EFFECTS OF SELECTED POLLUTANTS ON GRAZER UTILIZATION OF AUFWUCHS

by

James R. Clark

Dissertation submitted to the Graduate Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Zoology

APPROVED:

J. Cairns, Jr., Co-chairman	D. S. Cherry, Co-chairman
A. L. Buikema, Jr.	R. A. McNabb

J. R. Voshell

February, 1980

Blacksburg, Virginia

ACKNOWLEDGEMENTS

I would like to acknowledge the support and guidance of Dr. John Cairns, Jr., and Dr. Donald Cherry, the co-chairmen of my committee. They provided advice to assist me in my endeavors and encouragement to carry this project to completion. Dr. Arthur Buikema, Jr., Dr. Roger McNabb, and Dr. J. Reese Voshell provided direction and advice throughout my research and assisted in revising this manuscript. The direction and support of Dr. Kenneth Dickson is also greatfully acknowledged. I would like to thank the staff affiliated with the University Center for Environmental Studies for their cooperation. I thank Ms. Lisa Decker and Ms. Lynn Martin for their diligent laboratory assistance and Mr. Stephen Larrick for his counsel and review comments. Ms. Betty Higginbotham professionally typed the final draft of this manuscript.

A special thanks is due my wife, Joyce, for her love and understanding during my graduate career. Her patience and encouragement has sustained me, especially through the most difficult times.

This research was supported by a grant of the Energy Research and Development Administration, contract #E-(40-1) 4939.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Analysis of Aufwuchs Structure and Function - Perspective

Populations of microscopic organisms attached to but not penetrating submerged substrates in aquatic ecosystems comprise the Aufwuchs community (Ruttner 1953, Odum 1971). Although periphyton is a commonly employed synonym (Odum 1971, Weber 1973a, Clark et al. 1979), the German word Aufwuchs more precisely connotes the complex taxonomic composition of this community (Wetzel 1975, Clark et al. 1979). Analyses of the composition of communities through enumeration of individuals or species, taxonomic identifications, or quantification of biomass or cellular constituents are termed structural analyses (Odum 1962). These types of analyses form the information base pertaining to our current understanding of Aufwuchs communities (Weitzel 1979a). Functional analyses, which are investigations concerning the rates of dynamic processes within or between ecosystem components (Odum 1962, Margalef 1963), integrate the structural characteristics of communities with responses to abiotic ecosystem parameters (Odum 1977, Odum et al. 1979). The functional characteristics of ecosystem components have not been studied to the same extent as structural characteristics (Cairns 1971, Odum 1977) and this disparity is even greater with respect to Aufwuchs communities (Rodgers 1977, Rodgers et al. 1979, Weitzel 1979b). Of particular interest in characterizing ecological

systems is the relationship between structure and function within and among the ecosystem components (Odum 1962, 1971, Margalef 1963, Cummins 1974).

Investigations into the utilization of food resources by consumers and the flow of energy through an ecosystem can integrate the structural and functional aspects of ecological analyses (Lindeman 1942, Odum 1971). In order to investigate fully the flow of energy from producers to consumers, the ecosystem must be divided into its component systems, and comprehensive analyses of individual relationships must be performed. The research described herein emphasizes the interactions between Aufwuchs communities, at the producer trophic level, and snail grazers, at the consumer trophic level. Discerning what degree of change in Aufwuchs structure is necessary in order to alter the flow of energy to the grazer is important in assessing the sensitivity or response of aquatic ecosystems to perturbation. Since an ecosystem is ordered by the flow of energy (Odum 1971), its resistance to alteration in energy flow is an essential, primary factor in maintaining ecosystem stability (Cairns 1976, Cairns and Dickson 1976, Cairns et al. 1977).

This research is part of a program to develop and test methods for assessing impacts related to electrical power production. Since interactions between trophic levels are dynamic and complex (Lindeman 1942, Odum 1971), a series of

artificial streams were used to investigate the effects of low levels of perturbation on Aufwuchs food quality and grazer utilization of this food resource. Artificially simplified ecosystems (i.e. microcosms) can reduce natural complexity and assist in investigating trophic level interactions (Odum 1971, Warren and Davis 1971). The responses of Aufwuchs to two stresses, intermittent chlorination and copper sulfate, were chosen for study because of the routine use of these chemicals in power plant operations to control or prevent microfloral growth and biofouling (Becker and Thatcher 1973, E.P.A. 1976). Aufwuchs response to an organic enrichment was also studied to further evaluate the food quality and grazer utilization approach for impact assessment. Similar investigations were conducted in the receiving waters around a power plant discharge to verify the results of the artificial stream experiments.

Role of Aufwuchs in Aquatic Ecosystems

Since Aufwuchs communities are composed of numerous, complex, taxonomic groups, they enter into various functional processes within aquatic ecosystems. The algal component creates living matter or biomass from inorganic carbon through photosynthetic pathways, taking up other inorganic and organic nutrients in the process (Wetzel 1975). Autotrophic bacteria alter the chemical state of inorganic complexes utilizing

solar or chemical energy to increase organic carbon biomass and to cycle nutrients (Wetzel 1975). Other bacteria and fungi procure energy to sustain and increase their biomass through heterotrophic pathways; by decomposing organic matter they release inorganic nutrients and simple organic molecules into the surrounding medium (Brock 1974, Rheinheimer 1974). The remaining taxa within the Aufwuchs community (protozoans, microcrustaceans, and other microinvertebrates) consume living or dead organic matter and expel metabolic byproducts and smaller detrital particles (Odum 1971). Their growth and reproduction increase Aufwuchs biomass while contributing to the community functions of decomposition and nutrient cycling. The transfer of solar energy into photosynthetically fixed carbon coupled with the recycling of organic carbon and nutrients by heterotrophs produces a persistent and significiant food supply for grazing animals. The maintenance and growth of Aufwuchs communities and the utilization of this resource by consumers forms an important base for classical food chains in aquatic ecosystems (Hynes 1970, Odum 1971).

Since microflora dominate Aufwuchs communities, the impact of analyses of Aufwuchs for water quality assessment rarely will sway public sentiment. This is particularly true for the common structural parameters of Aufwuchs, such as diversity indices, algal species lists, or phytopigment

concentrations (Weber 1973a, Weitzel 1979a). Typical assessment or management objectives for determining or maintaining the health of aquatic systems to provide water resources suitable for human or industrial consumption, agricultural or recreational use, and commercial or sport fisheries enhancement, appear far removed from the composition of microfloral communities. The functional capabilities of Aufwuchs seem related more directly to water resources management. The production of oxygen through photosynthesis, the decomposition of organic wastes, and the cycling of nutrients sustain aquatic ecosystems and provide the essentials for the maintenance of higher trophic levels. However, as increasing pollutional stress is placed on aquatic ecosystems, the tradeoffs between decreasing water quality and industrial or residential development must be addressed (Cairns 1976, Cairns et al. 1977). While the effects of perturbations on Aufwuchs structure and function has been investigated (Dickson et al. 1978, Rodgers et al. 1979), the ultimate impact of these effects on higher trophic levels, particularly through food and energy transfers, will be the important component in decision making. The research outlined in this dissertation attempts to relate changes in Aufwuchs communities to the utilization rates of this resource by grazers and to the flow of energy through aquatic ecosystems.

Environmental Assessment Using Aufwuchs

The taxonomic complexity of Aufwuchs communities insures that some community component will persist under nearly any set of environmental conditions. Ecological studies of aquatic systems under pollutional stress may rely heavily on analysis of Aufwuchs. Under severe stress, Aufwuchs may be one of the few ecosystem components to survive and perhaps provide the only source of biological information. Qualitative and quantitative analysis of Aufwuchs species composition has been the most common approach to using these communities for assessing water quality and the health of aquatic systems in general (Patrick 1973a, 1973b, Cattaneo et al. 1975, Weitzel 1979a). A taxonomic approach is versatile in that comparisons and assessments can be made as to the diversity, presence-absence, and abundance of species and individuals (Cairns and Dickson 1971, 1973, Pielou 1975, Weitzel 1979a). However, since Aufwuchs communities are compositionally complex, such analyses are usually restricted to major divisions within the Aufwuchs (i.e. algae, bacteria, protozoans) and commonly to one or two groups within these divisions (diatoms, green algae, culturable heterotrophs) (A.P.H.A. 1976, Weitzel 1979a).

Other analyses of Aufwuchs have utilized the abundance and distribution of cellular constituents to assess community structure (Vollenweider 1974, Clark et al. 1979). A non-

taxonomic approach can incorporate a variety of parameters specific for divisions within a community (i.e. chlorophylls, carotenoids, or muramic acid for algae, microfauna, or procaryotic bacteria and algae) (Strickland and Parsons 1972, Weber 1973a, Moriarty 1975). Other parameters can encompass a community as a whole (ATP, ash-free dry weight) (Clark et al. 1979, Vollenweider 1974). Investigations into community function provide nontaxonomic information regarding the life processes of the organisms in the Aufwuchs (Odum 1977, Rodgers 1977). Previously, measurements of functional parameters have been restricted to basic ecological studies due to methodological problems (Dickson et al. 1978, Rodgers et al. 1979). Rates of primary production, nutrient cycling, and respiration (functional properties of Aufwuchs communities) are now of contemporary interest in assessing the impact of stresses on aquatic communities (Weitzel 1979b, Rodgers et al. 1979).

The analyses mentioned above deal with Aufwuchs communities as discrete entities containing specific functional processes. Few studies have investigated the functional interactions occurring between communities of an ecosystem as a means to assess water quality. The objectives of most ecosystem studies have been to quantify rates of energy flow or nutrient cycling in order to gain a better understanding of the ecosystem (Odum 1957, Teal 1957, 1962). Many eco-

system studies which have assessed perturbation impact, acid rain (Likens et al. 1977) or clearcutting (Swank and Douglass 1977), have dealt with ecosystem level inputs and outputs or turnover times within trophic levels rather than the interactions of the various component communities. Analyses of the internal functions of ecosystems are needed to explain changes at the ecosystem level. In identifying the impacts of pollutants on aquatic ecosystems, we should know what levels of pollutant will alter the flow of energy through the component communities to the point where total ecosystem processes are affected (Cairns 1970, 1976, Monk et al. 1977). We need to understand the mechanisms of inertia within the paths of energy flow through the ecosystem to determine acceptable levels of pollutant discharges into aquatic ecosystems (Cairns 1976, Cairns and Dickson 1976). Studies of trophic level interactions are necessary in addition to community function analyses.

Application of Feeding and Food Quality Studies to Environmental Assessment

Analysis of Aufwuchs food quality and the utilization of this food resource by grazers can be used to assess the impact of stresses at the population, community, and ecosystem level. While physical food quality is essentially a relative factor depending on the manipulative capabilities of the grazer consuming the food (Porter 1973, 1977, Patrick 1978),

chemical food quality is a structural parameter of an Aufwuchs community (Lund and Talling 1957, Odum 1962, Porter 1977). The ability of a grazer to consume and assimilate a food resource and the adequacy of that food in meeting the nutritional requirements for maintenance, growth, and reproduction will control the fate of the grazer population in that habitat. Any change in the Aufwuchs may cause a change in grazer utilization rates (Patrick 1978) and thus, the energy flow between producers and consumers in aquatic food chains. One set of experiments, as outlined in this dissertation, can provide insight at all of these levels of ecosystem organization. This approach can be used in conjunction with other measures of structure and function to better determine tolerable levels of perturbation in aquatic ecosystems.

Low levels of stress can change Aufwuchs species composition through shifts in competition as a result of toxic responses (Patrick 1970, 1978). These changes may not cause significant alterations of community function or affect grazer populations (Patrick 1970, Cairns 1976); however, the perturbation may require the utilization of stored photosynthetic products by surviving Aufwuchs components in order to cope physiologically with the stress. This decreases the chemical food quality of Aufwuchs by altering the caloric, carbohydrate, or protein content (Parsons et al. 1961, Eppley et al. 1971, Fogg 1975). If the food quality is nutritionally sufficient to support a grazer population, the food resource also must be

in a form acceptable to the grazer which manipulates and consumes it (Porter 1977, Patrick 1978). Any deleterious effect on Aufwuchs food quality may necessitate that grazers find alternate food sources or unperturbed habitats.

A change in the Aufwuchs food resource may elicit a change in food consumption at a constant assimilation efficiency so that grazers obtain sufficient nutrients to maintain their population (Grodzinski et al. 1975). Alternatively, a change in the grazers' assimilation efficiency may be evident after a change occurs in the chemical or physical food quality of the Aufwuchs (Edmondson and Winberg 1971, Porter 1973, 1977). Most likely, a change in the food resource will elicit changes in both the feeding rate and assimilation efficiency of the grazer. If a grazer is utilizing components of the Aufwuchs community selectively (e.g. diatoms between 50 and 100 um), alterations in the Aufwuchs composition which reduce the abundance of these specific food items may affect the grazer feeding characteristics (Patrick 1970, 1978). Under these conditions, sufficient nutrition might be obtained only by finding a suitable, alternate food source or by increasing forage activity to locate the scarcer food item. Although the same general relationships between food quality and grazer utilization rates apply to selective feeders, analysis of indiscriminate grazers is pursued in this research in order to simplify the

approach.

As part of the research for investigating grazer utilization of Aufwuchs from perturbed environments, food samples were labeled with sulfur-35. In aquatic ecosystems, this isotope has been used to estimate heterotrophic production (Monheimer 1972, 1974, 1978, Jassby 1975), estimate autotrophic production (Rodgers 1977, Campbell and Baker 1978, Jordan and Peterson 1978, Rodgers et al. 1979), and to follow components of the sulfur cycle (Howarth and Teal 1979). Sulfur is an essential, microfloral nutrient and readily assimilated as sulfate by autotrophic and heterotrophic microflora (Monheimer 1975a, 1975b, Rodgers 1977, Jordan and Peterson 1978). The sulfur is incorporated into the amino acids, cystein and methionine (Schiff and Hodson 1973) and commonly serves to form the tertiary structure of protein molecules through sulfhydryl bonding (Schiff and Hodson 1973, Lehninger 1975). Although sulfur-35 has been used to follow specifically labeled substrates in physiological studies of invertebrates (Grodzinski et al. 1975, Wang et al. 1975), its utility in estimating grazer consumption and assimilation of food resources in aquatic systems has not been tested.

The ultimate effects of changes in food resources due to low levels of perturbation will be demonstrated by changes in grazer population characteristics such as mortality and

survival rates, or fecundity and natality rates (Ricker 1968, McMahon et al. 1974). However, monitoring the health of aquatic ecosystems by these parameters would record detrimental changes only after they had occurred, resulting in a potential impairment of the grazer population. Analysis of the quality and utilization of available food resources may indicate whether the potential for detrimental effects exists prior to a demonstrable change in the grazer population. This information can be used to maintain energy flow in aquatic ecosystems, even at the expense of sensitive species or community components. Certainly, with societal pressures to sacrifice costly pollution abatement procedures to improve industrial operations or production, it is necessary to determine maximally permissable levels of pollutant impact at the community and ecosystem levels. Analysis of utilization of Aufwuchs food resources by grazers can play an important role in the assessment.

LITERATURE REVIEW

Aufwuchs Food Quality

Food quality concepts can be applied to both the physical and chemical characteristics of a food resource. In aquatic ecology, the physical quality of food consumed by filter feeders has received much attention (Porter 1977). This research has concentrated on the dimensions and geometry of planktonic food particles retained on an animal's feeding apparatus (Edmondson and Winberg 1971, Porter 1973, 1977, Nival and Nival 1976). For grazers, physical food quality also pertains to the size, texture, and configuration of the food particle with respect to the feeding appendages. Particles or filaments too large or too small may not be easily manipulated and consumed (Moore 1977a, 1977b). Food resource components with copious extracellular mucilage (heterotrophic bacteria, some blue-green algae) or sharp irregular surfaces (desmids, some diatoms) may be left ungrazed in preference for food items with more acceptable surfaces (Patrick 1970, 1978). Mucilage also may protect food items from digestive enzymes, decreasing the assimilation rate (Edmondson and Winberg 1971, Swiss and Johnson 1976). Shifts in taxonomic composition may produce a food resource which is more resistant to digestive enzymes of a particular grazer (Moore 1977a).

The role of chemical food quality in the diet of aquatic consumers has been investigated for a diverse group of freshwater invertebrates (Johannes and Satomi 1966, Paine and Vadas 1969, Arnold 1971, Calow 1975a, 1975b, Mason and Bryant 1975); however, the available data are not extensive for any single group of consumers or species. The nutritional quality of plankton has been reviewed by Corner and Cowey (1968) and expanded by Haug et al. (1973). Diatoms are highly digestible (Johannes and Satomi 1966) and high in carbohydrates (Haug et al. 1973). Many blue-green algae have high protein content owing to their extracellular mucilage (Wolk 1973, Stewart 1974), but may be indigestible or toxic (Arnold 1971, Edmondson and Winberg 1971). While other taxa seem to have some outstanding features, few generalizations can be drawn since the chemical food quality of aquatic microflora differs with taxonomic, culture, and environmental variables (Corner and Cowey 1968, Eppley et al. 1971, Haug et al. 1973, Fogg 1975). Detailed analyses of Aufwuchs food quality in relation to a grazer population are limited. Low C:N ratios (indicative of high protein content) have been found in Aufwuchs communities dominated by diatoms, blue-green algae, and bacteria with their associated proteinacious extracellular mucilage while higher C:N ratios were indicative of communities containing lower protein, such as those dominated by green algae with

a higher percentage of cellulose (McMahon et al. 1974). While both communities would seem to satisfy the protein requirements of aquatic grazers (McMahon et al. 1974), the lower C:N ratios are generally indicative of better food quality (Russell-Hunter 1970). Epilithic detritus has been characterized as an enriched nutrient source for animal grazers as a result of the associated bacterial component (Calow 1975a).

Chemical food quality most commonly is studied by evaluating the caloric content of food items (Paine and Vadas 1969, Edmondson and Winberg 1971, Grodzinski et al. 1975). Although this approach has allowed many generalizations on the flow of energy through ecosystems (Odum 1971), it has provided only limited insight into the relationship between the nutritional quality of food and consumer utilization rates (Paine and Vadas 1969). Other chemical food quality parameters, such as protein, carbohydrate, and lipid content, are important in most dietary studies (Grodzinski et al. 1975). These constituents form the classical nutrient groups in food utilization studies (Brody 1945), although little is known about the nutritional requirements of aquatic invertebrates (Corner and Cowey 1968). Aufwuchs vitamin or nutritive mineral content has not been extensively investigated and even less is known regarding the requirements of aquatic macroinvertebrates for these

food groups (Corner and Cowey 1968).

Several methods can be applied to evaluate the chemical quality of food resources. The caloric content can be estimated by conventional bomb calorimetry (Phillipson 1964, Edmondson and Winberg 1971) or chemical oxidative techniques (Maciolek 1962, Strickland and Parsons 1972). Although bomb calorimetry is frequently applied, the time needed to process individual samples may make chemical analyses more time effective (Strickland and Parsons 1972). Methods for protein analysis commonly involve extraction of proteins in a solute (water, saline, alcohol, alkali) and quantification by a staining procedure employing spectrophotometric analyses (Lowry, Coomassie, Biurette) (Lowry et al. 1951, Strickland and Parsons 1972, Grodzinski et al. 1975, Sedmak and Grossberg 1977). Since many proteins contain approximately 16% nitrogen, crude protein can be estimated by multiplying nitrogen content analysis (from Kjeldahl or CHN analysis) by a factor of 6.25 (Grodzinski et al. 1975); however, determination of carbon to nitrogen ratios only is often used to reflect the relative protein content of food samples (Russell-Hunter 1970, Grodzinski et al. 1975). Carbohydrate analysis commonly involves colorometric reactions with anthrone from water extracts of food samples (Strickland and Parsons 1972, Grodzinski et al. 1975). Lipid analysis is quite complex and seldom is used on plant samples since relatively few plants store extensive lipid reserves

(Corner and Cowey 1968, Haug et al. 1973, Fogg 1975, Grodzinski et al. 1975). In any food quality analysis the efficiency of extraction, combustion, or digestion must be considered. Some of these problems may be overcome if only one fraction of the food reserve is considered (i.e. water soluble portions on specific reactive components).

The food quality of Aufwuchs is dependent on the taxonomic composition of the sample and the physiological condition of the community. Various environmental factors can influence the morphological traits of autotrophic and heterotrophic microflora (Porter 1977). As these characteristics change within the assemblage of Aufwuchs component species, the physical quality of the food resource changes. The predominance of filamentous algae, mucilagenous heterotrophs, or colonial diatoms may alter the ability of a grazer to manipulate and consume the Aufwuchs food resource (Moore 1977a). Differential content of chemical constituents between species may also alter the food quality of Aufwuchs following shifts in taxonomic composition (Paine and Vadas 1969, Cummins and Wuycheck 1971, Mason and Bryant 1975). Since excess photosynthetic products are stored as food reserves, analysis for carbohydrates or oils in Aufwuchs samples may provide information on the physiological condition of the community (Corner and Cowey 1968, Eppley et al. 1971, Myklestad and Haug 1972, Haug et al. 1973, Fogg 1975).

Grazer Utilization of Aufwuchs

Many studies of aquatic ecosystems emphasize the importance of detritus in supporting higher trophic levels (Cummins et al. 1973, Cummins 1974, Wallace et al. 1977). This is based on a distinction of animal groups and the nature of the food resource they utilize (i.e. filter feeders, scrapers, grazers, etc.) (Cummins 1974). The importance of Aufwuchs in aquatic food chains often is overlooked due to the lack of distinction between living particulate organic matter and detritus (Minshall 1978). If Aufwuchs is sloughed from its substrate and consumed downstream as particulate organic matter, its utilization forms a link between primary producers and consumers within the aquatic system. Many researchers include this event in their definition of a detritus food chain although the Aufwuchs may remain viable and be capable of reattachment after sloughing (Minshall 1978). Therefore, either grazers or filter feeders can utilize Aufwuchs as a food resource. The relative importance of Aufwuchs as a food resource in aquatic ecosystems ranges from negligible to a major source of animal nutrition (Mann 1975, Minshall 1978). In this research, animals grazing directly on Aufwuchs were chosen for study.

Grazer utilization of Aufwuchs is a direct, readily observed transfer of energy in the form of organic matter

from a producer community to a consumer. Ingestion typically has been studied by gut analysis (Jones 1950, Chapman and Demory 1963, Winterbourn 1971, 1974, Shapas and Hilsenhoff 1976, Moore 1977a, 1977b, 1977c). While such studies may identify the important food sources for grazers, the assimilation of the food resource is important in the energy flow through aquatic ecosystems (Sorokin 1968). To differentiate between real and potential energy flow from producers to consumers, the processes of ingestion, assimilation, and egestion must be studied collectively (Edmondson and Winberg 1971). As an assessment tool, grazing and assimilation might be used to determine the degree of stress that a grazer can withstand before becoming physiologically incapable of obtaining or assimilating an adequate food resource. Analysis of Aufwuchs could determine the degree of change in the food resource under low levels of perturbation which may affect the efficiency of grazer feeding or assimilation. Considerations for this dissertation will be limited to analysis of changes in Aufwuchs community characteristics and the ability of unstressed grazers to utilize this food resource.

For any specific grazer, there are several characteristics of Aufwuchs which can influence the grazer's ability to consume and assimilate the food resource (Moore 1977a). The availability and suitability of Aufwuchs may have a direct

influence on consumption and assimilation rates (Paine and Vadas 1969, Monakov 1972, Calow 1975b, Mason and Bryant 1975). When food is scarce, the rate of consumption may decrease since more time is spent foraging. Abundant food supplies may increase or decrease feeding rates or assimilation efficiencies depending on an animal's ability to store excess energy (Paine and Vadas 1969, Monakov 1972, Calow 1975b, Swiss and Johnson 1976). Changes in the composition of aquatic food resources may reduce the grazer's ability to ingest or digest food (Paine and Vadas 1969, Calow 1975b, Swiss and Johnson 1976), perhaps due to changes in the physical or chemical characteristics of the food. Many studies of physical food quality (size, texture) have been carried out on planktonic filter feeders relative to their ability to retain and manipulate food items (Edmondson and Winberg 1971, Porter 1977, Bowers and Grossnickle 1978, Taghon et al. 1978). Few investigations have applied this concept to grazing macroinvertebrates (Moore 1977a). Chemical food quality is more likely to directly affect feeding rates if toxic or noxious characteristics are associated with the food resource (Swiss and Johnson 1976). Assimilation efficiency will be influenced by changes in the proportions of mucilage, recalcitrant constituents, or plant storage products in the food (Edmondson and Winberg 1971, Calow 1975b, Moore 1977b).

Measurements of feeding rates and assimilation efficiencies of aquatic organisms are inconvenient due to their small size. Grazing on Aufwuchs is especially difficult to measure since the food should be offered with the substrate (Trama 1957, Malone and Nelson 1969, Sedell 1972). The heterogeneous composition of Aufwuchs mandates that short duration feeding experiments (less than 24 hours) should be conducted to minimize the effects of rapid or differential turnover times of the food organisms (Sorokin 1968, Patrick 1978). All experiments must determine rates of ingestion, assimilation, egestion, and respiration to completely account for an animal's utilization of the food resource (Brody 1945, Edmondson and Winberg 1971). Loss of ingested or assimilated food through respiration or excretion can be minimized by using short duration feeding experiments (Hargrave 1971). This basic pattern of feeding studies has been incorporated into energy flow analysis for investigating ecological trophic dynamics (Lindeman 1942).

Using foods attached to a substrate, feeding rates can be measured as loss of weight from the substrate (Edmondson and Winberg 1971). Assimilation efficiency then can be computed as the weights ratio of food consumed minus the fecal material divided by the amount consumed. Changes in ratios of food constituents in the food and feces can assist in estimating assimilation efficiencies (Johannes and Satomi

1967, Edmondson and Winberg 1971, Fogal and Webb 1976); however, isotopic markers have been employed with greater success (Sorokin 1968, Edmondson and Winberg 1971). Through the use of radioisotopic nutrients (^{14}C, ^{32}P) or metals (^{60}Co, ^{137}Cs, ^{51}Cr), food resources can be followed during the assimilation process through the digestive system of the grazer (Trama 1957, Malone and Nelson 1969, Sorokin 1968, Hargrave 1970, 1971, Wilhm 1970, Sedell 1972, Calow 1975b). Assimilation efficiency can then be computed as the ratio of whole body counts (after gut clearance) to the radioactivity of the food ingested (Sorokin 1968, Edmondson and Winberg 1971). The use of radioisotopes in the previously cited studies has greatly facilitated research on the feeding and assimilation of small, aquatic animals (e.g. snails, insects and amphipods).

The choice of sulfur-35 to label the Aufwuchs samples in this research was made only after considering several possible isotopes. Studies of macroinvertebrate grazing rates and assimilation efficiencies have utilized a gamma ray or high energy beta particle emitting radioactive tracer in the food (Trama 1957, Malone and Nelson 1969, Elwood and Nelson 1972, Sedell 1972, Calow 1975b). Such isotopes allow the radioactivity of the food and the animal to be monitored without sample destruction. However, the radioactive metals commonly used, ^{60}Co, ^{137}Cs, ^{51}Cr, are not

physiologically incorporated into the food cells; rather they are absorbed into or adsorbed onto the food particles (Odum and Golley 1963, Grodzinski et al. 1975). Thus, digestive enzymes or pH changes in the gut may cause the radiotracer to be easily separated from the food particle, not accurately reflecting the digestion and assimilation of the food resource by the grazer (Grodzinski et al. 1975).

Three isotopes which can be incorported into microfloral biomass, ^{32}P, ^{14}C, ^{35}S, can be very useful in analyzing grazer utilization of Aufwuchs food resources. Inorganic phosphorus can be stored in excess quantities by some microfloral species or readily and rapidly excreted by others (Stockner and Armstrong 1971, Lean 1973, Wetzel 1975, Lean and Nalewajko 1976). In general, phosphorus is an active, labile nutrient in both plants and animals. The use of phosphorus-32 in feeding studies may capitalize on its incorporation into the plant cell, although many uncertainties may result as to the location and stability of the label in the food sample due to the cellular dynamics of phosphorus (Odum and Golley 1963).

In order to follow the flow of energy through aquatic food chains, the use of carbon-14 is widely accepted (Sorokin 1968, Edmondson and Winberg 1971). This tracer can be presented as inorganic or organic carbon allowing either autotrophs or heterotrophs to incorporate carbon-14 into

their cellular constituents (Grodzinski et al. 1975). From this approach, a thoroughly labeled food source is available to follow the flow of organic carbon through food chains. A major problem with using carbon-14 is continuous respiration of $^{14}CO_2$ by plants and animals. Accounting for this respiratory loss can be a difficult but necessary task to quantify all assimilated and non-assimilated food resources (Hargrave 1970, 1971).

Sulfur is not excessively taken up or readily excreted by aquatic microflora (Rodgers 1977, Jordan and Peterson 1978) which makes it a useful label for grazing studies. In addition, sulfur is incorporated in some proportion to the organic carbon present in the cells of aquatic microflora (Monheimer 1978), although carbon to sulfur ratios reported for aquatic microflora vary from 400 to 100 (Rodgers 1977, Jordan and Peterson 1978, Monheimer 1978). Perhaps the most useful aspect of employing sulfur-35 for feeding studies is that there are no respiratory losses, thus facilitating experimental computations of feeding parameters. Bacterial decomposition of fecal material may not result in the loss of sulfur-35 radioactivity from the sample. While carbon-14 may be respired rapidly by bacteria as they attach to and decompose a labeled fecal pellet, the sulfur-35 is more likely to be retained in the bacterial cells on the fecal pellet and included in the fecal sample.

There is an added benefit in using sulfur-35 in that an experiment need not be conducted in a restricted access, exteriorly ventilated system to avoid exposing a researcher to radioactive gases. The decay energy for the sulfur-35 beta particle is 0.167 Mev, and is almost identical in detection to carbon-14 (0.156 Mev) in liquid scintillation counting. Because the 87.9 day half-life of sulfur-35 is much shorter than that of carbon-14 (5730 years), sulfur can be used in most feeding experiments and greatly lessen human risk through accidental exposure or spills.

Research into producer-consumer interactions has taken many directions. In this dissertation, freshwater invertebrate grazers which utilize Aufwuchs food resources will be investigated since the role of this complex community in the nutrition of aquatic consumers has not been extensively studied. Previous research has emphasized grazer utilization of single species food offerings (Monakov 1972, Vivekanandan et al. 1974, Calow 1975b, Swiss and Johnson 1976) although in natural habitats a community of food organisms may be grazed upon. Grazing activities of aquatic insects typically have been evaluated through gut analyses (Moore 1977a). Grazing rates and assimilation efficiencies of the limnephilid caddis fly, Neophylax, were determined by Sedell (1972) through the use of cobalt-60 labeled Aufwuchs. Trama (1957) used phosphorus-32 to study the utilization of Aufwuchs food

resources by the mayfly, Stenonema pulchellum. The ingestion, egestion, and assimilation of benthic microflora by an amphipod, Hyalella azteca, has been intensively studied by Hargrave (1970, 1971).

Snails are particularly adapted to grazing Aufwuchs from smooth surfaces (Macan and Kitching 1976) and have been studied more extensively than other freshwater macro-invertebrates. However, several of these studies have emphasized the effects of snail grazing on Aufwuchs community composition or productivity (Castenholtz 1960, Kehde and Wilhm 1972, Doremus and Harman 1977) rather than the nutritional aspects of the producer-consumer interactions. Malone and Nelson (1969) used colbalt-60 labeled Aufwuchs to investigate the rate of gut clearance and daily cycle of feeding activity of the snail, Goniobasis clavaeformis. The effects of temperature on the utilization of Aufwuchs by this snail species has been studied using phosphorus-32 labeled food samples (Elwood and Goldstein 1975). Information from these snail feeding studies, when combined with the data generated in feeding studies using single specie food offerings, provides a general overview of factors influencing snail utilization of food resources. However, interactions between the quality of a complex food resource such as Aufwuchs and snail ingestion rates or assimilation efficiencies have not been extensively investigated.

OBJECTIVES

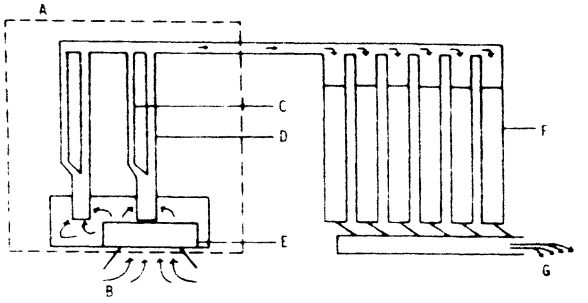
Three objectives associated with developing and testing structural and functional approaches for assessing the impact of power production-related perturbations on aquatic communities were addressed in this research. The first objective was to quantify cellular constituents of the microflora comprising Aufwuchs communities related to the nutrition of animal grazers in order to assess the impact of perturbations on Aufwuchs food quality. This objective was addressed by sampling Aufwuchs communities from artificial streams receiving low levels of perturbation and Aufwuchs in the New River below a power plant discharge. In the second objective, grazer utilization of Aufwuchs food resources from the stressed systems was investigated. The relationships between Aufwuchs food quality, grazer feeding rates and assimilation efficiencies were examined to determine the effects of low level perturbation on energy transfer between producers and consumers. Since sulfur-35 can be used to follow the uptake of inorganic sulfur in all components of the microflora, a third objective was to evaluate the use of this isotope in labeling Aufwuchs food sources to study the feeding and assimilation rates of grazers.

MATERIALS AND METHODS

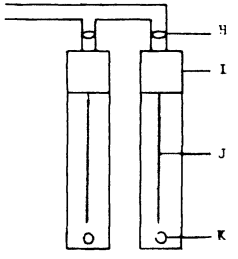
Aufwuchs Samples - Artificial Streams and New River

All Aufwuchs samples were obtained from New River water at the site of a 300 MW coal fired electrical generating plant located at Glen Lyn, Giles County, Virginia. This location was selected due to the energy production related perturbations of the power plant that were previously discussed. Aufwuchs communities were sampled from a series of artificial streams constructed at the power plant and from the New River in areas around the power plant discharges.

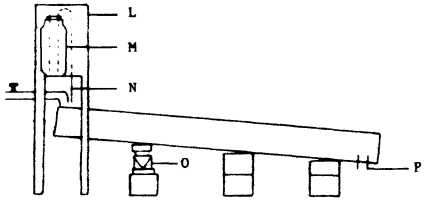
The artificial stream system consisted of a parallel series of six aluminum fish hatchery troughs (4 m x 35 cm x 39 cm) adjacent to the bank of the New River at the power plant pump house (Figure 1). The streams received untreated New River water on a once through flow at a rate of 30 l/min from two 1 hp Gould^{TM} submersible water pumps. A central, lengthwise, Plexiglas^{TM} partition formed two distinct and parallel channels with each stream. One channel of each stream was dosed with either chlorine, copper or dextrose while the opposite channel remained untreated. Stock solutions of the dosing chemicals were stored in boxes constructed over the influent end of the stream and delivered at controlled drip rates from marriotte bottles (Kubitschek 1970) or a peristaltic pump for chlorine solutions. Further details on the design and operation of these streams were reported in



A. Power Plant Screen House
B. New River Intake
C. Back-flush Line
D. Main Pump Line
E. Traveling Screen
F. Hatchery Trough
G. Outflow to New River



H. Inflow Gate Valve
I. Mixing Box
J. Plexiglas partition
K. Outflow



L. Chemical Dosing Box
M. Mariotte Bottle
N. Chemical Drip Tube
O. Automobile Jack
P. Outflow Drain

Figure 1. Schematic diagram of the artifical stream system at the Glen Lyn Power Plant.

Rodgers 1977, Clark et al. 1979, and Clark et al. (in press).

The dosing concentrations of chlorine, copper or dextrose were at levels known to cause toxicological impacts or changes in Aufwuchs structure and function (Becker and Thatcher 1973, Clark 1976, Rodgers 1977). Copper sulfate ($CuSO_4 \cdot 5H_2O$) was added to one stream channel to obtain a stream concentration of 0.05 ppm total copper. Water samples from this stream channel, preserved with acid, were analyzed by atomic absorption spectrophotometry. Another stream channel received intermittent chlorination (3 dosing periods per day, each lasting 20 minutes) to reach a stream concentration of 0.2 ppm total residual chlorine. The stock solution, made up from calcium hypochlorite (65% HTH), was recharged after 48 hours. Routine monitoring of stream chlorine concentrations was carried out by the amperometric titration procedure as described in Standard Methods (APHA 1976). Dextrose was added to a third stream channel to elevate the stream organic carbon levels by 1 ppm in experiments 1 and 2 and 2 ppm in experiments 3 through 7. Due to fluctuations in levels of organic carbon in the river source water and problems with analytical equipment, organic carbon levels were not monitored.

