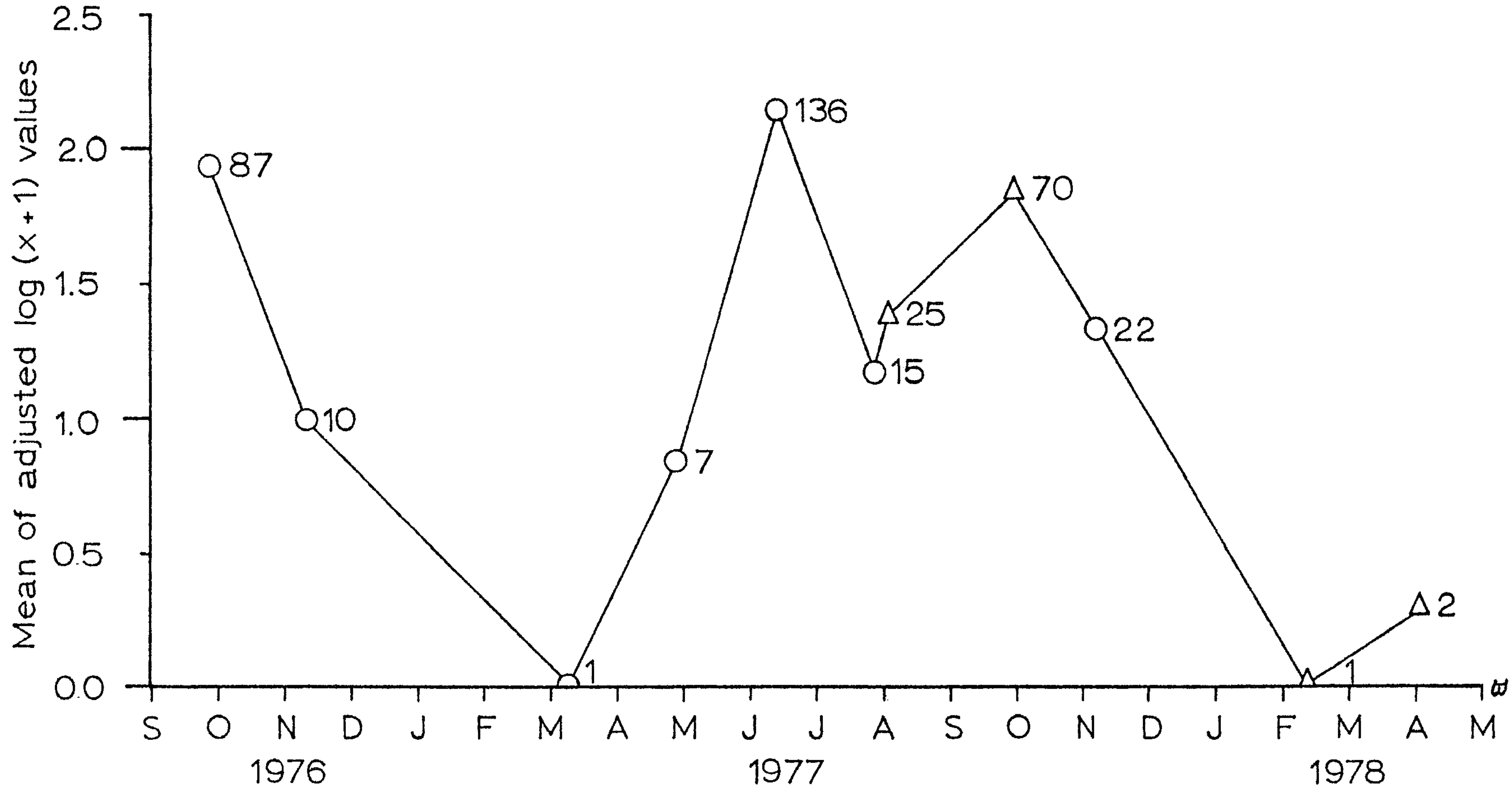


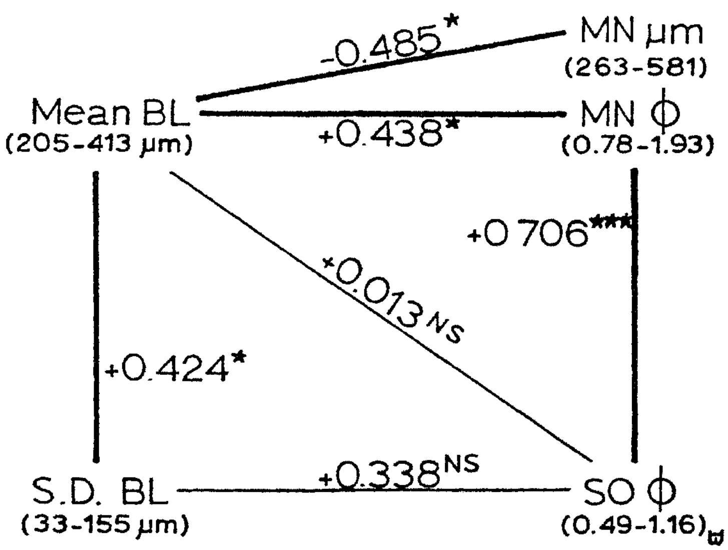
Fig. I



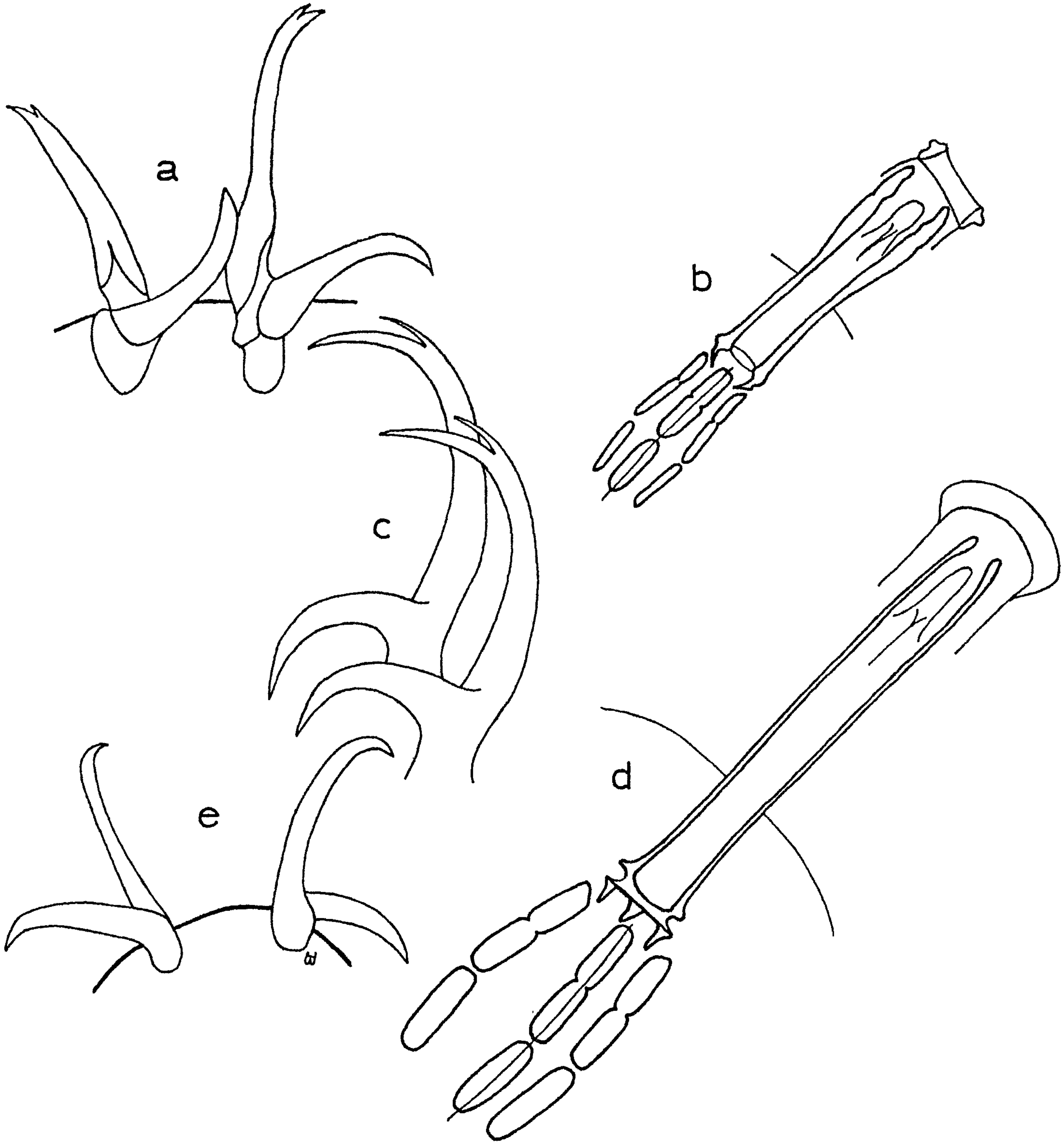
Warnberg & Henderson Fig. 2



Wasserberg & Henderson
Fig. 3



Wainberg +
Hummon



[illegible] +
[illegible]
Fig 5

Appendix C

Life table demography of the rotifer *Lecane tenuiseta* under culture conditions, and various age distributions.

