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The Bioenergetics of Three Aquatic Insects Determined by Radioisotopic Analyses

by Dale Alan McCullough

August 1975

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THE BIOENERGETICS OF THREE AQUATIC INSECTS DETERMINED BY RADIOISOTOPIC ANALYSES

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ABSTRACT

The bioenergetics of Simulium spp. and Cheumatopsyche analis from Rattlesnake Springs and Snively Creek, respectively, Benton County, Washington and Tricorthodes minutus from Deep Creek, Oneida County, Idaho were studied using a variety of techniques. Ingestion rates were measured using food sources (diatoms, finely ground watercress, bacteria, and blue-green algae) labelled with ^{51}Cr and ^{14}C. Theoretical ingestion rates were calculated from analyses of gut weights and digestion times. Assimilation efficiencies (AE) were determined using the ^{14}C and dual-label (^{51}Cr, ^{14}C) methods and the ash-ratio technique. The dual-label method provided reliable results when leaching of isotopes from food and feces were not significant. Controls for adsorption of ^{51}Cr and ^{14}C and assimilation of ^{51}Cr should also be used. Although Cr is a non-essential element in insect nutrition it is absorbed to a small extent. The dual-label method necessitates greater precautions and expense than the ^{14}C method but is advantageous when measuring AE of animals with rapid ingestion rates which could be adversely affected by handling. Provided the ^{51}Cr activity density of food is sufficient, the time required for digestion can also be more accurately determined with ^{51}Cr than with ^{14}C. The ash-ratio method provided a wide range of AE values and is not as reliable as the dual-label method because mineral assimilation is unpredictable. Assimilation rates were derived for these animals using the ingestion rate and AE, by several methods employing ^{14}C uptake curves, and by differences in ^{51}Cr- and ^{14}C-derived accumulation values. Methods used to measure other energy budget components are also given. A system was developed for combusting biological samples containing ^{14}C and determining cpm ^{14}C and total carbon from a single sample. This method employs Van Slyke wet oxidation, forced circulation and scrubbing of the gases of combustion, and collection of CO_2 in ethanolamine. Radioactivity in this system was determined by scintillation counting and total carbon by a gravimetric precipitation method.

Experiments were conducted on Simulium to determine the ingestion rates and assimilation efficiencies on three food sources (bacteria, diatoms, and finely ground watercress) using dual-label (^{51}Cr, ^{14}C) food. Three feeding chambers were employed and the effects of the physical environment created in these chambers on feeding rates were explored. Feeding chambers were a flow-through glass cylinder with a 6 cm/sec current speed and glass slide substratum; a plastic trough with current speeds ranging from 30 to 50 cm/sec and gravel substratum; and a circular plexiglass stream channel with large stones as substratum material. Ingestion was measured at experimental temperatures ranging from 17 to 24°C which generally corresponded to natural stream temperatures within 5°C. Grazing was shown not to be a means of feeding for Simulium. Ingestion rates on diatoms by filtration in the glass cylinder were approximately 1.4 to 3.0 μg DW/mg/h. The low current speed was probably responsible for these low feeding rates as larvae in the stream were found to be located in regions where speeds were

19 cm/sec or greater. It was found that Simulium larvae could filter suspended charcoal particles < 1 μm in diameter from the stream. The low (0.032 μg/mg/h) feeding rates on bacteria in the glass cylinder were again due to inadequate current speeds. The concentration of seston found in Rattlesnake Springs throughout the year was 0.97 to 2.5 mg/ℓ. Although food concentrations in glass cylinder and trough feeding chambers were 4 to 15 times greater than natural seston concentrations, the concentration of suspended food could not compensate for inadequate current. Ingestion rates on diatoms in trough experiments ranged from approximately 1 to 17 μg/mg/h even though current speeds were adequate. It is felt that in these experiments a combination of physical factors (current speed, water depth, and substratum shape) created excessive turbulence which impaired the effectiveness in food gathering by the cephalic fans of the larvae, especially of the larger size classes. Computation of the Froude number (F) for the physical conditions of feeding chambers is one means of estimating the degree of turbulence. Values of F > 1, indicative of turbulent flow, were calculated for the trough. Higher ingestion rates (43.2 to 55.0 μg/mg/h) were measured for larvae feeding on watercress in the plexiglass channel. In addition, F values were < 1, typical of streaming flow. Assimilation efficiencies of larvae feeding on diatoms measured by the dual-label method were 52.7 to 65.8% (mean 57.1%). Probably a combination of differential leaching of isotopes from food and feces and assimilation of ^{51}Cr made interpretation of results difficult in certain experiments. AE values measured by the ash-ratio method ranged from 7.6 to 56.1%. The time required for digestion, measured in the stream with a powdered charcoal marker, was 45 to 60 min. The gut weight fraction (gut DW/body DW) was 109 μg/mg; gut fractions calculated from the literature were 82-111 μg/mg. Based on observation and data from the literature the growth period of larvae was taken as 16 days. A linear increase in larval weight with mm-size classes to the maximum larval weight (1344 μg DW) was noted. Using a respiration rate from the literature for Simulium and an R.Q. of 0.88, assimilation during the 16 day growth period of an individual, determined as growth plus respiration, totaled 11.88 cal. A theoretical ingestion rate (μg/mg/h) for seston was determined from digestion time and gut fraction data and applied to the weights of larvae throughout the growth period to derive the total ingestion per individual. Total assimilation, determined using the mean AE (57.1%) derived by the dual-label method, was 11.76 cal for the growth period. The assimilation rate of larvae was 0.0439 cal/mg/h and the net growth efficiency was 49.5%.

Some workers, noting differences in relative proportions of algae between gut contents and suspended matter in streams, have felt that hydropsychids must supplement their food intake by grazing. Mean values of food accumulation by Cheumatopsyche in grazing experiments ranged from 0.38 to 0.74 μg/mg/h. It is probable that these values are primarily a result of surface adsorption of isotopes and not grazing. The range of

^{51}Cr- and ^{14}C-derived rates of filtration measured in a trough feeding chamber duplicating current speeds naturally experienced by larvae was 11.47 to 25.62 μg/mg/h. Based on a digestion time measurement of 2h for Hydropsyche occidentalis at Deep Creek and a gut fraction of 80 μg/mg, the theoretical ingestion rate is 40 μg/mg/h. The fact that measured values for filtration are comparable to the theoretical value indicates that the turbulence which adversely affected feeding by Simulium did not influence Cheumatopsyche. Ingestion rates by filtration at 6 cm/sec in a glass cylinder feeding chamber were comparable to the low rates obtained by grazing and probably indicate only adsorption. The lack of sufficient material for constructing a net probably is principally responsible for the low ingestion rates, but low current speeds may also play a role. The range of AE values determined in filtration experiments was 45.97 to 49.13% ($\bar{x}$ = 47.26). Using the mean ^{51}Cr-derived ingestion rate of 25.62 μg/mg/h and the mean AE, the assimilation rate on Rattlesnake Springs diatoms (18.47% organic) was 0.01011 cal/mg/h.

Experiments were conducted on Tricorythodes to determine ingestion and assimilation rates, and assimilation efficiencies on blue-green algae, diatoms, and finely ground watercress. Petri dish feeding chambers with a hypodermic needle aeration supply were used at temperatures ranging from 18 to 20°C. Curves depicting accumulation of radioactive food are a composite of gut-filling and assimilation rates. The accumulation curves for Tricorythodes feeding on blue-green algae were similar between experiments. Values of food material accumulated by two size classes after 22.5 h of feeding were nearly identical. In two experiments where feeding on diatoms was measured the amounts accumulated after 5.0 h of feeding were nearly identical (76.3 and 72.9 μg/mg, respectively). Nonetheless, considerable variability in rate of accumulation on a particular food source was evident between some experiments. Certain experiments indicated that within 1.0 h of feeding on diatoms or on blue-green algae that between 42.9 and 79.6 μg/mg were accumulated; other experiments indicated a lower range (15.2 to 34.0 μg/mg). Reasons for the variations are unclear but may be related to the rapid rate of gut-filling of Tricorythodes. An experiment in which food marked with charcoal powder was presented to nymphs preconditioned on unmarked food at 20°C showed that they could fill their guts within 7 min. Another experiment in which ^{14}C-labelled diatoms were presented as food showed that within 24 min nymphs had accumulated 60 μg/mg and that this level was not exceeded during the first hour of feeding. Gut weight fractions measured gravimetrically were 87 μg/mg; those calculated radiometrically (^{14}C) by subtracting tissue accumulation from the combined gut-plus-tissue accumulation were 64.9 to 78.1 μg/mg. Using a gut fraction of 80 μg/mg and a digestion time of 7 min, the theoretical ingestion rate would be 686 μg DW/mg/h. Although nymphs load their guts at this rate when food availability is great, these rates might not be sustainable. Evidence gained from ^{51}Cr- and ^{14}C-derived accumulation values on diatoms indicated that gut-filling occurs within 30 to 60 min.

The slopes of regression equations describing the cumulative assimilation of ^{14}C-labelled blue-green algae by 2.6-3.5 and 3.6-4.5 mm nymphs indicated assimilation rates of 12.4 and 14.7 μg DW/mg/h, equivalent to 0.0522 and 0.0619 cal/mg/h, respectively. Although these uptake curves for 22.5 h feeding were highly correlated with time, the value for accumulation at t = 22.5 h reduced the slope somewhat; the mean assimilation rate derived by averaging the hourly increments in accumulation during the first 6.0 h of feeding was 28.1 μg DW/mg/h. The values for accumulation at t = 22.5 h may be aberrant because of respiratory losses of ^{14}C; another experiment showed the loss of ^{14}C between 6.0 and 9.0 h after feeding on labelled food was slight. A quadratic equation fit the observed data for accumulation of blue-green algae vs. time better than a linear regression. Values of accumulation derived at t = 7 min from the quadratic equations were 15.2 and 19.6 μg/mg, respectively. These values correspond closely to values of accumulation after 7 min of feeding on diatoms in one uptake experiment. Assimilation rates derived from ^{14}C uptake curves during 5.0 h of feeding on diatoms ranged from 0.00768 to 0.0111 cal/mg/h. Assimilation efficiencies on blue-green algae in one experiment based on a 7 min digestion time ranged from 7.4 to 11.8%; AE values on blue-green algae in a second experiment ranged from 5.5 to 14.0% ($\bar{x}$ = 10.3%). AE values for diatoms calculated by the dual-label method averaged 37.6%. An energy budget was developed for Tricorythodes growing from the 3.0 to 4.0 mm size using data from R. L. Newell and D. Brass for growth rates and caloric values, respectively, of Tricorythodes from Deep Creek. Nymphs in this size range grew 0.522 mg in 7.59 days. The respiration rate was 2.51 μℓ O_2/mg/h. Assimilation rate derived by summing energy of growth, respiration and exuviae was 0.0369 cal/mg/h; of this amount growth (G), respiration (R), and exuviae (Ex) comprised 58.1, 33.3, and 8.6%, respectively, of the expended energy. With a gut fraction of 80 μg/mg theoretical ingestion rates on diatoms were derived for gut-filling times of 7, 30, and 60 min. With the assimilation rate calculated above, values of AE varied from 6.5 to 55.2% as gut-filling time varied from 7 to 60 min. Data supplied from some experiments showed that gut-filling occurred in 30 to 60 min. In addition, a 41-min gut-filling time would be indicated from AE derived by the dual-label method and the assimilation rate derived by summing G+R+Ex. If gut-filling times are nearer 60 min, then the AE values derived for blue-green algae and diatoms by methods based on a 7-min digestion time (methods other than the dual-label method) should be multiplied by 8.57.

INTRODUCTION

The objective of the present study was to determine the ingestion rates and assimilation efficiencies of Simulium spp. (Diptera) and Cheumatopsyche analis Banks (Trichoptera) from Rattlesnake Springs and Snively Creek, respectively, Benton County Washington and Tricorythodes minutus Traver (Ephemeroptera) from Deep Creek, Oneida County, Idaho. The species of Simulium studied were Simulium argus Williston and Simulium vittatum Zetterstedt. In collections of larvae only S. argus was found on March 9, 1973 and S. vittatum on July 23, 1973, although it is possible that other species existed concurrently with these. Tricorythodes experiments 1-5 were conducted in the Life Sciences Building at Idaho State University while Tricorythodes experiments 6-11 and the Simulium and Cheumatopsyche experiments were carried out at the Rattlesnake Springs laboratory on the Hanford AEC site. A variety of food sources were used: diatoms, blue-green algae, bacteria (Esherichia coli), and watercress detritus. Some effects of current speed, food concentration, and substratum were assessed in relation to the feeding of Simulium and Cheumatopsyche. Since grazing has been reported occasionally as a secondary means of feeding for both of these filter-feeders, their ability to scrape diatoms from slides was explored. In light of the present conflict over the utility of blue-green algae as a food for aquatic invertebrates (see Arnold 1971), feeding rates and assimilation efficiencies of Tricorythodes utilizing diatoms, watercress detritus, and blue-green algae were compared. In addition, the suitability of various methods for measuring energy budgets was examined and a method was developed for measuring radioactive ^{14}C and total carbon.

To date very few energy budgets have been developed for stream-dwelling invertebrates. Cummins (1973) gives an excellent review of the literature on relative percentages of various foods consumed by aquatic insects and ingestion rates in terms of percent dry body weight per day. Additional information on ingestion rates of invertebrates is summarized in Reichle (1967), Mason (1970a), McBrayer and Reichle (1971), Monakov (1972), and Mackay and Kalff (1973). Defecation rates in terms of percent body weight per day are summarized for deposit-feeders by Hargrave (1973). Notable energy budgets for aquatic insects are those by Trama (1957), McDiffett (1970), Sedell (1971), Lawton (1971a), Cummins et al. (1973a), Nielson (1974), and Otto (1974). Nevertheless, virtually nothing is known about the bioenergetics of desert stream insects.

The present study was conducted on aquatic insects from Deep Creek in southeastern Idaho and Rattlesnake Springs of southeastern Washington in an effort to derive energy budgets for insects of desert streams. Information gained on these invertebrates is useful in the context of data already available from the Deep Creek-IBP (International Biological

Program) studies, which have been in progress since 1969 (Minshall et al. 1973). As a preliminary investigation of the trophic dynamics of Deep Creek, Koslucher (Koslucher and Minshall 1973) examined the food webs on a seasonal basis. In addition Brass (1971) measured seasonal caloric values and respiratory rates for certain stream insects.