Prior to initiating an experiment in the artificial streams, silt and sediment in the channels were removed. Diatometers with clean glass microscope slides then were

placed on the bottom of the channels to be dosed as well as in the opposite, undosed channels. Each plexiglas diatometer held eight to ten vertically oriented microscope slides (Weber 1973b). The stream channels were dosed with their respective stock solutions and an experiment commenced.

Seven experiments were conducted in the artificial stream system from January through September, 1979 (Table 1). Mechanical problems as well as a six-week strike by the power plant personnel precluded any experiments between March and May 1979. The seven experiments encompassed a growing season with the expected fluctuations in temperature and biological communities. The glass slides were colonized by Aufwuchs until sufficient biomass accumulated to conduct an experiment (after 10 to 26 days depending on the season). Supplementary water chemistry (Table 1) characterized some water quality parameters from the artificial stream system which may have affected the degree of community response to the selected perturbations. Temperature and pH readings were from a calibrated thermister and an Orion pH meter, respectively. Alkalinity and hardness were analyzed according to Standard Methods (APHA 1976).

In sampling Aufwuchs from the New River, two types of diatometers with different glass substrates were used. In 1978, concrete slabs, 63.5 cm x 38 cm x 75 cm, with an inclined upstream facing plane, a horizontal top plane, and a

Table 1. Physical-chemical water quality of New River source water during Aufwuchs colonization for the seven artificial stream experiments.

Experiment Number	Colonization Dates	Temperature (°C)	pH	Alkalinity ($mgCaCO_3$ equiv)	Hardness ($mgCaCO_3$ equiv)
1	1/20/79 to 2/15/79	4.0[1] (3.0-4.5)[2]	7.2[1] (6.8-7.3)[2]	39[1] (37-43)[2]	47[1] (43-51)[2]
2	6/1/79 to 6/19/79	20.4 (18.6-22.8)	6.9 (6.8-7.3)	49 (42-56)	56 (50-65)
3	6/20/79 to 7/3/79	19.5 (15.6-22.2)	7.2 (6.8-7.4)	49 (41-58)	60 (51-76)
4	7/5/79 to 7/15/79	21.8 (20.2-23.7)	6.9 (6.7-7.0)	56 (50-63)	62 (55-69)
5	7/15/79 to 7/28/79	23.3 (21.3-24.8)	7.0 (6.9-7.2)	55 (48-65)	65 (57-73)
6	8/15/79 to 8/29/79	24.3 (22.2-25.8)	7.1 (7.0-7.4)	57 (46-61)	66 (54-71)
7	9/15/79 to 9/27/79	21.0 (19.3-22.2)	7.1 (6.8-7.3)	42 (36-50)	53 (47-65)

[1] means
[2] ranges

declined downstream facing plane were employed (Figure 2). Plastic bolts embedded in the concrete held glass plates (7.5 cm x 7.5 cm, with a hole in the center) to the diatometer. The entire unit (29.5 kg) held 24 glass plates in three rows of eight, one row in each plane of orientation to the current. The Aufwuchs samplers were placed on the river bottom inside a wooden sided cage (with open front, top and back) and covered with a fine mesh (2 mm x 2 mm) nylon screen to exclude grazers.

In order to compare more adequately the Aufwuchs samples from the New River with more conventional diatometer sampling devices, another diatometer design was employed in 1979. This unit consisted of a 12 cm by 7.5 cm plexiglas diatometer with eyebolts placed along the sides (Figure 3). The eyebolts were threaded over three upright reinforcement rods from a concrete filled cement block which allowed the diatometers to be incubated above the New River substrate to reduce grazing. Diatometers were incubated in the New River for four weeks prior to an experiment.

Three sampling stations were established in the New River to monitor the potential effects of the power plant discharge on Aufwuchs community food quality and grazer utilization of Aufwuchs (Figure 4). A reference station, station 1, was selected at the upstream end of a riffle across from the influence of the power plant chlorinated-thermal plume.

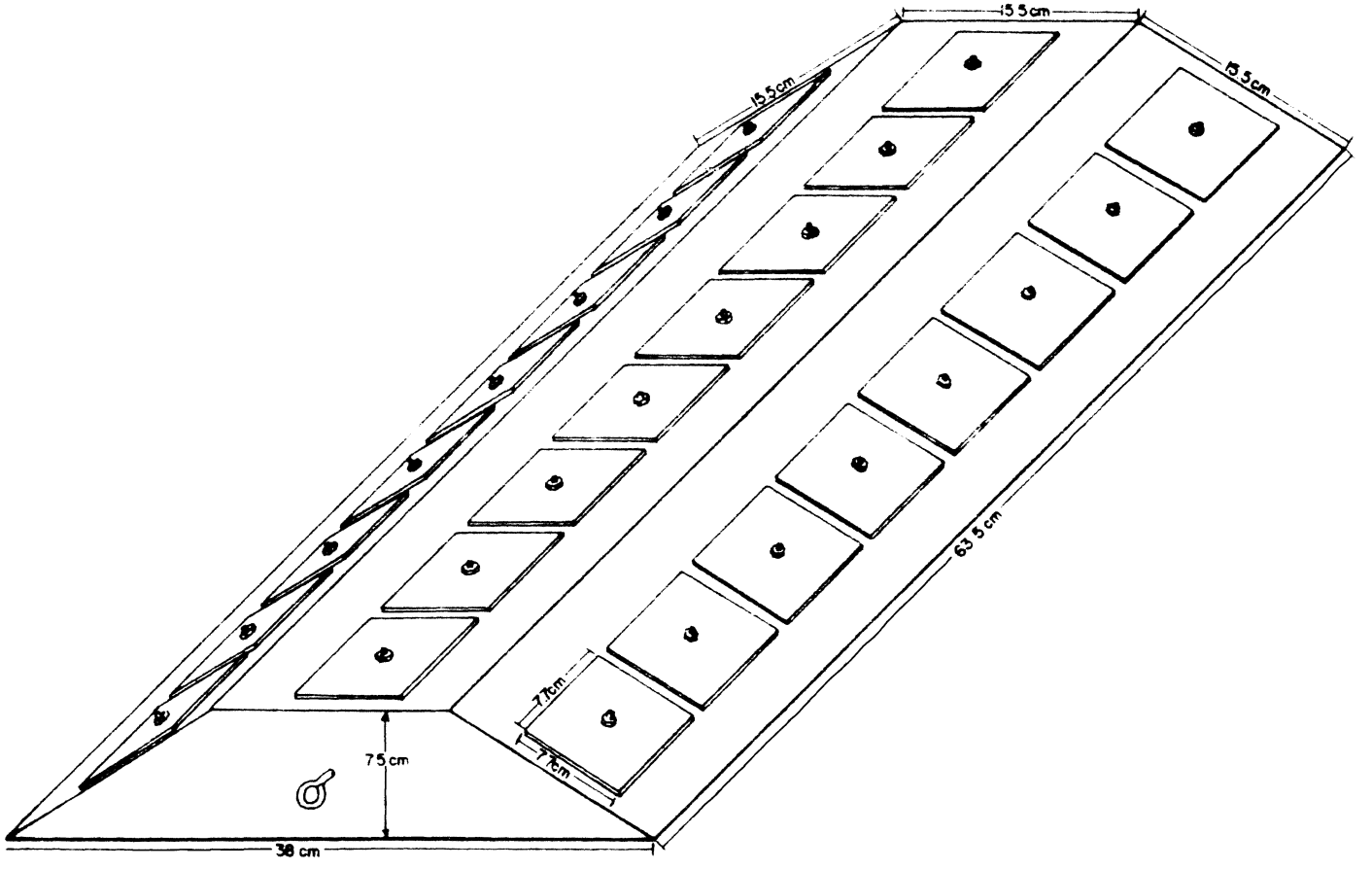


Figure 2. Benthic diatometer used in the New River during 1978 experiments.

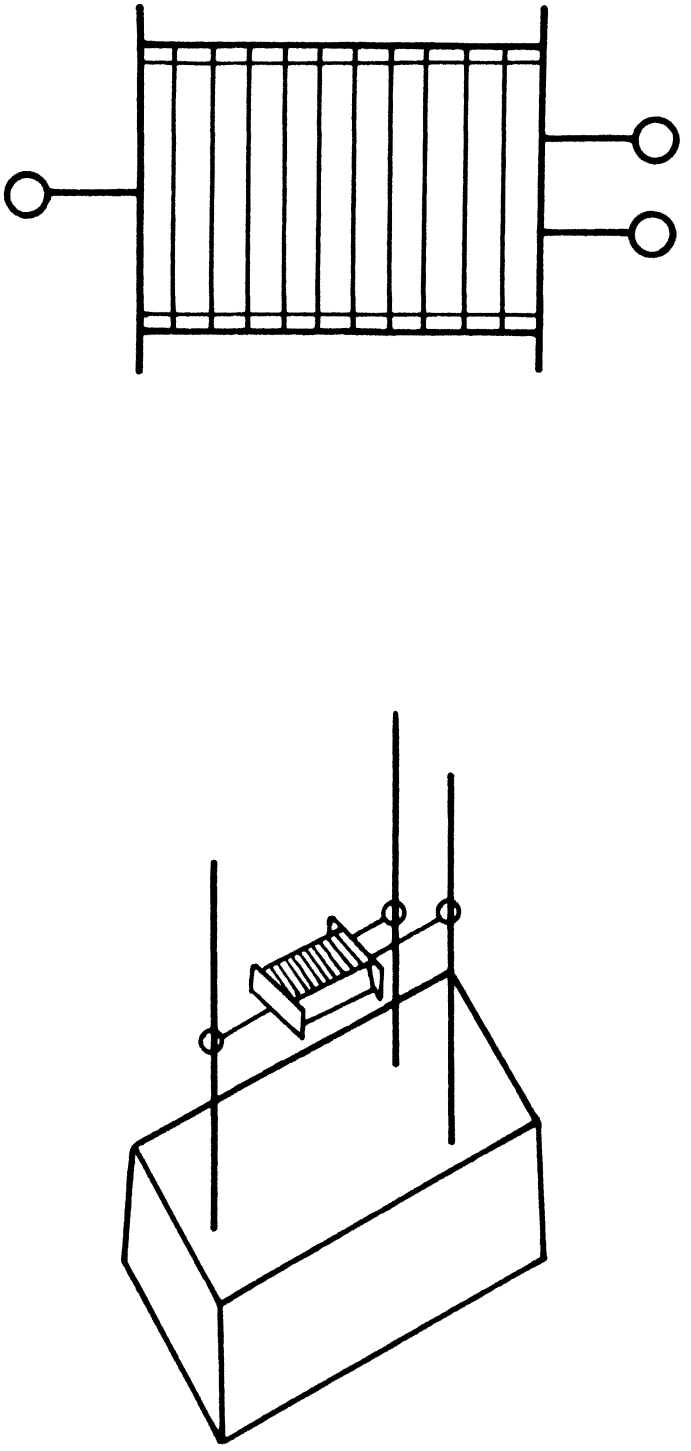


Figure 3. Diaometer used in the New River during the 1979 experiments.

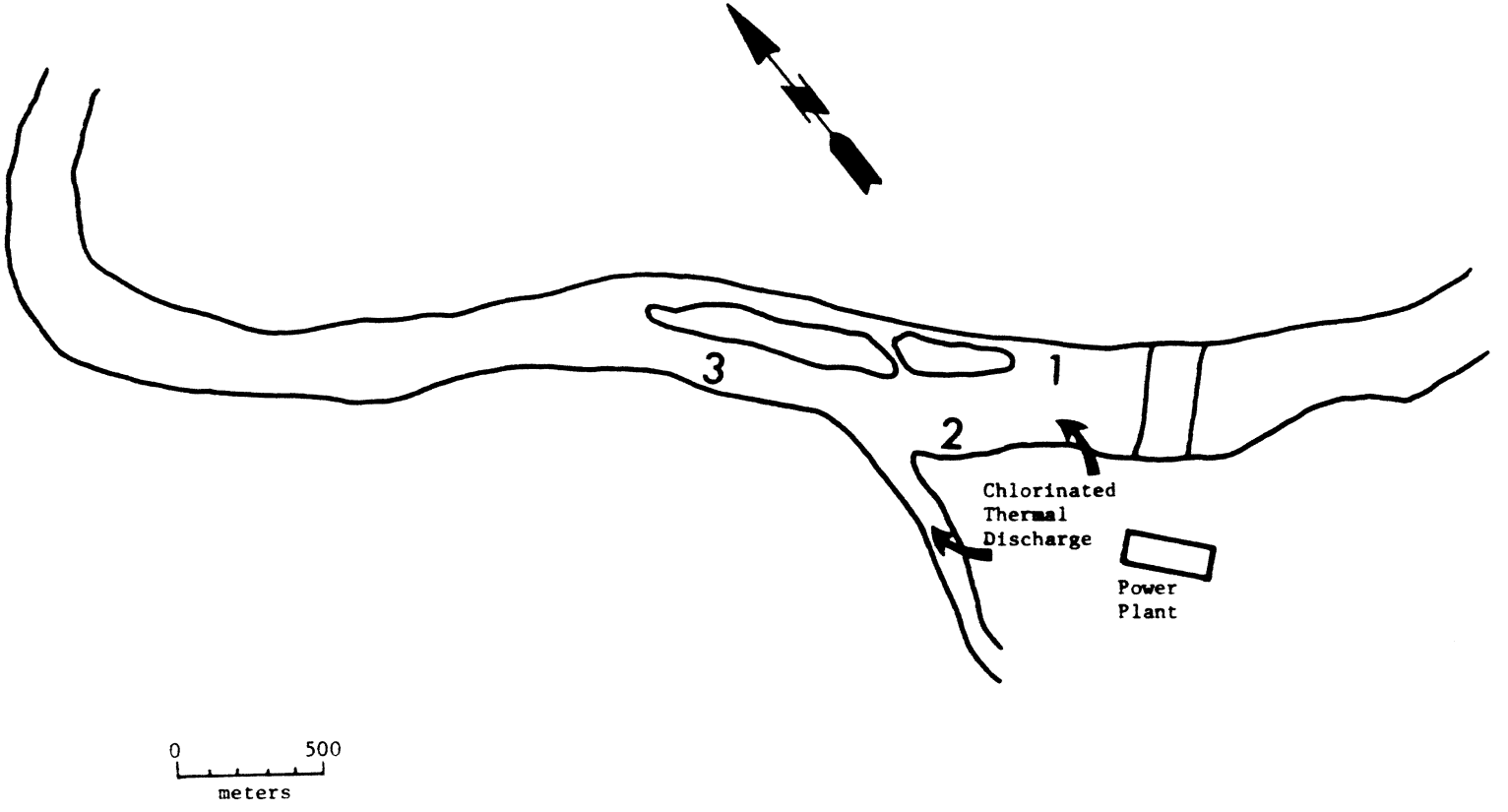


Figure 4. Schematic of the Glen Lyn Power Plant site and discharges into the New River respective to sample site locations.

Another sampling station, station 2, was set up within the effluent from one of the two generating units, 100 m upstream from the confluence of the New and East Rivers. Due to the separation of effluent waters, this station received approximately one-half to two-thirds of the chlorinated-thermal discharge (depending on plant operations), which elevated water temperatures 3 to 5^{o}C above ambient New River temperatures. The third sampling station was located approximately 500 m downstream from the confluence of the East River and within the combined plume of all thermal discharges. Chlorine residuals from the power plant discharge had been dissipated before effluent waters reached this sampling station (Cherry et al. 1977). Temperatures recorded at station 3 were usually 5 to 10^{o}C higher than the reference station (Stauffer et al. 1976).

Aufwuchs samples were obtained from the New River during extended periods of constant low flow in July and August of 1978 and 1979. Attempts to obtain samples at other times were unsuccessful due to vandalism, drastic changes in flow (flooding or low flow), or restricted access to the sample sites due to a strike by the power plant personnel. Three feeding experiments were conducted using Aufwuchs samples from the New River; two in August 1978 and one in August 1979. Since analytical procedures for food quality analyses were not perfected until after August 1978, analysis of food

quality for Aufwuchs from the New River was restricted to one experiment in August of 1979.

Food Quality Analysis

Aufwuchs samples for food quality analysis were obtained from glass slides incubated in the artificial stream system or New River stations previously described. All food quality and feeding studies were carried out simultaneously. Diatometers with colonized glass slides were transported to the laboratory in water filled buckets along with Aufwuchs samples for the feeding studies. A razor blade was used to scrape Aufwuchs from the glass slides into a sample vial. Slides for the food quality parameters were processed at the same time as Aufwuchs samples for the feeding studies so that the data could be interrelated.

For protein analysis, 4 glass slides from each artificial stream channel or river station were scraped into test tubes and stored at -15^{o}C for 3 to 10 days. Prior to analysis, the samples were thawed, augmented with 5 ml of a 2% sodium chloride (NaCl) solution, and ground 1 to 2 minutes in a Teflon^{TM} tissue grinder. The ground samples were stored overnight at 4^{o}C and assayed the next day.

Proteins were assayed using the Coomassie blue staining procedure of Sedmak and Grossberg (1977), using bovine serum albumin in 2% NaCl as standards. To a spectrophotometer cuvette containing 1 ml of the Coomassie blue stain, 1 ml of

sample extract or standard was added and read at 620 um absorbance after 1 minute on a Perkin-Elmer Model 55 spectrophotometer. Sample protein concentrations were calculated from a standard curve which had been computed using the GLM procedure of SAS 76 (Barr et al. 1976). Total protein estimates were obtained by multiplying the sample results by any dilution factors (if necessary) and the original extract volume of 5 ml. These estimates were restricted to NaCl soluble proteins in the extract. Proteins associated with cell fragments which had settled to the bottom of the test tube after grinding were not assayed due to turbidity interferences.

Four Aufwuchs samples from each artificial stream channel or New River station were scraped into test tubes and stored at -15^{o}C for carbohydrate analysis. Prior to analysis, 5 ml of distilled water was added to the frozen samples, which were then ground 1 to 2 minutes in a Teflon tissue grinder. The samples were then stored at 4^{o}C until assayed 1 or 2 days later.

Carbohydrate analyses were conducted following the general procedures of Strickland and Parsons (1972). An anthrone reagent was prepared by combining 8 ml of ethyl alcohol with 30 ml of distilled water and adding 0.2 g of anthrone, followed by addition of 100 ml of 36 N sulfuric acid. This reagent was prepared fresh for each assay. A

series of carbohydrate standards were prepared using glucose dissolved in distilled water. After vigorous shaking, 1 ml of sample suspension or standard solution was placed in a clean test tube followed by the addition of 10 ml of anthrone reagent and incubation in a 100°C waterbath for 7 minutes. Upon removal from the waterbath, samples were cooled to 20°C under running tap water. Samples were read in a Model 55 Perkin-Elmer spectrophotometer at 460 um. Total carbohydrate from each Aufwuchs sample was calculated from a standard curve, computed using the GLM procedure (Barr et al. 1976), and by multiplying by the total extract volume (5 ml).

Caloric estimates were obtained from two slides chosen randomly from each treatment group. Aufwuchs were scraped by razor blade from the glass slide into a 5 ml glass beaker and stored (up to 20 days) in a drying oven at 65°C until 15 to 25 samples had been collected from various experiments. There is no decrease in caloric estimates for samples stored in this manner (Maciolek 1962). The samples were assayed for caloric value by the dichromate oxidation procedures of Maciolek (1962). This method measured the oxygen consumed in oxidizing the sample using 2 N potassium dichromate, $K_2Cr_2O_3$, and 11 ml of 36 N sulfuric acid. By titration with 3 N ferrous sulfate the reduction of $K_2CR_2O_3$ during the oxidation of the organic material was computed. By quantifying the oxygen consumed as the sample was digested and

employing a conversion factor of 3.4 g-cal per mg oxygen consumed (Maciolek 1962), a measure of sample caloric content was derived. Further, known quantities of glucose were analyzed along with the Aufwuchs to serve as reference standards to check the caloric calculations.

The organic carbon content of the Aufwuchs colonizing the glass slides in the artificial streams and the New River was computed using gravimetric analysis. Four slides from each group were scraped into tared porcelain crucibles, dried overnight at 65^{o}C (Vollenweider 1974), then weighed to the nearest 0.1 mg on a Mettler analytical balance. Thereafter, the samples were fired at 550^{o}C for 1 hour, rewetted, and dried 24 hours at 65^{o}C (Vollenweider 1974). A final weighing of the samples was used to compute weight lost upon ignition, also known as ash-free dry weight (APHA 1976). This parameter is commonly used as a measure of the organic matter of a sample (Vollenweider 1974, Wetzel 1975).

One slide from each sample group was fixed in an algal preservative (5% formalin, 1% glycerol) to be examined microscopically at a later date to determine the relative composition of the Aufwuchs community. From 5 random fields of view (40x magnification), algae were counted and identified to the family or generic level. Bacteria were not counted but were deemed dominant when a heterotrophic mucilaginous matrix covered the surface of the colonized

glass slide.

Snails - Source and Acclimation

Snails used in the feeding studies were collected from the New River at stations 1 and 2. Representative individuals were identified as members of the genus Anculosa (Gastropoda:Pleuroceridae) using the key characteristics of Pennak (1978). According to Pennak, this genus synonymizes the genera formerly known as Nitocris, Spirodon, and Mudalia. Dillon (1977) conducted an extensive study of the distribution and abundance of gastropods in the New River and its tributaries above Claytor Lake, Virginia, and identified the species used in this study as Mudalia dilatata. This snail was one of the most common molluscs in the New River (Dillon 1977) and was easily distinguished from other snails in the New River by shell morphometry and operculate rings.

The shell size of snails used over all the feeding studies ranged from 6 to 20 mm while visceral dry weights ranged from 5 to 50 mg. This test population included both males and females since sex determination is not possible using external morphological traits (Pennak 1978). Snails collected in the New River were returned to the laboratory and held in aquaria at 20^{o}C under a 16- and 8-hour light/dark photoperiod. Snails were acclimated to laboratory conditions for at least two weeks prior to use in feeding experiments. During the holding period, snails were allowed

to graze on the aquaria walls with additional food provided from diatometers taken from the reference channels of the artificial streams. The snails readily adapted to the aquaria environment; the population death rate was less than 2% per week from January through September, 1979.

Feeding Studies

Each feeding study utilized Aufwuchs attached to glass slide substrates as food sources for the snails. Diatometers with colonized glass slides were collected at Glen Lyn and returned in river water to the laboratory where the feeding experiments were conducted. Seven experiments were designed to evaluate snail utilization of Aufwuchs from dosed artificial streams (Table 1). Snail utilization of food resources from natural habitats perturbed by power plant discharges compared to uninfluenced food resources was investigated using Aufwuchs communities from the New River sampling stations. Sulfur-35 was used to label the Aufwuchs samples for these feeding experiments.

Approximately 300 uCi of sulfur-35 (as $H_2{}^{35}SO_4$) were added to 5 l of dechlorinated tap water in a wash tub equipped with a magnetic stirrer. Diatometers with colonized glass slides were placed in predesignated positions in the tub such that the radiolabeled media flowed freely over all the glass slides during the mixing process. In order to achieve

a thoroughly radiolabeled food source of high specific activity, the Aufwuchs samples were incubated overnight (18-20 hours) in a controlled environment of 20^{0}C and a 16- and 8-hour light/dark photoperiod. During incubation the samples received 4 to 6 hours light, 8 hours dark, and then 6 hours light thereafter.

Snail guts were allowed to clear overnight prior to a feeding experiment. Preliminary data had shown that snail fecal output diminished after 8 hours which coincided with the clearance of radiolabeled food in starved and feeding snails (Figures A1 and A2, Appendix). Snails were allowed to graze 4 hours in experiments 1 through 4 and 6 hours in experiments 5 through 7. Although the 4 hour feeding experiments gave measurable results, the longer feeding time in the later experiments allowed for greater quantities of food to be eaten, assimilated, and fecal material formed. The food source was removed after the feeding time and snail guts were allowed to clear overnight again, this time to collect the feces formed from the unassimilated Aufwuchs consumed.

Plastic, 8 cm diameter petri plates, filled with 20^{o}C dechlorinated tap water, were used as test chambers. Snails were held individually in chambers overnight prior to an experiment to allow for adequate acclimation to test conditions and gut clearance. Immediately prior to commencing

a feeding experiment, the water was removed, the chamber rinsed to flush the accumulated snail feces, and fresh, dechlorinated tap water added. These preparation steps were accomplished with minor disturbance to the snail.

To initiate a feeding experiment, labeled Aufwuchs samples were removed from the isotopic media and rinsed in an aquarium of 0.1 M sodium sulfate (Na_2SO_4) in dechlorinated tap water to remove any adhering isotope solution from the Aufwuchs. In this manner, further uptake of isotopes and change in specific activity of the food was minimized (Jordan et al. 1978). Aufwuchs were then scraped from one side of the glass slide with a razor blade and deposited in a clean, numbered scintillation vial for subsequent weighing and isotopic analysis. This sample constituted a time zero measure of food dry weight and radioactivity. The slide was then placed, cleaned side down, into a prepared feeding chamber with a starved snail. The snail was placed on the Aufwuchs sample and the feeding time begun, while control slides were placed into chambers without snails.

During the feeding period, snails were checked each 30 minutes as to their position with respect to the food source. If the snails' grazing-exploratory movements had directed them off the glass slide and onto the bottom of the petri plate, they were lifted back onto the feeding surface. This maximized the feeding rate for each snail

since in preliminary experiments some snails grazed to the edge of the glass slide, then off onto the bottom of the feeding chamber, and failed to return to the food sample. Snails placed back onto the glass slide usually resumed feeding within one minute.

After the 4 or 6 hour feeding time, the Aufwuchs food sample was removed from the feeding chamber. The unconsumed Aufwuchs was scraped into a numbered scintillation vial for weighing and isotopic analysis. Any food particles which had been dislodged from the glass slide but not ingested were removed from the feeding chamber and added to the food samples. Fecal pellets, distinguished by their compact, regular shape, were left in the feeding chamber. They were collected, along with additional feces, after the snail guts had cleared overnight.

The following morning, each snail was removed from its feeding chamber and rinsed of any attached feces. It was dropped into a beaker of boiling water for one minute to facilitate removal of the snail and its shell. The snail body was then placed in a numbered scintillation vial for weighing and isotopic analysis.

Fecal samples were trapped on a numbered 0.45 um Millipore^{TM} membrane filter as the water from the feeding chamber was pulled through a 13 mm diameter filtration apparatus. The tared filter with accompanying fecal sample was

placed on a sheet of aluminum foil for drying. Samples from the control chambers were filtered in a similar manner. Finally, aliquots of filtered water were sampled for isotopic analysis. Liquid scintillation solution (as described below) was added directly to the water samples to prepare them for counting.

All samples, except water samples, were dried 24 hours at 65^{o}C and weighed to the nearest 0.1 mg on a Mettler analytical balance. All Aufwuchs and snail samples were returned to their respective liquid scintillation vials after weighing while fecal samples were subsequently placed in numbered vials. The samples were then prepared for liquid scintillation. Briefly, a small volume of perchloric acid (0.4 ml) and hydrogen peroxide (0.7 ml) was added directly to scintillation vials containing a dried sample. The vial was capped and placed in a 70 - 80^{o}C water bath for 1 hour to dissolve and bleach the organic matter (Wang et al. 1975). Upon cooling, 8 ml of ethylene glycol monoethylether and 12 ml of toluene counting solution were added (Bransome 1970). PPO (2,3 Diphenyloxazole) was added at a rate of 6 g per liter toluene to prepare the toluene counting solution (Bransome 1970). The sample was held overnight to reduce background luminescence, then counted on a Beckman model LS3150T liquid scintillation counter. Samples were corrected to 100% counting efficiency using a channels ratio quench

curve (Wang et al. 1975).

The specific activity of each food sample was calculated as total corrected sulfur-35 counts per minute (cpm) per milligram Aufwuchs dry weight. Although the isotope was incorporated into the living organic matter of the Aufwuchs community, only the dry weight, which included inorganic sediment, could be measured. Attempts to measure the specific activity of each sample on an organic matter basis would require volatilization of the organic matter with subsequent entrapment (Sorokin 1968), leaving the sulfur-35 with the inorganic material as a SO_4 salt.

The dry weight of Aufwuchs eaten by each snail was computed by summing the total corrected sulfur-35 counts per minute (cpm) from the snail body and the fecal material divided by the specific activity of the food sample. This is summarized as:

$$\text{Dry weight consumed (mg dry weight)} = \frac{\text{snail body (cpm)} + \text{fecal sample (cpm)}}{\text{Aufwuchs specific activity (cpm/mg dry weight)}}$$

The feeding rate was calculated as the dry weight eaten divided by the duration of the feeding time (4 or 6 hours depending on the experiment):

$$\text{Feeding rate (mg dry wt/hr)} = \text{dry weight consumed (mg dry weight)} / \text{feeding time (hr)}$$

Consumption of organic matter is a more appropriate

parameter for monitoring the utilization of Aufwuchs by grazers compared to dry weight consumed since the dry weight includes inorganic material which is of little or no nutritional value. Estimates of Aufwuchs consumed by each snail were corrected for bias due to the inclusion of inorganic matter by multiplying the estimates of Aufwuchs dry weight consumed by the percent organic matter in the Aufwuchs food sample (Malone and Nelson 1969). The percent organic matter in Aufwuchs samples processed for food quality analysis was used for this computation. There is only a small error in this calculation since the percent organic matter (computed as ash-free dry weight/dry weight) was closely replicated among samples of the individual Aufwuchs communities (median coefficient of variation 15%, range 6% to 70%). This computation is summarized as follows:

Aufwuchs organic matter eaten (mg) = dry weight consumed (mg) x % organic matter

Within each treatment group (i.e. snails feeding on Aufwuchs from a reference stream, a chlorinated stream, or a copper dosed stream, etc.), the data will have the same dispersion as the estimates of dry weight consumed since a mathematical constant (the appropriate mean percent organic matter for that food sample) was used to derive the estimate of organic matter consumed. However, differences between food samples in their percent organic matter may change the relative differences between feeding groups compared to data based on

Aufwuchs dry weight consumed. Division by the appropriate feeding time allows for a computation of the feeding rate on an organic matter basis.

Snail assimilation efficiences were calculated as 100% times the ratio of the snail body isotopic counts to the sum to the body and fecal counts (Grodzinski et al. 1975). This is formulated as:

$$\text{Assimilation efficiency} = \frac{\text{snail body (cpm)}}{\text{snail body (cpm)+fecal sample (cpm)}} \times 100$$

By utilizing only the sulfur-35 counts, this parameter most closely represents the efficiency with which the organic matter in the food sample was assimilated.

The final parameter computed from the feeding study data was the organic matter assimilated. This variable incorporates both the amounts of organic matter consumed and the grazers' ability to assimilate that organic matter from the various Aufwuchs communities. Milligrams organic matter assimilated was computed as the estimate of organic matter eaten multiplied by the measured assimilation efficiency:

$$\text{Organic matter assimilated (mg)} = \text{organic matter consumed (mg)} \times \text{assimilation efficiency (\%)}$$

Comparison of Feeding Parameters Based on Sulfur-35 or Carbon-14 Labeled Aufwuchs

Four experiments were conducted to compare estimates of snail consumption and assimilation of Aufwuchs based on sulfur-35 or carbon-14 labeled food samples. Aufwuchs samples

from a reference stream were used as the food source for these experiments. Diatometers with colonized glass slides were collected at Glen Lyn and returned in river water to the laboratory.

Test chambers and snail preparations for these experiments were the same as those previously described. In addition to preparing a sulfur-35 labeling medium, a carbon-14 labeling solution was prepared by adding 25 uCi of $NaH^{14}CO_3$ to 5 l of dechlorinated tap water in a tub equipped with a magnetic stirrer. Half of the glass slides from within a diatometer were placed in the sulfur-35 media while the other half were immersed in the carbon-14 media until a total of 10 slides were obtained for each test group. The solutions were then mixed overnight (18-20 hours) under the incubation conditions previously described. The same general experimental procedures were employed throughout these feeding experiments as has been described for the feeding experiments comparing snail utilization of Aufwuchs from variously stressed environments. Sulfur-35 labeled samples were processed in the same manner while some procedural changes were introduced for carbon-14 labeled samples.

In the first two experiments, carbon-14 labeled samples (food, snails, feces) were processed in a manner similar to sulfur-35 labeled samples. These samples were placed into 4 cm test tubes, deposited in numbered scintillation vials,

and placed in a drying oven (60°C) for subsequent weighing and isotopic analysis. The role of the 4 cm test tube will be explained below. In the third and fourth experiments, time zero Aufwuchs samples were processed in the same manner as in the first two experiments while the remaining samples were fixed with 1 ml of basic 3% formalin (pH 8.0) upon collection. They were then dried and weighed as done with the other samples in order to determine if carbon-14 was being lost through respiration as unfixed samples were drying. Also, three extra carbon-14 labeled samples were included in these experiments (making n=13 instead of 10). The extra samples (Aufwuchs, snails and feces) were frozen upon collection and prepared for liquid scintillation counting without drying or weighing. The carbon-14 radioactivity of these samples was compared to fixed and unfixed samples to determine if either fixing or drying would decrease the carbon-14 content of the samples.

The carbon-14 samples were prepared for liquid scintillation counting following the procedures by Rodgers (1977). After weighing, the sample was placed back into the 4 cm test tube. Two ml of 0.25 N sodium hydroxide (NaOH) were added to the scintillation vial (Rodgers 1977). One ml of cold (4°C) chromic acid was added to the sample and the test tube dropped into the scintillation vail which was then tightly capped (Rodgers 1977). This reaction vial

was placed in a boiling water bath for 3 hours to oxidize the labeled organic matter, volatilize the $^{14}CO_2$, and trap the isotope in the sodium hydroxide medium (Sorokin 1968). The sample was then cooled 2 to 5 hours at $4^{o}C$ and the oxidation tube removed. Twelve ml of triton X-100 counting solution (prepared by adding 333 ml of triton X-100 to 666 ml of toluene and adding 6 g PPO and 0.5 g POPOP [1,4-bis-2-(5-phenyloxazolyl)-benzene]) was added (Bransome 1970). The samples were held overnight to reduce chemoluminescence and radioactivity was determined on the Beckman LS-3150T liquid scintillation counter. Since all samples were counted in a clear solution of NaOH and the liquid scintillation cocktail, the data were corrected to 100% counting efficiency employing the 63% efficiency factor determined by carbon-14 toluene standards.

Data Analysis

Food quality parameters

To facilitate comparisons with data reported in the literature, square meter estimates of the food quality parameters from glass microscope slide substrates were obtained by dividing the raw data by 0.00375 (APHA 1976). Test for normality (Kolmogorov procedure, Helwig 1977) of the sample data from food quality analyses for each experiment indicated no significant differences from normal dispersion ($\alpha<0.07$ for each experimental test group). Differences

between means (Figures A3 to A9, Appendix) were tested by the analysis of variance procedure (Barr et al. 1976). If differences were indicated at a significance level of 0.1 or less, the Dunnett treatment versus control test (Steel and Torrie 1960) was employed at designated signficance levels of 0.1, 0.05, and 0.01 to identify the source and degree of significance.

Mean estimates of the food quality parameters were used to compute ratios of nutrient components to produce indices of food quality. The percent organic matter in the Aufwuchs samples was computed as mean ash-free dry weight/ mean dry weight. This index decreased as sediment and other inorganic matter accumulated within or on the colonized substrate. Protein and carbohydrate data were expressed as a percent of the organic matter in the Aufwuchs community by dividing means of these nutritional components by the mean ash-free dry weight estimate. In this manner, increases in protein or carbohydrate per unit organic matter might more accurately indicate changes in food quality. Caloric estimates per unit organic matter were computed in a similar manner.