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and

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Abstract

The rotifer *Lecane tenuiseta*, found contaminating an unpolluted culture water reservoir, was cultured in a dilute baker's yeast suspension using the same water. Ova, cultured individually in 10 μl wells at 20 C, were observed at half day intervals for hatching, daughter ova and death. Data were subjected to life table demographic analyses. With a stable age population of parthenogenic females, laying 6.8 ova per lifetime and increasing at 0.23 per individual day, mean life expectancy of a newly laid ovum was 26 days, generation time was 8.4 days and 11% of the population appeared as developing embryos. With a stationary age population calculated by the Leslie-Ranson method, there would be no change

in life expectancy but a decrease in embryos to 9%; a stationary age distribution calculated by the Hummon method would show a decrease in life expectancy to 4.9 days and a increase in embryos to 46%. Calculation by the latter method is more consistent with available field data for species of planktonic rotifers.

Introduction

While reproductive behaviour of Rotifera is not a new field of investigation, little has been done along the lines of life table demography. During a series of studies on freshwater meiofauna and their relation to acid mine pollution, we continually encountered a population of *Lecane tenuiseta* (Harring) contaminating our unpolluted culture water reservoir. The native habitat of the rotifers was not confirmed, though water in the reservoir was from Dow Lake, Athens County, Ohio.

Lecane, as with many other rotiferan genera, is heterogonous, having both asexual and sexual generations (Donner, 1956). Asexual females multiply exclusively by parthenogenesis and are by far the most common individuals found, males of this genus not having been found prior to 1930 (Miller, 1931). Subcultures with wheat grains and baker's yeast indicated that *L. tenuiseta* could be cultured rapidly and in such a manner as to provide demographic data.

Methods and Materials

Rotifers were subcultured with wheat grains in 60 mm diam plastic Petri dishes, using double filtered (Whatman No 1) lake water from which the animals had been obtained. Ova were removed from subculutres with a mouth pipette and transferred to a separate dish for rinsing before 120 of them were introduced individually into the truncatedly conical, 10 μl wells of two sterile Nuclon Delta microtest plates. Excess water was removed and each well filled with a highly dilute suspension of baker's yeast in double filtered lake water. Plates were checked every half day at 30-50X, using a Wild M8 dissecting microscope.

Data recorded were: time of hatching of original ovum, time of laying and hatching of each daughter ovum and time of death of the adult. An animal was considered dead if no movement could be induced by a current of water from the mouth pipette. Where multiple ova had been laid in a single well, it was assumed that ova hatched in the order they were laid. Juveniles were removed as soon as they hatched.

Between counting periods, test plates were fitted with their covers and placed in a covered plastic box, which contained a broad reservoir of distilled water to retard evaporation. Boxes were stored in a BOD box at 20 C. Evaporation loss from wells was replaced by double filtered lake water at the time of counting.

Standard life tables were computed as in Hummon (1974; see also Hummon and Hummon, 1975 and Faucon and Hummon, 1976), including l_x, d_x, L_x, T_x, e_x, m_x and $L_x m_x$. Net reproductive rate per individual lifetime (R_o), instantaneous rate of natural increase per individual day (r_{max}), generation time and stable age distribution were then calculated from the data. Also calculated were stationary age distributions after Leslie and Ránson (1940; see also Krebs, 1978) and Hummon (1974).

Results and Discussion

Chemical characteristics of the lake water were: pH 7.0; total conductivity 245 μmho/cm (25 C), total alkalinity 68 ppm, sulfates 34 ppm, total hardness 98 ppm and calcium hardness 77 ppm. The water had been coarsely filtered to remove flocculent material and most plankters, and then had been used periodically over 9 mo before cultures were begun.