Of particular ecological interest is the relative importance of allochthonous or autochthonous detritus versus periphyton in supplying energy to the animal populations. Gut analysis identifies which materials are consumed but not their relative importance nutritionally. A determination of ingestion rates, combined with the abundant data on population densities, can provide an estimate of the rate of processing of available food supplies. In the present study assimilation efficiencies of three herbivore-detritivores were examined and compared to other published energy budgets for aquatic animals.

Energy Flow in the Community

As a first step in tracing the flow of energy through a community, the trophic dependencies must be known. A number of investigators including Monakov (1972) have worked out detailed diagrams of community trophic structures. The principal feeding relations of invertebrates in Deep Creek have been similarly detailed (Koslucher and Minshall 1973). In such a scheme, herbivore-producer and predator-prey relationships are uncovered, along with the relative importance of various food sources on a diel and seasonal basis.

Analysis of gut contents combined with direct observations of the experimental animal's behavior and mouthparts enables one to classify the animal as a shredder, collector, scraper, or predator, with further subdivisions of these categories (see Cummins 1973). Insects studied in this research all were determined to be collectors. The collectors feed on algae and detritus with its microfloral community, as well as on the feces of other animals. Fine particulate matter in the water is filtered out by the cephalic fans of Simulium and by the silken nets of Cheumatopsyche. Tricorythodes is a bottom-dwelling mayfly that consumes the mixture of loose organic debris from the substratum. Contributions to the food supply of these collectors originate primarily from primary production within the stream system of Deep Creek and from a combination of autochthonous and allochthonous inputs in Rattlesnake Springs.

With knowledge of the animal's food habits, experimental feeding situations can be designed which permit natural feeding behavior. This knowledge also permits construction of the trophic spectrum (Darnell 1964) for a species in which the percent by volume (or dry weight) of each food component can be specified (Mecom and Cummins 1964, Coffman, Cummins, and Wuycheck 1971, and Cummins et al. 1973a). With information gained on the assimilation efficiencies for various food components at specific feeding rates, one can

obtain the tissue support structure (Coffman et al. 1971) for an animal and diagram energy flow between trophic levels.

Provided that a species belongs to only one trophic level, energy flow follows the conventional species oriented food chain. However, energy flow between trophic levels operates only within the superstructure of assembled populations. The actual energy transfer is between energy pools. In this system there can be no strict herbivore, for a herbivore draws energy from the decomposer pool as well as from energy of primary production. Coprophagy (see Frankenburg and Smith 1967) can be ecologically important in increasing the efficiency of energy extraction and processing of benthic food accumulations. Neess (personal communication, see Kozlovsky 1968) proposed this type of trophic scheme in which the regular primary consumers as well as the decomposers (bacteria and animals which operate on dead plant material and herbivore feces), and the intestinal parasites of herbivores are all thought of as herbivores. Carnivores are then consumers of herbivores, decomposers of animal flesh, internal parasites of herbivores, and intestinal parasites of carnivores. In this system food within the gut should be thought of as external to the animal and feces simply as energy not used (Odum 1968).

The Energy Budget

An energy budget for an animal is a mathematical expression of the inputs and outputs of energy. Such a budget for the aquatic stages of an insect may be based on energy flow in a short-term feeding study or throughout the life span in the aquatic environment. A short-term feeding experiment using ^{14}C-labelled food may neglect energy losses in exuviae, excretion, and secretions. Egg production may also be disregarded in the non-reproductive aquatic stages of insects. In a short-term energy budget derived with ^{14}C-labelled food all assimilated energy can be treated as one compartment without subdividing energy flow into compartments for reproduction, exuviae, excretion, secretion, etc. Nilsson (1974) found long-term field growth rates on Gammarus pulex (Amphipoda) to be much lower than laboratory short-term growth rates because animals in the field spent energy of production as exuviae and eggs. However, if assimilated ^{14}C-labelled organic matter is rapidly cycled and eliminated as excretory products during the feeding period, then assimilation must be corrected for this loss. Hargrave (1970) found that a considerable amount of assimilated energy is lost in excretion by Hyalella azteca. However, most published energy budgets depict assimilated energy divided between production and respiration. The principal aim in energy budget analysis is to determine the amount of energy utilized in production and respiration.

In an attempt to describe a complete energy budget for an animal it is useful to symbolize the various bioenergetic functions with letters and to

connect these energy compartments with arrows to describe the numerous energy pathways. A figure of this kind (Fig. 1) is presented here summarizing the probable energy pathways. Determining the rate of flow of energy through all pathways depicted is a complicated problem, and in a practical sense many of these pathways, though physiologically correct, represent such insiginficant energy losses or gains that they can be ignored. These energy relationships may be organized into energy balance equations:

1) $I = A + F$
   or $A = I - F$

2) $A = P + R + U$
   or $I - F = P + R + U$

3) $P = G + Rp + Ex + S$

The symbols used (as in Fig. 1) follow the suggestions of Ricker (1968) and Odum (1968).

I-- ingestion
F-- feces
A-- assimilation
P-- production
R-- respiration
U-- excretion (urine)
G-- growth
Rp-- reproduction
Ex-- exuviae (cast skins)
S-- secretion (mucus, peritrophic membrane)

Fig. 1 diagrams the flow of energy from the detritus food base to the level of the primary consumer. Energy flow between storage compartments is depicted in a way that emphasizes the basic principles of the ^{14}C tracer and material balance methods (Johannes and Satomi 1967). Energy is depicted as entering the gut in discrete bundles from the detritus pool where it is assimilated with 50% AE in the posterior region of the gut. Peritrophic membrane and excretions are added to the gut material; mucus is secreted through the body wall while some dissolved organic matter may be directly assimilated through the body wall (Stephens 1967). The gut is visualized as a conveyor belt which is external to the body tissue. Ingestion and defecation involve losses of dissolved organic matter from the food and feces which together with excretory products may be reincorporated into the food base via bacteria or algae (Adams and Angelovic 1970, Monakov and

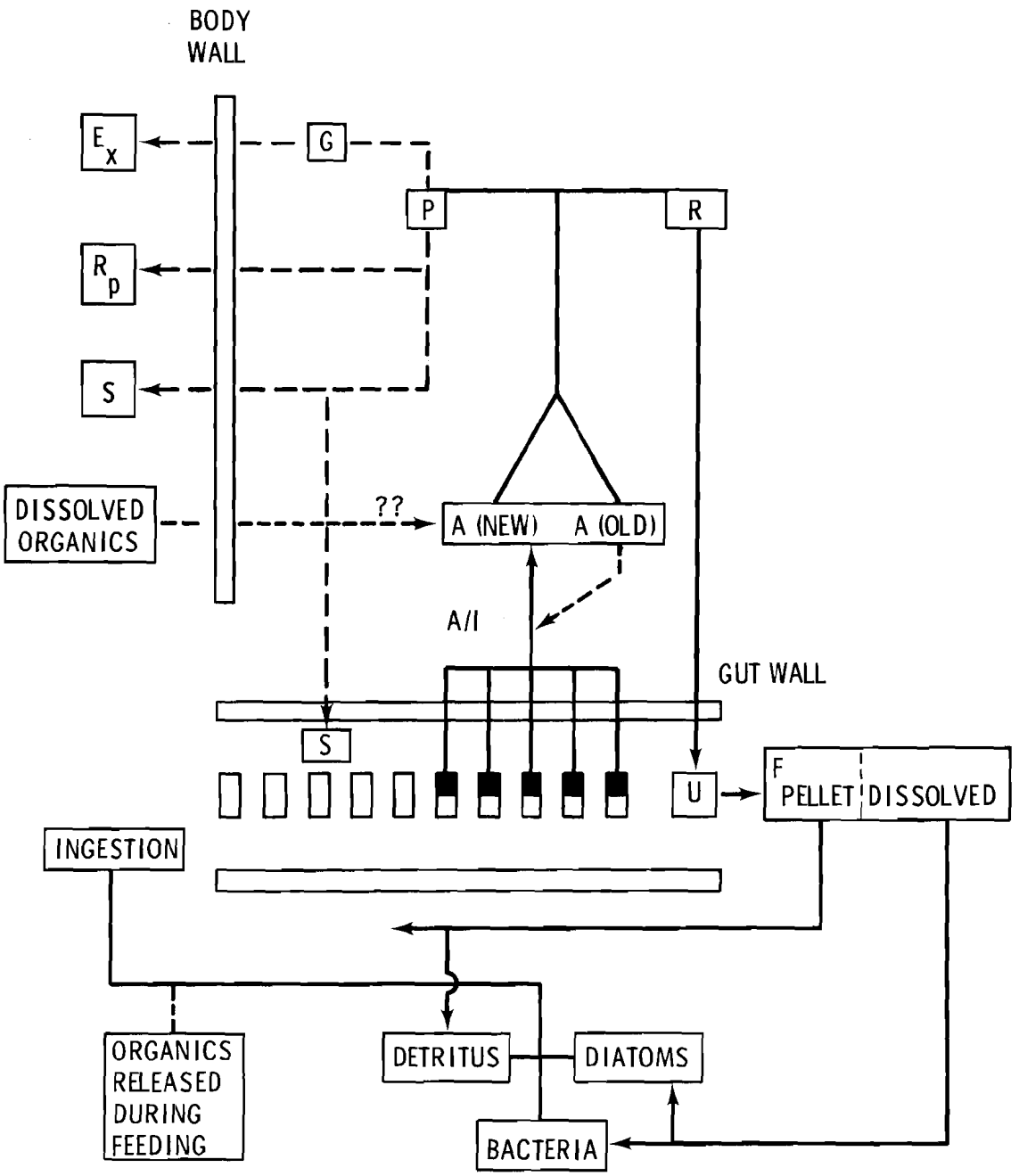


FIGURE 1. Diagram of Energy Flow Through an Insect Emphasizing Physiological Difficulties Encountered in Measuring Energy Flow.

Sorokin 1972). Feces may be reingested, increasing the degree of processing. Some material in the gut may be assimilated by an exchange reaction whereby previously assimilated material reenters the gut (Johannes and Satomi 1967). Assimilated energy is distributed between production and respiration; energy of respiration is derived from a combined pool of new and formerly assimilated material.

General Procedures for the Determination of Energy Budgets

Several methods for measuring ingestion and assimilation rates are available. These are divided into radiometric, gravimetric, and chemical analyses. One radiotracer method (see Edwards et al. 1970; Reichle and Van Hook, Jr. 1970) relies on the fact that when an equilibrium concentration of a radioisotope is established in an animal's body, the rate of absorption equals the rate of elimination. By determining the elimination rate (biological half-life), one can calculate the quantity of ingested food necessary to maintain the equilibrium level.

The ^{14}C method for measuring ingestion and assimilation (see Sorokin 1968) has been used by many authors (Rodina and Troshin 1954; Sorokin 1966, Sorokin and Panov 1968, Schindler 1968, Hargrave 1970, Arnold 1971, Schindler 1971). By this method the ^{14}C activity present in an animal's gut or tissue after a specified period of feeding on food of a known activity density, indicates the amount ingested or assimilated, respectively.

The dual-label method (Calow and Fletcher 1972) is another radiotracer method which allows independent ingestion rate measurements by two isotopes. In addition, assimilation efficiency is indicated by the change in $^{14}C/^{51}Cr$ activity ratios of samples of food and feces. Organic matter assimilation can be derived because one isotope (^{51}Cr) is unabsorbed while the other (^{14}C) acts as a tracer for uptake of organic matter.

The gravimetric technique (Mason 1970a, McDiffett 1970, Lawton, 1970, Stockner 1971, Mackay and Kalff 1973) is commonly used. By this method ingestion is measured as the difference of initial minus final dry weight of food offered; defecation is measured by collecting and weighing fecal pellets produced during feeding; assimilation is taken as the difference of ingestion minus defecation. Ingestion rates are also estimated gravimetrically by dividing the gut fraction (gut weight/body weight) by the digestion time.

Chemical analyses have been used to determine assimilation (see Conover 1966, Hargrave 1970, Pandian and Delvi 1973, Otto 1974) by measuring the change in concentration of some component of the food and feces such as

organic matter, nitrogen, or calories. Included in chemical analyses are the indicator methods (McGinnis and Kasting 1964, Edwards et al. 1970). The difference in concentration of a nonabsorbed substance (e.g., CrO_3) between food and feces indicates the degree of assimilation of food.

A schematic diagram for determining the flow of ^{14}C in a feeding experiment using Simulium is given in Fig. 2. In this experimental design all major energy pathways are measured; AE is calculated using the ^{14}C, ash-ratio, and dual-label methods, and corrections for ^{51}Cr absorption and adsorption and ^{14}C adsorption and losses due to respiration and excretion are made. Corrections are also made for leaching of ^{14}C and ^{51}Cr from feces, and procedures for reducing the dilution of food activity are given. Absolute amounts of energy in compartments such as respiration and excretion are not measured, but the amount of ^{14}C entering these compartments is calculated so that the total amount of organic material assimilated can be derived by summation of tissue accumulation, respiration, and excretion. Feces can be measured gravimetrically or radiometrically. Due to the variable ash content among and within food species (ash content of a species may vary threefold depending on the nutrient concentration of the water supply [Lowman et al. 1967, see Lowman, Rice, and Richards 1971]) all assimilation rates (and ingestion) should be expressed in terms of μg organic matter or carbon or calories.

By mathematical manipulation of the energy budget equations, assimilation (A) can be determined as a sum of P + R + U or as a difference of I - F. Absolute assimilation determined by the summation method (disregarding excretion) can be derived by standard respirometry and measurement of field growth, or by using ^{14}C-labelled food and determining the activity in the tissue (after defecation of labelled food) plus that lost in R and U.