Feeding experiments

The parameters of the feeding experiments reported and discussed in this dissertation were measured either directly from the raw data, (dry weight of Aufwuchs consumed, assimi-

lation efficiency) or computed from ratios of measured parameters (organic matter consumed, organic matter assimilated). Although other parameters were measured or could have been computed, the variables chosen for analysis and discussion represent the most pertinent information to address the objectives of this research. The means, medians, ranges and standard deviations of the dry weight and organic matter consumed, organic matter assimilated, and the assimilation efficiency for each test group of snails over all the experiments are presented in Figures A10 through A16 in the Appendix. In each experiment, the data were biased by snails in each test group that either did not eat, did not produce feces, ate voraciously, produced copious feces, or died. Since the propensity of a snail to ingest food was of central interest in this research, all data points obtained for analysis were included. Tests for normal distributions (Kolmogorov procedure, Helwig 1977) among the four parameters for each of the seven artificial stream experiments indicated only 4 of the 27 data sets were normally distributed. Median values were used to represent the central tendencies for each group. The Kruskal-Wallace rank sum test and the Dunn treatment versus control comparisons (nonparametric procedures) were employed to discern differences between groups since these measures are less sensitive to outlying data points (Hollander and Wolfe 1973).

Data variance resulted in few significant differences in all of the feeding study parameters. The variability was homogeneous between groups for each parameter in an experimental (Figures A10 to A16, Appendix). Thus, attempts to mathematically transform the data to decrease the variability would not allow any increased statistical sensitivity (Sokal and Rohlf 1969, Greene 1979). With such large variances due to the individuality of the test animal, the only way to increase statistical sensitivity would have been to increase the sample size or artifically reduce the variance. Considerations of centralizing the data for each experiment by "throwing out the highs and lows," even by some prescribed procedures (Greene 1979) are generally frowned upon and were not addressed. Since each group had one or two outliers, the overall trends would probably not be affected (Figures A10 to A16, Appendix). Increasing the sample size was considered in light of the total research effort. Each snail feeding chamber contributed five separate samples for gravimetric and isotopic analysis. Thus, with six replicate feeding chambers for each treatment or reference group, a total of over 120 samples were weighed and prepared for liquid scintillation counting. When this volume was added to the concomittent food quality analyses, it was not feasible to significantly increase the sample size in the feeding experiments. Estimates of the necessary sample size

to demonstrate a 25% change in Aufwuchs consumption due to a treatment effect ranged from 25 to 40 replicates per treatment group. Thus, at the end of experiment 2, a re-evaluation of the experimental design indicated that increasing the sample size to meet the statistical criteria would not be feasible. The sample size remained at five or six replicates for each test group. Results of the seven feeding experiments were examined for consistent trends or differences between experimental groups, as well as statistically significant differences, to provide a general test of the research hypotheses. For the seven artificial stream experiments, correlations among the mean food quality estimates from each treated and reference artificial stream were computed using the Spearman rank sum procedure (Barr et al. 1976). The food quality estimates were analyzed for correlations with the median estimates of the feeding parameters employing the same procedures.

Comparison of feeding parameters based on sulfur-35 or carbon-14 labeled Aufwuchs

The data from feeding studies comparing estimates of snail consumption and assimilation efficiency of Aufwuchs labeled with sulfur-35 or carbon-14 were also highly variable due to snail individuality. Differences between these two experimental groups were analyzed by the Kruskal-Wallace two sample rank sum test (Hollander and Wolfe 1973).

RESULTS AND DISCUSSION

Artificial Stream Experiments

Food quality analysis

The taxonomic characteristics of Aufwuchs community changed with seasons and dosing treatments over the sequence of seven experiments conducted (Table 2). Aufwuchs communities in reference streams were dominated by diatoms in all but one experiment (experiment 6), when blue-green algae became more prevalent. Temperature ranges in experiment 1 through 7 increased from 3.0^{o}C initially to a maximum of 25.8^{o}C during experiment 6 and decreased thereafter. Although the Aufwuchs communities in the chlorinated stream were similar to the reference communities in experiments 1 and 2 (3.0-22.8^{o}C), chlorine doses of 0.2 ppm TRC tended to favor growth and dominance by blue-green algae in subsequent experiments, especially in experiments 5, 6, and 7 (19.3-25.8^{o}C). Blue-green algae were most abundant in Aufwuchs communities developing under the copper treatment (0.05 ppm) in experiments 3 through 7. There was no copper treatment for experiment 2 due to mechanical problems. Diatoms dominated the copper treated Aufwuchs community in experiment 1. Dextrose additions stimulated heterotrophic growth most effectively in experiments 2 through 7, which were conducted at water temperatures of 15^{o}C or greater (Table 1). Heterotrophs, while stimulated above reference stream community

Table 2. Microfloral taxonomic divisions comprising ≥ 25% of the cell counts in Aufwuchs sampled from reference and treated artificial streams with approximate percent abundance (%) over the seven assessment experiments. [1]Bacterial dominance was determined by the relative extent of mucilage in the Aufwuchs sample while the relative frequency with which algal groups were observed within the mucilage was designated as often (oft), occasionally (occ) or seldom (sel) in the dextrose treated stream.

Experiment	Temperature Range (C)	Stream Treatment			
		Reference	Chlorine	Copper	Dextrose[1]
1	3 - 4.5	Diatoms (92%)	Diatoms (90%)	Diatoms (92%)	Bacteria Diatoms (oft)
2	18.6 - 22.8	Diatoms (80%)	Diatoms (76%)		Bacteria Diatoms (oft)
3	15.6 - 22.2	Diatoms (66%) Green algae (23%)	Diatoms (42%) Blue-green algae (40%)	Blue-green algae (50%) Diatoms (45%)	Bacteria Diatoms (oft)
4	20.2 - 23.7	Diatoms (60%) Green algae (24%)	Diatoms (46%) Blue-green algae (42%)	Blue-green algae (73%) Diatoms (25%)	Bacteria Green algae (occ)
5	21.3 - 24.8	Diatoms (72%) Blue-green algae (25%)	Blue-green algae (60%) Diatoms (40%)	Blue-green algae (73%)	Bacteria Diatoms (sel)
6	22.2 - 25.8	Blue-green algae (50%) Diatoms (42%)	Blue-green algae (74%) Diatoms (24%)	Blue-green algae (82%)	Bacteria Diatoms (sel)
7	19.3 - 22.2	Diatoms (55%) Blue-green algae (35%)	Blue-green algae (60%) Diatoms (37%)	Blue-green algae (72%)	Bacteria Diatoms (sel)

abundances, were less abundant in the dextrose treated stream during experiment 1 compared to other experiments. The shifts in structure demonstrated by this limited analysis of community composition were used to evaluate and explain the results of assessing the impact of perturbations through analyses of Aufwuchs chemical food quality.

The addition of chlorine caused dramatic changes in the food quality of the Aufwuchs community. In experiments 1 through 4, which were conducted when water temperatures were less than 21^{o}C, decreases in the accumulated dry weight and ash-free dry weight (a significant decrease in experiments 1 and 4) of Aufwuchs were recorded relative to reference stream communities (Figures 5 and 6). In these early experiments, the Aufwuchs communities developing under the chlorine stress maintained a composition similar to the reference stream community (Table 2). However, during experiments 5, 6, and 7 water temperatures exceeded 21^{o}C and blue-green algae dominated the chlorinated stream community. Under these conditions, dry weight estimates exceeded the reference stream values by 7.2 and 3.5 g/m^{2} for experiments 6 and 7 (significantly different in 6) (Figure 5). The organic matter on the glass slide substrates was 1.3 g/m^{2} less than reference stream values in experiment 7 and significantly less, (0.8 g/m^{2}) in experiment 5 (Figure 6). The percent organic matter (Figure 7) in the Aufwuchs samples from the chlorinated

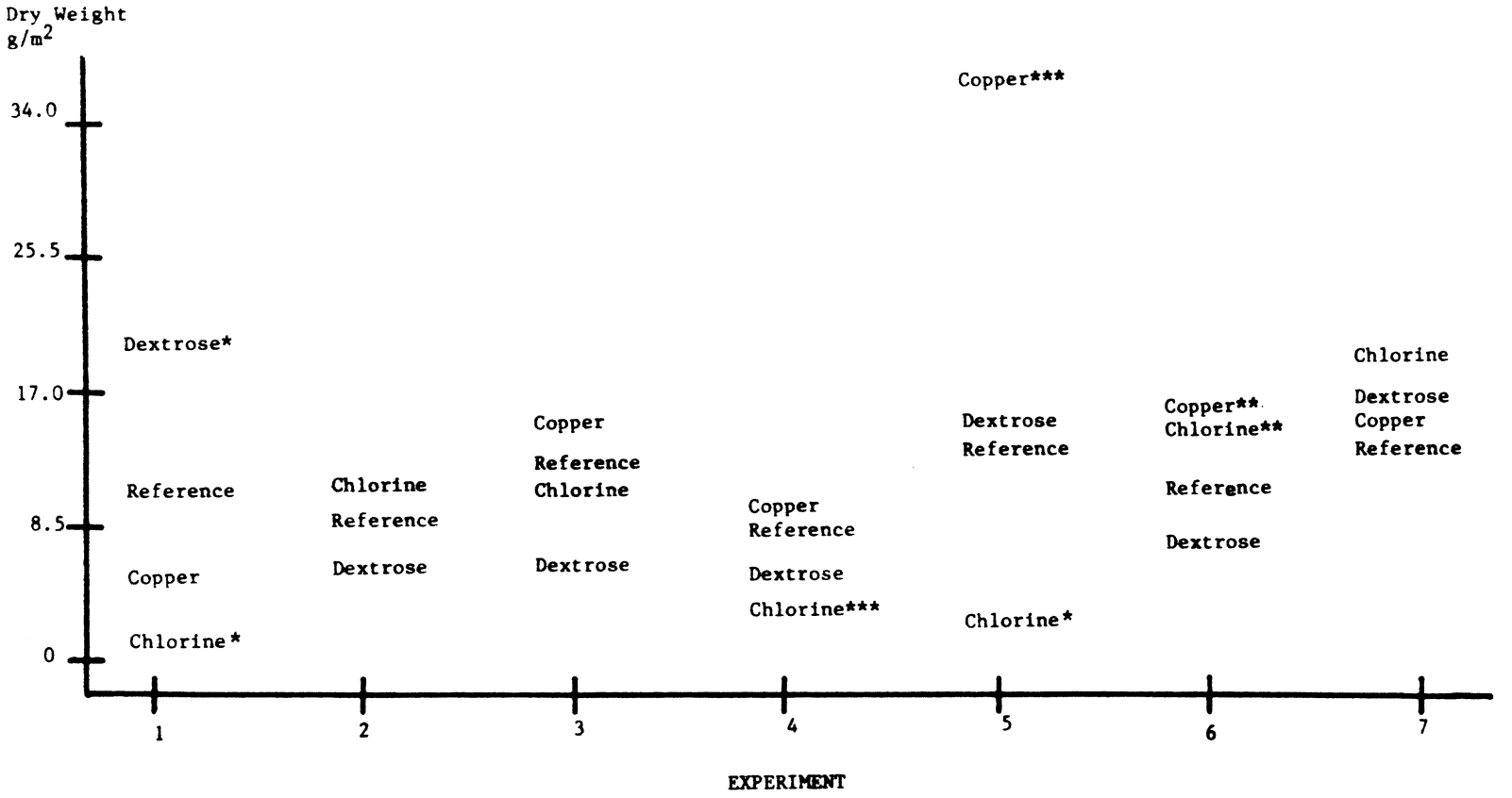


Figure 5. Aufwuchs dry weight accumulated during the artificial stream experiments. Mean values for each stream are plotted with results of the Dunnett treatment versus control test (* for α=0.10, ** for α=0.05, *** for α=0.01).

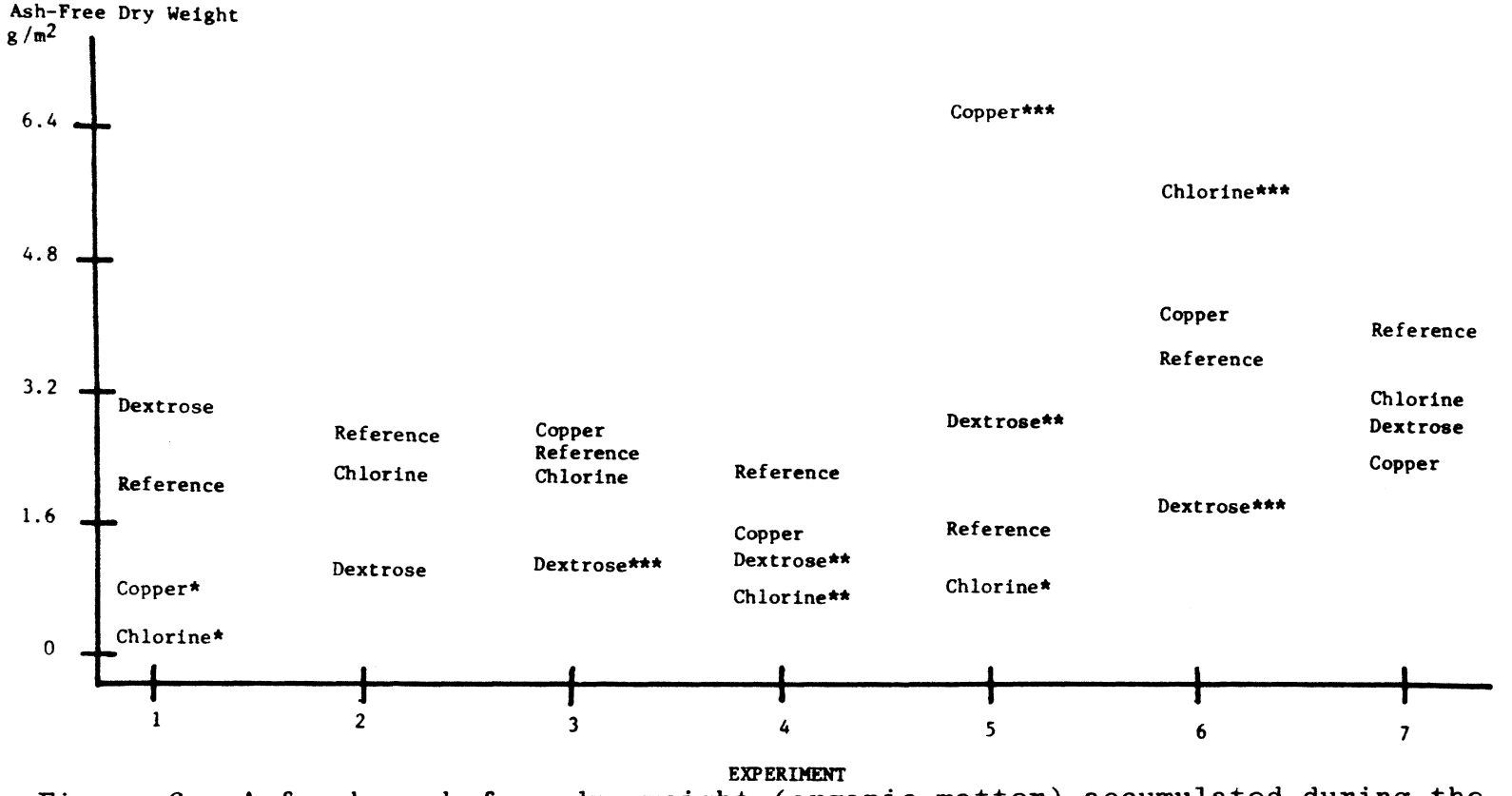


Figure 6. Aufwuchs ash-free dry weight (organic matter) accumulated during the artifical stream experiments. Mean values for each stream are plotted with results of the Dunnett treatment versus control test (* for α=0.10, ** for α=0.05, *** for α=0.01).

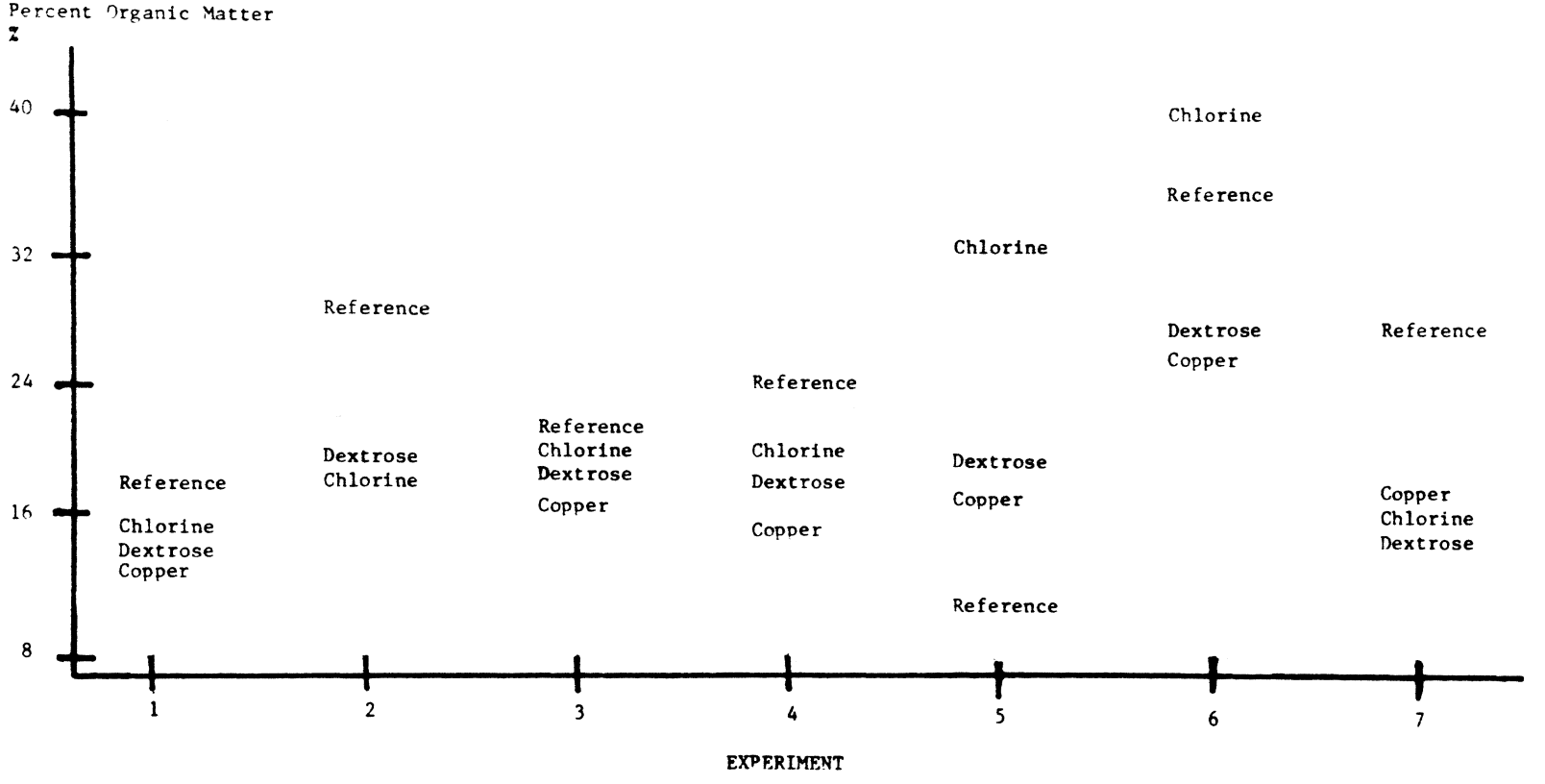


Figure 7. Percent organic matter in Aufwuchs sampled during the artificial stream experiments. Values represent mean ash-free dry weight/ mean dry weight.

stream was 1 to 10% less than the reference stream values in the 4 cooler (<21^{o}C) water experiments (1-4) and 3 to 21% greater in 2 of the 3 warmer (>21^{o}C) water experiments (5,6).

As Aufwuchs accumulated between chlorine doses, then died from chlorine stress, the empty diatom frustules and entrapped sediment which remained lowered the percent organic matter in the Aufwuchs sample. During the later experiments, the estimates of percent organic matter were increased by the accumulation of mucilage from sheathed blue-green algae. This was not expected since the blue-green algae tended to trap suspended sediment (increasing the dry weight) which should have decreased the estimate of percent organic matter for the sample. However, the Aufwuchs samples from the chlorinated stream contained 34% and 39% organic matter in experiments 5 and 6, respectively. These values are higher than those of the reference stream communities (11% and 36%, respectively) and higher than the estimates of percent organic matter for Aufwuchs from the chlorine treated stream in experiments 1 through 4 (15%, 19%, 21% and 19%), respectively).

Protein analyses of Aufwuchs developing under chlorine stress (Figure 8) followed the trends of the percent organic matter and the proliferation of blue-green algae. The estimates of protein as a percent of the organic matter sampled were approximately 1% above reference stream values in experiments 4, 5, 6, and 7 which correlated with the abundance of

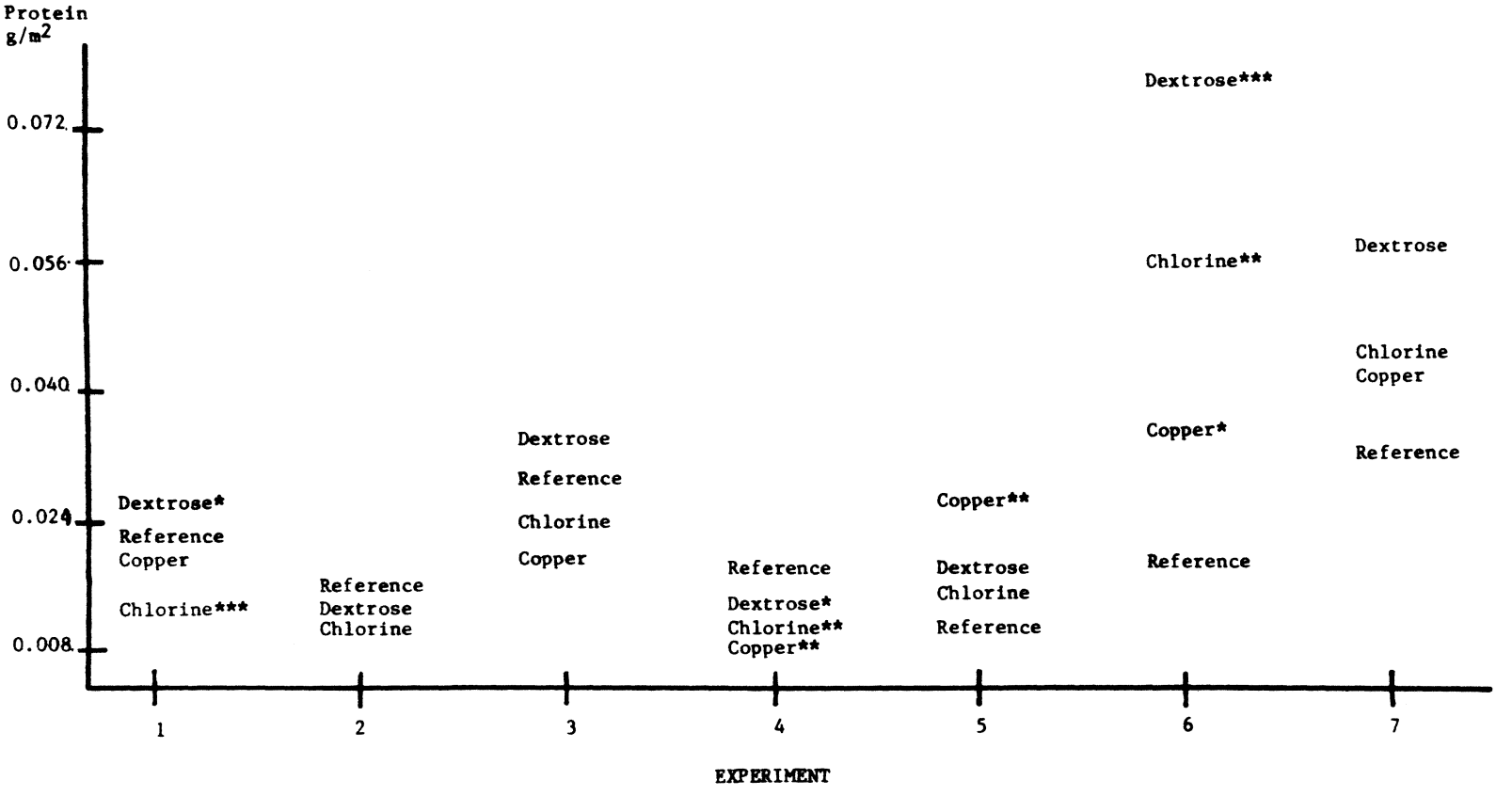


Figure 8. Aufwuchs protein content during the artificial stream experiments. Mean values are plotted for each stream with results of the Dunnett treatment versus control test (* for α=0.01, ** for α=0.05, *** for α=0.01).

blue-green algae (Figure 9). Aufwuchs carbohydrate content decreased by 0.01 to 0.48 g/m^2 in the chlorine treated stream in 6 of 7 experiments (1-6) with four of these decreases statistically significant (Figure 10). For experiments 6 and 7, in which the blue-green algae dominated the Aufwuchs, the carbohydrate values were either not significantly different or were greater than the reference stream values. Carbohydrate as a percent of the organic matter in the Aufwuchs sample showed no consistent trends (Figure 11). In experiments 5 and 7, the percent carbohydrate was greater by 8 and 23% compared to reference stream values. This parameter decreased between 3 and 20% under the chlorine dose in experiments 2, 4, 5, and 6 and had little change in experiment 1. Caloric estimates of Aufwuchs samples from the chlorine dosed stream were 4.2 and 2.5 $kcal/m^2$ above reference stream values in experiments 2 and 3, but were between 2.1 and 5.2 $kcal/m^2$ less than reference community estimates in experiments 4 through 7 (Figure 12). Expressed as kilocalories per gram ash-free dry weight (Figure 13), only data from experiments 6 and 7 were less than reference stream values, 2.1 and 0.47 kcal/g respectively. Caloric estimates per unit biomass exceeded reference community estimates from 0.8 to 10.6 kcal/g in experiments 2, 3, 4, and 5.

As microfloral cells died under the chlorine stress, non-structural proteins were rapidly lost while the more insoluble

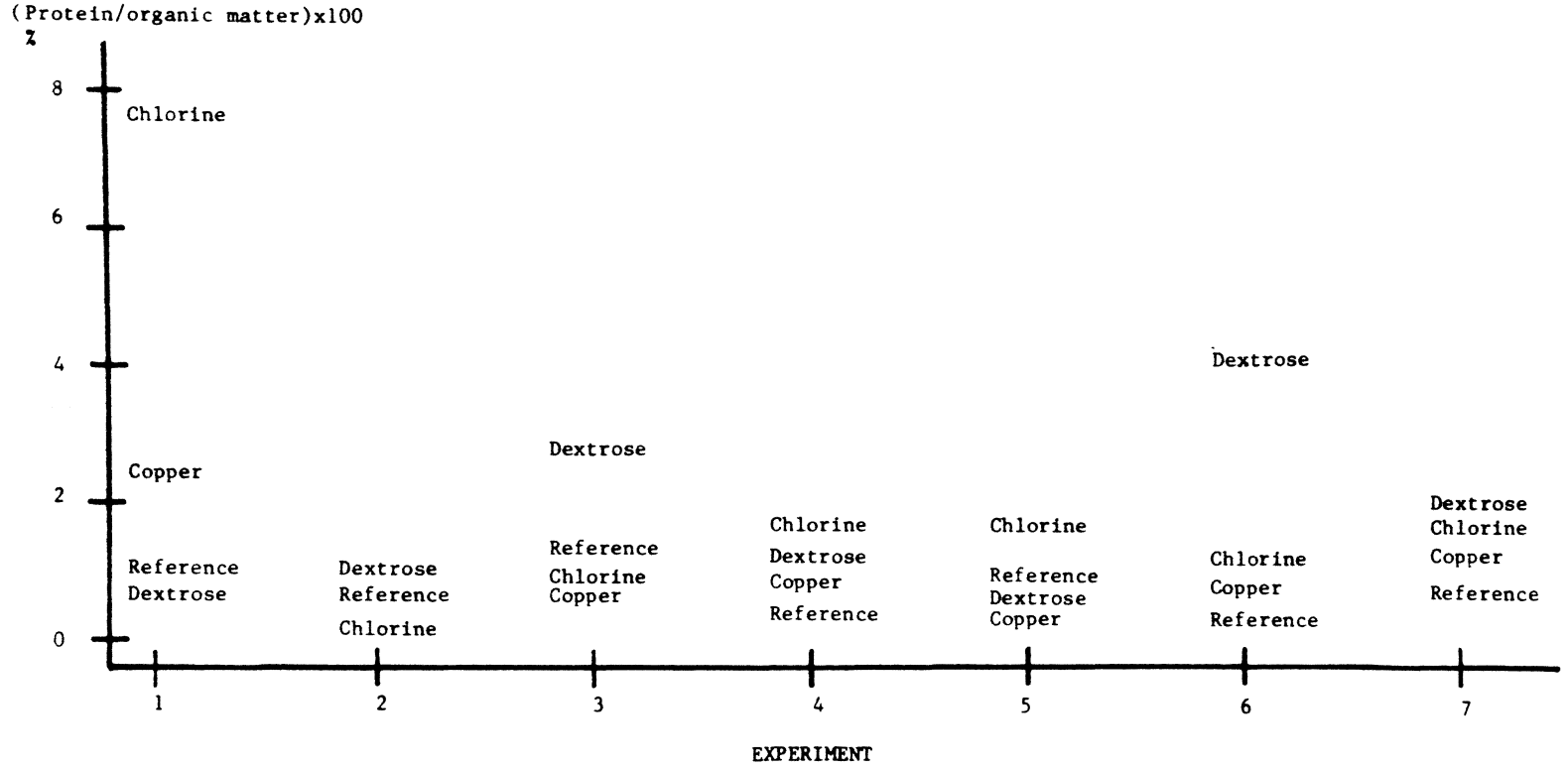


Figure 9. Protein estimates as a percent of the organic matter sampled during the artificial stream experiments. Values represent mean protein/ mean ash-free dry weight.

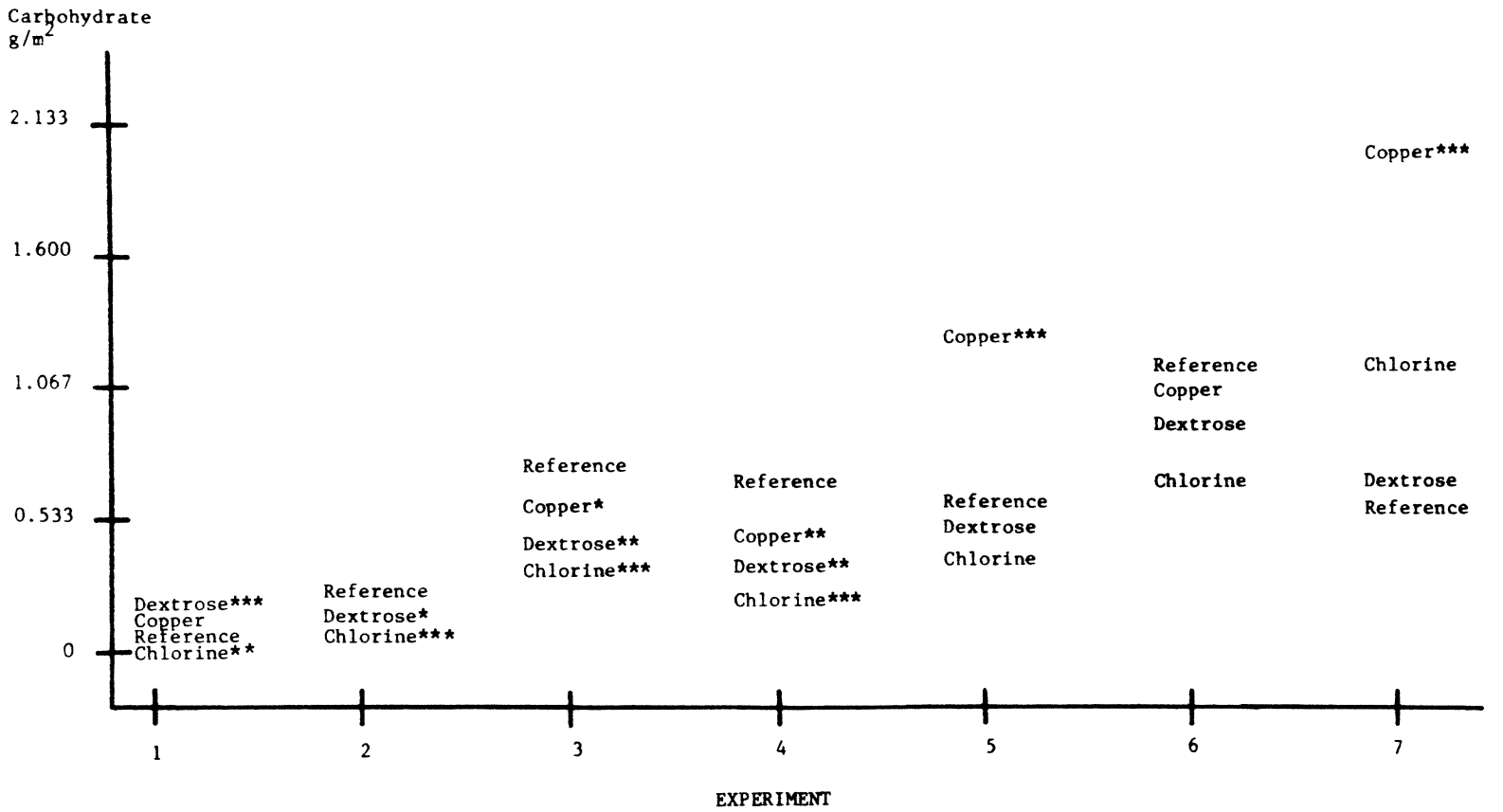


Figure 10. Aufwuchs carbohydrate content during the artificial stream experiments. Mean values for each stream are plotted with results of the Dunnett treatment versus control test (* for α=0.10, ** for α=0.05, *** for α=0.01).

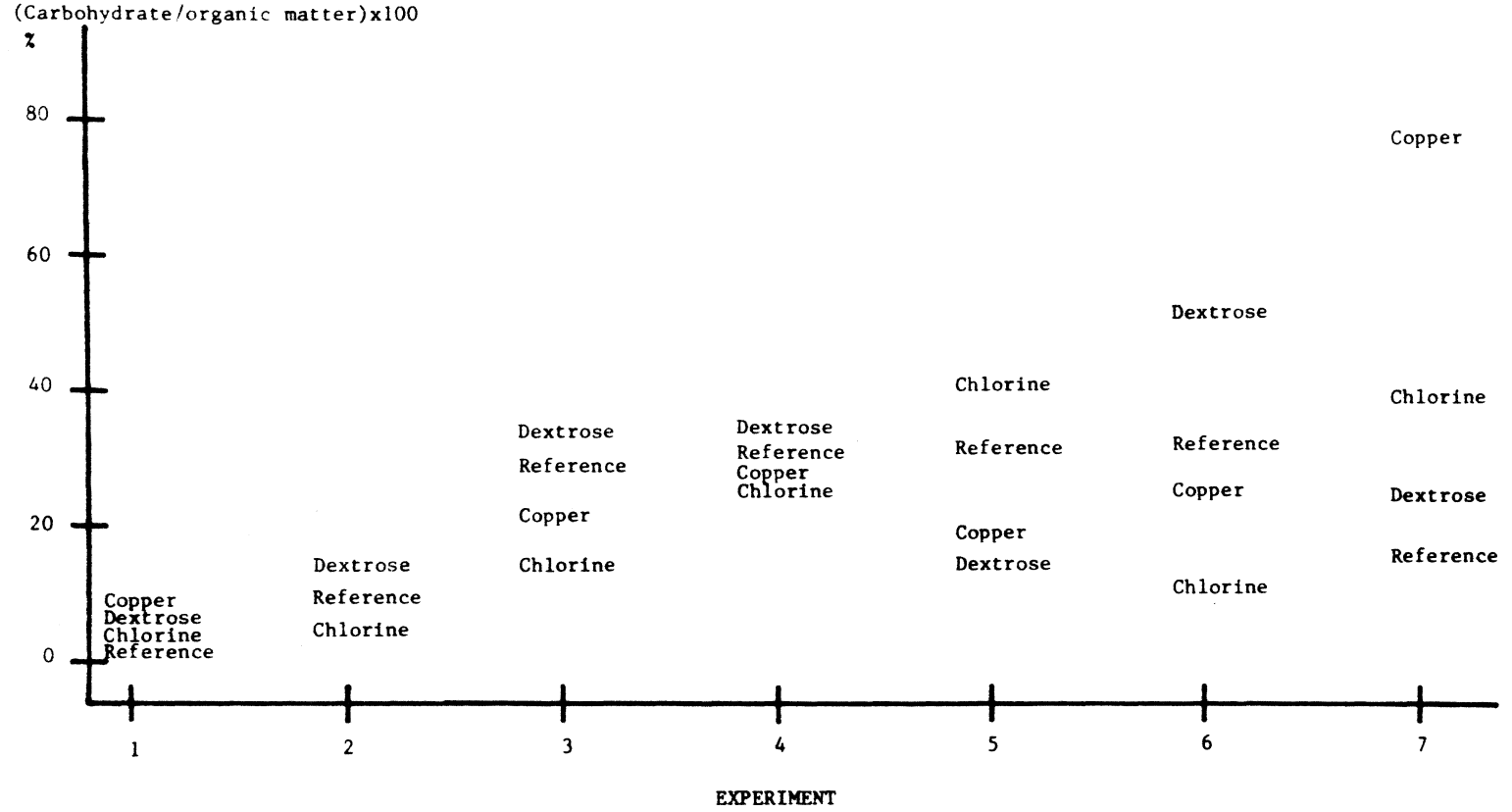


Figure 11. Carbohydrate estimates as a percent of the organic matter sampled during the artificial stream experiments. Values represent mean carbohydrate/mean ash-free weight.