Because of losses due to nonviable ova from bulk cultures and twice daily manipulations of well contents, calculations were based on 100 cultured individuals and their 687 ova laid in the test plate wells. Two problems were encountered while culturing the animals. It was relatively easy to distinguish adults from juveniles shortly after they hatched, because of size differences and the greater transparancy of juveniles. But juveniles, which hatched just after the culture was checked, nearly equalled the adult in size and body capacity after 12 hrs growth. To complicate matters further adults, which laid ova just before the culture was checked, gained in transparency with the resulting loss of opaque vitelline material. The second problem pertained to our death criterion; in a few instances, animals which had not responded to stimlulation at one counting were found freely swimming in the well at the next counting. Only early identification of both problems and subsequent care in decision-making prevented serious errors in the data.

Survivorship (l_x) and mean life expectancy (e_x) for Lecane tenuiseta are shown in Fig. 1. Two periods are noted when mortality dropped and the mean life expectancy of survivors increased. Curves are typical of those in the absence of predatory loss, representing physiological maximum survival under culture conditions.

Age specific natality (m_x) and reproductive value (v_x) are shown in Fig 2. Both exhibit distinct peaks between days 1 and 2 after hatching, indicating that the majority of animals quickly gained reproductive maturity. Thereafter the natality curve decreased until day 6 and then entered a long flat arc which was terminated with the last reproductive effort on day 53; actual points were somewhat scattered above and below the arc after day 6. What reproductive synchrony was present during the early period was soon lost. The quicker decline in reproductive value after day 10 results from its inclusion of both natality and mortality data.

A summary of culture and life table data is given in Table I. With a stable age population of parthenogenic females, laying 6.79 ova per lifetime, and increasing at a rate of 0.228 per individual day, the mean life expectancy of a newly laid ovum was 26.06 days, generation time was 8.40 days, and 10.90% of the population appeared as developing embryos for a ratio of 0.12 ova per adult.

Both methods of calculating stationary age distribution assume that net reproduction per individual lifetime is 1.00 and there is no change in population size. The mathematically elegant method of Leslie and Ranson (1940) further assumes that the birth rate changes to approximate the death rate, so that the mean life expectancy of a newly laid ovum remains unchanged and the age structures of the population takes up the L_x schedule, converted to percent. In this case the proportion which would appear as developing embryos would decrease slightly to 8.4% and the ratio of ova to adults to 0.10.

The Hummon (1974) method assumes that birth rate would be relatively unaltered and that the death rate would change to approximate the birth rate as a result of predatory mortality, under conjectured field conditions. In the calculations this is done by setting the age specific death rate (q_x) equal to the age specific birth rate (m_x) for each respective age class and superimposing this upon the age specific death rates observed in the culture to yield and adjusted age specific death rate (q'_x) schedule. An adjusted life table is then calculated, modifying the rates but not the schedule by a constant if necessary so that $\Sigma L_x m_x = 1.000$. In this case the constant was .795. The resulting age structure of the population would again take up the adjusted L_x schedule, but this time with the mean life expectancy of a newly laid ovum decreased to 4.93 days (of which 2.30 days would still be spent as a developing embryo). The proportion of the population that would appear as developing embryos increased markedly to 46.41% and the ratio of ova to adults to 0.87.

An egg ratio can be used to measure reprductive rates in natural populations of planktonic rotifers (Edmundson and Winberg, 1971, and included references). The number of ova per female (E) in preserved samples is divided by the development time in days (D) at the temperature prevailing at the time of sampling to give a finite rate of reproduction (E/D = B). Instantaneous birth rate is calculated by b = ln(B + 1) and the value of b inserted in the exponential growth equation ($N_t = N_o e^{(b-d)t}$) to obtain the instantaneous rate of natural increase of the population over time (r = b - d). No work on egg ratios for Lecane species has yet been published. But, our results suggest that under a single temperature regime, values of E varying from 0.12 to 0.87 could be accounted for simply by shifts from stable age distribution, under low predation pressure and rapid population increase, to stationary age distribution, under high predation pressure and no change in population size. These values represent a large proportion of the ranges noted for species of planktonic rotifers and, considering the available field data, provide support for the Hummon rather than the Leslie and Ranson method of calculating stationary age distribution.