Total production is measured as the proliferation of animal tissue due to eggs and exuviae as well as individual growth. Supplementary gravimetric measurements of egg and exuviae production complete the production estimate for the animal's growth period. Ingestion and defecation rate (I - F) for organic matter (i.e., ash-free dry weight) can also be measured with radiotracers by determining the activity density of the food (μg AFDW/cpm) and the cpm of food ingested and of feces. The weight of feces (μg AFDW) can then be determined simply by scintillation counting of a quantitative collection of feces after allowing time for gut clearance on nonlabelled food (and then multiplying cpm x μg/cpm) or by direct weighing. Again, feces production should be specified in terms of μg organic material, carbon, or calories because the activity density of the feces changes during assimilation due to an increase in the ash content. Compared to regular gravimetric analysis, the radiotracer estimate of I - F has the advantage of eliminating the interference caused by addition of peritrophic membrane to the feces since this material, assimilated prior to the experiment, will not be labelled to any extent. If the gravimetric approach is applied to

FOOD

GROW DIATOMS ON GLASS SLIDES - ALLOW ONLY 1 WEEK IN STREAM SO THAT NO ACCUMULATION OF DEAD CELLS DEVELOPS

20 SLIDES NON LABELLED

DETERMINE PERCENTAGE ASH AND PROTEIN, C/N RATIO, CALORIC CONTENT

USE AS FOOD FOR GUT CLEARANCE

20 SLIDES - LABEL WITH ^{14}C FOR 3 DAYS OR MORE; KEEP pH OF MEDIUM NEAR 7.5 TO INSURE GOOD CELL GROWTH

USE IN FEEDING STUDIES TO MEASURE INGESTION AND ASSIMILATION

ANIMALS

COLLECT 390 BLACKFLY LARVAE

PLACE LARVAE ON ROCKS IN 8 ℓ PLEXIGLASS STREAM CHANNEL; ALLOW DEFECATION FOR 30 MINUTES IN FILTERED STREAM WATER (0.45 μ); REPLACE WATER AFTER DEFECATION PERIOD WITH FILTERED WATER TO REDUCE CONTAMINATION BY NONLABELLED FECES

INTRODUCE EXCESS LABELLED FOOD SO THAT TOTAL CONCENTRATION IS NOT REDUCED MORE THAN 10% DURING INGESTION

SAMPLE FOOD (10 mℓ ALIQUOTS) AT t = 0, 0.25, 0.5 h AND DETERMINE MEAN μg/cpm

INGESTION RATE ANALYSIS (^{51}Cr, ^{14}C)

REMOVE 60 ANIMALS AT t = 0.25 h
REMOVE 60 ANIMALS AT t = 0.50 h
REMOVE 20 ANIMALS (KILLED AT t = 0 h IN 30% ETHANOL) FOR CONTROL ON ADSORPTION

FECES ANALYSIS

LET 100 ANIMALS DEFECATE IN PORCELAIN PAN WITH 150 mℓ FILTERED STREAM WATER; COLLECT ALL FECES FOR 0.5 h TO GET WEIGHT OF FECES PRODUCED (μg/mg/h); DETERMINE μg FECES/cpm; AE BY DUAL-LABEL METHOD; DETERMINE PERCENTAGE ASH AND PROTEIN, C/N RATIO, AND CALORIC CONTENT OF FECES; FILTER 5 mℓ ALIQUOTS OF WATER TO DETERMINE ^{51}Cr AND ^{14}C LEACHED FROM FECES (CORRECTION FOR LOSS FROM FECES); OBTAIN DRY WT (DW) OF ANIMALS

PLACE 150 ANIMALS IN A SECOND LABORATORY STREAM; FEED ON SUPERABUNDANT UNLABELLED DIATOMS TO CLEAR GUT (1.0 h) TO INSURE THAT LABELLED FECES PRODUCED MAKE AN INSIGNIFICANT PORTION OF UNLABELLED DIATOMS INGESTED

PLACE 50 ANIMALS IN SEALED 150 mℓ RESPIRATORY CHAMBER; AERATE USING CO_2-FREE AIR; COLLECT RESPIRED $^{14}CO_2$ IN EXHAUST GASES IN ETHANOLAMINE FOR 1 h PERIOD; ACIDIFY CHAMBER TO pH 3 AND BUBBLE FOR ADDITIONAL 10 MINUTES TO COMPLETE COLLECTION OF $^{14}CO_2$

SAMPLE THREE 1 mℓ ALIQUOTS OF CHAMBER WATER TO MEASURE ^{14}C EXCRETORY LOSS (cpm/mg/h)

COLLECT ALL 50 ANIMALS, DRY, WEIGH TO FIND RESPIRATORY LOSS AS cpm/mg/h

AFTER 1.5 h FEEDING

SAMPLE 50 ANIMALS TO DETERMINE ASSIMILATION (μg AFDW/mg/h) AND AE BY ^{14}C METHOD (CORRECT ASSIMILATION FOR RESPIRATORY AND EXCRETORY LOSSES)

SAMPLE 50 ANIMALS TO DETERMINE DEGREE OF ^{51}Cr ABSORPTION IN μg/cpm (DETERMINE PERCENT ABSORPTION PER FEEDING TIME AND CORRECT FOR THIS LOSS IN DUAL LABEL METHOD)

FIGURE 2. Measurement of Energy Flow in *Simulium* using ^{14}C, ^{51}Cr and ^{14}C, ash-ratio, and chemical methods

assimilation determinations based on I - F, then the organic matter retention scheme used by Johannes and Satomi (1967) should be applied, keeping in mind that an overall net assimilation (retention) is being measured and not an instantaneous assimilation.

The gravimetric method is often used by terrestrial ecologists (Waldbauer 1964, Mason 1970a, Mukerji and Guppy 1970, Latheef and Harcourt 1972) and frequently by freshwater ecologists (Hargrave 1970, Lawton 1970, McDiffett 1970, Triska 1970, Wallace, Woodall, and Sherberger 1970, Grafius 1972, Cummins et al. 1973b, Mackay and Kalff 1973, Otto 1974, Nilsson 1974), although the problems incurred in blotting and reweighing food for freshwater studies, variable decomposition, leaching, growth, water loss of food sources, and the construction of fresh-to-dry weight conversions make this method difficult. As can be seen in Hargrave's (1970) experiment using Chara as a food source, assimilation efficiencies by the gravimetric method can be extremely variable. This is due to the difficulty in accurately weighing the food before and after the feeding experiment. Food was weighed to the nearest 0.1 mg on a Mettler balance, while feces were weighed to the nearest 1.0 μg on a Cahn balance. In addition, epiphytes brushed off the Chara during grazing could result in considerable weight loss of the Chara, while disintegration of feces or the mingling of feces in the sediment introduce other possibilities for error. Unless the animal is quite large, gravimetric experiments (especially in water) must be extended over a greater time period than radiotracer experiments. During this time the quality of the food may change, leaching must be accounted for, and microbial growth on the food may increase or decrease the weight and caloric content and increase the protein content (Triska 1970, Kaushik and Hynes 1971). For these reasons food should be changed regularly, but the loss of weight within this time may be too small to measure.

The Gravimetric, Radiotracer, and Material Balance Techniques in Contrast

Basically two techniques are in common use for computing the rate of assimilation of energy: the gravimetric and radiotracer techniques. Each technique has specific limitations in its ability to estimate the actual quantity of material assimilated. The gravimetric technique, which uses the balance equation: A = I - F to derive assimilation, is based on two assumptions (Johannes and Satomi 1967): (1) that the feces contain organic matter entirely derived from food, and (2) that all unassimilated food is released in fecal pellets. Assumption 1 might not entirely be met because mucus and chitinous peritrophic membrane are released into the gut at a

constant rate in certain insects. Assumption 2 is also tenuous because some of the ingested organic matter can be released as dissolved organic matter of feces; or this dissolved organic matter could be derived from assimilated organic matter as secretions and excretions; and the process of chewing the food often releases dissolved organic matter.

Assimilation values calculated using the gravimetric and material balance methods differ considerably. The advantages and disadvantages of each method and the results obtainable by each can be most easily seen by constructing a sample problem. In the gravimetric method a quantitative collection of feces produced during 1.0 h is weighed on a foil square. The weight of feces produced during 1.0 h is subtracted from the weight of food ingested during 1.0 h to derive the weight assimilated during that time. Due to variability in the percent ash of food sources it is desirable to compute ingestion in terms of ash-free organic matter of food and feces when dry weights are used (Reichle 1971). Otherwise these rates are computed in terms of μgC/h or cal/h. Considering the possible magnitude of additions to feces weight from secretions and of losses of dissolved organic matter through secretions and excretions, it is seen that assimilation will be underestimated or overestimated, respectively. Due to this difficulty of measuring the actual amount assimilated, it is easier and more significant in a production sense to measure the net amount of organic material retained (assimilation corrected for losses) (see Johannes and Satomi 1967). This technique does not isolate the amount instantaneously assimilated but computes net assimilation in a long-term energy balance. The method creates additional problems though which are not explicated by Johannes and Satomi. Confusion arises because dissolved organic matter derived from material already assimilated is lumped with dissolved organic matter derived from non-assimilated sources.

These difficulties involved in calculating assimilation, shown in Fig. 1, can be quantitatively diagrammed in a representative problem (Fig. 3). If this problem (Fig. 3) were approached in the traditional gravimetric fashion, one would calculate assimilated material as I-F with no corrections for excretions, secretions, dissolved organic release with cell damage, or feces leaching. Assimilation then would be measured as 30-7.5 = 22.5, provided that all fecal pellets could be sorted out of the food. Ingestion is calculated as food (initial wt) - food (wt remaining) and only the solid food remaining can be determined by standard gravimetric methods. Feces are a combination of insoluble pellets and gut secretions.

Johannes and Satomi (1967) avoided the questions of rates of excretion, secretion, etc. by measuring the organic matter retained rather than the actual assimilation. Using the equation $X = I-F-E$ where X is the rate of retention of organic carbon, I is the rate of ingestion, F is the rate of release of feces, and E is the rate of release of assimilated organic carbon.

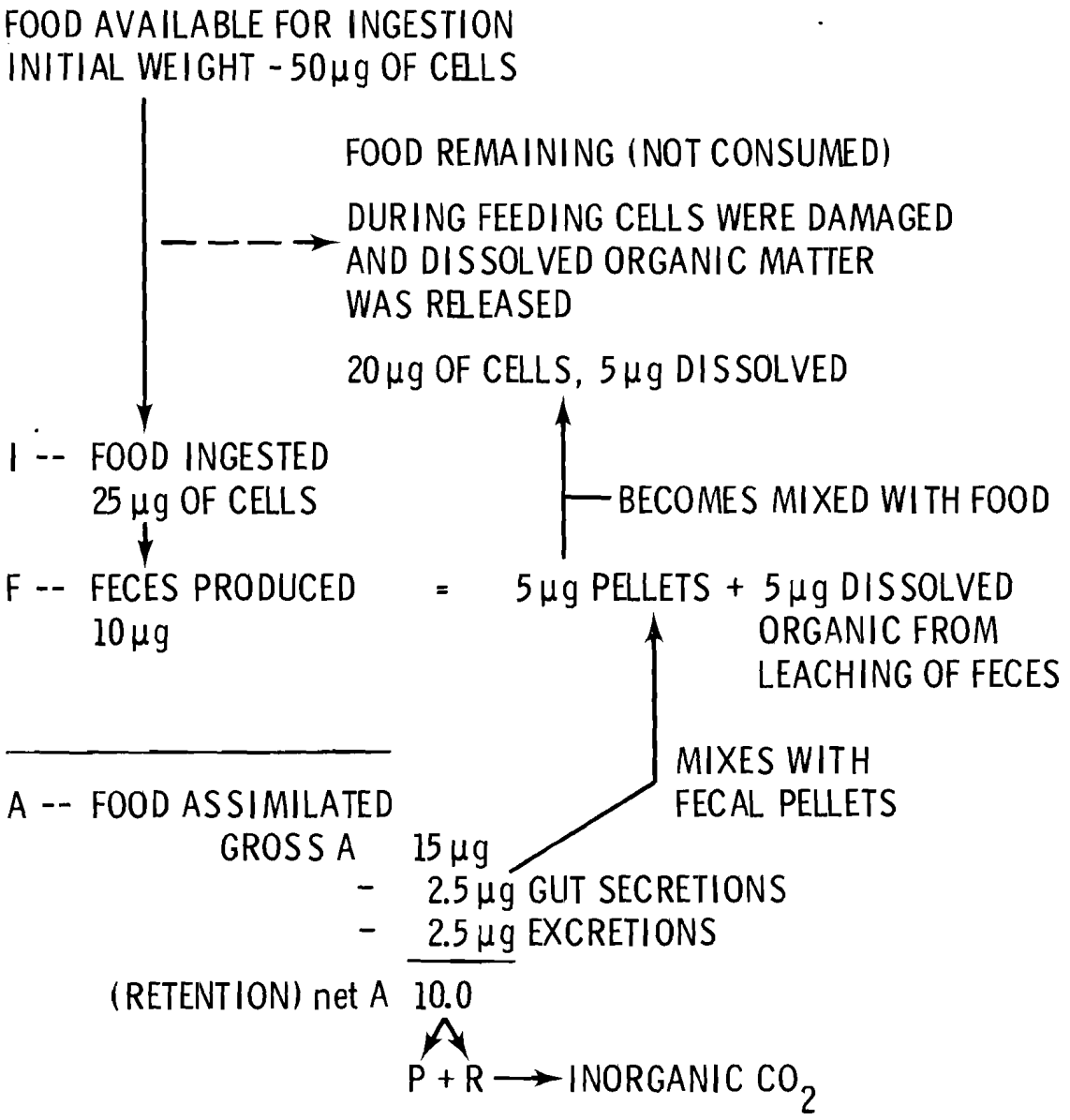


FIGURE 3. Hypothetical Diagram of Ingestion, Assimilation, and Defecation of Food Material Coupled with Leaching, Excretion and Secretion.

Approaching the same problem with this equation, one can calculate retention of organic matter.

$$X = I - F - E$$

$$= [(50-20) - 5] - [(5+2.5) + 5] - [2.5]$$

$$= 25-12.5-2.5$$

$$= 10$$

In essence the weight of fecal pellets (composite of undissolved pellets and secretions) plus all soluble organic matter is subtracted from the gravimetrically derived ingestion rate to yield retention. If the same problem is solved using the ^{14}C radiotracer method then many of these complicating functions can be ignored. The rupturing of cells during chewing, although probably a minor consideration using any technique, is less significant with the ^{14}C method because if all products are uniformly labelled then the counts which do appear inside the animal still represent a specific dry weight of cells (loss of labelled material through cell damage is automatically eliminated from the actual amount ingested). The amount assimilated can be estimated directly if it can be assumed that the secretions into the gut and the excretions are not labelled. Losses of labelled organic material from the animal can be ignored if the feeding experiment is short enough and the body burden of ^{14}C remains low. If excretions are largely in the form of NH_3, then these excretions will not be labelled, but the deamination of proteins occurs, with the resultant loss of metabolic CO_2; and if the newly assimilated labelled organic material is metabolized, this $^{14}CO_2$ can be measured and the assimilation value can be corrected. Labelled dissolved organics can also be measured with a scintillation counter by oxidizing a water sample in which these products were excreted using a special combustion apparatus.