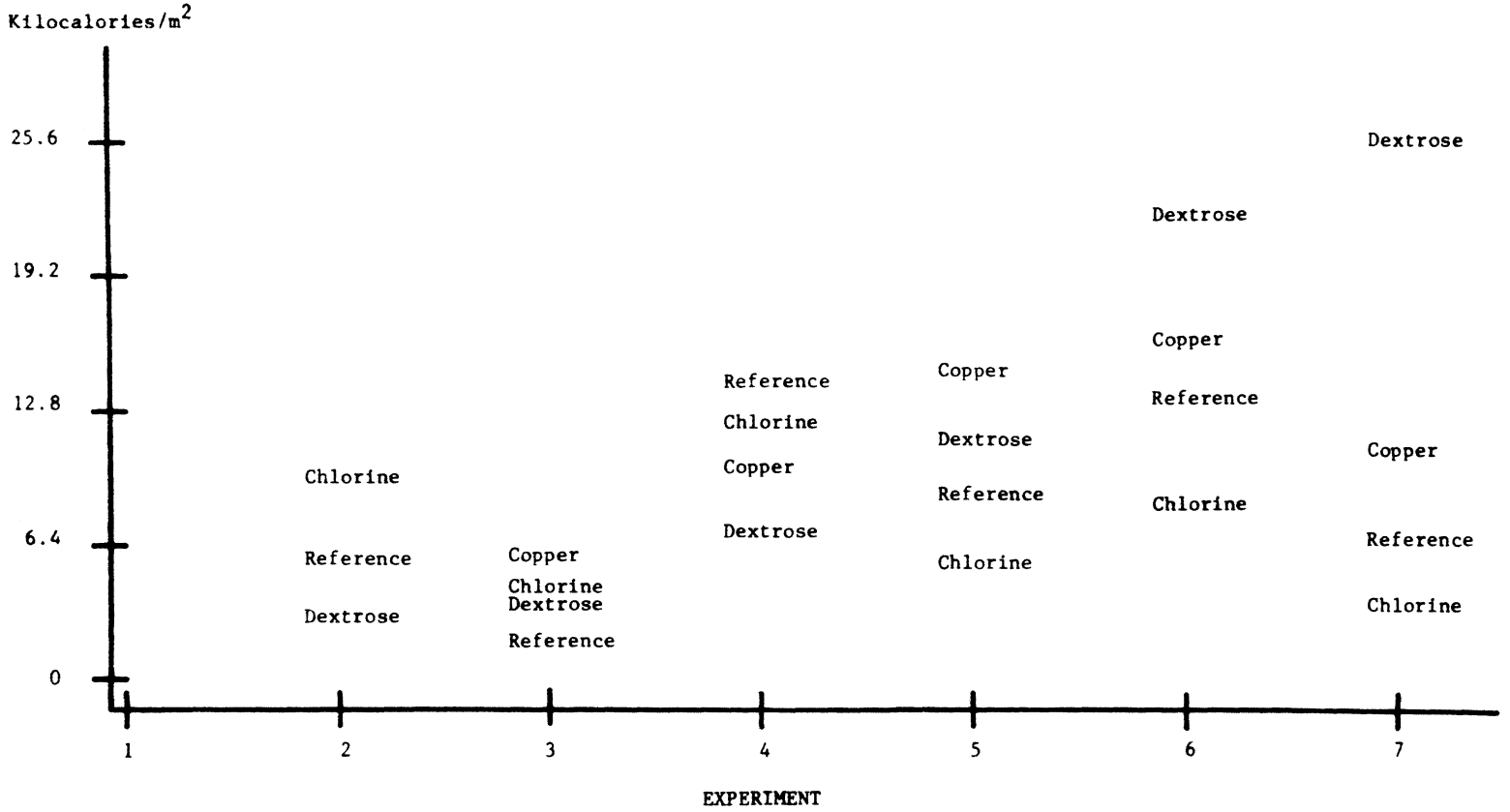


Figure 12. Caloric estimates from Aufwuchs sampled during the artificial stream experiments. Mean values are plotted for each stream. (No caloric samples were taken for experiment 1).

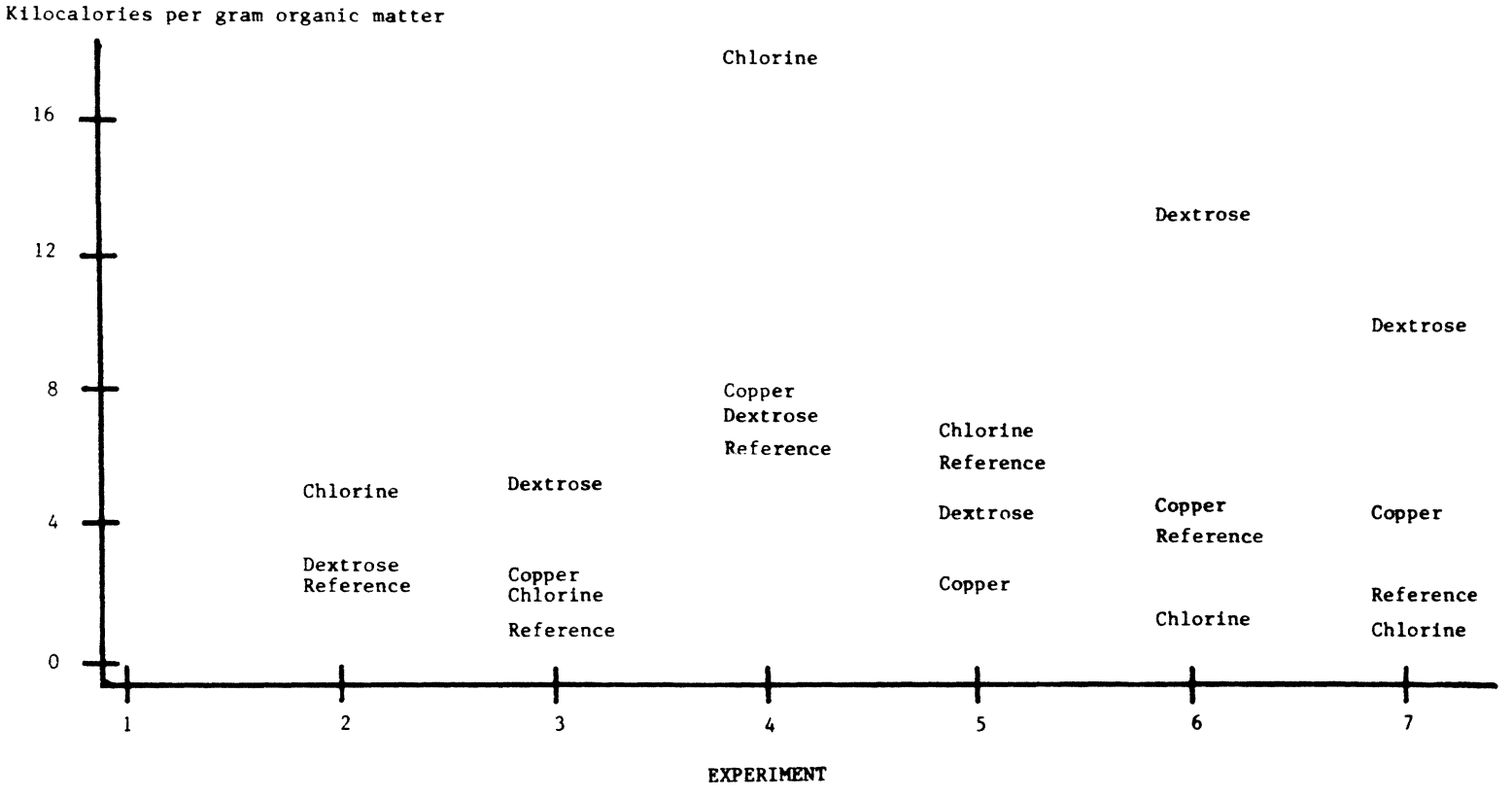


Figure 13. Kilocalories per gram ash-free dry weight for Aufwuchs sampled during the artifical stream experiments. Values represent mean kcal/mean ash-free dry weight.

protein structures which were not included in the protein assay (eg. those associated with membranes and cell walls) remained along with diatom frustules and entrapped inorganic sediment. Protein estimates, therefore, were expected to fluctuate in the same manner as estimates of percent organic matter in the Aufwuchs samples. However, protein expressed as a percent of the organic matter sampled was more representative of the chemical composition of the Aufwuchs community and fluctuated with the abundance of living matter. Increases in protein content of Aufwuchs dominated by blue-green algae reflect the high protein content of the extracellular sheath (Wolk 1973). Carbohydrate estimates decreased in experiments 1 through 5 as a result of the decreases organic matter present. Also, carbohydrates, which serve as immediate energy reserves for the microflora (Stewart 1974, Fogg 1975), probably were readily utilized in physiologically coping with the chlorine stress. This was seen when carbohydrates were expressed as a percent of the organic matter in experiments 2, 3, 4, and 6. Increases in percent carbohydrate in experiments 5 and 7 may reflect the greater relative abundance of blue-green algae compared to reference stream communities since the mucilage is high in carbohydrates as well as proteins (Wolk 1973, Stewart 1974). The higher calories per unit biomass relative to the reference communities in experiments 2 through 5 and decreases

in experiments 6 and 7 did not directly correlate with any observed changes in composition or other food quality parameters. The relative dominance of sheathed blue-green algae was much greater in Aufwuchs from the chlorinated stream in experiments 3, 4, and 5 while the chlorinated and reference communities were more alike in composition in experiments 6 and 7 (Table 2). The greater abundance of blue-green algal mucilage may have increased the caloric estimates, however, blue-green algae commonly contain less calories per gram ash-free dry weight compared to diatoms (Cummins and Wuycheck 1971).

Due to toxic interactions with the microflora, copper treatments were expected to decrease the rate of Aufwuchs accumulation on the glass slide substrates (Becker and Thatcher 1973, and Clark et al. 1979). Estimates of dry weight from Aufwuchs samples collected in the copper treated stream were 0.25 to 22 g/m^2 greater than reference stream values in experiments 3 through 7 (significantly greater in experiments 5 and 6) (Figure 5). Ash-free dry weight was increased by 0.5 g/m^2 above reference stream values in experiment 6 and significantly greater (5 g/m^2) in experiment 5. Results from experiments 1, 4, and 7 had decreases from 0.8 to 1.3 g/m^2, significantly lower in experiment 1 (Figure 6). Mean ash-free dry weight estimates were equal in experiment 3. In computing the percent organic matter

(Figure 7), only the results of experiment 5 were greater (7%) than reference stream values for the copper treated stream.

In experiments 5, 6, and 7, as the 21°C to 24°C water temperatures favored the proliferation of blue-green algae, the copper treatment reduced the other Aufwuchs community components, further stimulating the increase in blue-green algae (Table 2). Since Aufwuchs from the copper doses stream were predominantly blue-green algae, lower percent organic matter estimates were consistent with the observations that sheathed blue-green algae trap inorganic sediment. This accumulation of dry weight lowered the estimate of percent organic matter in the Aufwuchs sample.

The protein content of samples from the copper treated stream in experiments 5, 6, and 7 was 0.009 to 0.017 g/m^2 greater than reference community values with significant differences in the results of experiments 5 and 6 (Figure 8). Protein content was reduced by 0.002 to 0.010 g/m^2 in Aufwuchs samples under the copper stress in experiments 1, 3, and 4. Estimates of protein as a percent of the organic matter (Figure 9) in Aufwuchs samples from the copper treated stream were about 0.5% less than reference community values in experiments 3 and 5 and 0.2% to 1.5% greater in experiments 1, 4, 6 and 7. Carbohydrates (Figure 10) in Aufwuchs communities receiving copper doses were reduced 0.11 g/m^2

in experiment 6 and significantly reduced in experiments 3 and 4 (0.15 and 0.26 g/m^2, respectively). Carbohydrates were increased only 0.002 g/m^2 in experiment 1 but were significantly increased 0.76 g/m^2 and 1.34 g/m^2 in experiments 5 and 7, respectively. However, as a percent of the organic matter present, an increase in carbohydrates was found only in experiments 1 and 7, 1% and 63% respectively (Figure 11). Caloric estimates from Aufwuchs communities developing under the copper stress were greater than reference stream values by 1.2 to 5.9 $kcal/m^2$ in all experiments except experiment 4, where the caloric estimates were 3.8 $kcal/m^2$ less (Figure 12). As calories per unit organic matter (Figure 13), only the copper dosed stream in experiment 5 yielded data below reference stream values (0.9 kcal/g lower). All other estimates from the copper stream were 0.1 to 0.7 kcal/g greater than reference stream values.

In Aufwuchs samples from the copper dosed stream, estimates of protein exceeded the reference stream values when the copper stressed community was dominated by blue-green algae (Table 2). However, protein as a percent of the organic matter, was not a consistent indicator. The increase in carbohydrates relative to the reference community in experiments 5 and 7 may reflect the greater abundance of blue-green algae and the high carbohydrate content of their sheaths (Wolk 1973). When expressed as a percent of

the organic matter, the carbohydrate data indicate the Aufwuchs community had a decrease in carbohydrate reserves in order to meet the stress induced by copper. Both the caloric estimates and calories per unit ash-free dry weight were higher when the abundance of sheathed blue-green algae increased relative to the reference communities. As previously stated, this is presumably due to the greater organic content of the blue-green algal sheath.

The addition of 2 mg/l dextrose in an artificial stream caused a dramatic shift in the Aufwuchs community composition (Table 2). In experiments 2 through 7, heterotrophic bacteria (especially Sphearotilus natans) dominated the Aufwuchs community and formed a mucilaginous matrix upon the glass slide substrate. By visual assessment, the accumulation of material was greatly stimulated by this treatment. However, large sections of the community would often break away from the slide prior to or during sampling. This loss frequently reduced estimates of dry weight 3 to 6 g/m^2 and ash-free dry weight by 1 to 1.5 g/m^2 in the dextrose streams compared to reference streams (Figures 5 and 6). The 1 to 10% lower estimates of percent organic matter relative to reference communities (Figure 7) was a result of the abundance of inorganic matter trapped in the mucilage of the Aufwuchs from the dextrose enriched stream.

The loss of matter from the slides would tend to decrease

the other parameters along with the gravimetric measurements. However, the Aufwuchs community developing under the dextrose treatment produced such a copious mucilage that the protein estimates (Figure 8) were 0.002 to 0.06 g/m^2 above reference stream values in 5 of 7 experiments and were significantly greater in experiments 1 and 6. The protein estimate as a percent of the organic matter (Figure 9) was also higher by 0.25 to 4% 5 of 7 times, although not in all of the same experiments as the protein estimate. The estimates of carbohydrate from the dextrose treated stream ranged from equal to 0.36 g/m^2 lower than reference stream values in 5 of 7 experiments (Figure 10), perhaps due to the losses of organic matter due to sloughing. As a percent of the organic matter sampled (Figure 11), carbohydrate values were 3% to 20% greater in the Aufwuchs samples from the dextrose enriched stream in 6 experiments. This probably reflects the high carbohydrate content in the bacterial mucilage. Caloric estimates from the dextrose treated stream were also increased above reference values due to the development of copious bacterial mucilage (Figure 12), especially when expressed as calories per unit organic matter (Figure 13). Except for experiment 5, kilocalories per gram ash-free dry weight exceeded reference stream values, by as much as 8.6 kcal/g.

The treatment effects as well as the normal seasonal successions influenced the species composition and food

quality of the Aufwuchs community in all experiments. In addition, the treatment effects and resultant food quality changes varied with shifts in microfloral species colonizing the glass slide substrates. Water temperature was the major environmental change over the seasons and controlled the growth and competition between the microfloral species in the Aufwuchs communities of the reference streams. In the treated streams, the chlorine, copper, or dextrose treatments resulted in an additional factor that influenced the composition and food quality of the Aufwuchs community.

The low concentrations of chlorine, copper, and dextrose selected for application to the artificial streams were chosen to elicit a wide variety of responses in the Aufwuchs communities without causing an overt toxic response in the microflora. Chlorine, as an antifouling agent, should affect autotrophic and heterotrophic community components of the Aufwuchs community. The action of chlorine as a surface oxidant may stimulate the formation of microfloral extracellular mucilage or membranous sheaths to provide protection. Species living within the matrix of biomass accumulated on the substrate may avoid contact with the chlorine dose. Levels of chlorine reported to cause some adverse impact on microflora range from 0.18 to 20.0 ppm TRC (Becker and Thatcher 1973). The 0.2 ppm TRC dose level applied in this research is at the lower range of these microfloral toxicity

values. At the low dose level of 0.05 ppm copper used in this research, blue-green algae proliferated with respect to other algal groups, especially diatoms. This was expected since diatoms have been reported to be more sensitive to copper stress (Becker and Thatcher 1973). Copper is highly toxic to algae and has long been used as an algicide (McKee and Wolfe 1963, Becker and Thatcher 1973), since copper ions cause toxic reactions by interfering with algal enzyme systems (Bishop 1964, Steeman-Nielson and Brunn-Laursen 1976). The dextrose additions stimulated the growth of heterotrophs, mimicking the response of an Aufwuchs community to the addition of organic carbon from sewage outfalls, except at a more rapid rate. The 1 or 2 ppm of dextrose added to the stream provided a readily assimilated carbon source to stimulate the growth of heterotrophic bacteria and was highly effective except at cold water temperatures (experiment 1). The changes in the Aufwuchs community due to the treatment effects and the seasonal changes in composition allowed an evaluation of food quality analyses as structural parameters for assessing water quality.

The chemical constituents of Aufwuchs communities which relate to the nutritional needs of animal grazers are varied. Several easily measured and commonly employed nutritional components were investigated. While the specific results of the food quality analyses have been previously

discussed, this section presents a general discussion of the method employed, their specificities and biases, and an evaluation of their ability to assess pollutant impact.

The estimates of Aufwuchs dry weight (Figure 5) reflect the total accumulation of material on the glass slide substrates. The ash-free dry weight (Figure 6) represents the organic matter present and is independent of inorganic sediments or diatom frustules. These data are similar to those reported by Rodgers (1977) and Clark et al. (1979) and serve as a baseline for assessment since they are so commonly employed (APHA 1976, Clark et al. 1979, Weitzel 1979a). The ratio of ash-free dry weight to dry weight increased with the amount of inorganic matter trapped in an Aufwuchs sample and is an expression of the percent of organic matter in the Aufwuchs sample. This ratio ranged from 11 to 39% organic matter (Figure 7), again in agreement with literature data (Elwood and Nelson 1972, Vollenweider 1974, Clark et al. 1979).

There are several classes of carbohydrates and proteins which comprise microfloral cells (Stewart 1974) and complete carbohydrate or protein characterizations of Aufwuchs samples would require numerous extraction procedures (Strickland and Parsons 1972, Grodzinski et al. 1975). The carbohydrates and saline soluble proteins monitored in this research would be indicative of the most labile components of the total

carbohydrates and total proteins. These include most of the cytosol carbohydrate reserves and non-structural proteins which provide an indication of the general physiological health of the microflora (Eppley et al. 1971, Fogg 1975). Investigations into the more recalcitrant (alkaline or acid soluble) components might offer further insight into the impacts of perturbation on Aufwuchs community species composition (Stewart 1974), although the time involved in pursuing these parameters was prohibitive in light of an uncertain outcome.

The Coomassie staining procedure is commonly employed to quantify protein and is in general agreement with results of the Lowry method (Sedmak and Grossberg 1977, Esen 1978). Advantages of the Coomassie blue procedure include its rapidity and ability to accommodate a wide range of sample protein concentrations (Sedmak and Grossberg 1977, Esen 1978). Proteins which remained associated with cell fragments after the Aufwuchs samples were ground in 2% saline were not assayed in this research since the suspended particles interfered with spectrophotometric readings. The protein content of samples was computed from a standard curve using bovine serum albumin which is widely used and allows for data comparisons with other literature data (Grodzinski et al. 1975). Another choice of reference material may have been more representative of plant proteins (i.e. wheat flour, yeast

cells, specific extracts), however, there is no agreement on which plant protein might be used (Grodzinski et al. 1975, Esen 1978).

In a preliminary study, a 2% saline solution was used to extract approximately 15% more protein from Aufwuchs samples compared to extracts using water alone. The total protein content of algal cells can range from 70% dry weight for rapidly growing cells to less than 10% for older, nutrient deficient cells (Fogg 1975). Expressed as a percent of the organic matter, the protein values from all Aufwuchs samples ranged from 0.8% to 7% (Figure 9). These values are about 1/10th of those reported for the total protein content of algae (Fogg 1975), indicating the small proportion of the total protein present which was saline soluble. In addition, the accumulation of detritus within the community matrix, either as early colonizing cells which had subsequently died or from the settling of suspended, non-viable organic matter onto the substrate, contributed to the low estimate of protein content in the Aufwuchs sample. The detrital cells have low levels of non-structural proteins, these components having been leached from the cells after death.

Analysis of Aufwuchs community protein demonstrated fluctuations due to various physiological and morphological characteristics of the attached microflora and fluctuated widely over the seasons and perturbations. In assessing the

impact of the stressed applied to the artificial streams, protein analysis, as conducted in this research, was sensitive to changes in the mucilage content of the microflora comprising the Aufwuchs communities which developed under the chlorine, copper, or dextrose treatments. When expressed as a percent of the organic matter in Aufwuchs developing under the stresses, protein estimates increased with the greater abundance of blue-gree algae or bacteria compared to reference communities. This was attributed to saline soluble proteins in the sheaths of blue-green algae and bacterial mucilage. Decreases in the protein content were associated with the accumulation of detrital matter in Aufwuchs developing under intermittent chlorination. While saline soluble proteins only were assayed, their abundance relative to total proteins in the Aufwuchs may have provided greater insight into the dynamics of this protein group and the health of Aufwuchs communities in general.

The procedure used to assay for carbohydrate in Aufwuchs was rapid and sensitive (Strickland and Parsons 1972). The anthrone reagent reacts quantitatively with hexose sugars, mono-, and polysaccharides (Strickland and Parsons 1972, Grodzinski et al. 1975). Pentose or xylose carbohydrates are not assayed quantitatively, nor are the hexoseamine sugars such as chitin (Grodzinski et al. 1975). Since the assay reagent is dissolved in concentrated acid, grinding

the samples to facilitate extraction was not specifically mentioned in the procedural references of Strickland and Parsons (1972) and Grodzinski et al. (1975). In this study, Aufwuchs samples were ground to provide a more homogeneous sample and perhaps expose more reactive sites. Glucose solutions were used to generate standard curves since it is a highly reactive carbohydrate (Grodzinski et al. 1975) and a predominant polysaccharide algal constituent (Lewin 1974). The experimental results, expressed as glucose equivalents, represent a measure of mucilaginous or cytoplasmic storage carbohydrates, but do not include cellulose or lignin carbohydrate components (Grodzinski et al. 1975).

Estimates of carbohydrate content from the Aufwuchs samples, expressed as a percent of the organic matter, ranged from less than 1 to 78% (Figure 11), falling at the extremes of the percent carbohydrates reported by Fogg (1975). However, 90% of the estimates of percent carbohydrates were between 2 and 40%, precisely in the range of values reported by Parsons et al. (1961) and Fogg (1975). These fluctuations indicated changes in the physiological state of the microflora and changes in nutrient abundances attributed to the dosing and seasonal effects since algal groups are similar in their composition (Parsons et al. 1961, Eppley et al. 1971, Haug et al. 1973, Fogg 1975). Protein to carbohydrate ratios were about one order of magnitude lower than those reported

by Fogg (1975), a result of the low protein estimates (about 10% total protein). This ratio fluctuated widely since the carbohydrate cellular component responds more rapidly to stress (Myklestad and Haug 1972, Haug et al. 1973, Fogg 1975).

Analysis of carbohydrates was included in this research because of its potential for characterizing the physiological status of microfloral communities. Carbohydrates were frequently decreased in Aufwuchs communities sampled from chlorine or copper treated streams compared to reference stream communities. This was true for both total carbohydrate estimates and carbohydrates expressed as a percent of the organic matter, indicating a utilization of food reserves within the Aufwuchs community in coping with the environmental stressed used. However, when sheathed blue-green algae dominated Aufuchs community biomass (>70% cell counts), carbohydrate values for the copper and chlorine treated stream communities exceeded reference community values due to the high carbohydrate content in the extracellular mucilage. Heterotrophically dominated Aufwuchs communities, which developed under dextrose enrichments, also contained more carbohydrate per unit organic matter due to bacterial mucilage production. Thus, in assessing the impact of stresses on Aufwuchs structure through carbohydrate analysis, physiological stress as well as morphological and taxonomic characteristics must be considered.

The caloric estimates of Aufwuchs food samples are based on indirect, approximate calculations from dichromate oxidation procedures (Maciolek 1962, Grodzinski et al. 1975). This approach is subject to procedural errors if not carefully executed as well as underestimation due to incomplete oxidation of the sample (Maciolek 1962). However, it was necessary to use this procedure in order to expedite the analyses of numerous small samples (Strickland and Parsons 1972).

Estimates of the caloric content of glucose standards, based on the oxygen consumed during chemical oxidation, ranged from 14 to 1% below the complete oxidative caloric content of 3.6 kcal/g with a mean underestimate of 6%. All sample estimates of caloric content were then corrected for this 6% underestimation. The range of caloric values calculated for all Aufwuchs samples spanned almost 16 kcal/g ash-free dry weight (gAFDW) and ranged from 0.82 to 17.11 kcal/gAFDW (Figure 13). Mean caloric values for cultures of algae of 4.63 kcal/gAFDW and 4.52 for periphyton communities were reported by Cummins and Wuycheck (1971). Of the 23 caloric estimates computed for this research, 7 were within 25% of the Cummins and Wuycheck (1971) mean values while 14 were within a 50% interval of the mean caloric values reported by Cummins and Wuycheck (1971).

Caloric analysis of Aufwuchs communities provided

information on the energy content of the Aufwuchs community and the potential for energy flow through the ecosystem trophic structure. Total calories in an Aufwuchs sample did not produce consistent trends for interpreting perturbation impacts. Calories per unit ash-free dry weight (kcal/gAFDW) were higher than reference values in Aufwuchs from the dextrose treated stream, reflecting the production of mucilage. Although Aufwuchs developing under the copper treatment yielded higher kcal/gAFDW than reference communities, this parameter demonstrated considerable fluctuation over the seven artificial stream experiments for Aufwuchs from the chlorinated stream. As with the carbohydrate analyses, production of blue-green algal sheaths and bacterial mucilage increased caloric values of the organic matter. Although measurements of kcal/gAFDW were consistent in distinguishing copper and dextrose treated communities from reference communities, variability in the results from the chlorine stream decreased its reliability as an assessment method.

Correlation analysis of the mean estimates of the food quality parameters demonstrated several significant correlations over the seven experiments conducted in the artificial streams (Table 3). Both protein and carbohydrate were significantly correlated with dry weight ($r=0.520$, $P<0.01$; $r=0.548$, $P<0.01$) and ash-free dry weight ($r=0.538$, $P<0.01$; $r=0.621$, $P<0.01$). When expressed as a percent of the organic matter

Table 3. Spearman rank correlation coefficients, r, for mean Aufwuchs food quality estimates from the seven artificial stream experiments. The significance level, P, is given in parenthesis for each correlation. (N=27 for each correlation except for caloric data where N=23).

	Dry Weight	Ash-Free Dry Weight (AFDW)	Percent Organic Matter	Protein	Carbohydrate	Calories	Protein / AFDW	Carbohydrate / AFDW
Ash-Free Dry Weight	0.845^{r}_{p} (<0.01)	...	...	...	...	...	...	...
Percent Organic Matter	-0.200 (0.32)	0.270 (0.17)	...	...	...	...	...	...
Protein	0.520 (<0.01)	0.538 (<0.01)	0.134 (0.51)	...	...	...	...	...
Carbohydrate	0.548 (<0.01)	0.621 (<0.01)	0.274 (0.17)	0.588 (<0.01)				
Calories	0.234 (0.28)	0.234 (0.26)	0.008 (0.97)	0.210 (0.34)	0.396 (0.06)	...	...	...
Protein / AFDW	-0.395 (0.04)	-0.524 (<0.01)	-0.151 (0.45)	0.374 (0.05)	-0.125 (0.53)	-0.068 (0.76)	...	...
Carbohydrate / AFDW	-0.059 (0.77)	-0.076 (0.71)	-0.199 (0.32)	0.189 (0.35)	0.651 (<0.01)	0.128 (0.56)	0.222 (0.27)	...
Calorie / AFDW	-0.475 (0.02)	-0.640 (<0.01)	-0.176 (0.42)	-0.257 (0.24)	-0.248 (0.25)	0.548 (<0.01)	0.345 (0.11)	0.407 (0.05)

sampled, protein values were significantly and negatively correlated with dry weight ($r=-0.395$, $P=0.04$) and ash-free dry weight ($r=-0.524$, $P<0.01$), while carbohydrate estimates were not significantly related, although a negative correlation coefficient was calculated. Only when expressed as calories per gAFDW were mean caloric data significantly correlated with dry weight ($r=-0.475$, $P=0.02$) and ash-free dry weight ($r=-0.640$, $P<0.01$). There were no significant correlations between percent organic matter in the Aufwuchs samples and other chemical food quality parameters. Protein estimates were correlated with carbohydrate estimates, $r=0.558$, $P<0.01$. Since they were used in formulating the food quality per unit ash-free dry weight, mean protein, carbohydrate, and caloric data were positively correlated to their respective estimates as a percent of the organic matter sampled. Carbohydrate estimates were positively correlated with calorie estimates ($r=0.396$, $P=0.06$) as was carbohydrate as a percent of organic matter sampled correlated with calories per gram AFDW ($r=0.407$, $P=0.05$).

As colonized matter was the source of protein and carbohydrate, it was expected that as more Aufwuchs was sampled from the glass slide substrates, estimates of protein, carbohydrates, and calories would increase. This supports the correlations of proteins and carbohydrates with dry weight and ash-free dry weight data. The lack of correlation

between caloric estimates and the gravimetric parameters must be related to the greater importance of the overall chemical composition of the material sampled rather than its mass as well as the variability in the caloric estimates. The negative correlation coefficients between protein/AFDW, carbohydrate/AFDW, and calorie/gAFDW with Aufwuchs dry weight and ash-free dry weight reflect the accumulation of detrital material on the glass slide substrates which, while directly increasing organic matter, contained none, or limited amounts, of these food quality constituents. The fluctuations in percent organic matter for the Aufwuchs communities sampled over the seven experiments were related to natural and imposed environmental conditions (temperature, suspended sediment in the river water, treatment effects) causing changes independent of the chemical composition of the microfloral community and yielding no significant correlations. Positive correlations among caloric and carbohydrate parameters may be related to the proliferation of mucilage in the blue-green algae or bacterial dominated Aufwuchs communities.

Feeding studies

As reported in the methods section, results of the feeding studies will be examined according to trends in the median estimates for test parameters. A comparison of the means for each parameter may be obtained by referring to the

dissertation Appendix, Figures A10 to A16. Median estimates of dry weight consumed by snails feeding on Aufwuchs from the chlorine treated stream were 0.12 to 0.80 mg less than reference stream values in experiments 1, 3, 4, 5, and 6 (Figure 14). Organic matter consumed (Aufwuchs ash-free dry weight) was less than reference stream estimates by 0.04 to 0.18 mg in 5 of 7 experiments (1, 3, 4, 6, and 7) (Figure 15). The assimilation efficiency of snails feeding on Aufwuchs from the chlorinated stream was 5 to 26% greater than reference stream values in experiments 1, 2, 4, and 7 and 3 to 20% less in experiments 3, 5, and 6 (Figure 16). Computation of the median organic matter assimilated by snails during the feeding experiments (Figure 17) ranged from 0.05 to 0.15 mg less than assimilation of reference communities in four experiments (1, 3, 4, and 6) and 0.02 to 0.36 mg higher in experiments 2, 5, and 7.

The decrease in consumption of Aufwuchs from the chlorine dosed stream in experiments 1, 3, and 4 may reflect the decrease in biomass present on the glass slide substrates from this stream (Figures 5 and 6). The Aufwuchs community developing under the chlorine treatments in these experiments may have been attached more firmly to the substrate. Patrick (1978) reported that the snail, Physa heterostropha, selects against diatoms more firmly attached to the substrate. Due to the sparse food supply, the snails may have had to

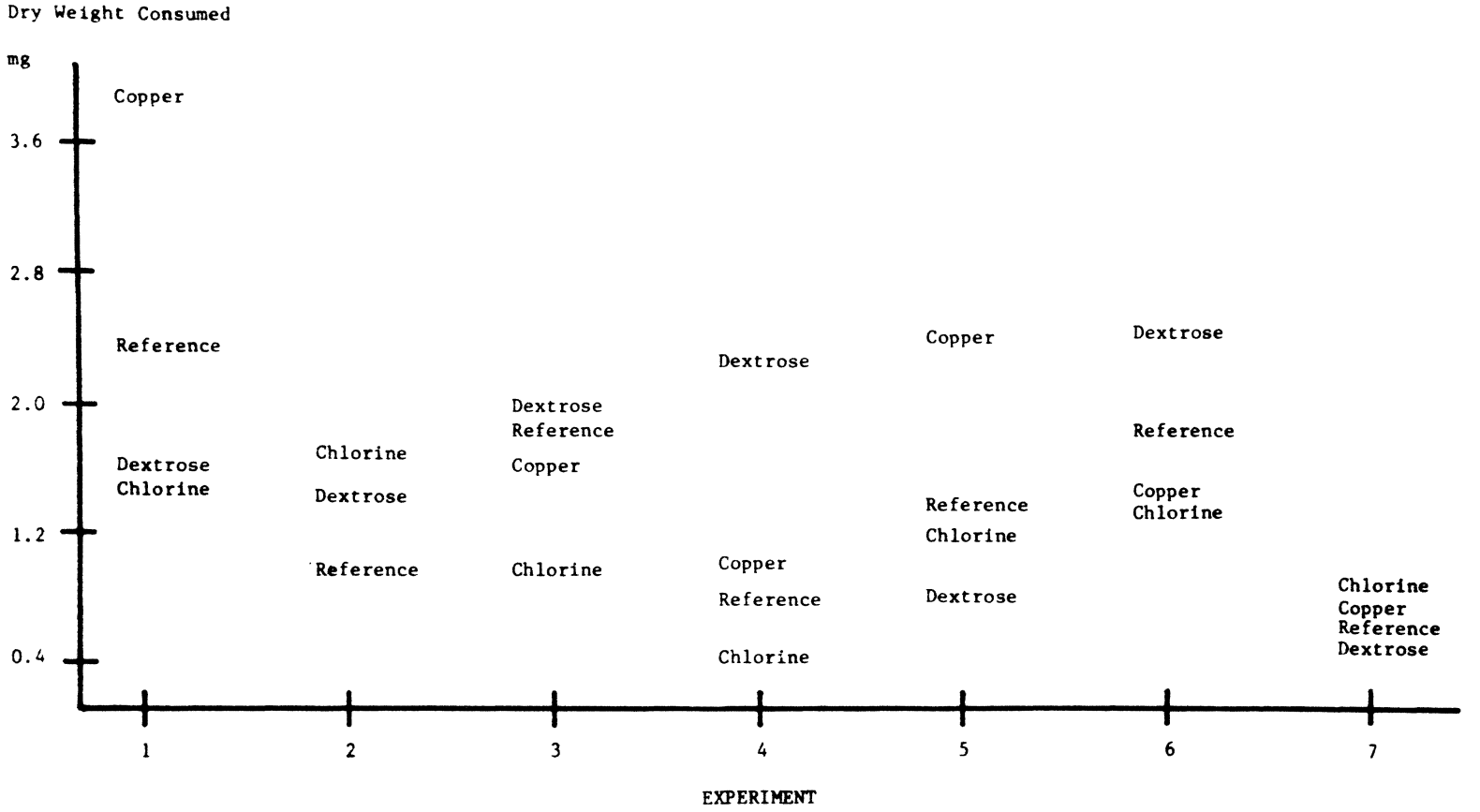


Figure 14. Aufwuchs dry weight consumed by snails during the artificial stream experiments. Median values for each stream are plotted.