Acknowledgements

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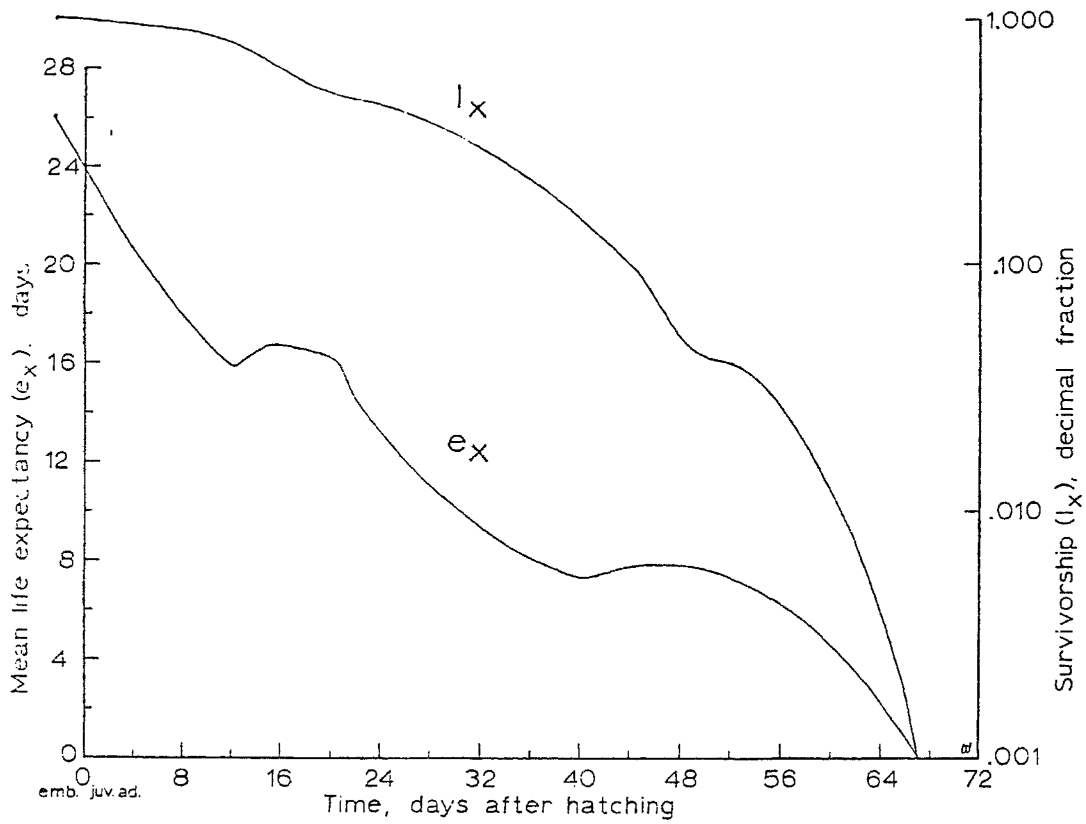
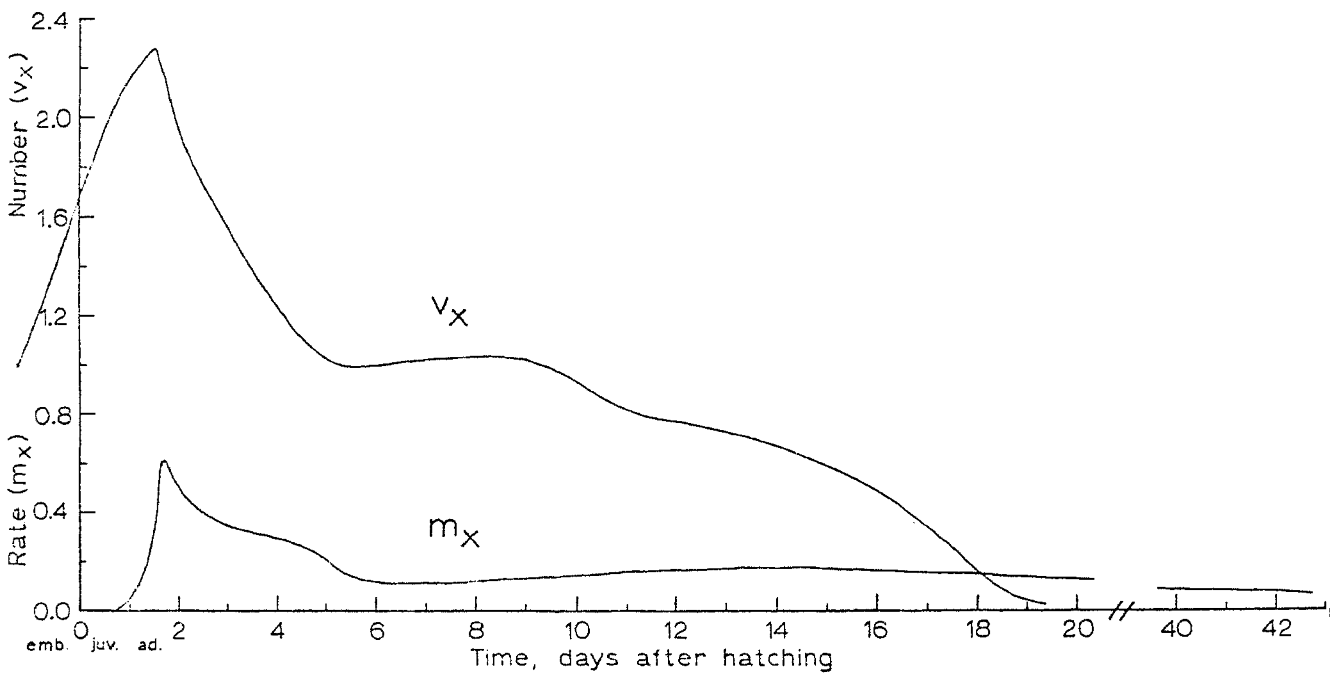
Figure Legends