The ^{14}C method is then used to calculate the amount assimilated in this problem.

$I = 25\ \mu g$

$A = 15\ \mu g$ calculated independently

If labelled dissolved organic matter is released to the water, then one calculates:

net A = 13

R = 1

U = 1

gross A = 15

AE = 15/25 = 60%

A possible danger involved in applying these corrections is that labelled organics from feces could be leached into the water and mistaken for excretions. However, when percent retention is calculated by Johannes and Satomi's (1967) method (in which percent retention of organic carbon = [(organic C ingested - particulate and dissolved organic carbon released) ÷ organic C ingested] x 100 a value of 33% [(30-20)/30] is obtained. If mechanical rupturing of cells is significant, then the real ingestion rate and the real defecation rate are unobtainable by this method, because dissolved material from feces, excretions, and cell contents and/or secretions are intermingled and inseparable. The actual amount ingested (25 μg). cannot be determined without knowing how much to correct the apparent ingestion rate (30 μg). The actual percent retention should be 10/25 = 40%. However, assimilation efficiency calculated from the standard formula (I-F)/I becomes (30-7.5)/30 = 75% or (25-7.5)/25 = 70% if the mechanical rupturing of cells is negligible, or (25-10)/25 = 60% if cell rupturing, feces leaching, gut secretions, and excretory losses do not occur. In this latter case assimilation efficiency determined gravimetrically is equal to efficiency calculated radiometrically. But if any losses occur, then AE calculated gravimetrically is in error (the degree of error depending on the actual rates of each of these functions), and in this case Johannes and Satomi's modification of the gravimetric method is essential in deriving a value with some application. Many of the errors described can be avoided by selecting the radiotracer technique for assimilation measurements. Any material retained in the animal through the feeding experiment can be used in growth and in respiration. If a balance were accurate enough to measure the increases in weight of an animal during continuous feeding (correcting for the increase in weight of the gut contents during growth) then this value would be growth (G). Since respiration continues throughout feeding at a constant rate, the factor R is removed from the amount of organic material retained (X), leaving G. Growth ideally is measured as a sum of instantaneous increases in dry weight (including cast skins), and respiration must be measured independently.

Environmental Considerations

Effects of the Environment on the Energy Budget of an Aquatic Animal

The food and feeding of aquatic insects is subject to a complex system of interacting biological, physical, and chemical variables. In addition, change in the level of one energy budget component (e.g., rate of respiration) results in the adjustment of all other bioenergetic components to effect a balance within limits determined by the energy intake. The rate of energy flow through aquatic animals is daily and seasonally "tuned" by the constant fluctuation and interplay of environmental variables. Laboratory experiments, which cannot duplicate changing conditions of this complexity, must at least measure the responses of an animal to average conditions about which these variables fluctuate. An understanding of factors which determine the distribution, abundance, behavior, and physiology of the animal will also aid in creating realistic experimental feeding situations or microcosms of the actual habitat.

As Cooke (1972) indicates, the effect of changing physical and chemical parameters may be studied very effectively in microcosm communities, but the responses measured for one species within the microcosm community may be expressed in proportion to the degree of stability or complexity of the community. That is, respiratory rates or growth rates measured in single species culture may differ greatly from the same rates measured in mixed culture. For example, Chua and Brinkhurst (1973) found differences in growth, respiration, and ingestion rates in pure versus mixed species cultures of tubificid worms. Cummins et al. (1973b) found significant differences in growth rate of stream detritivores with variations in animal density and species composition.

The difficulty in designing laboratory feeding experiments which provide data applicable to field situations is further emphasized by Hubbell, Sikora, and Paris (1965) and Lawton (1971b). Lawton found that actual feeding rates (in nature) were closer to maximum laboratory rates during the summer than in winter for *Pyrrhosoma nymphula* (Zygoptera). In addition, Lawton (1970) states that assimilation efficiencies derived in the laboratory can be applied to the field situation only for those animals in which assimilation efficiency (AE) is not affected by feeding rate. Temperature, larval size, and physiological state also can modify the AE but can be more easily controlled in the laboratory. A study of laboratory and field growth rates of the mayfly *Tricorythodes minutus* revealed that laboratory growth rates were often considerably less than field growth rates when measured under comparable temperature conditions (Newell, personal communication). This same relationship also was observed in laboratory and field growth studies on *Hydropsyche occidentalis* (Trichoptera) (McCullough, unpublished data).

The relationship of energy budget components to environmental variables is intricate. A decrease in temperature may limit the ingestion rate, thereby reducing growth rate and decreasing respiration rate. The growth and ingestion of some aquatic insects are curtailed below a low critical water temperature. Only long-term growth studies may uncover these effects. Environmental factors such as current speed, substratum, food supply, oxygen tension, nearby terrestrial vegetation, water chemistry, light intensity, and temperature can all influence the animal distribution within the stream community. Because these factors are tightly interconnected, a complex pattern of microenvironments is created in the stream (see Allen 1959) which causes animals to seek a specific set of variables within their range of tolerances to meet immediate needs for food. To some extent deficiencies can be compensated for by excesses elsewhere but a minimum requirement remains which must be met before any compensation can be of importance.

In order to understand the effects of interacting biological, physical, and chemical factors on the measured energy budget components, a compilation of primary biological, physical, and chemical factors was organized according to their effects on feeding, feeding rate, digestion time, growth, respiration, assimilation efficiency and defecation.

Biological Factors Which Affect the Energy Budget

Food and Feeding

Behavior of species and interactions among species influence food selection and feeding activity. For example, the oviposition site initially determines the food available to early instars. During the second instar *Simulium* larvae drift into the swift water sections of the stream from oviposition sites (Chutter 1972); predation on simuliids by the Hydropsychidae generally is high during this period. Cummins (1964) noted that *Pycnopsyche* larvae migrate from the center of the stream (where the eggs were deposited) to the margins during the life cycle. Competition for a limited food supply affects the availability of food per individual. A species excluded from its favored habitat may be able to obtain only a suboptimal amount or quality of food. Reduction in available food by floods may increase competitive interaction between related species (Mackay and Kalff 1973) and too great an increase in density of a species may cause intraspecific aggression or cannibalism, as noted for *Hydropsyche* (Williams and Hynes 1973). In the present study a small degree of predation upon *Simulium* by *Cheumatopsyche* was noted in joint feeding experiments. Mecom (1972) reported that physiological changes in certain caddisflies may cause them to become predaceous

during certain months. Experimental feeding of a herbivore under non-stressed (no predation) conditions may not be typical of the normal conditions, for under natural conditions each animal selects a habit where its behavior pattern enables it to effect the best compromise between obtaining food and being preyed upon too heavily (Macan 1961).

Feeding Rate

Suspended food concentration is important in determining distribution of filter-feeding insects (Cushing, Jr. 1963, Chutter 1968, Spence and Hynes 1971). Generally feeding rate increases with food concentration to a certain level, after which it remains constant despite further increases in food concentration. Filtration rate, on the other hand, remains constant at low food concentrations but declines after a critical high concentration is reached.

Innate biological rhythms of activity can cause the feeding rate to be greater under certain conditions. Among zooplankters, Calanus feeds more in the dark than in the light and Diaptomus graciloides feeds more in the light (Edmondson 1961). Hedriodiscus truquii (Diptera) feeds on top of the algal mat at night and hides within it during the day (Stockner 1971). Prus (1971) found that in the laboratory food consumption by Asellus aquaticus (Isopoda) was eight times greater in summer than winter, even though the animals were acclimated and fed at 10°C in both cases. This suggests that age or a biological clock governs feeding rate. Chaston (1968) found an activity rhythm in Simulium which causes drift of larvae to increase at night. If larvae move about on the substrate or drift at night, feeding rates extrapolated to a 24-h period from daytime feeding experiments would be too high.

Initial feeding rates may be greater than sustained rates. Holter (1973) found high initial rates of feeding by Aphodius (Coleoptera) larvae due to rapid feeding while burrowing into dung. Starvation has also been reported to affect the gut retention time. Reichle (1966) and Conover (1966) noted an increase in digestion time for forest floor detritivores and marine zooplankton, respectively, when food was scarce. The normal digestion time of Euphausia pacifica (Euphausiacea) is one hour but some food still remains in the gut after 40 h of starvation (Lasker 1966). Hubbell et al. (1965) found that retention of food by Armadillidium (Isopoda) in the field was much greater than in the laboratory because food concentrations in the field were lower. Defecation ceased when feeding ceased. This is an advantage to animals subject to variable food resources. Other investigators have assumed that starvation for up to 72 h completely clears the gut of all food. Calow and Fletcher (1972) determined extraordinarily high AE values for snails, which probably can be attributed to incomplete defecation. Wolda, Zweep, and Schuitema (1971) felt that high AE values for snails determined

by certain authors were erroneous because gut contents of snails are altered very little upon passage through the gut. Lawton (1971a) estimated field digestion time by measuring the time required for production of the last fecal pellet of Odonata nymphs collected in the field. The mean time required for complete defecation was assumed to be half the actual digestion time. Defecation rates were measured by McDiffett (1970) and Lawton (1971a) during starvation for 2.0 h and 6.0 h periods, respectively. In such studies it must be assumed that defecation continues normally even when feeding ceases. This assumption is probably less valid for continuous feeders (herbivores) than for intermittent feeders (carnivores).

Respiration

Diurnal activity rhythms have been reported in mayfly nymphs (Harker 1953, Elliot 1968). The activity pattern can exist independent of fluctuating lighting conditions, but greater diurnal fluctuations in activity occur under the added stimulus of a light-dark cycle. Ulanoski and McDiffett (1972) found a diurnal respiration rhythm in a mayfly known to have a diurnal activity pattern. Activity and respiration were significantly greater in the dark than in the light. However, McDiffett (1970) and Lawton (1971a) found no daily periodicity in oxygen consumption for Pteronarcys scotti (Plecoptera) and Pyrrhosoma nymphula (Odonata), respectively.

The life stage of an insect also influences the respiratory rate. Animals entering diapause generally show a reduced metabolic rate (Keister and Buck 1964). Lawton (1971a) noted a respiratory rate in stage 3 metamorphosing Pyrrhosoma which was two times greater than expected on the basis of size. Larvae in this state do not feed and with the great energy demand during metamorphosis, a gradual loss in weight was observed. Other physiologic states (quiescence, dormancy) can be expected to affect respiration.

Reichle (1968) has shown that metabolic rate varies with the metabolically effective body weight. Metabolic rate per unit weight tends to increase as body size decreases. Some workers have shown that daily ingestion rate is positively correlated with body surface area and is fairly comparable between animals of similar geometry. However, Reichle (1968) found a better correlation of food intake with dry body weight. Edwards (1958) found no correlation of O_2 consumption and surface area for Chironomous riparius. Stockner (1971) noted that respiration rate relative to body weight of the terminal instar of Hedriodiscus was significantly lower than in early instars due to an accumulation of metabolically inert lipids. Although mean ingestion per day (μg/ indiv/day) is greater for larger instars (instar 4>3>2), the ingestion rate in terms of μg/mg/day is greatest for smaller instars (instar 2>3>4).

Assimilation Efficiency

Biological conditions such as size, age, density, developmental stage, or reproductive state can affect the assimilation efficiency. Pyrrhosoma showed a decreased efficiency with increasing size, ranging from 92.9% for 4 mm animals to 84-86% for 13-14 mm individuals (Lawton 1970). McDiffett (1970) stated that assimilation efficiency of Pteronarcys decreases with age. Stockner (1971) noted a decrease in assimilation efficiency of Hedriodiscus truquii from instars 2 and 3 to instar 4 related to onset of pupation and decreased activity. However, diapause and metamorphosis in Pyrrhosoma (up to the point where feeding ceases) had no effect on assimilation efficiency relative to values for nonmetamorphosing terminal instars (Lawton 1970).

Mukerji and Guppy (1970) found that assimilation efficiency in the larval stage of a moth decreased with age due to less discriminatory food selection in the larger larvae. Portions of the leaf of highest food quality were selected by young instars. McDiffett (1970) noted a similar behavior in Pteronarcys, which skeletonized leaves. Prus (1971) found that the reproductive condition of Asellus aquaticus determined assimilation efficiency. Ovigerous females had a higher AE than males or non-ovigerous females, which allowed them to compensate for a lower ingestion rate. A partial explanation for the low ingestion rate of ovigerous females is that half the body weight was composed of metabolically independent eggs. Prus tested the effect of density (1, 5, 10, and 20 individuals per petri dish) on assimilation efficiency and found significantly greater efficiencies in group cultures (34.9%) compared to single cultures (26.3%).

Physical Factors Which Affect the Energy Budget

Feeding

It is conceivable that natural feeding responses from an animal can be obtained only in a favorable habitat. Popham (1941) (see Macan 1961) indicated that Corixa distincta chooses a background which it matches and is restless on a background of different intensity than itself. Maciolek and Tunzi (1968) noted that blackfly larvae generally choose rocks of a dark color as attachment sites although they occasionally found large numbers of larvae on light objects. In the present research animals in feeding experiments were placed against a dark background or upon food.

Habitat also partially determines the food selection of an animal. Although Simulium, Cheumatopsyche, and Tricorythodes are all collectors, they have distinctly different microhabitats and different food habits. Simulium larvae, attached to the tops of smooth stones are able to filter seston from the water; they are not likely to ingest large amounts of coarse

sand, which generally would not be in suspension. The food of Tricorythodes however is mixed with nonnutritive sediment, indicative of their existence within the algal mat covering coarse gravel in riffles and within loose detritus in slower waters. Cheumatopsyche lives under stones and is more apt to collect detritus particles, along with some algae which settle between the stones. Some foods of suitable nutritional value are not eaten because of mechanical features of the consumer's mouthparts. Among filter-feeding invertebrates particle size selection has been found to be a function of the mechanical structure of the filtration apparatus and certain behavioral features (Arnold 1971, Jorgensen 1955, Wilson 1973). The combination of filtration effectiveness and food concentration and quality determines whether an animal can meet its energy requirements.

Williams and Hynes (1973) determined the mesh size of the net created by the fifth (terminal) instar Hydropsyche sparna (Trichoptera) to be 190x300 μm and that for the second instar to be 30x55 μm. Mesh size increases with each instar due to the increase in size of the mouthparts, which create the net. With the increase in net size there undoubtedly is a change in food gathering capabilities with age. The net mesh dimensions of another filter-feeding caddis larva (Chimarra aterrima) were much smaller (6x70 μm) and consequently extracted a smaller range of particles from the water. Selection for food of a particular size range could simultaneously discriminate for food of a certain quality. Other authors have described the net mesh dimensions of Hydropsychidae (Edington 1968, Maciolek and Tunzi 1968, Hynes 1970). Williams and Obeng (1961) found that Simulium can trap a wide range of particle sizes but select most heavily for particles in the range 6-14 μm. A brief study on particle size selection was done in this research.