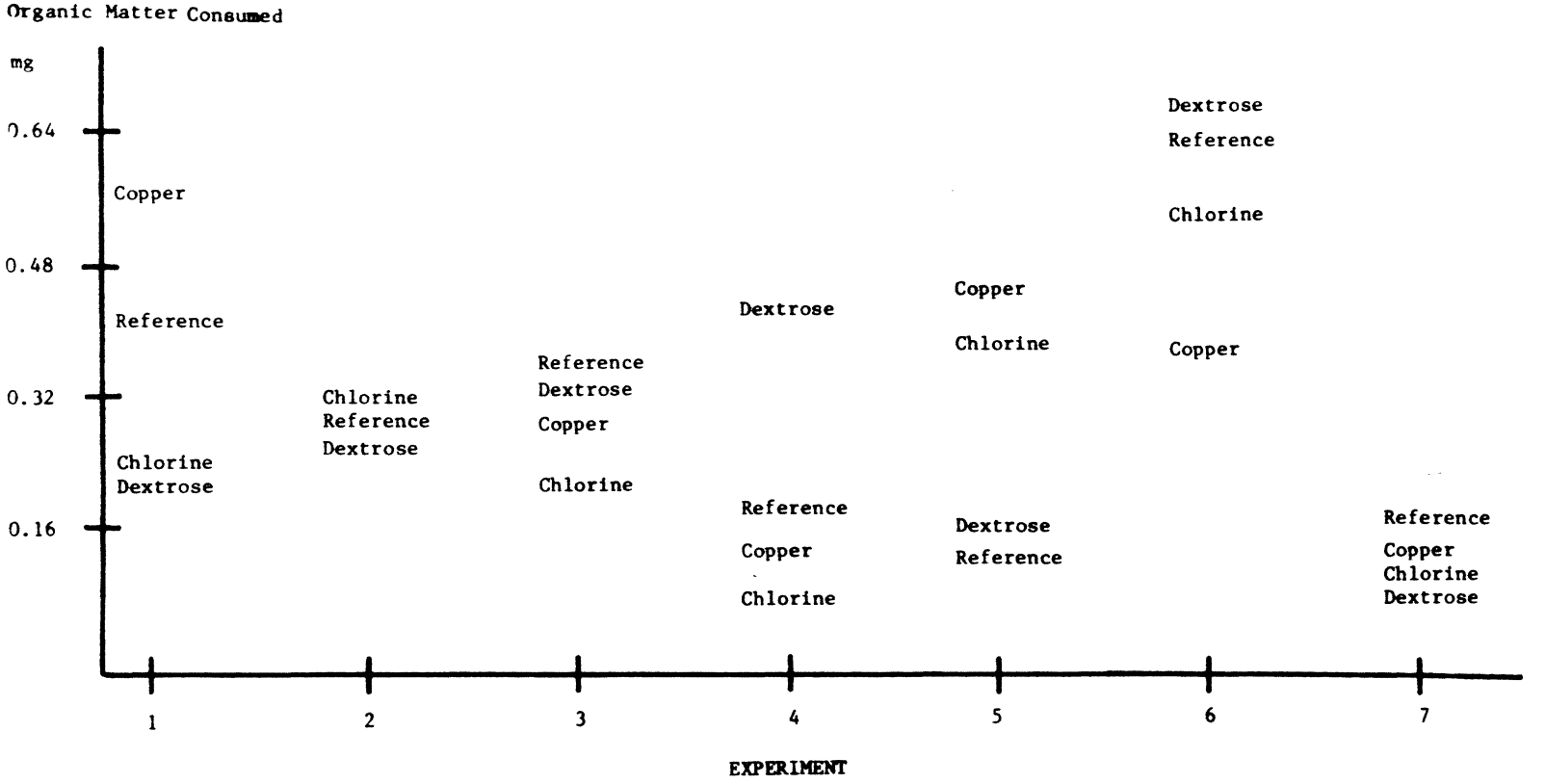


Figure 15. Aufwuchs organic matter (ash-free dry weight) consumed by snails during the artificial stream experiments. Median values for each stream are plotted.

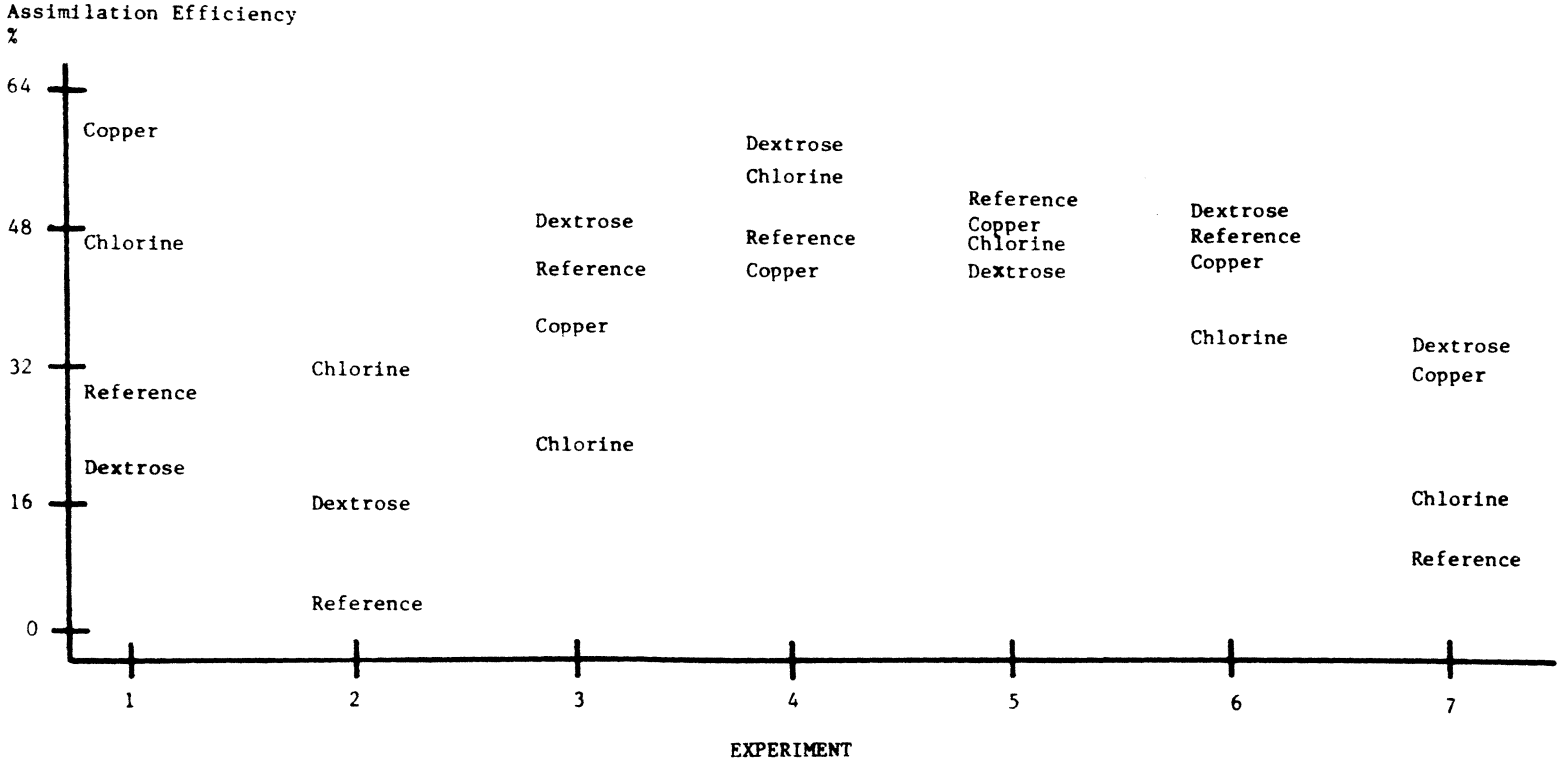


Figure 16. Assimilation efficiency of snails feeding on Aufwuchs during the artificial stream experiments. Median values for each stream are plotted.

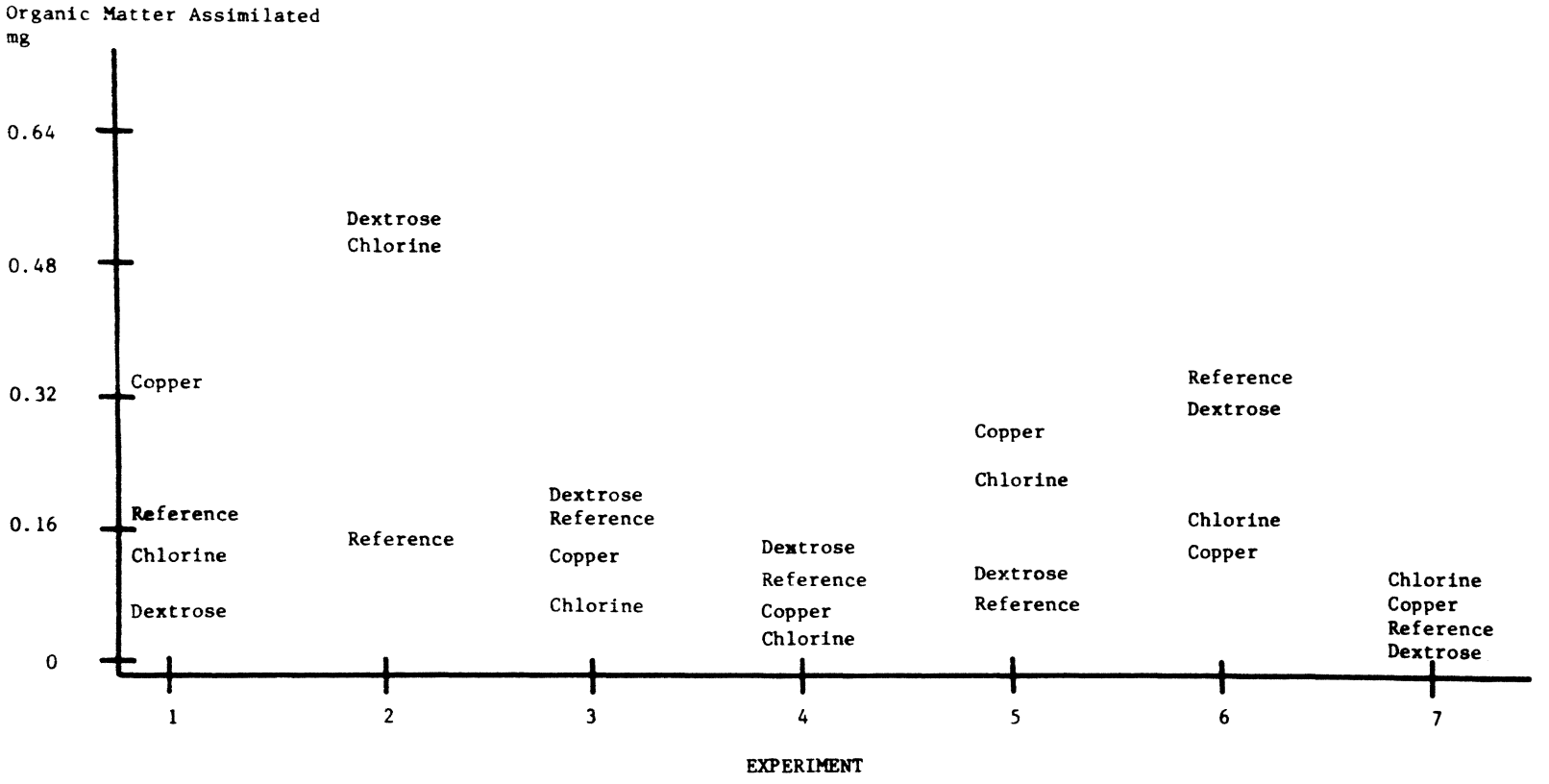


Figure 17. Aufwuchs organic matter assimilated by snails during the artificial stream experiments. Median values for each stream are plotted.

cover a greater area to consume an amount of food equivalent to the snails feeding on Aufwuchs from the reference stream. Alternative explanations may involve the fact that the percent organic matter in the Aufwuchs was less than reference stream communities (Figure 7) and therefore serve as an inadequate food resource. In experiments 1, 2, and 4, increases in snail assimilation efficiencies for Aufwuchs from the chlorine treated streams relative to the reference communities may have resulted from the decreased amounts of food consumed, allowing for a greater percent of the food to be digested and absorbed (Brody 1945, Monakov 1972, Calow 1975b). The assimilation efficiencies in experiments 5 and 6 were 3% and 12% lower than the reference stream values and may be related to the increased predominance of blue-green algae in Aufwuchs from the chlorine stream compared to reference communities (Table 2). Low macroinvertebrate assimilation efficiency of blue-green algae has been reported by Swiss and Johnson (1976) and Edmondson and Winberg (1971).

Snail consumption of Aufwuchs from the copper dosed stream ranged 0.17 to 1.53 mg greater than reference stream values for dry weight consumed in 4 of 6 experiments (1, 4, 5, and 7)(Figure 14). However, when corrected for the inorganic content, median estimates of snail consumption of Aufwuchs were 0.04 to 0.24 mg less for food from the copper

dosed stream in 4 of 6 experiments (3, 4, 6, and 7)(Figure 15). The assimilation efficiency (Figure 16) was lower by 2 to 6% for snails feeding on the copper dosed Aufwuchs in experiments 3, 4, 5, and 6 while assimilation efficiencies were increased 30% in experiment 1 and 21% in experiment 7. Organic matter assimilated was 0.05, 0.07, 0.19 mg lower in experiments 3, 4, and 6 and 0.17, 0.19, and 0.09 mg higher in experiments 1, 5, and 7 relative to the amount or organic matter assimilated by snails feeding on Aufwuchs from the reference stream (Figure 17).

Blue-green algae dominated the Aufwuchs community developing under the copper treatments in all experiments except 1 (Table 2). The mucilaginous sheaths of these algae are generally sticky and entrap suspended sediment as it is carried by the current past the cells. The sheaths may also enable blue-green algae to persist in spite of the copper doses and are suspected as a reason for the lower digestibility of this algal group (Edmondson and Winberg 1971). This possibility is supported by the lower assimilation efficiencies of snails feeding on Aufwuchs from the copper dosed streams in experiments 3, 4, 5, and 6. The increase in organic matter assimilated by snails feeding on these Aufwuchs communities in experiments 5 and 7 may reflect the retention of blue-green algal mucilage or cell fragments.

The dextrose enrichment produced an Aufwuchs community

dominated by heterotrophic bacteria (Table 2). Estimates of snail consumption of Aufwuchs from the dextrose dosed stream were higher (0.22 to 1.48 mg) than reference stream values during 4 of 7 experiments based on Aufwuchs dry weight (experiments 2, 3, 4, and 6)(Figure 14). Snail consumption of Aufwuchs ash-free dry weight (Figure 15) was greater than reference community values in experiments 4, 5, and 6 (by 0.25, 0.02, and 0.04 mg, respectively). Median estimates of organic matter consumed in experiments 2 and 3 were less than 0.02 mg below reference stream values while there was a 0.17 and 0.07 mg decrease in experiments 1 and 7. The snails were able to assimilate communities from the dextrose treated stream with 1 to 25% greater efficiency in 5 of the 7 experiments (2, 3, 4, 6, and 7)(Figure 16). Overall, with these small differences in ash-free dry weight consumed and slightly increased assimilation efficiencies, the snail's ability to assimilate organic matter from this heterotrophically dominated community was within 0.04 mg of reference stream values 5 of 7 times (experiments 3, 4, 5, 6, and 7)(Figure 17).

The heterotrophic Aufwuchs community that developed under the dextrose enrichment also entrapped sediment. However, snails consumed the bacterially dominated communities to a greater measure (or only slightly less than) and assimilated them more readily than reference stream communi-

ties. These results are consistent with literature data reporting greater assimilation efficiencies for bacteria compared to algae or other plant material (Sorokin 1968, Hargrave 1970, Calow 1975b, Baker and Bradnam 1976), which demonstrate that bacteria can be an important food source for snails.

The amount of Aufwuchs consumed during the feeding experiment was a parameter of central interest in the feeding studies. This is reported and discussed on both a dry weight and ash-free dry weight (organic matter) basis. The dry weight of Aufwuchs consumed is reported since it was measured directly. However, this variable includes, by necessity, inorganic matter which did not incorporate the isotopic label and is not of utmost concern in snail feeding and ecological trophic dynamics. Since the sediment entrapped in the Aufwuchs community contributes to the texture and physical composition of the food resource, grazer utilization of Aufwuchs on a dry weight basis can provide important information. In five comparisons of snail consumption of Aufwuchs from treated streams and reference communities, the estimates based on dry weight indicated more Aufwuchs from the stressed streams was consumed while the organic matter based values indicated less Aufwuchs was consumed. These differences in impact assessment were attributed to the greater amount of inorganic sediment present in the Aufwuchs

from the treated streams.

The milligrams of Aufwuchs organic matter consumed, that is, consumption on an ash-free dry weight basis, was computed from the feeding data based on dry weight estimates and the ash-free dry weight/dry weight ratio from Aufwuchs samples taken for food quality analyses. This parameter is ecologically important and pertains to the actual flow of organic matter and energy from producer to consumer. The organic matter consumed should be emphasized in analyzing the results since the organic matter contained the isotopic label. In addition, the organic matter consumed is comparable to data reported in the literature for other feeding studies (Trama 1957, Malone and Nelson 1969, Sedell 1972, Calow 1975a, 1975b). On an hourly basis, the median feeding rates of Anculosa used in this study ranged from 0.11 to 0.02 mg Aufwuchs organic matter consumed per hour (Figure 18). These values are greater than the 0.018 mg/hr feeding rates reported for Goniobasis claeviformis by Malone and Nelson (1969) and similar to the feeding rates reported for Planorbis contorus by Calow (1975b). Snails consumed less Aufwuchs organic matter in eight of ten samples from copper and chlorine dosed streams where blue-green algae were dominant in the community (experiments 3-7) compared to reference stream communities. These results agree with literature data suggesting blue-green algae are less preferred food

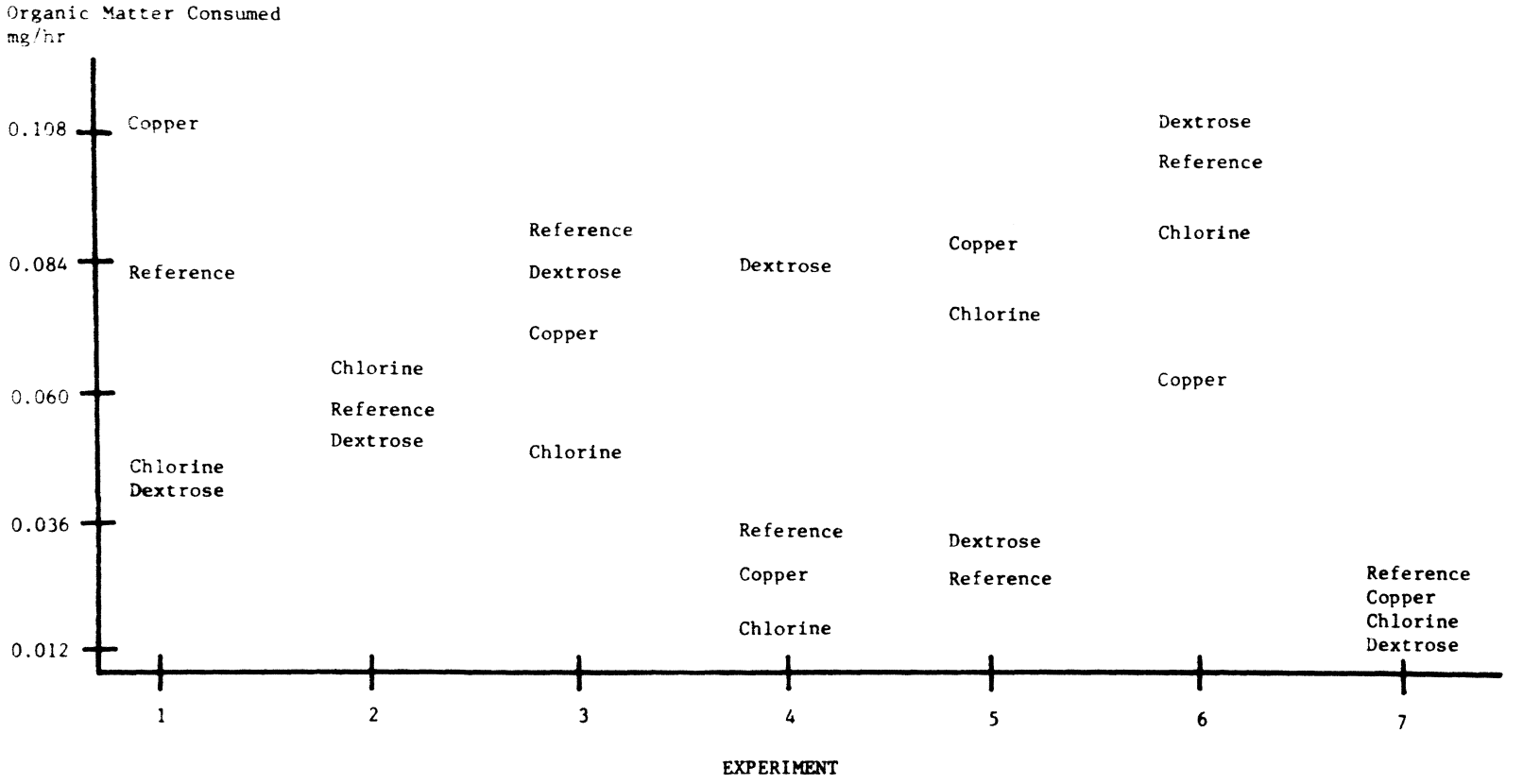


Figure 18. Rate of Aufwuchs organic matter consumed by snails during the artificial stream experiments. Median values for each stream are plotted.

types for grazing macroinvertebrates (Edmondson and Winberg 1971, Swiss and Johnson 1976, Moore 1977a). There were no consistent trends in snail consumption of Aufwuchs from the dextrose enriched stream relative to reference communities and the values were usually within 0.05 mg.

Bioenergetic studies commonly report animal food consumption on a weight specific basis (i.e. mg food consumed/ mg animal body weight) to reduce the variability in animal metabolism as a function of size (Brody 1945, Prosser 1973). Weight specific feeding rates have been reported from studies of the caloric or nutrient requirements of aquatic invertebrates and the utilization of single species food resources (Carefoot 1970, Vivekananden et al. 1974, Swiss and Johnson 1976). Ecological investigations into grazer consumption of available food resources and the environmental factors influencing the utilization of food resources have reported feeding rates for individual animals without corrections for size (Malone and Nelson 1969, Hargrave 1970, 1971, Calow 1975b, Elwood and Goldstein 1975). The data reported in this dissertation were computed without corrections for snail size since the information was evaluated in an ecological context and similarly sized snails were used for each test group in an experiment.

There were no consistent correlations between snail body weight and Aufwuchs organic matter consumed over the

seven experiments (Table A1, Appendix). An evaluation of the feeding data on a weight specific basis (computed in spite of the lack of association) produced results generally in agreement with the previous conclusions regarding snail consumption of Aufwuchs from the dextrose enriched stream. However, similar estimates of snail consumption of organic matter for Aufwuchs from the chlorine and copper treated streams exceeded reference values in 7 of 8 comparisons for experiments 4 through 7. These apparent relative increases in consumption during the later experiments are inconsistent with the results presented in Figure 15 and inconsistent with literature reports of decreased grazer consumption of blue-green algal dominated food resources (as was present in the chlorine and copper treated communities for experiments 4 through 7). For these reasons, snail consumption of Aufwuchs on an individual basis was used to evaluate the effects of pollutants on grazer utilization of Aufwuchs.

The amount of Aufwuchs organic matter assimilated during the feeding study was computed from the organic matter consumed and the estimate of assimilation efficiency for each snail. This variable encompasses all the physical and chemical characteristics of the Aufwuchs community as well as the snail's ability to consume and retain the organic matter. A median value was computed for each experimental

group and the data ranged from 0.02 to 0.60 mg Aufwuchs organic matter assimilated (Figure 17). While this information is rarely reported in the literature, it represents the actual flow of organic matter and energy up the trophic chain. Out of 20 total comparisons between snail utilization of Aufwuchs from treated and reference communities, estimates of organic matter assimilated provided 5 conclusions that were opposite of ash-free dry weight eaten, 3 of these from snail feeding activity on Aufwuchs from dextrose treated streams. Disagreement of this sort suggests that consumption data alone may not reflect all trophic dynamic or energy flow interactions, especially regarding the importance of heterotrophic microflora in the diet of snails. However, estimates for this parameter were computed by indirect methods using two conversion factors and a previously calculated consumption rate. The results are biased by the variability of these measurements and decrease its reliability in assessing perturbation impacts.

Assimilation efficiency was a directly measured parameter, computed as the ratio of isotopic counts retained in the snail body to the isotope in both the body and fecal sample, which is independent of gravimetric units (Edmondson and Winberg 1971). This parameter is frequently reported in the literature and serves as an index of the ability of the consumer to digest and absorb the food resource. Median

assimilation efficiencies in the seven experiments ranged from 7 to 61% (Figure 16). These are within the range of values reported for grazing macroinvertebrates (Carefoot 1967, 1970, Hargrave 1970, 1971, Vivekanandan et al. 1974, Calow 1975b). Assimilation efficiencies were less for Aufwuchs with a greater abundance of blue-green algae compared to reference communities (in 7 of 10 comparisons). Aufwuchs dominated by bacteria from the dextrose enriched stream were assimilated with greater efficiency 5 of 7 times compared to reference communities.

Feeding chambers without snails were used as controls to account for Aufwuchs sloughed or dislodged from glass slides during handling and to monitor any change in the specific activity of the food supply during the feeding experiment. Sloughing of Aufwuchs was less than 2% of the Aufwuchs biomass or radioactivity. By comparing the specific activity of the food sample at time zero with the ungrazed food left at the end of the experiment, the consistency of the food sample radioactivity and the thoroughness of the sulfur-35 label in the food could be evaluated. If the Aufwuchs food sample was not uniformly labeled, its specific activity would be expected to change during the duration of the feeding experiment (4 or 6 hours) as any unassimilated isotope was transferred between species prior to arriving at some equilibrium in the community (Sorokin 1968). Since

there were no significant differences between the specific activity of the food at time zero and at the end of the feeding experiment, it was assumed that the Aufwuchs community was thoroughly saturated with sulfur-35.

The isotopic methods and computations used in estimating snail assimialtion efficiencies assumed the sulfur-35 was uniformly incorporated into the Aufwuchs community biomass and assimilated into the snail tissues in the same manner as the Aufwuchs organic matter. Isotopic labels which are absorbed or adsorbed to food samples provide an estimate of food absorption efficiency (Calow 1975b, Elwood and Goldstein 1975) however true assimilation efficiencies are best estimated by isotopes which are incorporated into the structure of the food tissues (Trama 1957, Odum and Golley 1963, Hargrave 1970, 1971, Grodzinski et al. 1975). The expression "assimilation efficiency" was employed in this dissertation based on such a distinction of terms. Sulfur-35 is incorporated into the protein components of living microfloral tissues through assimilatory sulfate reduction (Schiff and Hodson 1973, Monheimer 1974, Rodgers 1977, Campbell and Baker 1978). Aufwuchs which had been incubated in sulfur-35 enriched media were rinsed in a 0.1 M sodium sulfate solution to remove as much adsorbed sulfur-35 as possible (Jordan et al. 1978). Since the entire Aufwuchs sample was digested for liquid scintillation counting, inclu-

sion of sulfur-35 which had adsorbed to clay or other inorganic materials cannot be ruled out. No attempt was made to determine the precise size fraction or material in the Aufwuchs sample which contained the sulfur-35 isotope. However, passive absorption of sulfate is negligible compared to active microfloral assimilation (Rodgers 1977). Thus the assumption that at least the bulk of the label was incorporated into the viable microbial tissues would appear sound.

A question remains concerning the uniformity of the label within the various species composing the Aufwuchs community sample. There was no way to test whether one component, such as bacteria, had accumulated more or less radioactivity per unit biomass. Since the radioactive counts in the snail bodies and feces were used to represent quantities of food ingested, assimilated, and egested, variations in the distribution of these counts could lead to misinterpretations regarding feeding and assimilation rates. It is expected that the differential metabolic rates between groups within the microflora may contribute to heterogeneous levels of specific activity within the Aufwuchs sample. This problem has rarely been addressed in the literature.

In this research, the food samples were allowed to incorporate inorganic sulfur-35 for 20 hours prior to the experiment. This incubation process insured a thoroughly labeled food sample of high specific activity to follow

small amounts of organic matter transferred between producers and consumers (down to 0.005 mg). Further, inorganic sulfur is assimilated by both autotrophic and heterotrophic microflora (Monheimer 1972, 1974, Jassby 1975, Rodgers 1977, Campbell and Baker 1978) which will insure that both of these components in the Aufwuchs community will be labeled. Since sulfur is found in fairly constant ratios to organic carbon in Aufwuchs communities (Rodgers 1977) or microfloral samples (Jordan and Peterson 1978, Monheimer 1978), excessive concentrations of the sulfur isotope within any community component is not expected. It was then assumed that the Aufwuchs food samples were uniformly labeled, at least as well as food samples used in previously published feeding studies using isotopes.

Correlations among feeding and food quality results

Correlation coefficients between the median estimates for the snail feeding studies and the mean food quality estimates for Aufwuchs from the artifical streams encompass a variety of seasonal and treatment effects over the seven experiments (Table 4). None of the food quality parameters were significantly correlated with estimates of Aufwuchs dry weight consumed by snails. Ash-free dry weight eaten and organic matter assimilated by snails were significantly correlated with the estimates of dry weight consumed ($r=0.791$, $P<0.01$; $r=0.626$, $P<0.01$), although this trend was expected

Table 4. Spearman rank correlation coefficients, r, for median snail feeding parameters with mean food quality measurements for Aufwuchs sampled from the seven artificial stream experiments. The significance level, P, is given in parenthesis for each correlation. (N=27 except for caloric data where N=23).

	Aufwuchs Dry Weight Consumed per hour	Aufwuchs Organic Matter Consumed per hour	Aufwuchs Organic Matter Assimilated per hour	Snail Assimilation Efficiency
Aufwuchs Organic Matter Consumed per hour	0.791[r] (<0.01)[P]	...	...	...
Aufwuchs Organic Matter Assimilated per hour	0.626 (<0.01)	0.766 (<0.01)	...	...
Snail Assimilation Efficiency	0.334 (0.09)	0.331 (0.09)	0.140 (0.48)	...
Aufwuchs Dry Weight	-0.148 (0.46)	-0.223 (0.26)	-0.344 (0.08)	-0.405 (0.04)
Aufwuchs Ash-Free Dry Weight (AFDW)	-0.201 (0.32)	-0.013 (0.95)	0.156 (0.44)	-0.446 (0.02)
Aufwuchs Percent Organic Matter	-0.200 (0.32)	0.338 (0.95)	0.323 (0.10)	-0.068 (0.73)
Aufwuchs Protein	-0.019 (0.93)	0.122 (0.54)	-0.156 (0.44)	-0.173 (0.39)
Aufwuchs Carbohydrate	-0.269 (0.18)	-0.010 (0.95)	-0.217 (0.28)	0.037 (0.85)
Aufwuchs Calories	-0.055 (0.35)	-0.051 (0.82)	-0.219 (0.32)	0.409 (0.05)
Aufwuchs Protein/AFDW	0.029 (0.88)	-0.001 (0.99)	-0.100 (0.62)	-0.230 (0.25)
Aufwuchs Carbohydrate/AFDW	-0.210 (0.29)	-0.045 (0.82)	-0.186 (0.35)	0.379 (0.05)
Aufwuchs Calories/AFDW	-0.146 (0.51)	-0.175 (0.42)	-0.195 (0.37)	0.590 (<0.01)

since they were derived from these estimates. Snail consumption of Aufwuchs organic matter was significantly correlated ($r=0.338, P=0.08$) with the percent organic matter in the Aufwuchs community; however, this ratio (ash-free dry weight/ dry weight) was used to calculate the ash-free dry weight eaten. The correlation between the median estimates of snail assimilation efficiencies and snail consumption of Aufwuchs (dry weight, $r=0.334, P=0.09$; organic matter, $r=0.331, P=0.09$) demonstrates some positive interaction between the snails' inclination to consume a food resource and the ability to digest and adsorb it. Negative correlation coefficients were calculated between snail assimilation efficiency and Aufwuchs dry weight ($r=-0.405, P=0.04$) and Aufwuchs ash-free dry weight ($r=-0.446, P=0.02$). Median assimilation efficiency estimates were also correlated with carbohydrate expressed as a percent of the food organic matter ($r=0.379, P=0.05$) and caloric estimates of the food samples, both as total calories available in the food resource ($r=0.409, P=0.05$) as well as calories per gram organic matter ($r=0.590, P<0.01$).

Correlations between the various measures of Aufwuchs consumption by snails were expected since the same raw data were used to compute these parameters through various conversion factors. The lack of consistent correlations between estimates of snail consumption and measures of food quality agrees with previous studies of marine snails grazing on

algae. Carefoot (1967, 1970) reported no trends associating snail feeding rates or growth rates with algal food quality. Paine and Vadas (1969), working with another species of marine snail, found that neither caloric, carbohydrate, protein nor lipid content alone could account for the preference in food species recorded by gut analyses for marine benthic grazers. They concluded that the availability of the food types and the generally opportunistic feeding habits of the grazers contributed to this lack of association. Freshwater snails have been classified as opportunistic feeders (Malone and Nelson 1969, Cummins 1973) and the results of this research support that generalization. However, Pip and Stewart (1976) reported the grazing rate of the freshwater snail Physa was highest when the carbohydrate content of the aquatic macrophyte food resources peaked, during the early and midsummer. Snail growth rates and reproductive activity were also maximal at this time (Pip and Stewart 1976), placing additional energy demands on the snails which necessitated increased consumption. Thus, a true cause and effect relationship may not be present.

Estimates of snail assimilation efficiency rarely have been directly correlated with various chemical constituents of the food resource. In this research, assimilation efficiency was correlated with the measure of calories and carbohydrates per unit organic matter in the food sample ($r=0.590$

and 0.379 respectively, Table 4). Carefoot (1967, 1970) found that foods of high caloric content were usually assimilated with great efficiency, further facilitating the growth rate of marine snails. Most investigators chose to monitor growth rates and assimilation efficiency, reporting positive correlations above maintenance feeding levels (Carefoot 1967, 1970, Welch 1968, Vivekananden et al. 1974, Grodzinski et al. 1975, Swiss and Johnson 1976). McMahon et al. (1974) reported higher growth and fecundity rates for freshwater snails feeding on Aufwuchs communities of lower C:N ratios (higher protein content).

New River Experiments

Food quality analysis

Food quality constituents demonstrated changes in Aufwuchs communities which developed on glass slides at sample stations in the New River near the Glen Lyn Power Plant (Table 5). At station 2, a station intermediately influenced by chlorinated-thermal discharge ($\Delta T=3-5^{o}C$) (Figure 4), dry weight and ash-free dry weight estimates of accumulated Aufwuchs were more than twice the reference station values while the percent organic matter was 8% higher. Protein estimates were also more than twice reference community values. When expressed as a percent of the organic matter present, the protein content of the Aufwuchs community was nearly equal to that of the reference community. Carbohydrate estimates were only 0.2 g/m^2 less than the reference community, however, when expressed as a percent of the organic matter, the carbohydrate content was reduced by 3.2%.