Figure 1. Survivorship (l_x) and mean life expectancy (e_x) curves for individually cultured *Lecane tenuiseta*.

Figure 2. Curves of age-specific natality (m_x) and reproductive value (v_x) for individually cultured *Lecane tenuiseta*. Age-specific natality is measured as ova laid per individual day, reproductive value as ova yet to be produced per individual entering an age class relative to the number of individuals entering that age class.



28
24
20
16
12
8
4
0
Mean life expectancy (e_x), days
1.000
.100
.010
.001
Survivorship (l_x), decimal fraction
l_x
e_x
0
8
16
24
32
40
48
56
64
72
emb. juv. ad.
Time, days after hatching



2.4
2.0
1.6
1.2
0.8
0.4
0.0
Number (v_x)
Rate (m_x)
v_x
m_x
0
2
4
6
8
10
12
14
16
18
20
40
42
emb. juv. ad.
Time, days after hatching

Table I. Summary reproductive data for individually cultured *Lecane tenuiseta*.

Culture data:

Ova laid per individual	0 - 16, $\bar{x}$ = 6.87 (SD = 3.79)
Hatching percent	98.8
Embryonic duration, of hatched individuals, days	1.0 - 5.5, $\bar{x}$ = 2.30 (SD = 0.39)

Life Table data:

	Age Distribution		
	Stable	Stationary Leslie and Ranson, 1940	Stationary Hummon, 1974
Mean life expectancy (e_x) of newly laid ovum, days	26.06	26.06	4.93
Ova as percent of population	10.90	8.78	46.41
Net reproduction per individual lifetime (R_o)	6.79	1.00	1.00
Intrinsic rate of natural increase per individual day (r)	0.228	0.000	0.000
Generation time, days	8.40	___	___

Appendix D

Respiration of aquatic insect larvae (Ephemeroptera, Plecoptera) in acid mine water.

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Abstract

Warburg manometry was used to assess the effect of acid mine water on respiratory processes in three species of aquatic insect larvae. Field collections and laboratory toxicity tests indicated short longevity under strong acid mine conditions. Mixed results were found with respect to weight-dependent respiratory rates. Sequential respiration determinations, under control-control or control-treatment fluids, indicated that acid mine water did not consistently alter rates. Animals maintained in mine water until death showed gradual decreases in respiratory rates over time, rather than stepwise drops that would accompany ionic interference. For these species the toxic mode of action of acid mine water does not appear to operate through mechanisms that are detectable by respirometry.

Introduction

Ecotoxicology (Truhaut, 1975) is the study of the harmful effects of natural substances and artificial pollutants experienced by organisms in the environment. The degree of response exhibited by an organism toward the presence of noxious substances can often be determined by monitoring a physiological parameter. One such parameter is respiration (Cheng and Rodrick, 1974; Kawatski et al., 1974). The majority of studies dealing with the biological impact of acid mine drainage have been ecological surveys. No studies have been reported which deal with the physiological response of an organism to acid mine water other than acute toxicities of the various components expected in effluent stream (Bell and Nebeker, 1969; Kimmel and Hales, 1973; Warnick and Bell, 1969). These studies did not consider possible synergistic effects between individual components of acid mine water or the mode of action of pollutants involved. The work we report was undertaken to determine whether the toxic mode of action of an acid water effluent involves any aspect of the respiratory processes in three species of aquatic insect larvae. Although respirometry can be valuable in detecting signs of metabolic involvement, one must be aware that it is not a technique for the identification of specific toxic mechanisms (Cheng and Rodrick, 1974).