Although it generally is assumed that aquatic invertebrates ingest leaves to obtain the associated fungal growth, Cummins et al. (1973b) found that the mayfly Stenonema was unable to feed on a thick fungal mat due to mechanical interference with the feeding mechanism. Stockner (1971) found that feeding rate of Hedriodiscus was reduced on blue-green mats in a hot spring compared to the feeding rates on filamentous Oedogonium sp. and epiphytic diatoms, despite a greater caloric content in the blue-green mat. The smooth, fibrous blue-green mat apparently made feeding difficult. Wilhm (1970) was able to note differences in particle size selection between Limnodrilus (Oligochacta) and Stictochironomus (Diptera) feeding on detritus. But Liminodrilus accumulated radioisotopes faster than Stictochironomus by feeding selectively on smaller (more contaminated) particles. The snail Ancylus fluviatilis was shown to have preference for particular species of periphytic algae and for small-sized cells (Calow 1973).

Feeding Rate

The feeding rate of filter-feeding zooplankton is linearly related to the concentration of food until a critical high concentration of food is reached, after which ingestion rate usually is stable (Rigler 1971). Secondary productivity is therefore controlled by primary productivity as well as mechanical ability of the organism to utilize food.

A variety of mathematical functions describe the relationship between body size and filtering rate for zooplankton (Buikema 1973). The maximum ingestion rate for aquatic invertebrates (e.g., Pyrrhosoma nymphula) is a function of larval size (Lawton 1971b). It is obvious that larger animals have a greater ingestion rate (μg/indiv/h). McDiffett (1970) discovered a linear relationship between $\log_{10}$ ingestion rate and $\log_{10}$ dry weight at 5, 10, and 15°C. The 5 and 10°C regression coefficients (0.65 and 0.89) were significantly different; coefficients of regression for 10 and 15°C were not significantly different. Reichle's (1968) regression coefficient (0.68) for ingestion rate of 11 terrestrial arthropods is very similar to that of McDiffett.

Temperature is known to affect the feeding rate. The maximum feeding rate for Pteronarcys dorsata occurred between 15-20°C (Nebeker 1971), while no feeding took place at 1°C or 35°C. Feeding at 25°C was erratic and less was eaten than in the 15-20°C range. Lawton (1971b) described the maximum feeding rate (mg/indiv/deg) for Pyrrhosoma between 4°C and 15°C as a percent of the rate at 15°C. There was a 6.2%/°C reduction in feeding rate from 15°C to 4°C for all larval sizes. Ladle, Bass, and Jenkins (1972) found that feeding in Simulium was suppressed between 5 and 8°C; between 8 and 21°C there was no difference in feeding rate.

Fredeen (1964) (see Ladle et al. 1972) noted that the feeding rate of Simulium was related to rate of water flow. The Simulidae and Hydropsychidae nearly always are observed in fast flowing waters but increased food availability is not the only beneficial effect of rapid current. Feeding rates of invertebrates may also be expected to vary diurnally and monthly (Malone and Nelson 1969).

Digestion Time

Gut-filling time is not necessarily equal to the time required for digestion. Initial feeding rates may be exaggerated so that rapid gut-filling is followed by long gut retention times. This is especially true of carnivores which feed sporadically on prey (see Lawton 1971a). For herbivores continuous steady feeding and a constant food supply are usually the rule so that digestion time and gut-filling time probably are equal. Lawton (1971a) calculated digestion time as the time between ingestion and defecation of a particle. Ladle et al. (1972) found no difference in digestion time for Simulium relative to larval size or species. In all instances digestion time was 20-30 minutes. This was corroborated by Fredeen (1964).

Growth

Growth rate is regulated by several environmental factors. Certain factors relative to the animal itself are specified by Hynes (1961), who categorized many species according to growth types ("slow" and "fast" growth species). Ulfstrand (1968) found several species of Simulium have a one-year cycle in which certain species grow rapidly after oviposition, leading to large slow-growing larvae, and others exhibit intense growth just before emergence. A lowered rate of metabolism in winter would be an advantage in streams of low productivity where food is scarce, but when food is abundant some species could be expected to have a rapid winter growth if temperatures exceed 8°C. Mackay (1972) followed instantaneous growth rates (IGR) of three species of caddisfly and found that for Pycnopsyche gentilis and P. luculenta the IGR decreased through autumn and winter (although mean individual weight steadily rose). IGR began to rise with the water temperature in spring; a spring thaw in April removed much of the available food and the IGR showed a corresponding dip. Another species, P. scabripennis, had a much reduced IGR in winter compared to the other species, reducing competition for a limited food supply. Although physical factors may direct the growth rate, growth responses by individuals are uniquely characteristic of the species.

Combinations of light and temperature are also known to affect larval development. Corbet (1959, see Cummins 1964) found that rising water temperatures in the spring synchronize growth. In "summer" species (forms that overwinter in stages other than the terminal instar) synchrony is obtained because the Terminal-2 stage has a lower temperature threshold than the Terminal-1 stage and commences growth first. Longer days at each of the experimental temperatures promoted more rapid growth than short days.

Respiration

The respiration rate of an animal is a good indicator of its activity or physiological state (intermolt, premolt, diapause, instar, life stage). Within a single instar metabolic rate is also found to vary depending on a variety of physical conditions.

Temperature is an important control on respiratory rate. Lawton (1971a) found an increase in the $\log_{10}$ respiratory rate with an increase in temperature for Pyrrhosoma between 5 and 16°C. McDiffett (1970) found that for Pteronarcys a linear dependence of $\log_{10}$ respiratory rate on $\log_{10}$ dry weight (expressed as $Y = aX^b$) exists within the same temperature range but could find no significant difference in slopes for regressions at 1 and 10°C. Stockner (1971) found only slight variation in respiratory rate for Hedriodiscus between 10-40°C.

Eriksen (1968) showed the effect of substrate particle size on respiratory rate of two species of mayflies. Oxygen consumption was lowest on the preferred particle size. Nymphs acted as respiratory regulators on an optimal

substrate, maintaining a constant rate of respiration by adjusting gill beat frequency, and acted as conformers on unsuitable substrata. McDiffett (1970) found that Pteronarcys scotti was extremely active if no substrate was provided in the respiratory chamber. Selection for a particular size substratum is influenced by the level of O_2 concentration in the interstitial water. Oxygen tension can influence respiration rate, as shown by Lawton (1971a). Respiratory rate of Pyrrhosoma declined gradually with O_2 tension between 100-50% saturation and then dropped drastically. Most pond invertebrates have respiratory rates which are independent of O_2 concentration down to very low O_2 levels (see Lawton 1971a). McDiffett (1970) found O_2 consumption by Pteronarcys to be constant down to 70% depletion of O_2 in water.

Current speed has a considerable influence on respiration. Respiration rates of some aquatic insects increase with current speed (Phillipson 1956, Eriksen 1966), probably due to the phenomenon of physiological enrichment of the medium (Ruttner 1963) as the boundary layer is reduced and the O_2 gradient is increased at greater current speed. Oxygen requirements in an experiment can be satisfied by providing two of the following three factors: adequate current speed, high O_2 content, or low temperature (Hynes 1970).

Assimilation Efficiency

Since assimilated energy is the sum of energy of growth, exuviae, respiration, eggs, and excretion, absolute assimilation will vary as its components vary under the influence of physical factors. Assimilation efficiency, however, being the ratio of assimilation to ingestion, may not change under the influence of these factors provided that the ingestion rate varies directly with the assimilation rate. Since feeding often is correlated with temperature changes, temperature could influence assimilation efficiency. McDiffett (1970) found no seasonal change in assimilation or growth efficiencies for Pteronarcys, while Prus (1971) showed that AE varies seasonally for Asellus aquaticus but consumption per unit weight remains constant. Conover (1966) and Lawton (1970) found that assimilation efficiency was not correlated with temperature or feeding rate for Calanus (Copepoda) and Pyrrhosoma, respectively. Mason (1970a) found that assimilation efficiency was independent of temperature for seven species of snails but that both ingestion rate and absolute assimilation increased with increasing temperature. Schindler (1968) found temperature to markedly increase assimilation efficiency of Daphnia magna, while feeding rates were unaffected.

Defecation

Hargrave (1972) compared the relationship of $\log_{10}$ dry body weight egested/animal/day versus $\log_{10}$ dry body weight for values reported by 17 workers and found that no single regression line could be given for all species of deposit feeders. However, there appeared to be a regular relationship between egestion and body weight regardless of species.

Lawton (1971a) found that gut clearance time of Pyrrhosoma was correlated with size (head width) and that gut clearance time of all sizes of larvae varied inversely with temperature. These relationships were described mathematically so that ingestion rates of larvae from the field could be predicted if head width, egestion rate, AE, and water temperature were known.

A linear relationship exists between fecal pellet production and temperature for Hyalella (Hargrave 1972). Most workers have found linear increases in egestion rate within a narrow temperature range or, alternately, no change in egestion rate within a certain range, followed by elevated rates with further temperature increase (reviewed by Hargrave 1972). However, Rhoads (1963) found that a slight temperature increase (from 16 to 21°C) caused a five-fold increase in the rate of sediment egestion by Yoldia limatula (Protobranchia).

Chemical Factors Which Affect the Energy Budget

In a gross manner, the amount of energy stored in plant tissue during primary production determines the amount of energy which can flow into the consumer levels, according to thermodynamic restrictions at each level of transfer. Although one seldom sees an instant in nature where plant matter is not available to aquatic herbivores, the availability of digestible organic matter may be limiting, and in this way food quality (and not simply energy stored in plant biomass, as measured by calorimetry) acts as a direct controlling agent on the amount of energy which is passed on to consumers. The complex of factors which regulate the species of plants and the variation in food quality within a species governs the rate of uptake and release of energy from this energy pool (Boyd and Goodyear 1971).

Food and Feeding Preference

In order to adequately evaluate food supply of aquatic animals (herbivores and detritivores) one must know: (1) the percentage composition of food materials in the gut (or some other meaure of what is eaten). (2) the digestive capabilities of the organism - i.e., whether cellulose is digestible and the efficiency of utilization of the organic components; and (3) the percentage composition of these organic components in the food consumed. Cellulose may be useless as a food by a herbivore, but when partially degraded by microbial action the food may increase in quality for detritivores. Protein is essential for growth and due to the large interspecific variations in protein content of different leaves (see Kaushik and Hynes 1971) feeding rate and growth potential of consumers are therefore directly affected. Again the food quality (protein content) can be improved by the action of microbes. Numerous studies reveal that live leaf material may be rejected because of toxic chemicals (Latheef and Harcourt 1972), that fresh leaves with no toxic

effect may be rejected because of low food value (see Boyd and Goodyear 1971), or that the effect of microbial action increases the feeding rate and energy assimilation on dead leaves (Triska 1970, Mackay 1972, Mackay and Kalff 1973). For these reasons it is desirable to measure total protein (total N x 6.25) and the amount of noncell wall material (see Boyd and Goodyear 1971) as an index of the food quality. Generally, if protein levels are adequate, then other essential nutrients also are present in required levels.

Selection for food of high quality has even been demonstrated in tubificid worms feeding on sediment (see Monakov 1972). Selection for the leaf parts of highest food quality by early instars has been noted (McDiffett 1970, Latheef and Harcourt 1972). Lasker (1966) showed that for Euphausia pacifica, an omnivorous crustacean, diatoms or Artemia nauplii are suitable foods, but Artemia is preferred. Certain diatoms also are selected over others and if an undesirable diatom species is presented as food one day, a 1-2 day lag phase occurs before the animal will feed when a desirable food is presented.

Feeding Rate

Feeding rate is determined to a great extent by the quality of food. Probably the most extensive investigation of the feeding rates on various food types is the Russian work reported by Monakov (1972). They found that as the availability of suitable food declines in mud worked by Limnodrilus (Tubificidae), feeding rate increases for a period, then declines and finally stops. As discussed before, a temperature increase can greatly speed up ingestion of mud, but increased quality of the food reduces the ingestion rate (Monakov 1972, Chua and Brinkhurst 1973).

A similar relationship was found by House (1965) with Celerio euphorbiae (Lepidoptera) larvae. The larvae ate progressively more food as the percentage of nutritive materials was decreased by the addition of nonnutritive cellulose. Increased consumption acted as a compensating mechanism for a decrease in food quality. Only a slight increase in assimilation efficiency was detected as food quality decreased. When the same insects were presented with an unbalanced diet (excess amino acids and vitamins), ingestion rate declined so that metabolic imbalance was avoided. As a result, growth rate decreased, too. On the contrary, Conover (1966) and Reichle (1967) found that gut retention time was increased when not enough energy could be supplied by the available food. This is an alternative form of compensation for low food quality.

Growth

House (1965) indicated that growth rate is determined largely by the adequacy of the diet. Slow growth is a reflection of a poor diet. It is also a response to a seasonal lack of food of good quality. Stockner (1971) observed that with enrichment of the algal mat of a hot springs Hedriodiscus had three generations per year instead of two, due to greatly increased growth

rates. Latheef and Harcourt (1972) found assimilation efficiencies of a beetle to be nearly identical on potato and tomato leaves, although the net growth efficiency (G/A) on potato leaves was two to four times greater than on tomato leaves. Presumably a greater proportion of the energy was used in respiration when the beetles fed on tomato leaves. Greater fecundity was observed when the food was adequate, while greater mortality and lower fecundity were observed when the food was inadequate.

Respiration

Levanidov (1949) found that the metabolic rate of Asellus aquaticus increases after food is ingested. Dietary protein causes a 30% increase in in metabolism, while fats and carbohydrates cause a 4% increase. Protein, fat, and carbohydrate have caloric values of 5.50, 9.30, and 4.10 kcal/g, respectively (see Boyd and Goodyear 1971). The physiologic value of protein is 4100 kcal/g due to its greater specific dynamic action (SDA) (Nelson and Scott 1962). Because of the greater energy expenditure required in assimilating proteins, Welch (1968) found that net growth efficiency of carnivores is lower than for herbivores, while assimilation efficiencies for carnivores are higher.