From station 3, which was located within all the power plant discharges ($\Delta T=5-10^{o}C$) (Figure 4), estimates of dry weight and ash-free dry weight for Aufwuchs samples were about equal to reference community values; percent organic matter was only 5% less. Protein estimates from station 3 were approximately 5 times below reference community values while carbohydrates were 3 times greater. This converted to a decrease in percent protein as organic matter of 2.2%

Table 5. Mean food quality estimates for Aufwuchs communities from New River sampling stations above and within the chlorinated-thermal discharge of the Glen Lyn Power Plant on 8/10/79. (Refer to Figure 4).

Food Quality Parameters	Sample Site Station 1 (reference)	Station 2 ($\Delta T=3-5^{o}C$)	Station 3 ($\Delta T=5-10^{o}C$)
Dry Weight (mg/m^2)	6.37	13.01	7.41
Ash-free Dry Weight (AFDW) (mg/m^2)	2.13	5.33	2.11
Percent Organic Matter	33%	41%	28%
Protein (g/m^2)	0.061	0.144	0.013
Protein/AFDW	2.8%	2.7%	0.6%
Carbohydrate (g/m^2)	0.101	0.083	0.301
Carbohydrate/AFDW	4.8%	1.6%	14.3%

and an increase of 9.5% in the percent carbohydrate.

The habitat in the area of station 2 supported an algal population similar to the uninfluenced area relative to the dominance of diatoms (90%), green algae, and blue-green algae; however, shifts occurred in the component species (Cairns et al. 1978). The Aufwuchs community at station 3 had a relatively larger blue-green algal component (45%), with reductions in diatom (40%) and green algae abundances (person observations, Cairns et al. 1978). The increased biomass at station 2 was a result of increased growth due to thermal enrichment while at station 3 the additional thermal load caused a shift in the community composition, as better adapted microfloral species probably outcompeted other Aufwuchs components. Decreases in carbohydrate per unit ash-free dry weight at station 2 relative to reference communities while maintaining similar protein to ash-free dry weight ratios indicated that this community was utilizing a larger fraction of stored carbohydrates to cope with power plant effluents. At station 3, the higher carbohydrates and lower protein per unit organic matter reflect an Aufwuchs community with copious mucilage and senescence or low metabolic activity (Fogg 1975). Due to the limited sampling, a more comprehensive discussion of the impacts of the power plant discharges on New River Aufwuchs communities should be reserved.

Feeding studies

In August, 1978, and August, 1979, Aufwuchs samples were obtained from sample sites in the New River to conduct three snail grazing experiments (Table 6, Figure 4). In the 1979 experiment, snails feeding on Aufwuchs samples from station 2 ($\Delta T=3-5^{o}C$) consumed more than twice the Aufwuchs dry weight and organic matter compared to those grazing the reference community. Aufwuchs samples from station 3 ($\Delta T=5-10^{o}C$) were grazed only slightly less (0.02 mg dry weight and 0.015 mg organic matter) than the reference station samples. In 1978, snails consumed 0.174 mg less Aufwuchs from station 2 in one experiment and 0.123 mg more in the other compared to dry weight consumed by snails feeding on Aufwuchs from the reference station. Median estimates of assimilation efficiency were consistently higher for snails feeding on Aufwuchs from the reference station compared to Aufwuchs from station 2 (25% in 1979, 5% in both 1978 experiments) and station 3 (16% in 1979). The estimate of organic matter assimilated by the snails feeding on Aufwuchs from station 2 was approximately double that of the reference station in 1979, even though the assimilation efficiency was lower by 25%. For station 3, the lower amounts of organic matter eaten and lower assimilation efficiency combined to decrease the estimate of organic matter assimilated by 0.023 mg below the reference station values. There were no esti-

Table 6. Snail consumption, assimilation, and assimilation efficiency for Aufwuchs communities from sampling stations above and within the chlorinated-thermal discharge of the Glen Lyn Power Plant in the New River. (Refer to Figure 4)

Date of Experiment Sample Site	Median Dry Weight Consumed (mg)	Median Organic Matter Consumed (mg)	Median Organic Matter Assimilated (mg)	Median Assimilation Efficiency (%)
8-10-79				
Station 1 (reference)	0.200	0.066	0.048	73
Station 2 (ΔT=5 C)	0.434	0.173	0.099	48
Station 3 (ΔT=10 C)	0.180	0.051	0.025	57
8-20-78				
Station 1 (reference)	1.858	...	...	61
Station 2 (ΔT=5 C)	1.684	...	...	56
8-5-78				
Station 1 (reference)	0.357	...	...	75
Station 2 (ΔT=5 C)	0.480	...	...	70

mates of percent organic matter in the Aufwuchs sampled in 1978 so calculations of organic matter consumed or organic matter assimilated were not possible.

The experiments conducted in 1978 were intended as preliminary studies, but are included since Aufwuchs sampling from the New River was restricted to a single successful effort in 1979 and these preliminary findings support the results of the later study. The abundance of sheathed blue-green algae at station 3 (45% cell counts) may be responsible for the lower estimates of snail consumption of Aufwuchs and assimilation efficiencies compared to the reference station community (which contained over 90% diatoms). Reasons for the lower assimilation efficiencies and higher consumption rates of snails feeding on Aufwuchs from station 2 compared to reference station Aufwuchs communities are unclear. Although both communities were dominated by diatoms, differences in the diatom species may be responsible. This may have lead to decreased feeding rates if snails had more difficulty in scraping the diatoms from the substrate. Differences in the species composition between Aufwuchs from station 2 and the reference station may also have accounted for the 5% decreases in assimilation efficiency recorded in the 1978 experiments. However, as diatoms are generally easily digested (Edmondson and Winberg 1971), other factors are probably responsible for the 25% decrease in assimila-

tion efficiency recorded in the 1979 experiment. Perhaps, as was reported by Vivekanandan et al. (1974), the greater organic matter present in the Aufwuchs samples from station 2 (Table 5) stimulated snail feeding activity but assimilation occurred less efficiently. As recorded in Table 6, the snails still managed to assimilate twice as much organic matter as those feeding on Aufwuchs from the reference station.

Comparison of Feeding Parameters Based on Sulfur-35 or Carbon-14 Labeled Aufwuchs

Using Aufwuchs samples from untreated artificial streams, estimates of snail consumption of Aufwuchs were 5 to 42 times grater for carbon-14 labeled Aufwuchs compared to sulfur-35 labeled food samples (Table 7) with all of these increases significantly different (α=0.01). Estimates of snail assimilation efficiency for food samples labeled with carbon-14 were 5, 22, and 6% greater than sulfur-35 labeled Aufwuchs in experiments 1, 2, and 4 and 1% less in experiment 3. Only in experiment 2 was the difference between estimates of snail assimilation efficiencies (22%) significantly different (α=0.05). Aufwuchs samples within an experiment were from the same source although changes in the composition of Aufwuchs communities between experiments may have led to fluctuations in the feeding parameters for each labeled food type between experiments. Since all Aufwuchs samples used in an experiment contained the same percent organic matter, employing a single constant to compute estimates of organic matter consumed or assimilated for each experiment would not change the magnitude of differences between snail utilization of sulfur-35 or carbon-14 labeled Aufwuchs.

The assimilation efficiency was computed as the ratio of snail body isotopic counts to the sum of the body and fecal sample counts. Since this ratio was about the same for both carbon and sulfur labeled samples, it was assumed that no differential loss of the isotopes occurred in ex-

Table 7. Median estimates of snail feeding parameters using Aufwuchs labeled with sulfur-35 or carbon-14 with results of the Kruskal-Wallace Rank Sum test ($\alpha<0.01$***;$\alpha<0.05$**)

Experiment	Isotope	Median Aufwuchs Dry Weight Consumed (mg)		Median Assimilation Efficiency (%)		Median Food Specific Activity (cpm/mg dry weight)	
1	sulfur-35	0.20	***	30		887	***
	carbon-14	8.50		35		112	
2	sulfur-35	0.58	***	45	**	235	**
	carbon-14	9.34		67		104	
3	sulfur-35	0.27	***	19		674	***
	carbon-14	10.80		18		97	
4	sulfur-35	0.27	***	26		1297	***
	carbon-14	1.51		32		123	

tracting snails from their shells, in collecting fecal samples, or preparing for liquid scintillation counting. Furthermore, various subsets of samples were fixed with basic (pH>7.5) formalin prior to processing in order to decrease any respiratory losses of carbon-14. The results of these procedures demonstrated no significant difference in the carbon-14 content between fixed and non-fixed samples. Thus the reason for higher estimates of consumption for the carbon-14 labeled Aufwuchs samples may be related to problems in obtaining an accurate estimate of the carbon-14 content in the food sample or to the distribution of the isotope in the food sample.

All carbon-14 samples (food, snails, feces) were prepared for liquid scintillation counting in the same manner. This method has been successfully employed in Aufwuchs primary productivity studies (Rodgers 1977, Clark unpublished). When the carbon-14 counts obtained for the Aufwuchs food samples used in this research were compared to primary productivity data, computed specific activity levels were similar for both groups. This negated the potential bias from improper sample preparation.

The objective of using carbon-14 in primary productivity studies was to add small amounts of labeled bicarbonate to the inorganic carbon pool so that the reservoir of inorganic carbon remained essentially unchanged. Under these

conditions, the isotopic uptake of carbon-14 represented the rate of inorganic carbon fixation. Algal photosynthesis usually accounts for over 90% of the fixed carbon while heterotrophic fixation of inorganic carbon-14 bicarbonate is commonly 10% or less of Aufwuchs photosynthetic fixation (Wetzel 1975, Rodgers 1977). The specific activity of the food sample (carbon-14 cpm/mg Aufwuchs dry weight) is based mostly on the algal component of the Aufwuchs community when inorganic carbon-14 is used. Inorganic sulfate on the other hand, is assimilated by both autotrophs and heterotrophs (Monheimer 1974, Jassby 1975, Rodgers 1977), leading to a more thoroughly labeled Aufwuchs food sample when sulfur-35 sulfate is used.

This information alone may not account for the large differences between the estimates of snail consumption of Aufwuchs, since the Aufwuchs communities were dominated by autotrophs, largely diatoms, in each of the experiments. Therefore, the only other apparent difference between the raw data in these experiments is the greater specific activity of the sulfur labeled food (Table 7).

The difference in the amount of isotope used to label Aufwuchs (60 uCi/l sulfur-35 and 5 uCi/l carbon-14) was considered incidental when designing the experiments since the photosynthetic uptake of carbon-14 was sufficient to provide food samples with enough isotope incorporated into their biomass to follow the path of the food through and into

the snail body. However, the specific activity of the carbon-14 labeled food was well below that of the sulfur-35 labeled food (Table 7). When the specific activity of the carbon-14 labeled food increased from 97 to 123 cpm/mg (apparently due to greater metabolic activity in the Aufwuchs communities sampled), estimates of food consumed by the snails decreased from 10.80 to 1.51 mg. As the specific activity of the sulfur-35 labeled food doubled from 674 to 1297 cpm/mg in experiments 1, 3, and 4 (again due to increased metabolic activity), no dramatic changes in the feeding rate was observed. The higher estimate of Aufwuchs consumed in experiment 2 may be related to the lower specific activity in the sulfur-35 labeled food sample.

Since the estimates of snail feeding rates from the artificial stream experiments based on sulfur-35 labeled Aufwuchs are close to values reported in the literature for other snail feeding studies, the carbon-14 based feeding data are suspect. The 18 to 20 hour labeling time was thought to encompass a sufficient time span to insure complete labeling by either isotope. Although the inorganic carbon may have been incorporated into simple algal carbohydrates and the sulfate assimilated into microfloral proteins, the similar estimates of snail assimilation efficiency negated the possibility that differential component labeling led to the differences in snail feeding rates. The sulfur-35 isotope

was apparently more thoroughly incorporated into the Aufwuchs biomass than carbon-14 in all four experiments and provided a more reliable estimate of snail consumption of Aufwuchs in these experiments.

SUMMARY AND CONCLUSIONS

1. A series of artificial streams was used to evaluate the impact of intermittent chlorination (0.2 ppm TRC) and continuous exposures to copper (0.05 ppm) and dextrose (1 or 2 ppm) on Aufwuchs food quality and utilization by grazers (snails). Seven experiments were conducted from January through September, 1979. The chemical food quality of Aufwuchs communities was determined through quantification of the organic matter, protein, carbohydrate, and caloric contents. Sulfur-35 was used to monitor snail consumption and assimilation of Aufwuchs from the variously dosed and reference artificial streams to assess the impact of altered food resources on the energy transfer from producer to consumer trophic levels.

2. Aufwuchs communities developing under the copper, chlorine, and dextrose treatments contained a lower percent organic matter than reference stream communities in 5 of the 7 experiments. Percent organic matter decreased under the chlorine treatment as microflora, which had accumulated between applications, died during chlorine dosing. Sheathed blue-green algae dominated the Aufwuchs community under copper stress. As the blue-green algae proliferated, suspended inorganic sediment was entrapped in the sheath, decreasing the organic carbon content of the Aufwuchs samples when compared to reference communities. Less organic matter

in Aufwuchs samples from the dextrose enriched stream, which were composed predominantly of bacteria, resulted from a similar accumulation of inorganic sediment.

3. In experiments 1 through 4, less protein was obtained from Aufwuchs samples that were treated with chlorine and copper relative to the reference stream since Aufwuchs biomass was decreased. In experiments 4 through 7, protein content (expressed as a percent of the organic matter sampled) increased in Aufwuchs from the chlorine and copper streams as blue-green algae proliferated. The sheaths of these algae contained more protein compared to reference community biomass. The bacterial mucilage in Aufwuchs from the dextrose treated stream also had increased protein content. As a nontaxonomic parameter for evaluating environmental perturbation, the protein content of Aufwuchs communities was most affected when the microfloral groups comprising the Aufwuchs community changed.

4. Carbohydrate analyses also were closely associated with the extent of biomass accumulated and the microfloral composition in the Aufwuchs. Reductions in the carbohydrate content of Aufwuchs from the chlorinated and copper treated streams were attributed to the reduced biomass present as well as the utilization of algal food reserves in physiologically adapting to stress. Increases in Aufwuchs carbohydrate content in the dextrose enriched stream were caused

by the high carbohydrate content in the bacterial mucilage. Since blue-green algal sheaths are also high in carbohydrate, the increased carbohydrate content of Aufwuchs from the chlorine or copper treated streams in experiments 5 to 7 may be related to the abundance of blue-green algae. Although carbohydrate analysis was selected for inclusion as a measure of Aufwuchs structure, the results demonstrated both physiological and morphological characteristics of the microflora. As these factors may counteract each other, carbohydrate analyses may be less reliable in assessing perturbation impacts compared to more traditional methods.

5. Calories per unit organic matter were higher in the chlorinated stream when compared to reference communities in experiments 2 through 5 with a decrease in experiments 6 and 7. When sheathed blue-green algae dominated the Aufwuchs community under the copper stress (experiments 3-7), caloric analyses were consistent in distinguishing these communities from reference communities, presumably a result of the higher organic matter in the mucilaginous sheath. Caloric analyses best assessed the impact of the dextrose enrichments with the resulting copious bacterial mucilage, especially when expressed as calories per unit organic matter, compared to the protein and carbohydrate methods. Caloric analyses provided useful information on the changes in the chemical constituents in Aufwuchs communities developing under copper

and dextrose treatments but the results were variable in assessing the impact of chlorine on Aufwuchs community structure.

6. Snails consumed less organic matter in 8 of 10 Aufwuchs samples from chlorine and copper dosed streams when blue-green algae dominated the community (experiments 3-7). Results of these experiments agree with literature data that suggest blue-green algae are less preferred food types for grazing macroinvertebrates. Slightly less Aufwuchs from the dextrose enriched stream was consumed by snails in 4 of the 7 experiments compared to reference communities. Heterotrophically dominated Aufwuchs were shown to be possible food resources for grazers. Snail feeding studies demonstrated that low levels of stress may not alter grazer consumption of Aufwuchs until substantial changes in algal composition occur.

7. Snail assimilation efficiencies for Aufwuchs communities from the chlorine and copper dosed streams, where blue-green algae predominated, were generally 3 to 5% less than reference values. Heterotrophically dominated Aufwuchs from the dextrose enriched stream were assimilated with 1 to 15% greater efficiency compared to snail assimilation efficiencies for reference communities. These data were consistent with published literature. Snail assimilation efficiency was generally a sensitive parameter in assessing

trophic level effects of changes in Aufwuchs communities resulting from pollutant impacts.

8. The lack of significant correlations between Aufwuchs protein, carbohydrate, or caloric content and the estimates of snail consumption of Aufwuchs was attributed to the opportunistic feeding strategy of the snails. In laboratory feeding experiments, the grazer consumed any available food resource. Snail assimilation efficiencies were correlated with higher caloric content ($r=0.590$, $\alpha<0.01$) and with increased carbohydrate content ($r=0.379$, $\alpha=0.05$) in the Aufwuchs samples. These associations represented a greater assimilation efficiency of bacterially dominated Aufwuchs from the dextrose treated stream and high caloric and carbohydrate content associated with bacterial mucilage.

9. Compared to Aufwuchs from a New River reference station, the biomass and protein content of Aufwuchs was stimulated under a chlorinated-thermal ($\Delta T=3-5^{o}C$ stress) power plant effluent. The lower carbohydrate content of this community was presumably a result of adaptations to cope with the chlorinated-thermal stress. Although Aufwuchs communities from this habitat were consumed by snails to a greater extent than reference communities, snail assimilation efficiencies were 5 to 25% less. Aufwuchs developing under a thermal effluent at 5 to $10^{o}C$ temperature increases exhibited a decrease in protein content and an increase in carbohydrates.

Changes due to this higher temperature stress reflected a shift toward blue-green algal predominance and presumably altered metabolic activity. Snail consumption and assimilation efficiency for this community were lower than reference communities. Analysis of Aufwuchs food quality and associated grazer utilization distinguished the New River habitats receiving power plant discharges from reference communities and allowed an evaluation of the impact on producer-consumer interactions.

10. Snail consumption estimates for Aufwuchs labeled with sulfur-35 were similar to other literature data for snail grazing rates while carbon-14 labeled Aufwuchs produced snail feeding estimates 5 to 42 times greater. Estimates of snail assimilation efficiency of Aufwuchs labeled with sulfur-35 or carbon-14 were similar. The extent of isotope labeling per unit biomass in the Aufwuchs food sample influenced the estimates of snail consumption, apparently due to a heterogenous distribution of the label among the Aufwuchs biomass. Since inorganic sulfur-35 is directly incorporated into both autotrophs and heterotrophs, its use may be more appropriate than inorganic carbon-14 in labeling Aufwuchs communities for feeding studies.

LITERATURE CITED

American Public Health Association. 1976. Standard methods for the examination of water and waste water. 14th edition. American Public Health Association, Washington, D. C.

Arnold, D. E. 1971. Ingestion, assimilation, survival, and reproduction by Daphnia pulex fed seven species of blue-green algae. Limnology and Oceanography 16:906-920.

Baker, J. H., and L. A. Bradnam. 1976. The role of bacteria in the nutrition of aquatic detritivores. Oecologia 24:95-104.

Barr, A. J., J. H. Goodnight, J. P. Sall and J. T. Helwig. 1976. A user's guide to SAS 76. SAS Institute, Raleigh, N. C.

Becker, C. D., and T. O. Thatcher. 1973. Toxicity of power plant chemicals to aquatic life. Battelle Labs, Richland, Washington.

Bishop, N. I. 1964. Site of action of copper in photosynthesis. Nature 204:401-402.

Bowers, J. A., and N. E. Grossnickle. 1978. The herbivorous habits of Mysis relicta in Lake Michigan. Limnology and Oceanography 23:767-778.

Bransome, E. D., Jr., editor. 1973. The current status of liquid scintillation counting. Grune and Stratten, Inc. New York.

Brock, T. D. 1974. Biology of microorganisms. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Brody, S. 1945. Bioenergetics and growth. Reinhold Publishing Co., New York.

Cairns, J., Jr. 1970. Ecological management problems caused by heated waste water discharge into the aquatic environment. Water Resources Bulletin 6:868-878.

Cairns, J., Jr., editor. 1971. The structure and function of fresh-water microbial communities. Research Division Monograph No. 3. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Cairns, J., Jr. 1976. Heated waste-water effects on aquatic ecosystems. Pages 32-38 in G. W. Esch and R. W. McFarlane, editors. Thermal Ecology II. Technical Information Center, Springfield, Virginia.

Cairns, J., Jr., and K. L. Dickson. 1971. A simple method for the biological assessment of the effects of discharges on aquatic bottom dwelling organisms. Journal of the Water Pollution Control Federation 43:755-772.

Cairns, J., Jr., and K. L. Dickson, editors. 1973. Biological methods for the assessment of water quality. ASTM STP 528. American Society for Testing and Materials, Philadelphia.

Cairns, J., Jr., and K. L. Dickson. 1976. Elasticity, inertia, and resiliency of water ecosystems. Pages 371-381 in Proceedings of the symposium on terrestrial and aquatic ecological studies of the northwest. Eastern Washington State College Press, Cheney, Washington.

Cairns, J., Jr., K. L. Dickson, and E. E. Herricks, editors. 1977. Recovery and restoration of damaged ecosystems. University Press of Virginia, Charlottesville, Virginia.

Cairns, J., Jr., K. L. Dickson, E. Benfield, and A. Buikema. 1978. The development of biological indices for identifying the evaluating impacts of pollutants on freshwater ecosystems. Pages 1-161 to 1-176 in Summary Report to DOE, June 1978.

Calow, P. 1975a. On the nature and possible utility of epilithic detritus. Hydrobiologia 46:181-189.

Calow, P. 1975b. The feeding strategies of two feshwater gastropods, Ancylus fluviatilis Mull. and Planorbis contortus Linn. (Pulmonata), in terms of ingestion rates and absorption efficiencies. Oecologia 20: 33-49.

Campbell, P. G., and J. H. Baker. 1978. Measurements of sulfate uptake in the dark by suspended microorganisms: application to running waters. Internationale Vereinigung fur Theoretische und Angewandte Limnologie 20:1423-1428.

Carefoot, T. H. 1967. Growth and nutrition of Aplysia punctata feeding on a variety of marine algae. Journal of the Marine Biological Association of the

United Kingdom 47:565-590.

Carefoot, T. H. 1970. A comparison of absorption and utilization of food energy in two species of tropical Aplysia. Journal of Experimental Marine Biology and Ecology 5:47-62.

Castenholtz, R. W. 1960. Seasonal changes in the attached algae of freshwater and saline lakes in the lower Grand Coulee, Washington. Limnology and Oceanography 5:1-28.

Cattaneo, A., S. Ghittori, and V. Vendegna. 1975. The development of benthic phytocoenosis on artificial substrates in the Ticino River. Oecologia 19:315-327.

Chapman, D. W., and R. L. Demory. 1963. Seasonal changes in the food ingested by aquatic insect larvae and nymphs in two Oregon streams. Ecology 44:140-146.

Cherry, D. S., R. C. Hoehn, S. S. Waldo, D. H. Willis, J. Cairns, Jr., and K. L. Dickson. 1977. Field-laboratory determined avoidances of the spotfin shiner and the bluntnose minnow to chlorinated discharges. Water Resources Bulletin. 13:1047-1055.

Clark, J. R. 1976. Estimating the biomass of Aufwuchs using adenosine triphosphate assays. Masters thesis. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Clark, J. R., K. L. Dickson, and J. Cairns, Jr. 1979. Estimating Aufwuchs biomass. Pages 116-141 in R. Weitzel, editor. Methods and measurements of periphyton communities: a review, ASTM STP 690, American Society for Testing and Materials, Philadelphia.

Clark, J. R., J. H. Rodgers, Jr., K. L. Dickson, and J. Cairns, Jr. (in press). Using artificial streams to evaluate perturbation effects on Aufwuchs structure and function. Water Resources Bulletin.

Corner, E. D. S., and C. B. Cowey. 1968. Biochemical studies on the production of marine zooplankton. Biological Review 43:393-426.

Cummins, K. W. 1974. Structure and function of stream ecosystems. BioScience 24:631-641.

Cummins, K. W., and J. C. Wuycheck. 1971. Caloric equivilents for investigations in ecological energetics. Internationale Vereinigung fur Theoretische und Angewandte Limnologie, Mitteilung No. 18.

Cummins, K. W., R. C. Peterson, F. O. Howard, J. C. Wuycheck, and V. I. Holt. 1973. The utilization of leaf litter by stream detritivores. Ecology 54:336-345.

Dillon, R. T., Jr. 1977. Factors in the distributional ecology of upper New River mollusks (Virginia/North Carolina). Biology honors thesis. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Dickson, K. L., J. Cairns, Jr., J. R. Clark, and J. H. Rodgers, Jr. 1978. Evaluating pollution stress on ecosystems. Pages 80-83 in K. C. Flynn and W. T. Mason, editors. The freshwater Potomac:aquatic communities and environmental stresses. Interstate Commission on the Potomac River Basin, Washington, D. C.

Doremus, C. M., and W. N. Harman. 1977. The effects of grazing by physid and planorbid freshwater snails on periphyton. The Nautilus 91:92-96.

Edmondson, W. T., and G. G. Winberg, editors. 1971. A manual on methods for the assessment of secondary productivity in fresh waters. I.B.P. Handbook No. 17, Blackwell Scientific, Oxford.

Elwood, J. W., and R. A. Goldstein. 1975. Effects of temperature on food ingestion rate and absorption, retention, and equilibrium burden of phosphorus in an aquatic snail, Goniobasis clavaeformis Lea. Freshwater Biology. 5:397-406.

Elwood, J. W., and D. J. Nelson. 1972. Periphyton production and grazing rates in a stream measured with a ^{32}P material balance method. Oikos 23:295-303.

Environmental Protection Agency. 1976. Quality criteria for water. USEPA, Washington, D. C.

Eppley, R. W., A. F. Carlucci, O. Holm-Hanson, D. Kiefer, J. J. McCarthy, E. Venrick, and P. M. Williams. 1971. Phytoplankton growth and composition in shipboard cultures supplied with nitrate, ammonium or urea as the nitrogen source. Limnology and Oceanography 16:741-751.

Esen, A. 1978. A simple method for quantitative, semi-quantitative, and qualitative assay of protein. Analytical Biochemistry 89:264-273.

Fogal, W. H., and D. P. Webb. 1976. Cellulose content of food and feces of Diprion similis as an internal marker for determining food consumption and digestibility. Canadian Journal of Zoology 54:571-575.

Fogg, G. E. 1975. Algal cultures and phytoplankton ecology. Second edition. University of Wisconsin Press, Madison, Wisconsin.

Greene, R. H. 1979. Sampling design and statistical methods for environmental biologists. John Wiley and Sons, New York.

Grodzinski, W., R. Z. Klekowski, and A. Dunkan, editors. 1975. Methods for ecological bioenergetics. IBP Handbook, No. 24, Blackwell Scientific, Oxford.

Hargrave, B. T. 1970. The utilization of benthic microflora by Hyalella azteca (Amphipoda). Journal of Animal Ecology 39:427-437.

Hargrave, B. T. 1971. An energy budget for a deposit-feeding amphipod. Limnology and Oceanography 16:99-103.

Haug, A., S. Myklestad, and E. Sakshaug. 1973. Studies on the phytoplankton ecology of the Trondheimsfjerd. I. The chemical composition of phytoplankton populations. Journal of Experimental Marine Biology and Ecology 11:15-26.

Helwig, J. T., editor. 1977. SAS supplemental library user's guide. SAS Institute, Raleigh, N. C.

Hollander, M., and D. A. Wolfe. 1973. Nonparametric statistical methods. John Wiley and Sons, New York.

Howarth, R. W., and J. M. Teal. 1979. Sulfate reduction in a New England salt marsh. Limnology and Oceanography 24:999-1013.

Hynes, H. B. N. 1970. The ecology of running waters. University of Toronto Press, Toronto.

Jassby, A. D. 1975. Dark sulfate uptake and bacterial productivity in a subalpine lake. Ecology 56:627-636.

Johannes, R. E., and M. Satomi. 1966. Composition and nutritive value of fecal pellets of a marine crustacean. Limnology and Oceanography 11:191-197.

Johannes, R. E., and M. Satomi. 1967. Measuring organic matter retained by aquatic invertebrates. Journal of the Fisheries Research Board of Canada 24:2467-2471.

Jones, J. R. E. 1950. A further ecological study of the River Rheidol:the food of the common insects of the main stream. Journal of Animal Ecology 19:159-174.

Jordan, M. J., and B. J. Peterson. 1978. Sulfate uptake as a measure of bacterial production. Limnology and Oceanography 23:146-150.

Jordan, M. J., R. J. Daley, and K. Lee. 1978. Improved filtration procedures for freshwater ^{35}S (SO_4) uptake studies. Limnology and Oceanography 23:154-157.

Kehde, P. M., and J. L. Wilhm. 1972. Effects of grazing by snails on community structure of periphyton in laboratory streams. American Midland Naturalist 87:8-24.

Kubitschek, H. E. 1970. Introduction to research with continuous cultures. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Lean, D. R. S. 1973. Phosphorus dynamics in lake water. Science 179:678-681.

Lean, D. R. S., and C. Nalewajko. 1976. Phosphate exchange and organic phosphorus excretion by freshwater algae. Journal of the Fisheries Research Board of Canada 33:1312-1323.

Lehninger, A. L. 1975. Biochemistry. Second edition. Worth Publishers. New York.

Lewin, R. A. 1974. Biochemical taxonomy. Pages 1-39 in W. D. P. Stewart, editor. Algal physiology and biochemistry. University of California Press, Berkeley.

Likens, G. E., F. H. Bormann, R. S. Pierce, J. S. Eaton, and N. M. Johnson. 1977. Biogeochemistry of a forested ecosystem. Springer-Verlag, New York.

Lindeman, R. L. 1942. The trophic-dynamic aspect of ecology. Ecology 23:399-418.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry 193:265-275.

Lund, J. W. G., and J. F. Talling. 1957. Botanical limnological methods with special reference to the algae. Botanical Reviews 23:489-583.

Macan, T. T., and A. Kitching. 1976. The colonization of squares of plastic suspended in midwater. Freshwater Biology 6:33-40.

Maciolek, J. A. 1962. Limnological organic analyses by quantitative dichromate oxidation. Bureau of Sport Fisheries and Wildlife. Research Report, No. 60.

Malone, C. R., and D. J. Nelson. 1969. Feeding rates of freshwater snails (Goniobasis clavaeformis) determined with cobalt-60. Ecology 50:728-730.

Mann, K. H. 1975. Patterns of energy flow. Pages 248-263 in B. Whitton, editor. River ecology. University of California Press, Berkeley.

Margalef, R. 1963. On certain unifying principles in ecology. The American Naturalist 97:357-374.

Mason, C. F., and R. J. Bryant. 1975. Periphyton production and grazing by chironomids in Alderfen Broad, Norfolk. Freshwater Biology 5:271-277.

McKee, J. E., and H. W. Wolfe. 1963. Water quality criteria. Second edition. California State Water Quality Control Board, Sacramento, California.

McMahon, R. F., R. D. Hunter, and W. D. Russell-Hunter. 1974. Variation in Aufwuchs at six freshwater habitats in terms of carbon biomass and carbon:nitrogen ratio. Hydrobiologia 45:391-404.

Minshall, G. W. 1978. Autotrphy in stream ecosystems. BioScience 28:767-771.

Monakov, A. V. 1972. Review of studies on feeding of aquatic invertebrates conducted at the Institute of Biology of Inland Waters, Academy of Sciences, USSR. Journal of the Fisheries Research Board of Canada 29:363-383.

Monheimer, R. M. 1972. Heterotrophy by plankton in three lakes of different productivity. Nature 236:463-464.

Monheimer, R. H. 1974. Sulfate uptake as a measure of planktonic microbial production in freshwater ecosystems. Canadian Journal of Microbiology 20:825-831.

Monheimer, R. H. 1975a. Sulfate uptake by microplankton communities in western Lake St. Clair. Limnology and Oceanography 20:183-190.

Monheimer, R. H. 1975b. Effects of three environmental variables on sulfur uptake by aerobic bacteria. Applied Microbiology 30:975-981.

Monheimer, R. H. 1978. Difficulties in interpretation of microbial hetertrophy from sulfate uptake data: laboratory studies. Limnology and Oceanography 23: 150-154.

Monk, C. D., D. A. Crossley, Jr., R. L. Todd, W. T. Swank, J. B. Waide, and J. R. Webster. 1977. An overview of nutrient cycling research at Coweeta hydrologic laboratory. Pages 35-49 in D. L. Correll, editor. Watershed research in eastern North America. Volume 1. Chesapeake Bay Center for Environmental Studies, Edgewater, Maryland.

Moore, J. W. 1977a. Some aspects of the feeding biology of benthic herbivores. Hydrobiologia 53:139-146.

Moore, J. W. 1977b. Some factors effecting algal consumption in subarctic Ephemeroptera, Plecoptera and Simuliidae. Oecologia 27:261-273.

Moore, J. W. 1977c. The importance of algae in the diet of subarctic populations of Gammarus lacastris and Pontoporeia affinis. Canadian Journal of Zoology 55: 637-641.

Moriarty, D. J. W. 1975. A method for estimating the biomass of bacteria in aquatic sediments and its application to trophic studies. Oecologia 20:219-229.

Myklestad, S., and A. Haug. 1972. Production of carbohydrates by the marine diatom Chaetoceros affinis var. Willei (Gran) Hustedt. I. Effect of the concentration of nutrients in the culture medium. Journal of Experimental Marine Biology and Ecology 9:125-136.

Nival, P., and S. Nival. 1976. Particle retention efficiencies of an herbivorous copepod, Acartia clausi

(adult and copepodite stages): effects on grazing. Limnology and Oceanography 21:24-38.

Odum, E. P. 1962. Relationships between structure and function in ecosystems. Japanese Journal of Ecology 12:108-118.

Odum, E. P. 1971. Fundamentals of ecology. Third edition. W. B. Saunders Co., Philadelphia.

Odum, E. P. 1977. The emergence of ecology as a new integrative discipline. Science 195:1289-1293.

Odum, E. P., and F. B. Golley. 1963. Radioactive tracers as an aid to the measurement of energy flow at the population level in nature. Pages 403-410 in V. Schultz and A. W. Klement, Jr. editors. Radioecology. Proceedings of the First National Symposium on Radioecology.

Odum, E. P., J. T. Finn, and E. H. Franz. 1979. Perturbation theory and the subsidy-stress gradient. BioScience 29:349-352.

Odum, H. T. 1957. Trophic structure and productivity of Silver Springs, Florida. Ecological Monographs 27:55-112.

Paine, R. T., and R. L. Vadas. 1969. Calorific values of benthic marine algae and their postulated relation to invertebrate food preference. Marine Biology 4:79-86.

Parsons, T. R., K. Stephens, and J. D. H. Strickland. 1961. On the chemical composition of eleven species of marine phytoplankters. Journal of the Fisheries Research Board of Canada 18:1001-1016.

Patrick, R. 1970. Benthic stream communities. American Scientist 58:546-549.

Patrick, R. 1973a. Diatoms as bioassay organisms. Pages 139-152 in G. E. Glass, editor. Bioassay techniques and environmental chemistry. Ann Arbor Science, Ann Arbor, Michigan.

Patrick, R. 1973b. Use of algae, especially diatoms, in the assessment of water quality. Pages 76-95 in J. Cairns, Jr., and K. L. Dickson, editors. Biological methods for the assessment of water quality. ASTM STP 528, American Society for Testing and Materials, Philadelphia.

Patrick, R. 1978. Effects of trace metals in the aquatic ecosystem. American Scientist 66:185-191.

Pennak, R. W. 1978. Fresh-water invertebrates of the United States. John Wiley and Sons, New York.

Phillipson, J. 1964. A miniature bomb calorimeter for small biological samples. Oikos 15:130-139.

Pielou, E. C. 1975. Ecological diversity. John Wiley and Sons, New York.

Pip, E., and J. M. Stewart. 1976. The dynamics of two aquatic plant-snail associations. Canadian Journal of Zoology 54:1192-1205.

Porter, K. G. 1973. Selective grazing and differential digestion of algae by zooplankton. Nature 244:179-180.

Porter, K. G. 1977. The plant-animal interface in fresh-water ecosystems. American Scientist 65:159-170.

Prosser, C. L. 1973. Comparative animal physiology. W. B. Saunders Co., Philadelphia.