Methods and Materials

Mayfly larvae (Ephemeroptera; two species of the genus *Ameletus*) (Edmunds et al., 1976) were collected on a twice weekly basis for a six week period during April and May, 1978. Stonefly larvae (Plecoptera; *Acroneuria xanthenes*) (Hitchcock, 1974) were collected every other day for a two week period in mid-June, 1978. Animals used in this study were collected from Trace Run, a tributary of Hewett Fork and Raccoon Creek, located near the town of Hocking in southeastern Ohio (SW ¼, SE ¼, Sec. 1, T12N, R16W). They were readily found in the unpolluted stream portions but not in zones below which mixing had occurred with an acid water effluent. Neither sex nor instar stage of the larvae were determined. To minimize handling and possible damage to individuals, species identifications were not made until after rate determinations had been completed. Collections were halted by larval emergence.

In the laboratory, larvae were starved and kept in 25 ml beakers containing non aerated, non agitated stream water cooled to 15 ± 0.5°C in a constant temperature, circulating waterbath. No tests were begun before an elapsed time of 24 hours. Organisms were subjected to flourescent room lighting for an approximately natural photoperiod (13L:11D).

Mine water used in this experiment was collected from a flooded, abandoned coalmine shaft, located 50m downstream from the collection site for larvae at Trace Run. The upper unpolluted stretch of Trace Run from which larvae were collected served as the source of control water. Analyses for pH, conductivity, and particular ions (Mn, Fe, SO_4) were done respectively with an Orion Research Ionanalyzer Model 404, YSI Model 33 S-C-T meter, and Bausch and Lomb Spectronic 20 with the aid of prepackaged reagents (Hach Chemical Co.). Alkalinity was measured through titration with 0.02N H_2SO_4 (APHA 1976).

Time to mortality studies were performed at 15°C in fingerbowls of acid mine water on the two species of _Ameletus_ to determine the median lethal time (LT_{50}) for the population. An insufficient number of specimens precluded comparable tests on _Acroneuria_ _xanthenes_. Animals in which a response could not be elicited by tactile stimulation over a one-min period were considered dead.

A circular Warburg apparatus was used in determining O_2 consumption rates by the method of Umbreit _et._ _al._ (1964), the apparatus being calibrated by the method of Lazarow (1949). Gaseous volumes of manometer flasks were 18-21ml with a fluid volume in each of 2.2ml (2ml of test fluid in the main compartment; 0.2ml CO_2 absorber (10% KOH) in the center well). Experiments were conducted at 15 ± 0.5°C. Warburg flasks were kept in a constant linear shaking motion, utilizing a rate of 60 strokes/min over a cyclic distance of 2.5cm. No substratum was employed during the measurements. Rates were determined on solitary individuals. Manometers were read to the nearest 0.5mm.

All control respiratory rates in unpolluted water were determined from a period of measurement of 3 hr in duration. After the initial 3 hr period, control water was removed from all flasks. Fresh control water was returned to half of the flasks while the remainder were supplied with acid mine water. Determination of respiratory rates was then continued for a period of 3 hr. Subsequent to this portion of the study, respiratory rate determinations were also conducted for individuals considered dead because of mine water toxicity. Time intervals between manometer readings on a particular day were set at either 10 or 15 min. After rate determinations had been completed, animals were sacrificed and dried at 80°C for 24 hrs. Dry weights were the mean of three readings obtained on a Mettler H20T Analytic balance to the nearest 10 μg.

Calculation of LT_{50} values was performed through probit analysis (Goldstein, 1964; Finney, 1971). Hourly rates of oxygen consumption were determined through calculation of regression coefficients by least squares. Comparison of rates in unpolluted and acid mine water was performed through the use of the F-test for differences between two regression coefficients (Sokal and Rohlf, 1969). Significance in all tests was set at the 5% level.

Results

Table 1 provides chemical characteristics of the control and treatment fluids utilized in these experiments. Severity of pollution represented by the treatment fluid is indicated by a partial list of chemical parameters used as guidelines in classifying acid mine pollution.

Time to mortality studies on Ameletus indicated LT_{50} levels of 21.4 hrs for A. lineatus and 29.0 hrs for Ameletus sp. No correlation was found between time of death and weight of individuals for either species.