Assimilation Efficiency

Monakov (1972) found the index of assimilation (percent of body carbon assimilated daily) for Paramecium caudatum to vary from 0.9 to 13 to 74.4%, depending on whether the animals were feeding on Anabaena, bacteria, or Chlorella, respectively. The relationship between feeding rate and assimilation efficiency is not always positive. For example, with some filter-feeding crustaceans, ingestion rate on Chlorella is high but assimilation efficiency is low. Also the efficiency of utilization of certain algal species varies widely among invertebrates (see Monakov 1972). Lawton (1970) found that assimilation efficiencies for Pyrrhosoma feeding on different prey varied only from 79.9 to 90.6%. Presumably since carnivores feed on prey which vary little in caloric content and contain a high percentage of protein, assimilation efficiencies do not experience the extremes found in herbivores.

Schindler (1968) found assimilation efficiency of Daphnia magna to be directly related to caloric content. He found fresh algae, with a high caloric content was assimilated more efficiently than was detritus, with a lower caloric content. A concomitant reduction in feeding rate was noted when the Daphnia were feeding on detritus. Similar results were obtained by Bell and Ward (1970). Assimilation efficiency may be small, however, even on food of high caloric content if the food is indigestible (Boyd and Goodyear 1971). Hyalella azteca is unable to digest cellulose and lignin. A wide variation in assimilation efficiency (5-75%) was noted for several species of algae. In addition, assimilation efficiency determined on the basis of total organic matter was significantly less than that based on changes in protein content between food and feces (Hargrave 1970). Bocock (1963, see Hargrave 1970) found assimilation efficiency of a millipede to vary between

10.5% for dry matter and 43.2% for crude fats. Hubbell et al. (1965) and Reichle (1967) noted that there was a selective assimilation of organic matter of higher caloric value. Reichle found that for two litter feeders the caloric value of food digested was 1.15-1.17 times higher than that of the food ingested.

Humic substances leached from the leaves can inhibit assimilation of food in the gut (Blazka 1971). Antibiotics can be applied to the medium to limit the growth of bacteria (Hargrave 1970, Kaushik and Hynes 1971) but the possible effects on the gut microflora of the organism are uncertain. Certain insects which feed on leaf material have a symbiotic intestinal microflora which converts cellulose to assimilable products (Mason 1970b, Bjarnov 1972).

Interrelationships of Energy Budget Components

Energy flow of a population or a community as defined by Stockner (1971) is production + respiration during time (t). Because of the relation (production + respiration = assimilation), the absolute amount assimilated can be expected to vary considerably with changes in biological or physical conditions during the season; but as Lawton (1970) found, assimilation efficiency may remain constant during these changes. Temperature, a primary physical factor, controlled feeding rate but not assimilation efficiency. *Pyrrhosoma*, however, being a carnivore, is less subject to great seasonal fluctuations in caloric content or abundance of food than some herbivores might be, so chemical changes in food quality are not so significant.

McDiffett (1970) shows one of the clearest examples of the dynamic relationships between energy budget components in a graph of cumulative energy requirements (growth and respiration vs. time) for *Pteronarcys* during a 17-month period. Curves for cumulative ingestion and egestion are parallel during this 17-month period although the slopes change throughout. This is an indication that assimilation efficiency is nearly constant during this period. The curves for growth and respiration show that respiratory expenditures of energy become greater in proportion to growth with nymphal age. A gradual decrease in net production efficiency (P/A) also shows that a smaller percentage of assimilated energy is diverted to growth as the animal ages. Stockner (1971) showed an interesting relationship of growth to respiration in *Hedriodiscus* populations inhabiting two hot springs. Larvae in one spring (never exposed to temperatures greater than 27°C) put only 61% of assimilated energy into respiration, while larvae in the other spring at 35°C diverted 76% of this energy to respiration and the remainder to growth.

A good description of the direct relationship between ingestion and egestion rates is from Hargrave (1972). Ingestion rates calculated from knowledge of assimilation efficiencies and egestion rates with specific food sources were very similar to ingestion rates calculated independently by radiotracer experiments. Egestion rate is then a useful index of ingestion rate. The similarity of these rates was also shown by McDiffett (1970), Lawton (1971), Stockner (1971), and Mukerji and Guppy (1973).

It is interesting to note the similarity of energy distribution in the budgets of different species. Table 1 presents a synopsis of selected studies of individual or population energy flow. Values from studies by Stockner, Teal, McDiffett, Lawton, and Lasker are means of monthly studies conducted on populations for at least one year. Hargrave only studied the adult stage of H. azteca. Only Hargrave and Levanidov measured excretory losses. If the importance of this loss is as great as indicated, 36% of assimilated energy may be lost as excretion (Hargrave 1971). Studies summarized from Coffman et al. (1971) are mean values of G/A for the herbivore and carnivore populations of several aquatic communities.

There is little evidence to support Welch's (1968) statement that carnivores tend to have lower net growth efficiencies (G/A) than herbivores. Nonetheless, a generally applicable relationship can be found between growth and respiration. A comparison of many studies of populations of short-lived aquatic and terrestrial invertebrates showed that P/R values range from 0.2-0.4 (Hargrave 1971). Hargrave found a P/R ratio of 0.31 for Hyalella. Animals with a long life span would be expected to have lower P/R ratios because they exhibit growth curves with gradual slopes. However, the P/R ratios during the rapid growth phase might be comparable.

Mukerji and Guppy (1970) and Latheef and Harcourt (1972) found a direct linear relationship between feeding rate and growth. The presence of an alkaloid in tomato leaves caused a reduction in feeding rate by Leptinotarsa (Coleoptera) so that the threshold level of energy demand was met (basal metabolic rate or BMR) and a small amount of energy was available for growth (Latheef and Harcourt 1972). When the preferred food was supplied, a greater amount of the assimilated energy was diverted to growth. Lasker (1966) showed that a temperature increase of only 3.5°C caused a reduction in feeding rate coupled with an increase in metabolic demands, resulting in drastic reduction in the growth rate of Euphausia.

The numerous Russian studies reviewed by Monakov (1972) clearly show the link between food concentration and type, feeding rate, and assimilation and growth. In filter-feeding Cladocera, the daily ration (amount ingested) and absolute amount assimilated increase with food concentration until unusually high food concentrations are reached, after which point the ration increases and assimilation efficiency decreases. Assimilation efficiency generally is constant for a specific food type until the optimal food concentration is reached; absolute assimilation reaches a maximum at this value. With certain other Cladocera (e.g., Polyphemus) the index of assimilation (percent of body carbon assimilated/day) does not form a plateau but falls sharply at high food concentrations. The same relationships of food concentration and index of assimilation are observed in copepods. The index of of assimilation is stable at natural food concentrations.

TABLE 1. Energy Distribution in Populations and Communities from the Literature

Populations	G/A	R/A	Ex/A	S/A	U/A	Rp/A	Investigator
Hedriodiscus truquii (Diptera)	21.9-39.1	60.0-77.1					Stockner 1971
Pteronarcys scotti (Plecoptera)	31.9	65.8	2.3				McDiffett 1970
Hyalella azteca (Amphipoda)	15.0	48.8			36.2		Hargrave 1971
Pyrrhosoma nymphula (Odonata)	48.3-49.3	47.9-49.0	2.7-2.8				Lawton 1971
Euphausia pacifica (Euphausiacea)	9.4	66.4	15.3			8.9	Lasker 1966
Leptinotarsa decemlineata (Coleoptera)	15.2-63.7						Latheef and Harcourt 1972
Armadillidium vulgare (Isopoda)	~31	~80				6	Reichle 1967
Cylisticus convexus (Isopoda)	~17	~80				9	Reichle 1967
Chrysomela knabi (Coleoptera)	28						Crossley 1966 (see Reichle 1967)
Orchelimum fidicinium (Orthoptera)	37						Odum and Smalley 1959
Tubifex tubifex (Oligochaeta)	62	38					Ivlev 1939 (see Welch 1968)
Asellus aquaticus (Isopoda)	16.4	81.3	2.3				Levanidov 1949 (see Welch 1968)
Lestes sponsa (Odonata)	63.7	36.3					Fischer 1967 (see Welch 1968)
Daphnia pulex (Cladocera)	56-73	27-44					Richman 1958
Potamophylax cingulatus (Trichoptera)	35.6	56.7	1.9	5.8			Otto 1974
Hydropsyche fulvipes (Trichoptera)	53.0	47.0					Edington and Hildrew 1973
Diplectrona felix (Trichoptera)	51.6	48.4					Edington and Hildrew 1973

(G/A calculated from data summarized by Coffman et al. 1971)

Communities	Trophic level Herbivore-detritivores	Carnivores	
Linesville Creek	42.92	42.83	Coffman et al. 1971
Silver Springs	78.20	21.36	Odum 1957
Root Spring	31.73	133.6	Teal 1957
Cone Spring	35.63	87.23	Tilly 1968

At optimal food concentrations, where absolute assimilation is maximal, growth probably is also maximal. During periods of low food supply respiration rate drops (see Stockner, 1971), the rate increases with heavy feeding (see Lawton, 1971). At any temperature within the normal feeding range, respiration rate reaches a plateau depending on the level of feeding, physiological state, and food concentration; energy assimilated in excess of metabolic requirements is stored as growth. During metamorphosis and molting, P/R is zero or very low. Only mean P/R ratios obtained over the life span can be meaningfully compared. Lasker (1966) found that occasionally the percentage distribution of assimilated energy between respiration and growth measured by short-term ^{14}C experiments was far different from the percentage distribution between growth and respiration measured by other means over a longer time span. The effect of feeding rate on excretion rate may also have to be considered, as Hargrave (1971) suggests that starvation may lower the excretion rate.

DESCRIPTION OF STUDY AREAS

Rattlesnake Springs and Snively Creek

In this research Simulium spp. were collected in Rattlesnake Springs and Cheumatopsyche analis were collected in Snively Creek. These two small spring-fed desert streams are located in the 33,350 ha. Rattlesnake Hills Research Natural Area on the Atomic Energy Commission's Hanford Works Reservation in Benton County, Washington (46°30' N and 119°40' W). The research area, also known as the Arid Lands Ecology (ALE) Reserve, has been studied as part of the IBP Grasslands Biome Project by the Pacific Northwest Laboratory (Battelle Memorial Institute). Watercress (Rorippa) contributes most of the autochthonous production in each stream. Cladophora, Spirogyra, and diatoms are seasonally abundant in Rattlesnake Springs but periphytic algae is very sparse in Snively Creek. Very few diatoms were carried in the suspended load, which was primarily inorganic sediment. The study area of Snively Creek was shaded in places by low riparian shrubs while that of Rattlesnake Springs was closed by willows through most of the study area. The surrounding desert vegetation of these streams was an unlikely source of input.

Rattlesnake Springs arises as seeps in a canyon bottom at approximately 213 m above sea level and flows easterly approximately 1.5 km before being consumed by riparian vegetation and influent seepage (Brown 1970). The stream is characterized principally by reaches and occasional areas with shallow slow-flowing pools choked by emergent vegetation. Several short riffles are found having substratum materials composed of pebbles, fine gravel, and some larger stones. Simulium were collected from riffles next to the laboratory and also below a pond further downstream. The mean discharge was 105-110 m^3/day (E. Wolf, unpublished data). The minimum and maximum daily water temperatures were 2.8 and 22.3°C recorded on December 13 and May 17, 1973, respectively. The maximum diel temperature change was 6.7°C. Temperature changes near this magnitude (6.0-6.7°C) were found from March 23 to June 6. The minimum diel water temperature change was 0.1°C.

Snively Creek arises 5.8 km south of the Rattlesnake Springs laboratory from springs at an elevation of about 427 m, flows north and finally percolates into the ground before reaching Rattlesnake Springs (Brown 1970). Cheumatopsyche were found in riffles which had substratum materials composed of sand, gravel, pebbles, and many coarser stones (fist-sized or smaller).

Deep Creek

Tricorythodes minutus were collected from station 2 of the IBP-Desert Biome study at Deep Creek. The study area is located in Oneida County in southeastern Idaho (42°8' N, 112°40' W) at an elevation of 1406 m above sea level. The principal substratum materials in this study area are gravel, clay, and silt. Water flow arises at the head of the study area from Holbrook Springs. Mean discharge is 0.7 m^3/sec; the mean width and depth are 5.9 m and 0.42 m, respectively. Due to diversion of the stream water at the springs for irrigation, the discharge is periodically reduced severely during spring and summer months. The relatively constant 17°C water arising from the springs may increase in temperature to around 29°C during low flow periods and may drop to 3°C during snow-melt runoff. Mean annual precipitation is 40.6 cm. The average stream gradient in the 363 m study area is 0.240%.

The major macrophytic species in the stream are Potamogeton pectinatus, P. filiformis, and Eleocharis macrostachya. Cladophora contributed the largest percentage of all plant production during the year. Periphytic diatoms were abundant on the riffles in spring and autumn. The invertebrate species most abundant by numbers per meter square in the benthos were Optioservus divergens, Hyalella azteca, Tricorythodes minutus, Chironomidae, Hydropsyche occidentalis, and Baetis tricaudatus, respectively (Minshall et al. 1973).

METHODS

Outline of Procedures for Analysis of the Energy Budget

The methodology for determining energy flow through an organism using a radioactive tracer can be subdivided into several basic procedures: (1) selection of a suitable animal; (2) determination of food availability and foods of major dietary importance; (3) growth of a suitable food and its labelling with radioisotopes; (4) presentation of this food to the organism; and (5) measurement of radioactivity of food, animal tissue, and feces of the animal, plus metabolic or physical losses of activity from food, animal, or feces. The overall approach used in this study was to create a laboratory microcosm which reproduced essential physical variables of the natural stream environment so that realistic rates of ingestion and assimilation could be determined. Ingestion rates determined with isotopes in these experiments were compared with one another to assess the effect of the physical variables and were also compared with rates obtained by non-radiotracer methods. Two radiotracer methods (the ^{14}C and dual-label ^{14}C-^{51}Cr method) and the ash-ratio technique were used in determining assimilation efficiency. Four types of food sources were used in feeding experiments: bacteria (*Esherichia coli*), diatoms (assorted species cultured on slides), blue-green algae (*Lyngbya* and *Anabaena*), and watercress detritus (a finely ground suspension). The algal or detrital suspensions were not bacteria-free, so it is possible that some microbial organic material was ingested and assimilated in these cases too. It was considered of greater value in these experiments to measure ingestion and assimilation of a normal (mixed) algal community than that of a pure culture of a single species.