Ricker, W. E., editor. 1968. Methods for assessment of fish production in fresh waters. I.B.P. Handbook, No. 3, Blackwell Scientific, Oxford.

Rheinheimer, G. 1974. Aquatic microbiology. John Wiley and Sons, New York.

Rodgers, J. H., Jr. 1977. Aufwuchs communities of lotic systems - nontaxonomic structure and function. Doctorate dissertation. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Rodgers, J. H., Jr., K. L. Dickson, and J. Cairns, Jr. 1979. A review and analysis of some methods used to measure functional aspects of periphyton. Pages 142-167 in R. Weitzel, editor. Methods and measurements of periphyton communities: a review. ASTM STP 690, American Society for Testing and Materials, Philadelphia.

Russell-Hunter, W. D. 1970. Aquatic productivity. McMillan Co., London.

Ruttner, F. 1953. Fundamentals of limnology. University of Toronto Press, Toronto.

Schiff, J. A., and R. C. Hodson. 1973. The metabolism of sulfate. Annual Review of Plant Physiology 24:381-414.

Sedell, J. R. 1972. Feeding rates and food utilization of stream caddisfly larvae of the genus Neophylax (Trichoptera:Limnephilidae) using cobalt-60 and carbon-14. Radioecology. Proceedings of the Third National Symposium on Radioecology.

Sedmak, J. J., and S. E. Grossberg. 1977. A rapid, sensitive, and versatile assay for protein using Coomassie brilliant blue-G250. Analytical Biochemistry 79:544-552.

Shapas, T. J., and W. L. Hilsenhoff. 1976. Feeding habits of Wisconsin's predominant lotic Plecoptera, Ephemeroptera, and Trichoptera. Great Lakes Entomologist 9: 175-188.

Sokal, R. R., and F. J. Rohlf. 1969. Biometry. W. H. Freeman and Co., San Francisco, California.

Sorokin, J. J. 1968. The use of carbon-14 in the study of nutrition of aquatic animals. Internationale Vereinigung fur Theoretische und Angewandte Limnologie. Mitteilung No. 16.

Stauffer, J. R., Jr., K. L. Dickson, J. Cairns, Jr., and D. S. Cherry. 1976. The potential and realized influences of temperature on the distribution of fishes in the New River, Glen Lyn, Virginia. Wildlife Monographs No. 50, Supplement to the Journal of Wildlife Management.

Steel, R. G., and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw Hill, Inc., New York.

Steeman-Nielson, E., and H. Brunn Laursen. 1976. Effect of $CuSO_4$ on the photosynthetic rate of phytoplankton in four Danish lakes. Oikos 27:239-242.

Stewart, W. D. P., editor. 1974. Algal physiology and biochemistry. Botanical Monograph. Volume 10. University of California Press, Berkeley.

Stockner, J. G., and F. A. J. Armstrong. 1971. Periphyton of the experimental lakes area, northwestern Ontario. Journal of the Fisheries Research Board of Canada 28:215-229.

Strickland, J. D. H., and T. R. Parsons. 1972. A practical handbook of seawater analysis. Second edition. The Fisheries Research Board of Canada, Bulletin No. 167.

Swank. W. T., and J. E. Douglass. 1977. Nutrient budgets for undistrubed and manipulated hardwood forest ecosystems in the mountains of North Carolina. Pages 343-364 in D. L. Correll, editor. Watershed research in eastern North America. Volume 1. Chesapeake Bay Center for Environmental Studies, Edgewater, Maryland.

Swiss, J. J., and M. G. Johnson. 1976. Energy dynamics of two benthic crustaceans in relation to diet. Journal of the Fisheries Research Board of Canada 33:2544-2550.

Taghon, G. L., R. F. Self, and P. A. Jumars. 1978. Predicting particle selection by deposit feeders: a model and its implications. Limnology and Oceanography 23: 752-759.

Teal, J. M. 1957. Community metabolism in a temperate cold spring. Ecological Monographs 27:283-302.

Teal, J. M. 1962. Energy flow in the salt marsh ecosystem of Georgia. Ecology 43:614-624.

Trama, F. 1957. The transformation of energy by an aquatic herbivore, Stenonema pulchellum. Doctorate dissertation. University of Michigan, Ann Arbor, Michigan.

Vivekanandan, E., M. A. Haniffa, T. J. Pandian and R. Raghuramam. 1974. Studies on energy transformation in the freshwater snail Phila globosa. 1. Influence on feeding rate. Freshwater Biology 4:275-280.

Vollenweider, R. A., editor. 1974. A manual on methods for measuring primary production in aquatic environments. Second edition. I.B.P. Handbook, No. 12, Blackwell, Oxford.

Wallace, J. B., J. R. Webster, and W. R. Woodall. 1977. The role of filter feeders in flowing waters. Archives fur Hydrobiologie 79:506-532.

Wang, C. H., D. L. Willis, and W. D. Loveland. 1975. Radiotracer methodology in the biological, environmental, and physical sciences. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Warren, C. E., and G. E. Davis. 1971. Laboratory stream research: objectives, possibilities, and constraints. Annual Review of Ecology Systematics 2:111-144.

Weber, C. I. 1973a. Biological field and laboratory methods for measuring the quality of surface waters and effluents. USEPA Cincinnati, Ohio.

Weber, C. I. 1973b. Recent developments in the measurement of the response of phytoplankton and periphyton to changes in their environment. Pages 119-138 in G. E. Glass, editor. Bioassay techniques and environmental chemistry. Ann Arbor Science, Ann Arbor, Michigan.

Weitzel, R. L., editor. 1979a. Methods and measurements of periphyton communities: a review. ASTM STP 690, American Society for Testing and Materials, Philadelphia.

Weitzel, R. L. 1979b. Periphyton measurements and applications. Pages 3-33 in R. Weitzel, editor. Methods and measurements of periphyton communities: a review. ASTM STP 690, American Society for Testing and Materials, Philadelphia.

Welch, H. E. 1968. Relationships between assimilation efficiencies and growth efficiencies for aquatic consumers. Ecology 49:755-759.

Wetzel, R. G. 1975. Limnology. W. B. Saunders Co., Philadelphia.

Wilhm, J. L. 1970. Transfer of radioisotopes between detritus and benthic macroinvertebrates in laboratory microecosystems. Health Physics 18:227-284.

Winterbourn, M. J. 1971. The life histories and trophic relationships of the Tricoptera of Marion Lake, British Columbia. Canadian Journal of Zoology 49:623-625.

Winterbourn, M. J. 1974. The life histories, trophic releations and production of Stenoperla prasina (Plecoptera) and Deleatidium sp. (Ephemeroptera) in a New Zealand River. Freshwater Biology 4:507-524.

Wolk, C. P. 1973. Physiology and cytological chemistry of blue-green algae. Bacteriological Reviews 37:32-101.

APPENDIX

Table A1. Spearman rank correlation coefficients, r, for snail body dry weights and Aufwuchs organic matter consumed with the significance level, P.

Experiment #	N	Correlation Coefficient r	Significance Level p
1	19	0.618	0.005
2	18	0.042	0.868
3	24	0.574	0.004
4	20	-0.358	0.140
5	20	0.226	0.337
6	24	0.079	0.713
7	24	0.284	0.178
all data	149	0.228	0.005

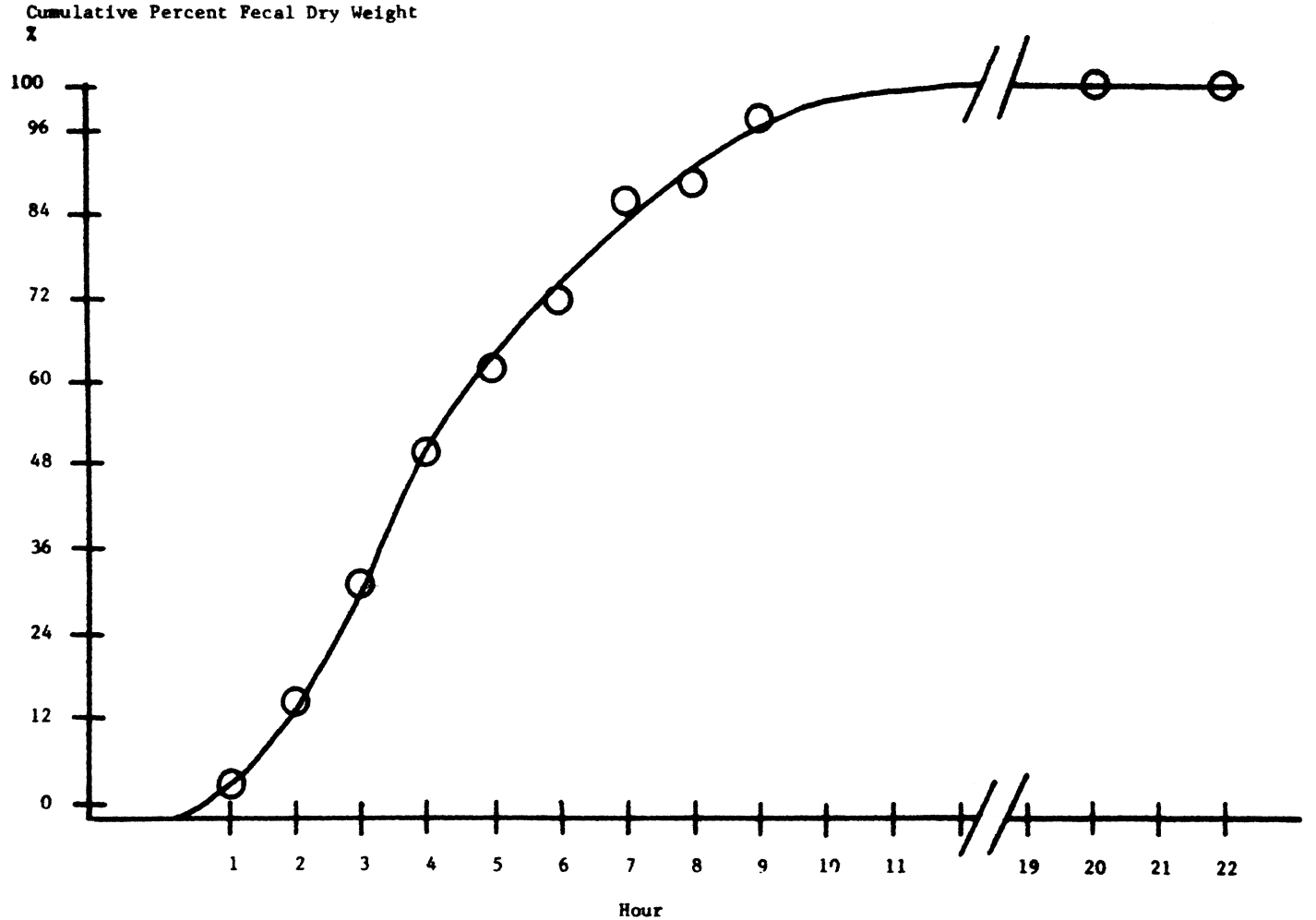


Figure A1. Accumulation of snail fecal material during a feeding experiment. Feeding occurred during hours 1 through 4, snails starved thereafter.

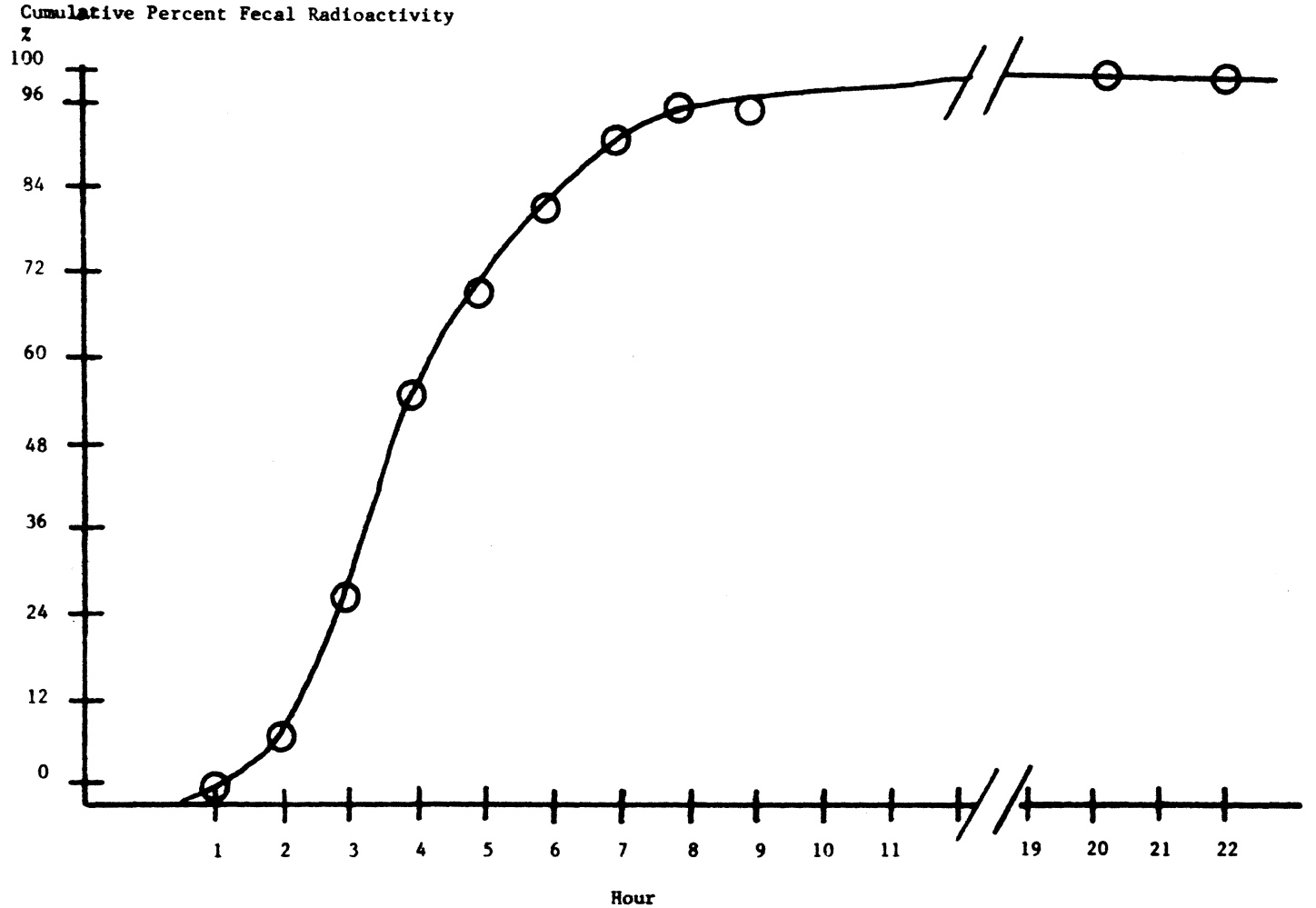


Figure A2. Accumulation of sulfur-35 in fecal samples of snails fed sulfur-35 labeled Aufwuchs. Feeding occurred during hours 1 through 4, snails starved thereafter.

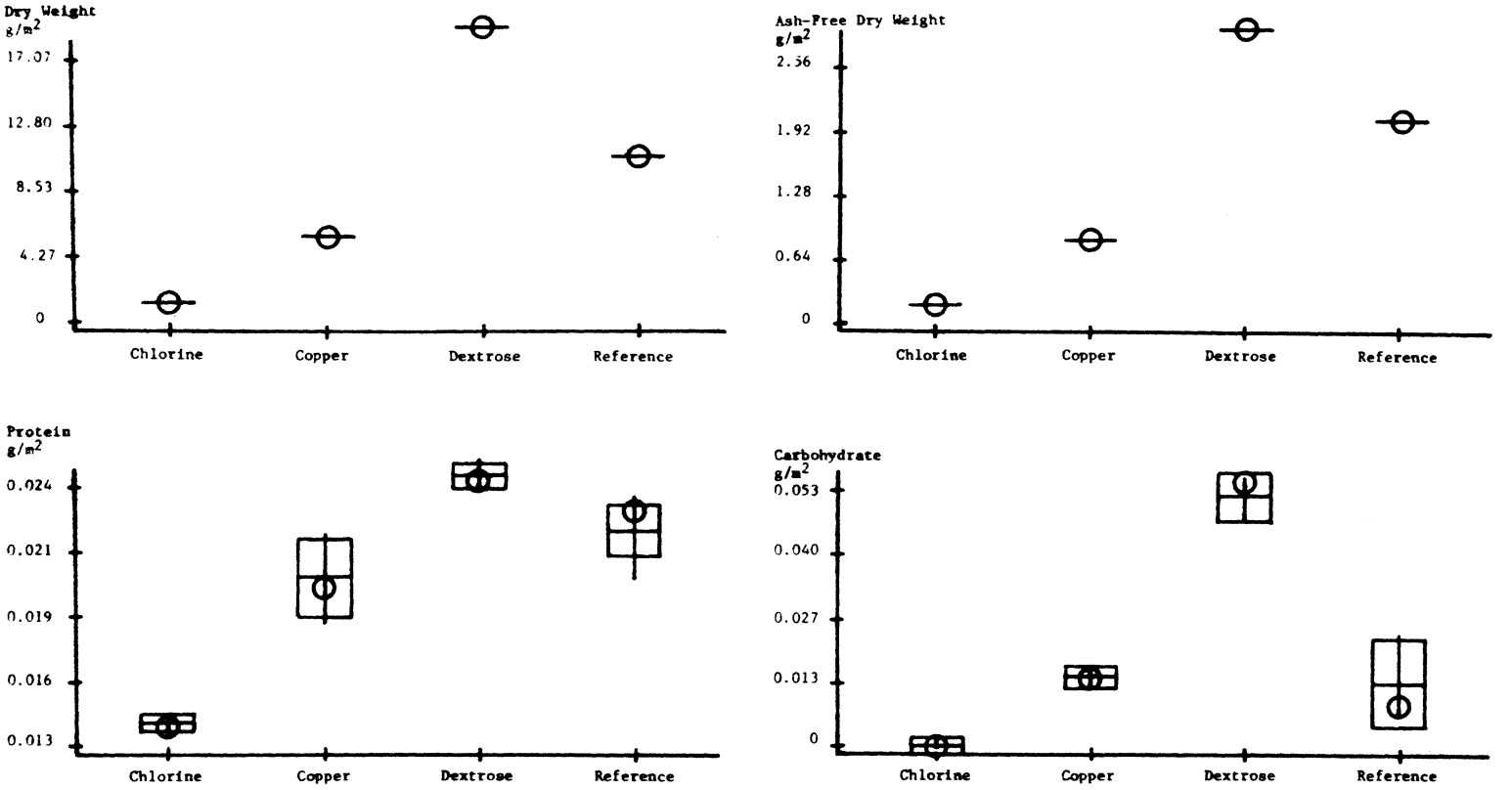


Figure A3. Food quality parameters measured from Aufwuchs for experiment 1 with means (-), medians (O), ranges (|), and standard deviations (±□). Each group was composed of four replicates except dry weight and ash-free dry weight which were from a single composite for each stream. There were no caloric samples for this experiment.

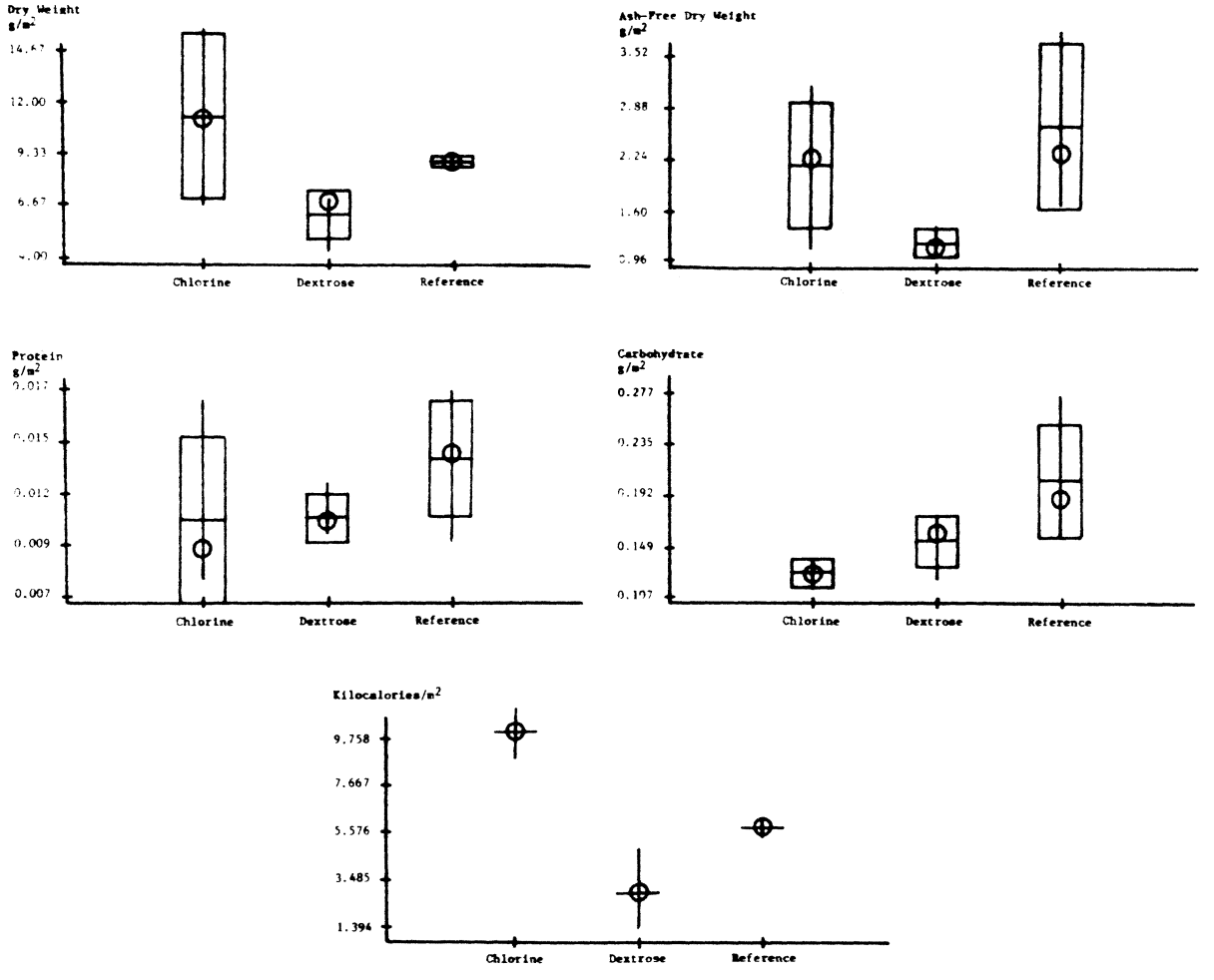


Figure A4. Food quality parameters measured from Aufwuchs for experiment 2 with means (-), medians (O), ranges (|), and standard deviations (±□). Each group was composed of four replicates except caloric samples where n=2. There was no copper treatment during this experiment.

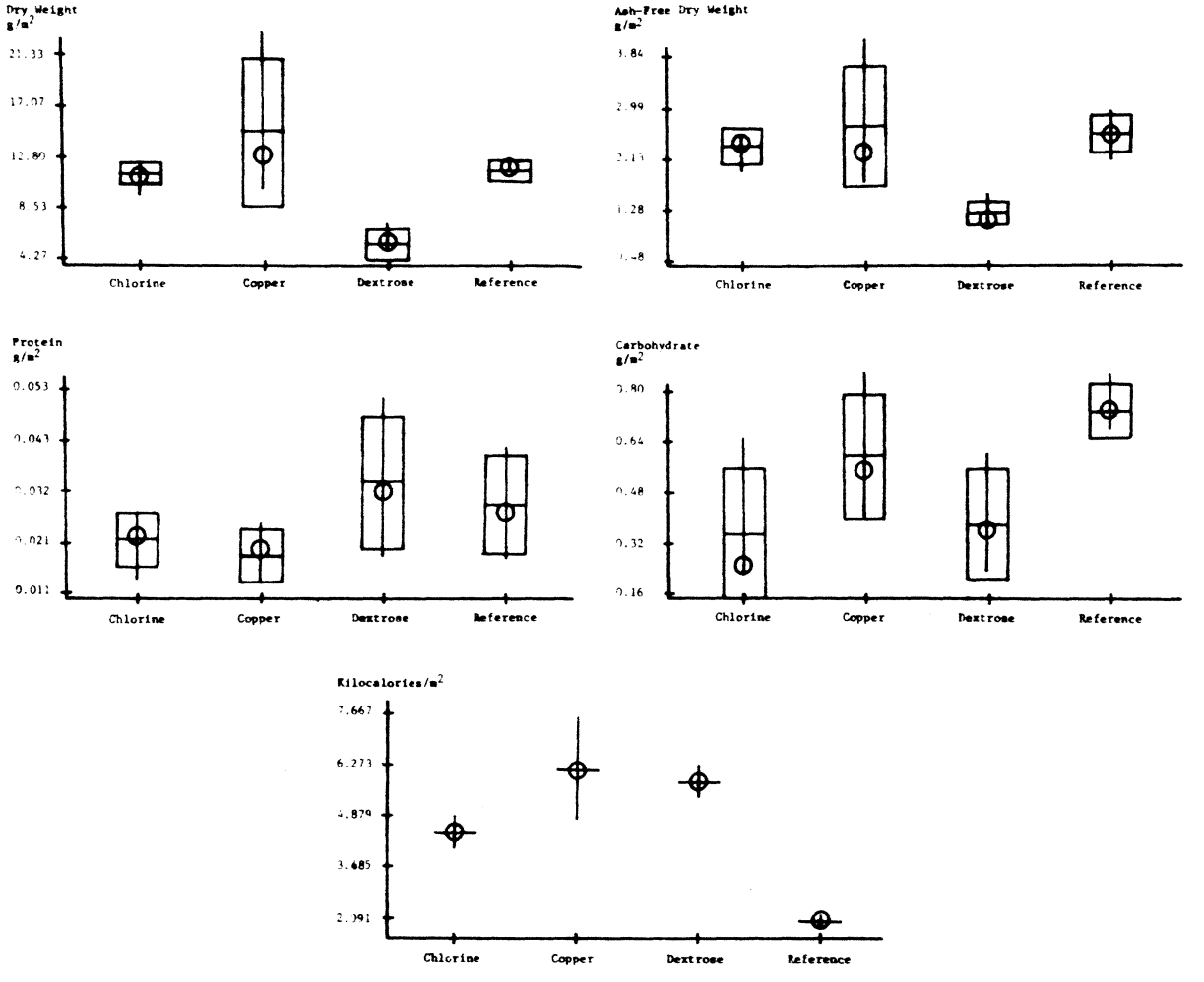


Figure A5. Food quality parameters measured from Aufwuchs for experiment 3 with means (-), medians (O), ranges (|), and standard deviations (±□). Each group was composed of four replicates except caloric samples where n=2.

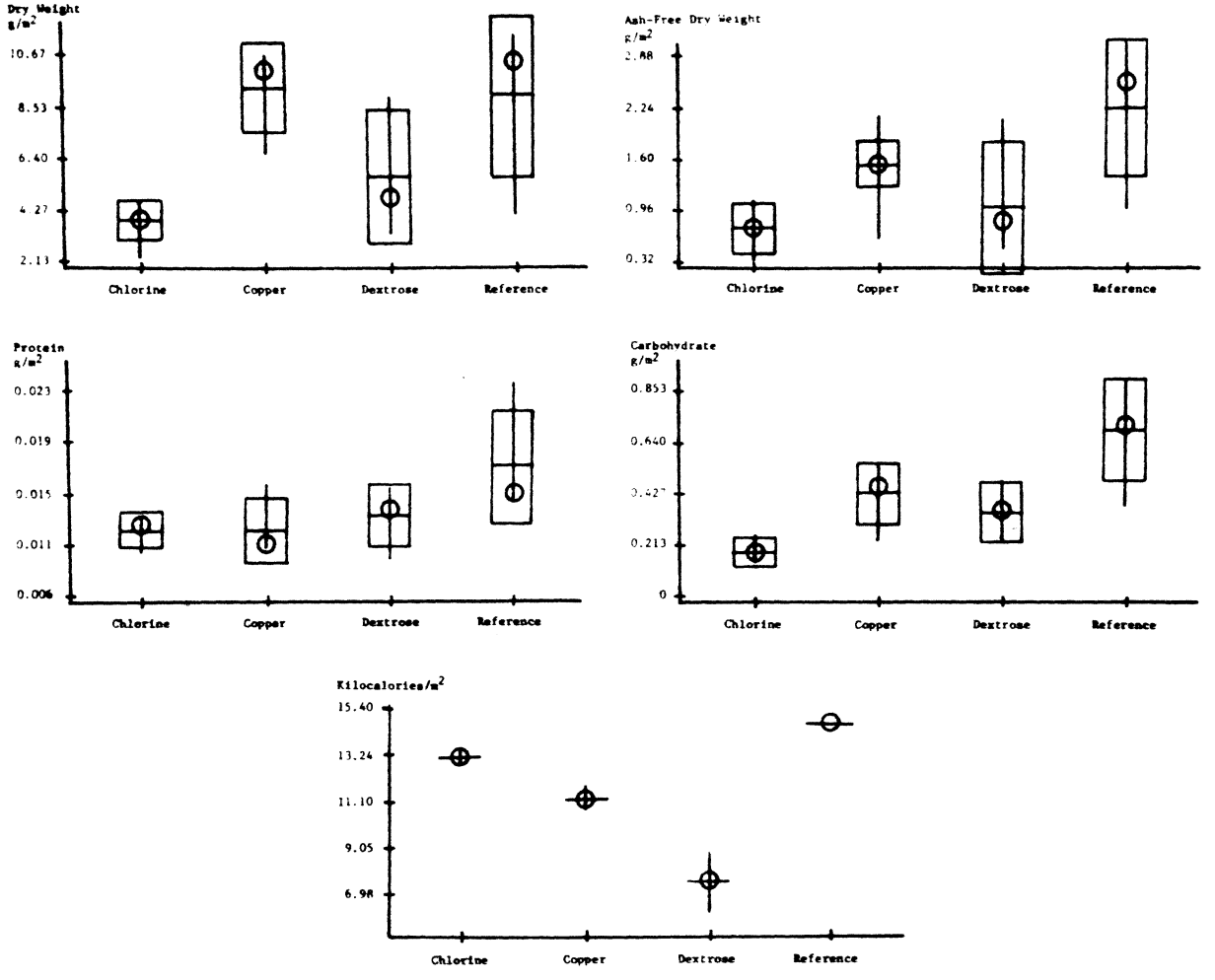


Figure A6. Food quality parameters measured from Aufwuchs for experiment 4 with means (-), medians (O), ranges (|), and standard deviations ($\pm\square$). Each group was composed of four replicates except caloric samples where n=2.

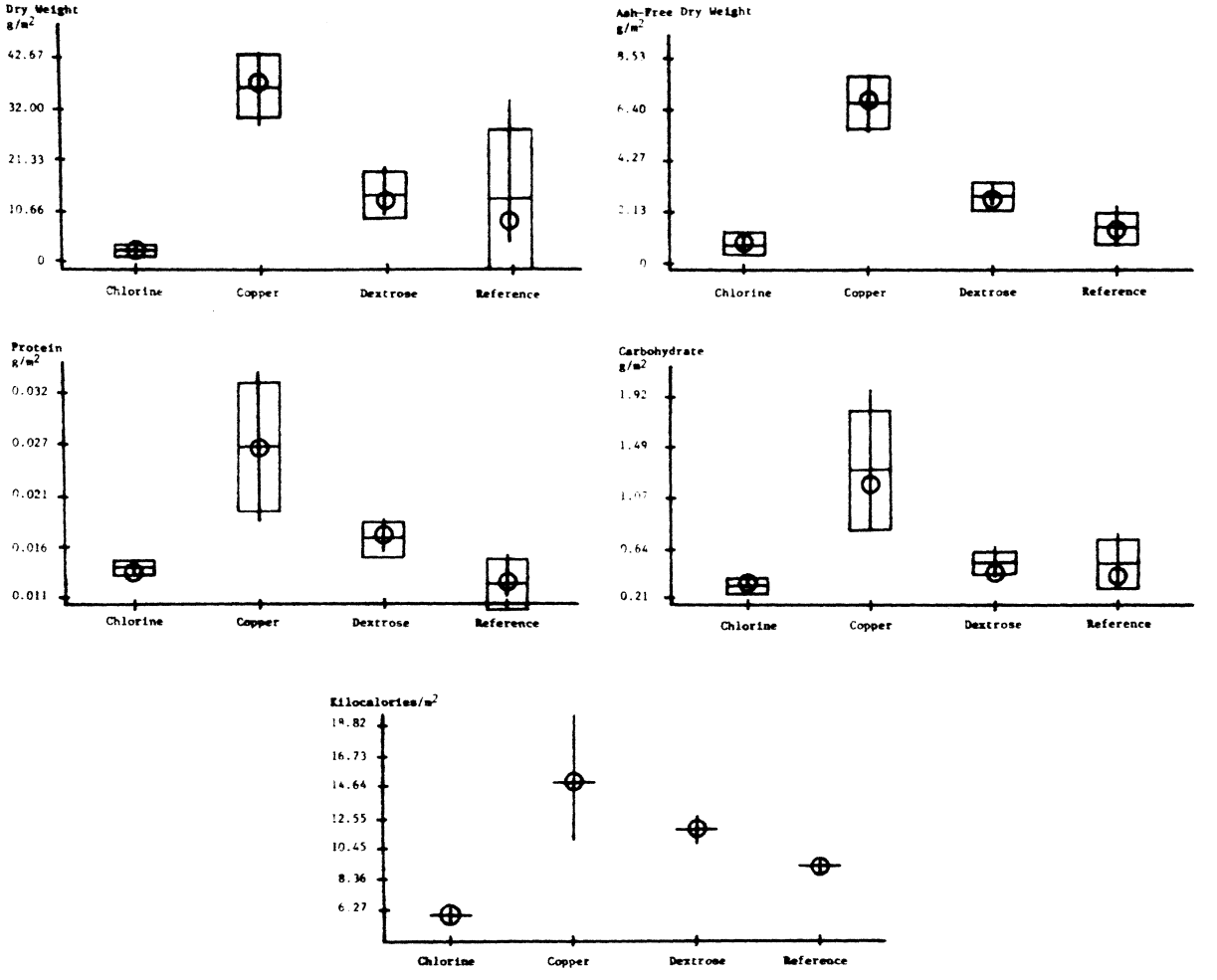


Figure A7. Food quality parameters measured from Aufwuchs for experiment 5 with means (-), medians (O), ranges (|), and standard deviations (±□). Each group was composed of four replicates except caloric samples where n=2.