In each species it was found that log transformation of O_2 consumption ($\mu l\ O_2$/g hr) decreased as individual dry weight (mg) increased. The regression coefficient b, representing relative O_2 uptake per unit weight, was -0.074 (s_b = 0.021, n = 14) for Ameletus lineatus, -0.022 (s_b = 0.012, n = 11) for Ameletus sp., and -0.0056 (s_b = 0.0026, n = 19) for Acroneuria xanthenes. Body weight for the two species of Ameletus ranged form 1 to 7 mg, that for Acroneuria xanthenes from 14 to 50 mg. Only rates of the first and third species differed significantly from zero.

The outcome of statistical comparisons between regression coefficients obtained from sequential determinations of respiratory rates on solitary individuals is presented in Table 2. Results indicate that a majority of larvae exhibited similar sequentially determined respiratory rates regardless of the solution, control or acid mine water, that was utilized in the second rate determination. Similar patterns of respiratory rate increases and decreases between control and treatment groups suggest intervention in the respiratory process of extraneous factors not under the scope of these experiments.

Respiratory rates for individuals considered dead by the criteria given above decreased gradually with time, but for the first few hours still lay within the 95% confidence limits of the regression lines. Oxygen consumption rates for control and acid waters in the absence of test animals were negligible, indicating no bacterial contamination.

Discussion

Bell and Nebeker (1969) have shown that aquatic insects are tolerant of acidic conditions down to pH 4 for short periods of time. Tolerance below this level is dependent both on species and stage of life history involved (Kimmel and Hales, 1973). The absence of Ephemeroptera and Plecoptera from zones of

Trace Run polluted by acid mine water and the results of laboratory tolerance tests both indicate that high concentrations of mine acid are toxic to these animals.

The toxic mode of action of acid mine water has received much discussion. One theory suggests that mucus normally present on the gills of fish is precipitated in solutions of high acidity or heavy metal concentrations. Permeability of the gill membrane system is thus presumably impaired, the fish eventually succumbing to anoxia (Westfall, 1945; McKee and Wolf, 1963; Skidmore, 1970; Nichols and Bulow, 1973). A similar mechanism has been suggested for benthic organisms (Nichols and Bulow, 1973), high levels of acidity affecting animals directly through attack on their gills. An alternate theory of toxicity involves displacement of functional cations from active sites of enzymes by heavy metal ions, resulting in failure of life processes such as respiration (Eichorn, 1975; Webb, 1977). Various studies (Jackim et al., 1970; Brown, 1976) have reported the deleterious effects of heavy metals on the activities of liver and on nitrogen metabolizing enzymes, while Hiltibran (1971) illustrated alterations in oxygen and phosphate metabolism of bluegill liver mitochondria by heavy metals.

Results of our study support neither of these theories for aquatic insect larvae. Acid mine water did not consistently alter the respiratory rates of species studied. Observations on individuals, sacrificed in acid mine water and subsequently monitored, indicated a gradual decrease in respiratory rate with the loss of life processes, supporting a claim of non-interference by noxious ions. Had anoxia due to ionic interference been a major cause of death, the decrease in respiratory rate would have been more precipitous. We conclude, therefore, that the toxic mode of action of acid mine water on these aquatic insect larvae does not operate through mechanisms that can be detected by means of respirometry.

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Table 1

Chemical Characteristics of Control and Treatment Fluids

	Control	Acid Mine Water	Parameter Levels Characteristic of Acid Mine Drainage*
pH	8.3	2.5	<6.0
Conductivity (μmhos/cm)	280	4400	------------------
Mn (mg/l)	0.01	6.95	>0.5
Fe (mg/l	0.0	1.17	>0.5
SO_4 (mg/l	60.4	3110	>75.0
Alk (mg/l	85.0	0.0	------------------

*Cited in Nichols and Bulow (1973). U S. Government classification criteria for acid mine water drainage.

Table 2

Tablular presentation of the outcome of statistical comparisons of sequentially determined respiratory rates in unpolluted and acid mine water.

	Acroneuria xanthenes		*Ameletus lineatus** *Ameletus* sp.	
	Control+ (n)	Treatment± (n)	Control (n)	Treatment (n)
Significant Increase:	1	1	1	1
N.S.:	6	6	3	10
Significant Decrease:	3	3	0	1
Total:	10	10	4	12

*Pooled data for genus *Ameletus*.

+Signifies those individuals whose rates were determined in control water for both periods of measurement.

±Signifies those individuals whose rates were first determined in control water and subsequently in acid mine water.