Selection of the Animal

Animals used in this research were considered to be prime energy movers in their communities on the basis of high population densities in certain stream sections throughout the year. *Simulium* spp. from Rattlesnake Springs and *Cheumatopsyche analis* from Snively Creek made convenient animals to study because larvae were found in abundance throughout the study period. Since both species are filter feeders a water current was required for feeding. This mode of feeding permitted the animals to become acclimated to conditions in the feeding chamber before introduction of food. Food concentrations could be measured easily. Feces collections from *Simulium* were readily obtainable but collection of feces during feeding on nonlabelled food was not possible. The feeding behavior of *Simulium* permits transfer of larvae to a suspension of nonlabelled food

for gut clearance but this is not possible with Chematopsyche, which requires up to 24 h to establish a net for feeding. Chematopsyche were able to survive at least seven days at room temperature in still water but the tolerance of Simulium to still water was much less.

Tricorythodes minutus from Deep Creek has been identified from the Desert Biome-IBP studies as an organism contributing heavily to the secondary production of the stream and as having a high turnover rate (Minshall, personal communication). T. minutus was found in Rattlesnake Springs also but was a minor constituent of the mayfly fauna. A study of the energetics of this species could be reinforced by food habit studies of the major aquatic insects of Deep Creek conducted by Koslucher (Koslucher and Minshall 1973) and growth and life history studies on Tricorythodes of Deep Creek conducted by R. L. Newell (unpublished data). Tricorythodes is abundant in shallow slow-moving backwaters where it collects diatoms and detritus (largely inorganic material) from the sediments. T. minutus moves slowly through the deposited sediments gathering material toward its mouth with its forelegs. This mode of behavior makes Tricorythodes an especially easy experimental animal to feed. This robust mayfly was found to be very resistant to temperature change and reduced oxygen tension and could be successfully maintained in still water at room temperature for several weeks. Under these conditions nymphs were easily cultured through emergence. Nymphs blotted dry and exposed to the air for 15 min resumed activity when rewetted. These animals were also unaffected by handling and resumed eating immediately after disturbance. T. minutus required no elaborate culturing equipment. Ingestion rates measured on this animal in the laboratory are more apt to correspond to field values than are rates determined for a species with more complex demands.

Determination of Food Habits

For the purposes of this work only the relative abundance of algae vs. detritus (organic or inorganic) in the guts of consumers was considered important. In addition, the major algal taxa found in the guts of Simulium were identified to genus. An extensive survey of the diatoms of Rattlesnake Springs by Lippert (unpublished data) aided the identification. Food habits of Tricorythodes are described by Koslucher and Minshall (1973). Examination of gut contents in my study was done by severing the head and a small portion of the tip of the abdomen above the anus to free the gut, followed by easing the gut out of the body with fine-tipped forceps and a dissecting needle. Guts were removed quite easily from fresh Simulium larvae, but it was helpful to store Tricorythodes and Cheumatopsyche briefly in formalin to harden the gut lining before dissection. Occasionally the gut contents could be pushed out without tearing the gut wall, which was then removed. Otherwise, quantitative removal of

the gut contents without damage to the gut wall was done by applying a small amount of 3% H_2O_2 to one end of the gut with a fine paint brush; this procedure created oxygen bubbles, which pushed the gut contents neatly onto the coverslip. Gut contents were then examined in a wet mount or by use of a semi-permanent (glycerin-gelatin) mounting medium. This was ideal for examining material which could not be dried prior to mounting. Lowering the slide onto the coverslip minimized the movement of material to the edge of the coverslip.

Microbes associated with gut material were not counted. The importance of bacteria and fungi cannot be disregarded as a source of food but probably made up only a small fraction of gut load. However, preferential digestion of these foods would make them significant regardless of their relative abundance.

Preparation of Food for Use in Experiments

Algae were cultured on a medium designed for freshwater diatoms. The pH of the medium must be adjusted to near neutrality as it is slightly basic after preparation (Kates and Volcani 1966). This medium was selected in preference to others, in which the exact concentration of elements is indefinite due to use of a soil extract to supply certain minor elements and vitamins. Vitamins, however, are not essential for the growth of most types of freshwater diatoms (Altman and Dittmer 1968).

Two methods were used in preparing food for *Tricorythodes minutus* in experiments 1 through 5. An initial attempt to culture diatoms involved scraping and washing them from rocks and lowering the pH of the diatom suspension to 3.5 for 5 min to eliminate the microconsumers. The suspension was then filtered, washed, and resuspended in a small amount of water and mixed for 2 min at high speed in a Waring blender to break up colonies. This suspension was painted on a sheet of styrofoam (Sperling and Grunewald 1969), dried slightly to attach cells and then placed in a specially designed aquarium which accommodated the 12 cm x 23 cm sheet with only a small volume remaining for the circulation of medium over the face of the styrofoam. A second styrofoam sheet was suspended vertically in midwater at station 2 of Deep Creek and allowed to colonize for one week. Loose sediment and detritus were rinsed off the sheet which was then incubated in the aquarium to culture periphytic algae. Algae were grown under continuous illumination (approx. 450 ft.c.) from a bank of cool white fluorescent bulbs and an incandescent bulb to supply the longer wavelengths. Each aquarium was spiked periodically with 50 μCi of $NaH^{14}CO_3$ and the medium was changed every two weeks during the six-week culturing period. After a culturing period of three to four weeks in a diatom medium, the styrofoam sheet in one aquarium held a nearly pure culture of *Lyngbya*. The other sheet held primarily *Anabaena* and *Achnanthes*

lanceolata, with minor amounts of Phormidium retzii, Schizothrix callsicola, Cosmarium, Nitzchia, and Cocconeis. Although the brief lowering of pH eliminated most microconsumers, it appears that the shift to a predominance of blue-green algae was a normal outcome or that the effect of low pH on diatoms was adverse. Subsequent experimentation revealed that pure cultures of diatoms were easily isolated after culturing mixed diatom suspensions streaked on agar plates with the algal medium. Techniques for creating single species algal cultures are given by James (1971).

In the Tricorythodes experiments 1-3 the food texture was tough and leathery as it grew on the styrofoam. This material was broken into smaller pieces as it was peeled off the styrofoam support but the texture was unchanged. Animals clung tenaciously to these pieces when placed in petri dishes for feeding. In experiments 4 and 5 the food sources used were Lyngbya and Anabaena, respectively. These foods were blended thoroughly in a Waring blender until a homogeneous cottony consistency was obtained. Microscopic examination revealed that trichomes were broken up into short units but cells within the shortened filaments were intact. A superabundance of food was present in each petri dish for these experiments and a great many more habitat sites were created by increasing the surface area of the food.

Three food sources were prepared at Rattlesnake Springs for feeding experiments: diatoms, watercress, and bacteria. A natural diatom community was cultured in Rattlesnake Springs and in an outdoor artificial stream with flowing Columbia River water on glass slides which had been inserted vertically into slits cut at intervals around the circumference of a large rubber stopper (Sladeckova 1962). Slides were suspended in the stream for 1 week, after which they were freed of invertebrates and transferred to a 10-place glass slide holder and immersed in a 500-ml beaker filled with filtered diatom medium. Diatoms were grown in a Hot pack incubator at 18°C on a 16:8 h light-dark cycle. Light from six cool white fluorescent bulbs diffused through a sheet of white translucent plastic illuminated the chamber. As a rule 60 μCi of $NaH^{14}CO_3$ was added initially to the culture medium, followed in 4 days by a second dose of 20 μCi. After one additional day of incubation the slides were removed, rinsed gently in distilled water and placed on a sheet of Parafilm. Then 6 to 8 ml (240 to 320 μCi) of a $^{51}CrCl_3$ solution was applied evenly over the slides, which were then stacked, wrapped in the Parafilm and sealed in a plastic bag. Slides were held in the dark at 3°C to limit respiratory losses of $^{14}CO_2$ from the cells. A greater degree of labelling was achieved by direct application of ^{51}Cr than by adding 1 μCi/ml of cell suspension as specified by Calow and Fletcher (1972). Before use diatoms coating the slides were either rinsed with distilled water or were scraped off the slides, suspended in filtered creek water for 15 min and then washed down several times on a membrane filter to remove any unbound ^{51}Cr.

Diatoms from the Rattlesnake Springs inoculum found on slides after one week culturing in the laboratory were primarily Achnanthes lanceolata and Synedra ulna. Those cultured from the Columbia River water were primarily Melosira and Cymbella with minor numbers of Fragilaria sp., Gomphonema sp., Synedra rumpens, Epithemia turgida, and Navicula sp.

To label watercress, small immature plants were removed from the stream and placed side by side in a desiccator jar in 2 cm of water so that the bottom of the container was covered but shading was minimal. The jar was then placed without a lid in an incubator at 18°C. After two days of growth the plants became erect and neatly arranged in the jar. At this time 80 μCi of $^{14}CO_2$ was released with H_2SO_4, from its bicarbonate salt along with carrier CO_2 from 100 mg Na_2CO_3 to bring the total CO_2 content in the jar to approximately 0.5%. The jar was placed in the stream at 3:45 pm on May 17, 1973 and incubated for 3.0 h under natural lighting conditions. The jar was rotated every 15 min during this period to eliminate shading effects. At night much of the newly synthesized ^{14}C products probably travelled to the roots. An additional 80 μCi was released about 12 noon the next day and 3.0 h were allowed for photosynthesis, with rotation of the jar as before. After this last incubation a Geiger-Mueller counter showed younger leaves to have 2 to 3 times more activity than older leaves. Only upper healthy leaves were collected; stems, roots, and petioles were discarded. The collected leaves were dried at 60°C in a 50-ml Erlenmeyer flask and were crushed into fine fragments with a glass rod. Approximately 6 cm^3 of wet watercress were inoculated with 51 μCi (6 ml) of $^{51}CrCl_3$ solution three days before Tricorythodes experiment 9 and with 33.8 μCi (or 4 ml, corrected to counting date) before experiment 10. Before each use, the watercress was washed several times with distilled water, soaked 20 min in distilled water and drawn down on a 5-μm membrane filter.

Bacteria were also used as a food source. Fredeen (1964) successfully cultured Simulium on bacterial suspensions and found E. coli, a gram-negative microbe, to be a very suitable food. E. coli was grown on an M-9 salts medium (see Appendix A) using dextrose as a carbon source (Roberts et al. 1957) at 37°C in a shaker bath. To avoid carmelization, the dry dextrose should be autoclaved alone and then added aseptically to 50 ml of the salts medium. Five ml of the culture was added to each of two 100-ml volumes of the salts medium in 250-ml flasks equipped with rubber stoppers fitted with intake and exhaust ports. In addition to vigorous shaking, a steady flow of sterile air (passed through a dense bed of sterile cotton) was bubbled through the solution. Exhaust gases passed through a dilute KOH trap to catch respired $^{14}CO_2$. Twelve hours were allowed for cell growth after which an additional 50 ml of medium was added to each flask with 10 μCi of sterile glucose-^{14}C and 4 hours were allowed for assimilation of the ^{14}C. Immediately the culture was heated to 80°C for 10 min to kill the cells. The suspensions were stored in the dark at 3°C. It was advantageous to collect the cells by centrifugation rather than by filtration,

wash them to remove salts, and then inoculate them with approximately 100 μCi $^{51}CrCl_3$. In this way the labelling solution was more concentrated and loss of ^{51}Cr by precipitation in the salts medium was avoided.

Since maximum and uniform ^{14}C labelling of cells occurs in the log-phase of growth, it might be advantageous in the future to monitor growth by following the increase in turbidity in bacterial cultures, the increase in chlorophyll of diatom cultures, or possibly the increase in protein with bacteria and diatoms. Protein analysis could be performed using standard micro-Kjeldahl analysis or the method of Lowry et al. (1951).

Feeding Experiments

Although individual feeding experiments differed from one another with respect to certain physical conditions (e.g. current speeds, duration of feeding), the general procedure followed in each was routine. The basic components of feeding experiments (physical aspects of feeding chambers, preparation of consumer organisms, introduction of the food, sampling the animals and feces, and determination of feeding rates) will be described to show the degree of similarity between experiments.

Feeding Chambers

Several different types of chambers were assembled to fit specific feeding requirements of the animals being studied (see Fig. 4). Tricorythodes from Deep Creek was found at the sides of the stream in vegetation and in the slower moving waters of reaches and silted margins of riffles. It feeds on detritus and diatoms which it collects on the bottom. Excellent results have been obtained in culturing this animal in the laboratory. R. L. Newell (personal communication) has been successful in rearing Tricorythodes to the adult stage using a deep plastic petri dish with a hypodermic needle inserted through the side and connected to an air source for aeration. This method of maintaining Tricorythodes was followed in all my experiments. Water temperatures were maintained at 21-23°C throughout all experiments conducted on Tricorythodes at ISU and at 18°C in the Rattlesnake Springs laboratory. Animal density per dish was varied according to the apparent number of sites available for occupation; i.e., when food was extremely abundant in a dish, the number of habitat sites was large, whereas when only a small amount of food covered the dish, fewer sites were available. Animal densities were subjectively adjusted so that animals spent little time searching or competing for food. With fewer animals per dish there is less chance of an animal reingesting feces, thereby resulting in elevated AE values as the ^{14}C activity of food is reduced. Animal density in Tricorythodes experiments