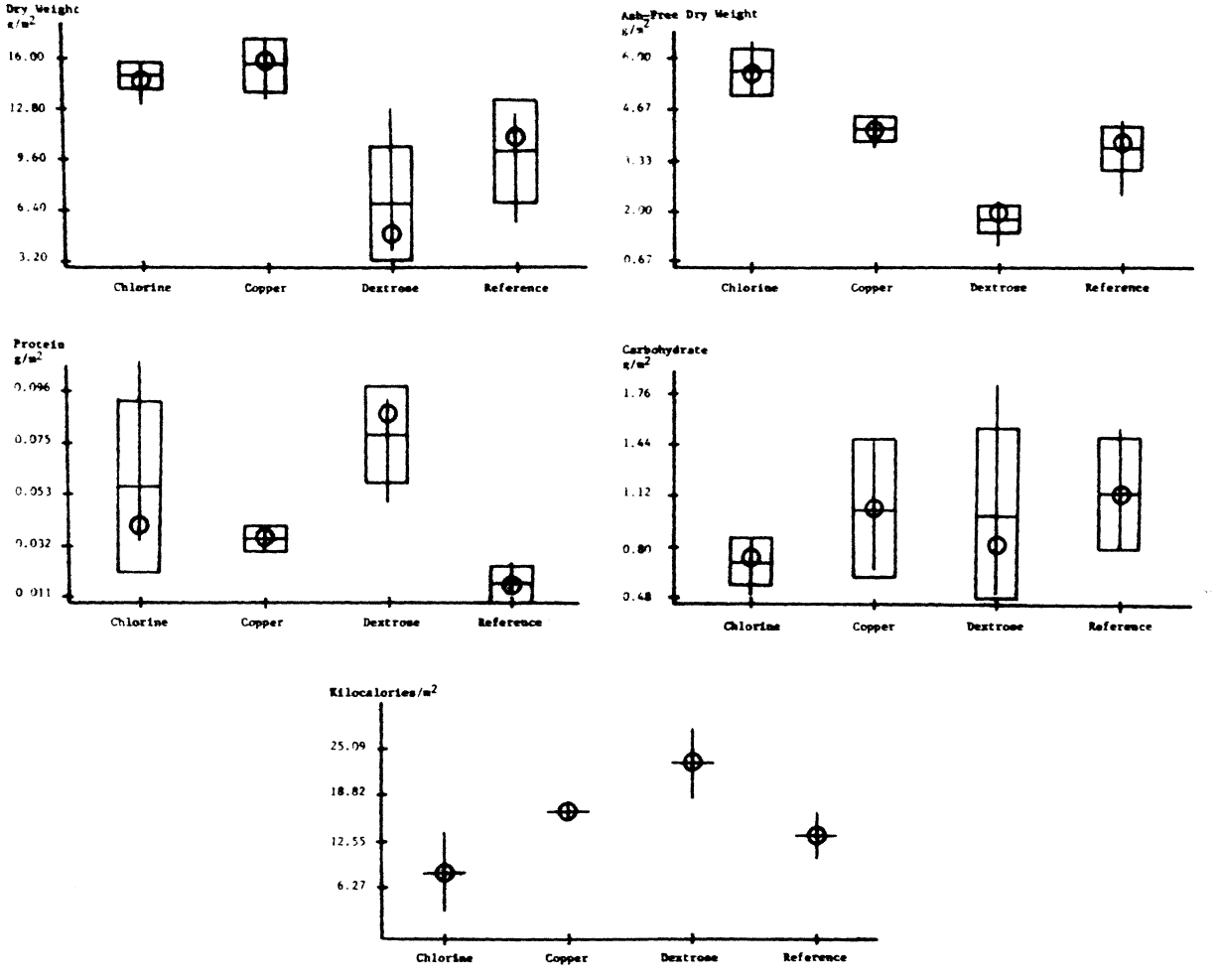


Figure A8. Food quality parameters measured from Aufwuchs for experiment 6 with means (-), medians (O), ranges (|), and standard deviations (±□). Each group was composed of four replicates except caloric samples where n=2.

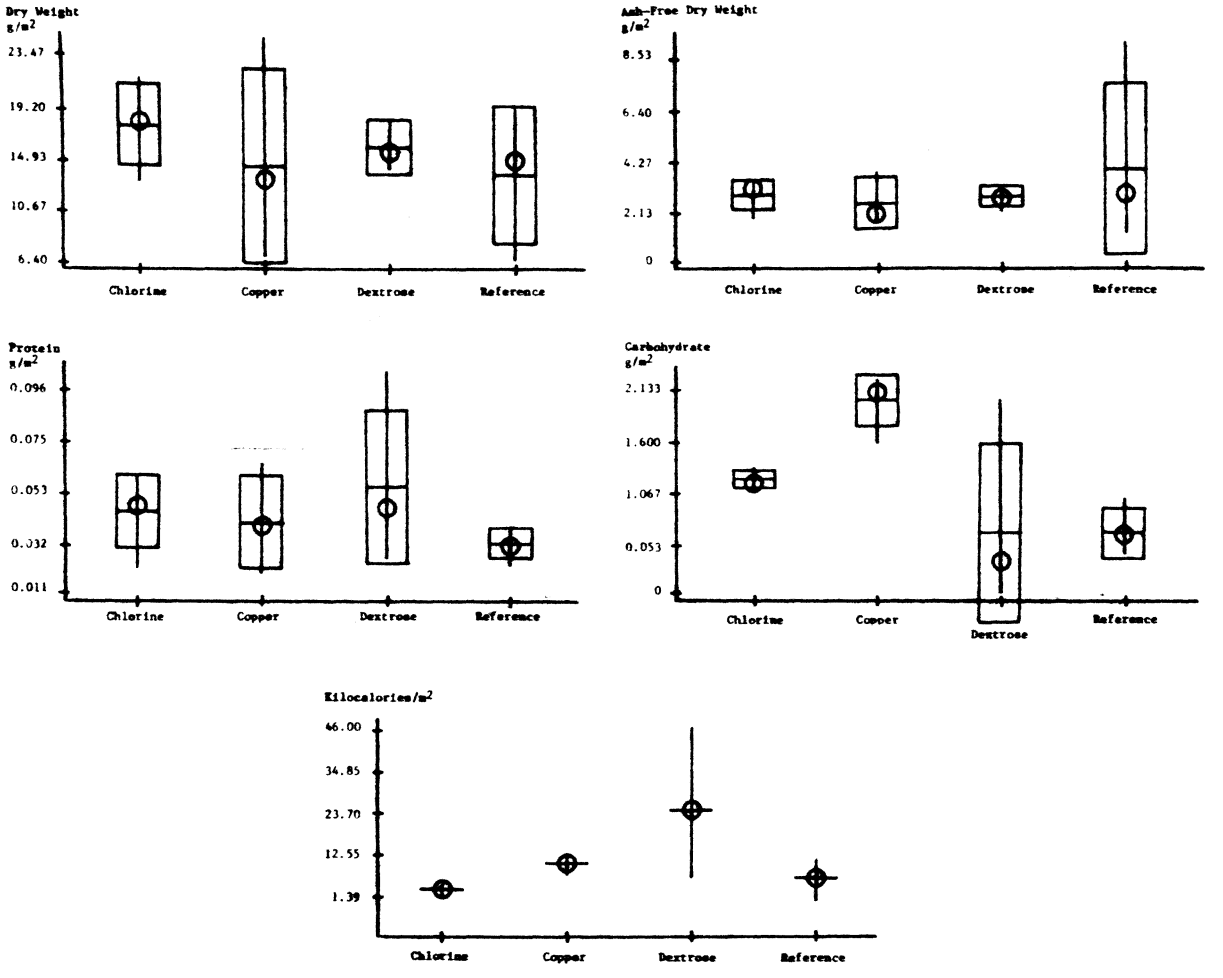
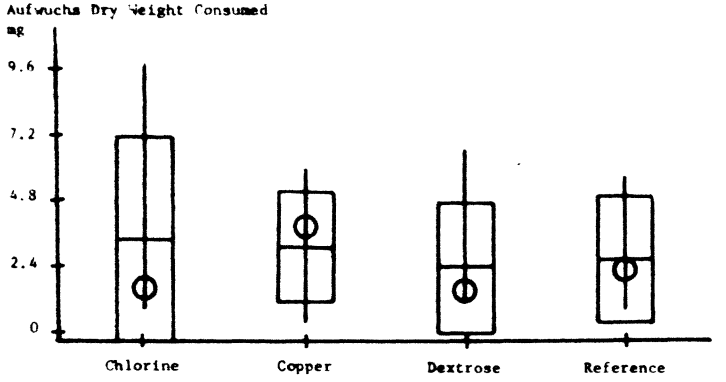
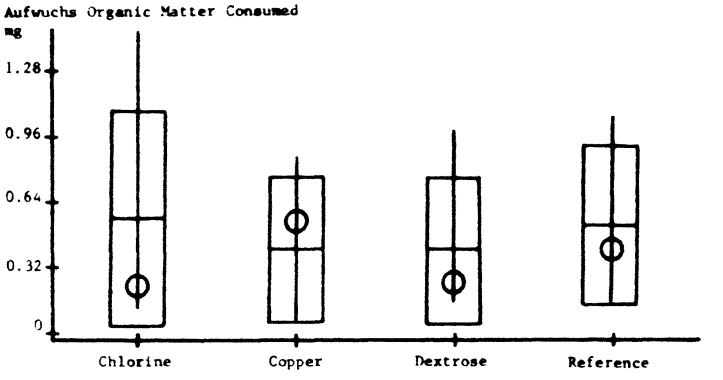
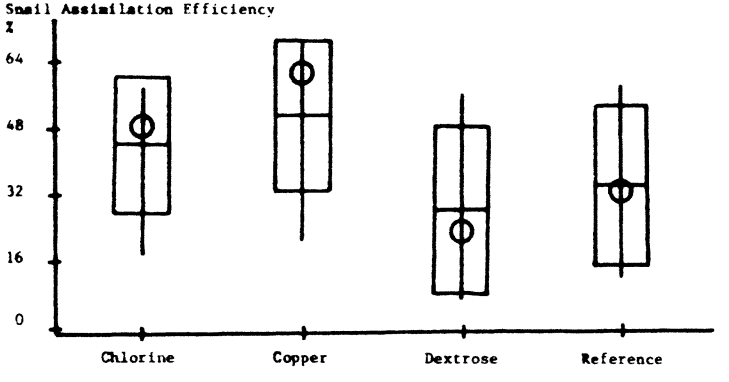


Figure A9. Food quality parameters measured from Aufwuchs for experiment 7 with means (-), medians (O), ranges (|), and standard deviations (±□). Each group was composed of four replicates except caloric samples where n=2.







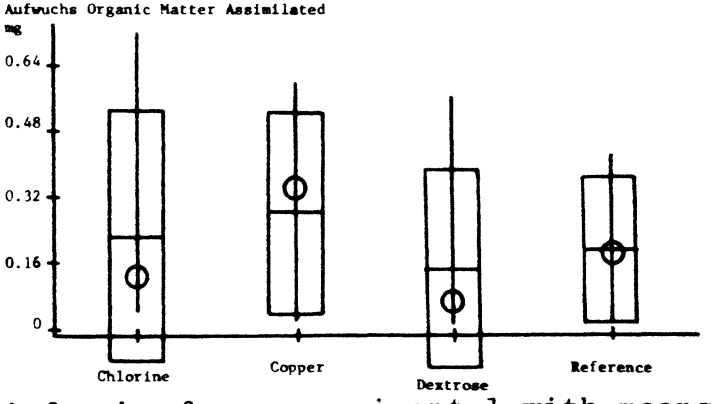


Figure A10. Snail feeding parameter for Aufwuchs from experiment 1 with means (-), medians (O), ranges (|), and standard deviations (±□). Each group was composed of five replicates.

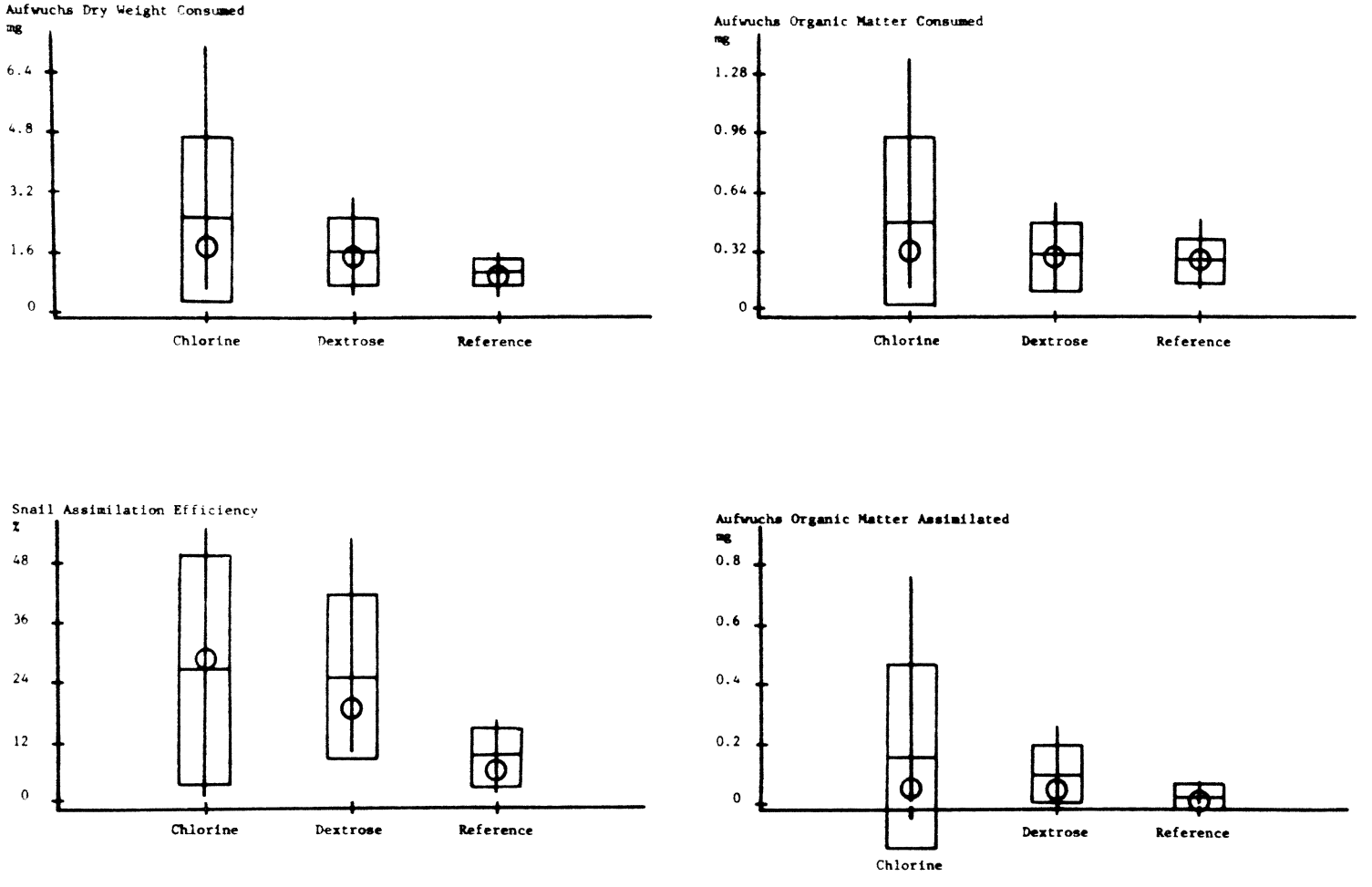


Figure A11. Snail feeding parameters for Aufwuchs from experiment 2 with means (-), medians (O), ranges (|), standard deviations (±□). Each group was composed of six replicates.

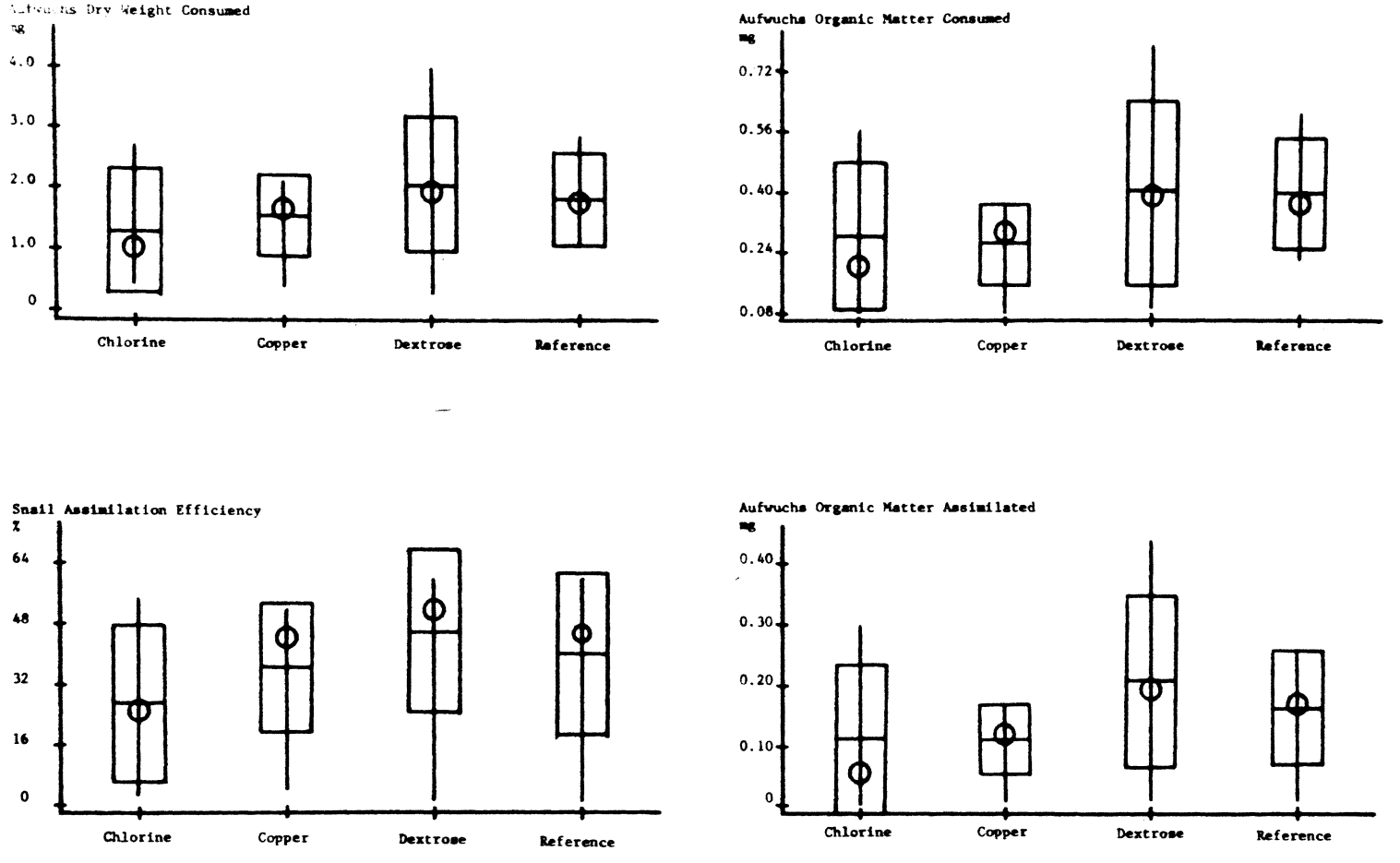


Figure A12. Snail feeding parameters for Aufwuchs from experiment 3 with means (-), medians (O), ranges (|), and standard deviations (±□). Each group was composed of six replicates.

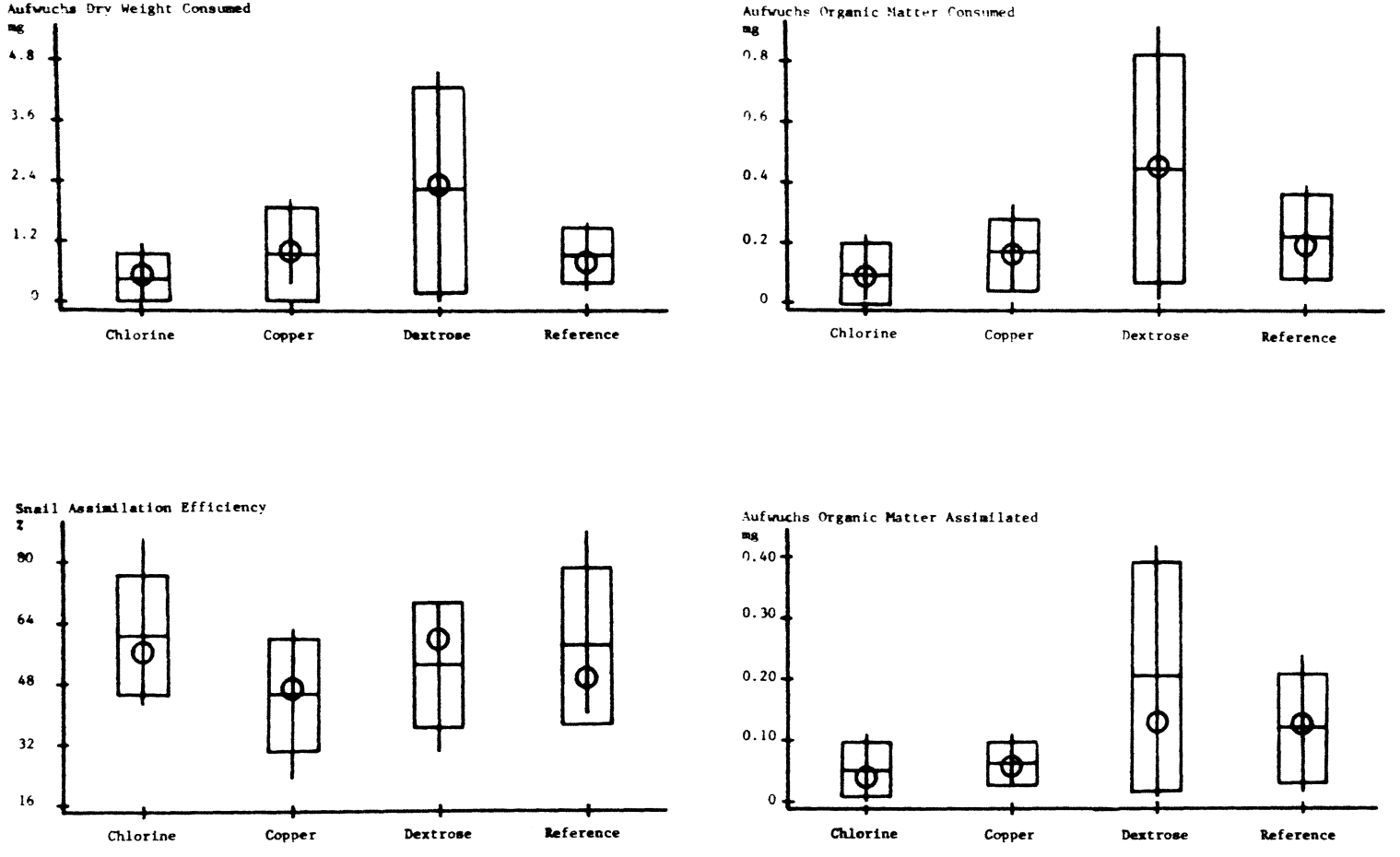


Figure A13. Snail feeding parameters for Aufwuchs from experiment 4 with means (-), medians (O), ranges (|), and standard deviations (±□). Each group was composed of five replicates.

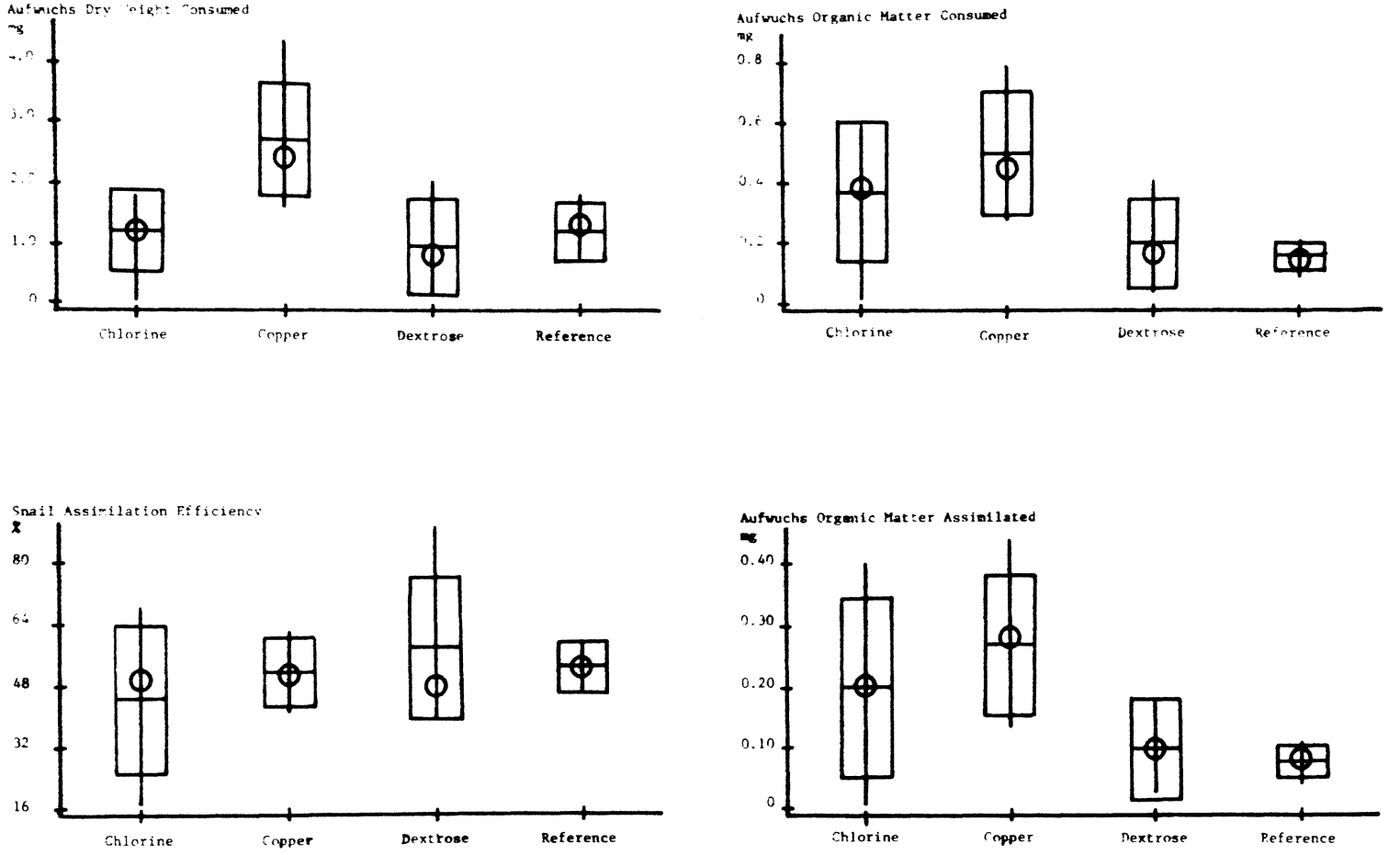


Figure A14. Snail feeding parameters for Aufwuchs from experiment 5 with means (-), medians (O), ranges (|), and standard deviations (±□). Each group was composed of five replicates.

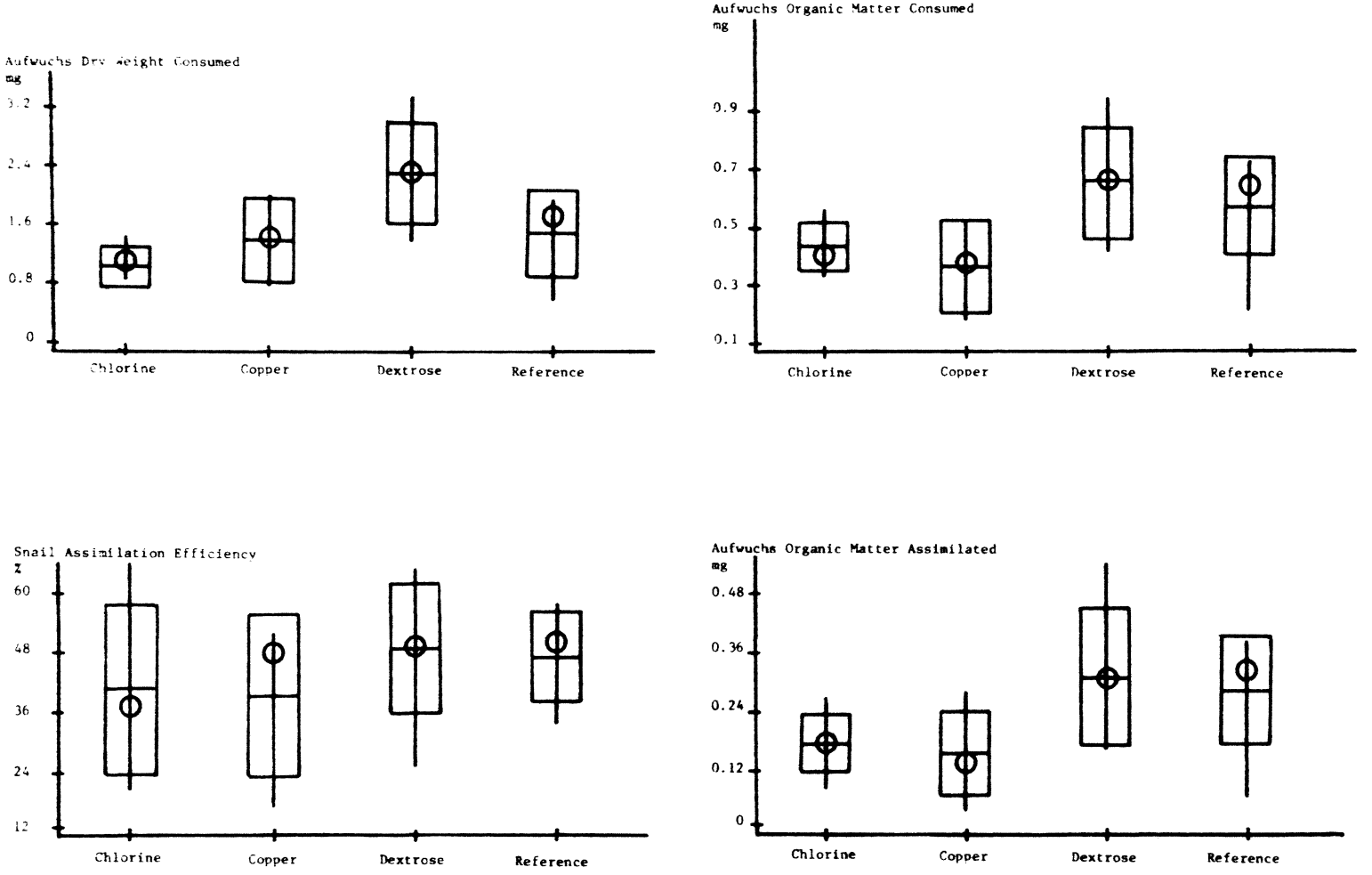


Figure A15. Snail feeding parameters for Aufwuchs from experiment 6 with means (-), medians (O), ranges (|), and standard deviations (±□). Each group was composed of six replicates.

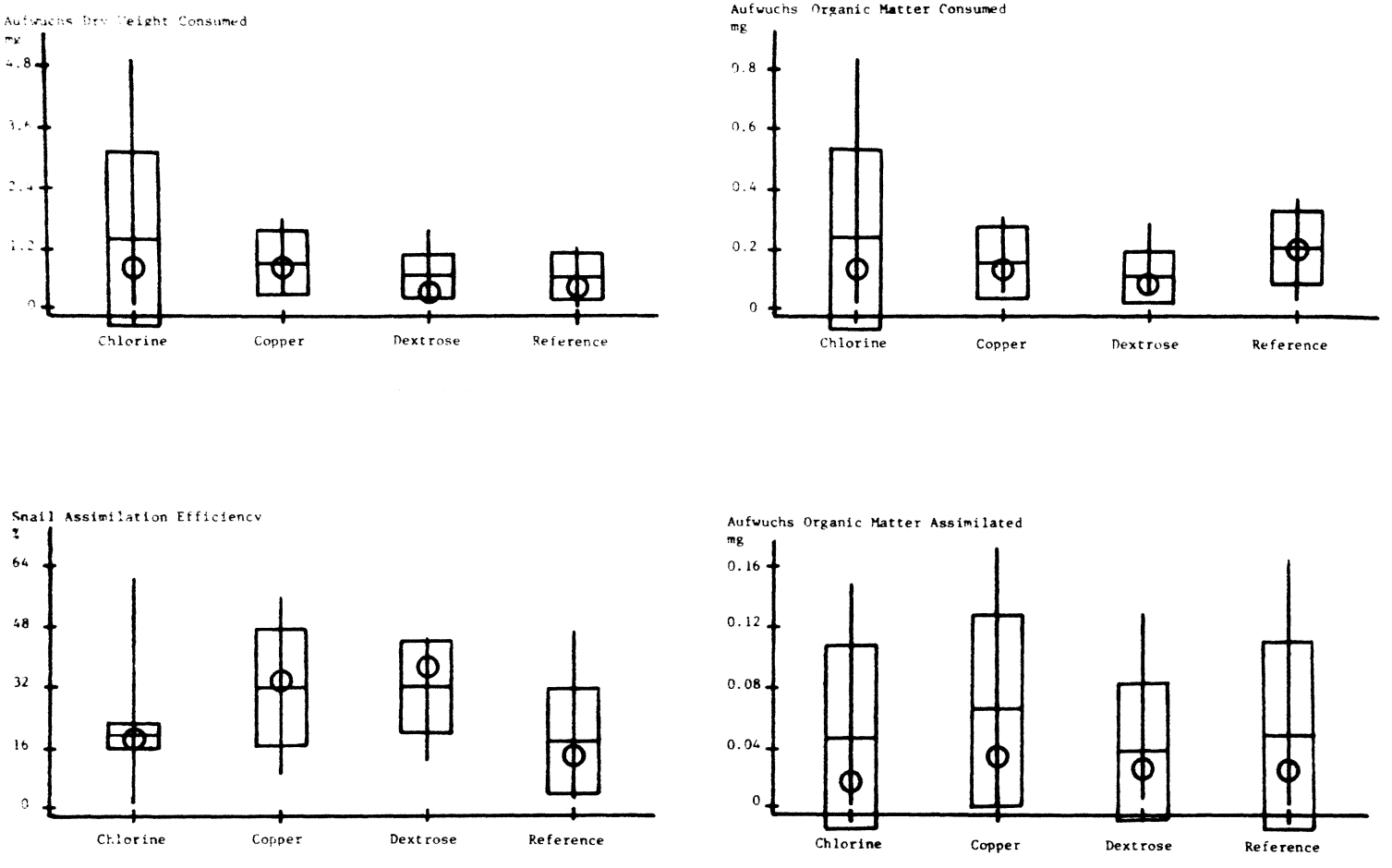


Figure A16. Snail feeding parameters for Aufwuchs from experiment 7 with means (-), medians (O), ranges (|), and standard deviations (±□). Each group was composed of six replicates.

The 4 page vita has been removed from the scanned document

The _4_ page vita has been removed from the scanned document

The _4_ page vita has been removed from the scanned document

The _4_ page vita has been removed from the scanned document

Effects of Selected Pollutants on Grazer Utilization of Aufwuchs

by

James R. Clark

(ABSTRACT)

The trophic level impact of structural changes in Aufwuchs communities resulting from low levels of stress can be assessed through analyses of the nutritive value of the microfloral community in conjunction with measurements of grazer consumption rates and assimilation efficiencies. Artificial streams dosed with either intermittent chlorination (20 minute doses 3 times per day) or continuous treatments of copper (0.05 ppm) or dextrose (1 or 2 ppm) were used to obtain Aufwuchs communities from stressed environments. Aufwuchs communities were also sampled from the New River within and around a chlorinated-thermal, power plant discharge. Food quality analyses included quantification of organic carbon, protein, carbohydrate, and caloric content through gravimetric, Coomassie blue staining, anthrone staining, and wet chemical oxidation procedures, respectively. Snail (Pleuroceridae:Anculosa) utilization of Aufwuchs from the variously perturbed environments was assessed through laboratory feeding studies employing radiolabeled (sulfur-35) Aufwuchs to determine snail feeding rates and assimilation efficiencies.

The heterotrophically dominated Aufwuchs communities

developing under the dextrose enrichment contained more protein, carbohydrate, and calories compared to reference communities. These increases were attributed to the copious extracellular mucilage associated with the bacteria. There was no significant change in snail consumption of Aufwuchs from the dextrose enriched streams, although this community was consistently assimilated with greater efficiency relative to reference communities. When Aufwuchs developing under the chlorine or copper treatments became dominated by blue-green algae, the protein content of these communities increased as a result of the algal proteinatious sheath. Carbohydrate content was generally less than reference values for Aufwuchs developing under chlorine or copper stress. Aufwuchs from these treated streams were consumed to a less extent than reference communities and assimilated with 2 to 12% less efficiency. These results agree with literature reports that blue-green algae are a less preferred food for aquatic grazers and are assimilated with less efficiency. Aufwuchs sampled from habitats influenced by the power plant discharge were of less nutritive value compared to samples from uninfluenced stations. Snails consumed more of the reference communities and assimilated them with greater efficiency compared to Aufwuchs influenced by the power plant discharge.

Both the autotrophic and heterotrophic components of

the Aufwuchs community were labeled with sulfur-35, allowing sensitive measurements of the feeding parameters. Snail feeding studies demonstrated that low levels of stress may not alter grazer consumption of Aufwuchs until substantial changes in algal composition occur. Snail assimilation efficiency was generally a sensitive parameter in assessing trophic level effects of changes in Aufwuchs communities resulting from pollutant impacts. Food quality analyses provided useful information regarding changes in Aufwuchs community structure. A lack of correlation among Aufwuchs food quality parameters and snail utilization measurements reflected the opportunistic feeding strategy of the grazer.