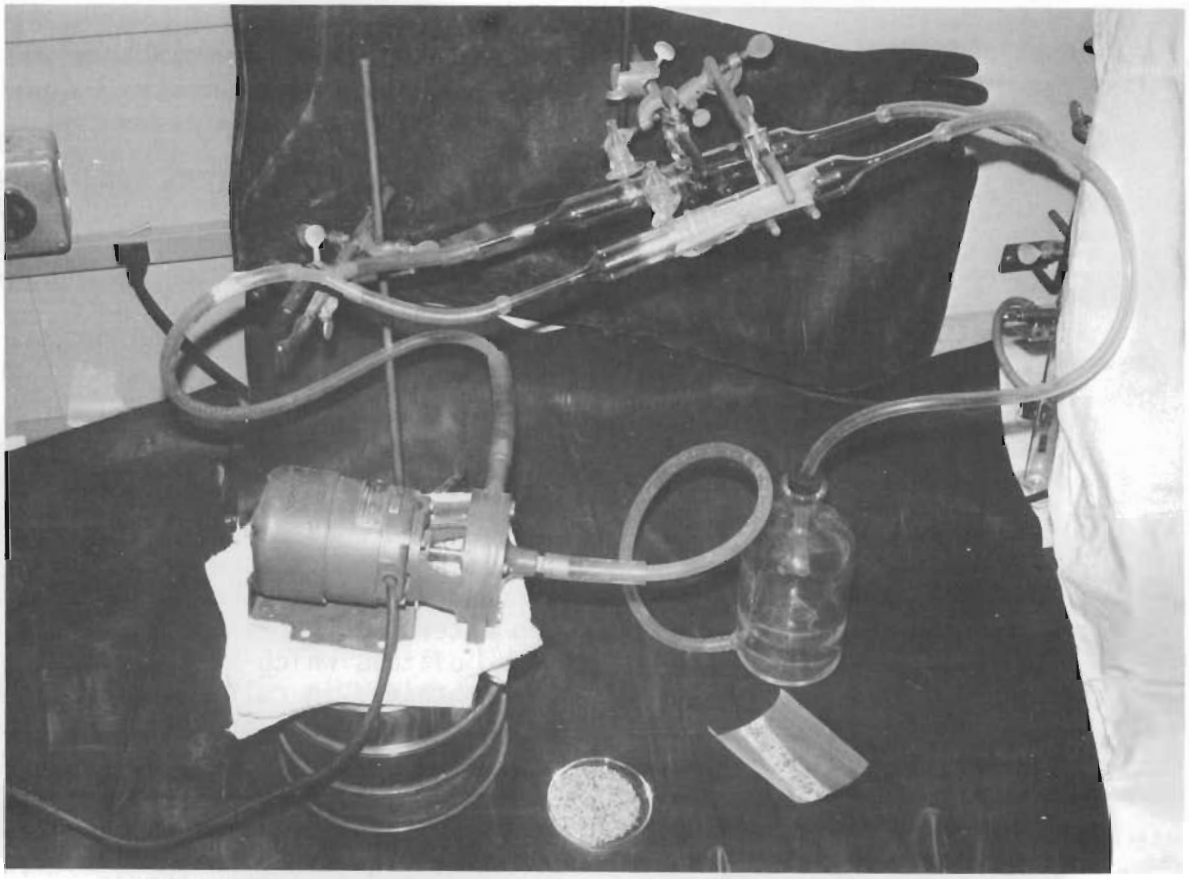


FIGURE 4a. Feeding Chambers (Glass Cylinder Chamber)

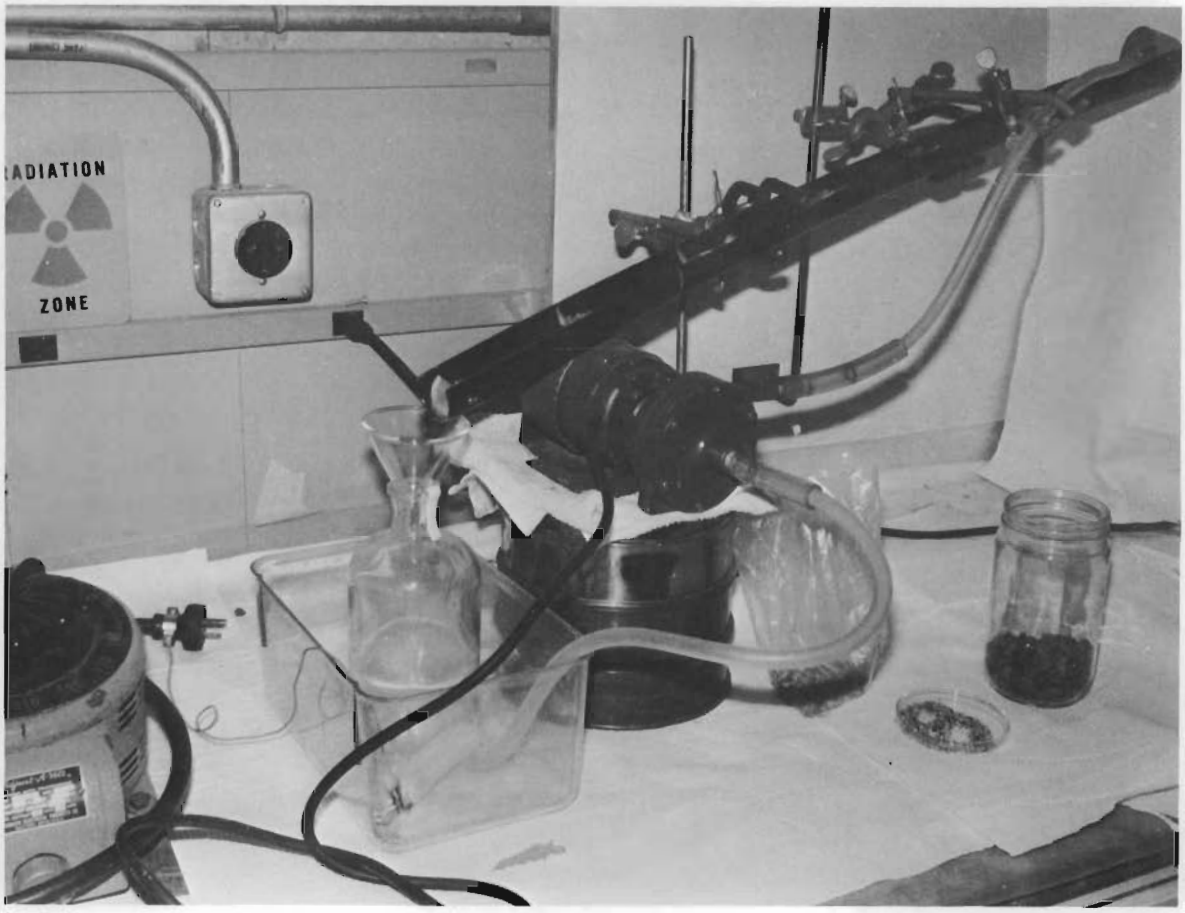


FIGURE 4b. Feeding Chambers (Trough Feeding Chamber)

FIGURE 4c. Feeding Chambers (Plexiglass Channel)

1-5 in which the blue-green algae filled 75% of the petri dish were approximately 8037/m^2. This density was about one-third the mean Tricorythodes densities in Deep Creek (station 2) (Minshall et al. 1973) and far less than total animal densities, which were about 120,000/m^2.

Small gravel (2-5 mm diam.) was scattered over the bottom of each dish to provide a suitable substratum. The gravel covered about 20% of the bottom of the dish and food generally covered the remainder. The dishes were placed on a dark background to avoid a negative reaction by the nymphs to unnatural lighting conditions. A mesh screen divided each dish in Tricorythodes experiment 1 but this was removed when it was found that some animals clung to the screen throughout the experiment in preference to feeding. For Tricorythodes ingestion rate experiment 6 the nymphs were allowed to feed directly on the diatom-covered slides (2 slides per dish). In experiments 7, 8, and 9 animals were allowed to feed directly on the slides (3 per dish) and on a 47-mm membrane filter on which had been filtered a thick layer of cells which had fallen off the slides. The membrane filter was placed upside down because it was found that the animals preferred to cling on the underside of the filters. Slides were supported on gravel so that the nymphs could move underneath the slide. The diatoms on the slides formed a fairly thin covering; generally it was not possible for an animal to burrow into the mass. In experiment 11 the diatoms were scraped off the slides, rewashed in distilled water and placed loose in the dishes so that an animal could cling to clumps of the diatoms.

Three types of feeding chambers were employed in Simulium and Cheumatopsyche experiments at Rattlesnake Springs. A summary of the primary physical parameters in Simulium and Cheumatopsyche feeding experiments is given in Table 3 and 4, respectively. Both animals are filter feeders, requiring food particles of appropriate size in suspension. Both animals show a preference for rapid current. The first feeding chamber used was a glass cylinder (23.0 cm x 2.9 cm) with two halves joined by 40/50 ₮ ground glass joints (Figure 4a). The ends of the tube were drawn out to fit into 10-mm I.D. tygon tubing, which led to a D-6 Eastern pump. Water flowing through the chambers entered a 1-liter reservoir immersed in an ice bath. With this arrangement water temperatures were held between 17-18°C (experiments 2-6, see Table 3). Blackfly (Simulium) larvae were placed on two glass slides, which were then inserted into the cylinder. The cylinder was filled slowly with water to allow animals to attach securely to the slide before the pump was started. A cone-shaped piece of monofilament netting (Nitex) (390 μm) covered the outlet port of the chamber to prevent animals from being swept away. Approximately 700 ml of water was needed to operate this system. Under proper conditions blackfly larvae can be collected directly on slides held in the stream for a 3-day period. These slides can then be inserted directly into the chamber. Fredeen (1964) stated that if experimental feeding conditions

TABLE 3. Summary of Essential Experimental Conditions for *Simulium* Experiments 1-12

Experiment	Food	Acclimation Time	Feeding Time	Water Temperature (°C) Experimental	Water Temperature Stream Daily Mean	Water Temperature ΔC°	Experimental Device	Water Volume in System (ml)
Simulium 1 (January 29, 1973)	*Escherichia coli*	5 min	30 min	24	--	--	Glass cylinder (1 chamber)	500
Simulium 2 (February 14, 1973)	Diatoms	45 min	45 min	18	11.7	0.3	Glass cylinder (2 chambers)	700
Simulium 3 (February 20, 1973)	Diatoms	10 min	1.0 h	18	11.6	3.1	Glass cylinder (2 chambers)	700
Simulium 4 (February 27, 1973)	Diatoms	30 min	45 min	17-18	13.7	1.6	Glass cylinder (2 chambers)	700
Simulium 5 (March 10, 1973)	Diatoms	none	1.0 h	17	13.2	2.3	Glass cylinder (2 chambers)	650
Simulium 6 (March 23, 1973)	*Escherichia coli*	1.5 h	1.0 h	18	14.3	6.3	Glass cylinder	710
Simulium 7 (April 23-24, 1973)	Diatoms	21.0 h	3.0 h	17.5	15.6	3.9	Black plastic trough	--
Simulium 8 (May 10, 1973)	Diatoms	2.0 h	1.0 h	15-17	16.5	5.0	Black plastic trough	--
Simulium 9 (May 26, 1973)	Diatoms	1.5 h	1.5 h	18	16.1	3.7	Black plastic trough	900
Simulium 10 (June 1, 1973)	Diatoms	0.5 h	20.0 h	17-22.5	16.7	5.0	Black plastic trough	820
Simulium 11 (June 14, 1973)	Watercress	0.5 h	6.0 h	17.5-22	17.3	3.5	Black plastic trough	--
Simulium 12 (July 26, 1973)	Watercress	1.0 h	0.75 h[a] 1.75 h[b]	21.5-23.5	19.0	3.8	Plexiglass channel	8000

(a) Feeding time for analysis of ingestion. (b) Feeding time for analysis of assimilation.

TABLE 4. Summary of Essential Experimental Conditions for *Cheumatopsyche* Experiments 1-5.

Experiment	Food	Acclimation Time	Feeding Time	Water Temperature: Experimental	Water Temperature: Stream Temp. During Collection	Experimental Device	Water Volume in System (ml)
Cheumatopsyche 1 (March 27-28, 1973)	Diatoms	21.0 h	1.5 h	17	12.3	Glass cylinder (2 chambers)	760
Cheumatopsyche 2 (April 5, 1973)	Diatoms	none	1.5 h	14-15	14	Plexiglass channel	10,000
Cheumatopsyche 3 (April 12, 1973)	Diatoms	none	3.0 h	19	14.7	Plexiglass channel	10,000
Cheumatopsyche 4 (April 23-24, 1973)	Diatoms	21.0 h	3.0 h	16.5	14.3	Black plastic trough	1,095
Cheumatopsyche 5 (May 10, 1973)	Diatoms	24.0 h	1.0 h	15-17	13.8	Black plastic trough	1,110

are suboptimal, it may be necessary to feed Simulium in the dark or in diffuse light. Larvae in the stream were exposed to bright light, but while feeding in glass cylinders lighting conditions were low and uniform.

The second type of feeding chamber was a piece of black irrigation pipe (5 cm diam) cut in half length-wise to form a trough. The trough (Fig. 4b) was divided into five sections with Nitex net (390 μm) and the D-6 Eastern pump was used to circulate water between the trough and reservoir. Large (1 to 2 cm) stones and small gravel (2 to 5 mm) were placed in each compartment as a substratum. The total water volume required in this system was 1 liter.

The third feeding chamber was a plexiglass stream channel (60 cm long) (Fig. 4c). Water was circulated by a Cole-Parmer pump. Pump inlet and outlet ports were fitted with 16-mm I.D. tygon tubing. Adjustment of the relative position of these tubes in the channel permitted control of current speed. In experiments with Cheumatopsyche, larvae were placed in petri dish chambers with Nitex net lids to allow water circulation into the dish. Dishes were provided with gravel as a substratum and glass slides covered with labelled diatoms as a food source. For work on Simulium in the plexiglass channel, four sterile, smooth-surfaced rocks (8 x 13 cm) were butted against one another in a series to provide a substratum for attachment.

In the plastic trough and glass cylinder feeding chambers water was pumped with a D-6 Eastern pump connected to a variable transformer so that pump speeds could be controlled by adjusting the voltage. Pumping rates and current speed at various voltages in a single glass cylinder are given in Table 5. The glass cylinder had a volume of 118 cm^3 and was 23 cm long. Current speeds were calculated by dividing the pumping rate (cm^3/sec) at selected voltages by the volume of the cylinder and multiplying this figure by 23 cm. Due to the design of the cylinder, current speeds probably were much greater toward the center than the outsides of the chamber. A great amount of turbulence was created in the anterior half of the chamber (slides with Simulium were in the posterior half) as high velocity water in the intake port entered the larger diameter chamber. Simulium larvae lodged in this half of the cylinder were buffeted severely by the currents and often had their abdomens swept back toward the inlet port. In all Simulium experiments in the glass cylinders 80 V was used and current speeds were adjusted by the use of one or two chambers. When two chambers were used in parallel the speeds were half that of one chamber alone. Therefore, the current speed in Simulium experiment 1 was 12.2 cm/sec while speeds in experiments 2-6 were 6.1 cm/sec. In Cheumatopsyche experiment 1 the pump was operated at 70 V, providing a current speed of 4.5 cm/sec. In experiments conducted in the plastic trough, 80 V generally was used with this pump but the use of 100 V had no

TABLE 5. Pumping Rates and Current Speeds in the Glass Cylinder Feeding Chamber at Various Voltages

Voltage	Pump Rate (ml/sec)	Current Speed in Single Glass Cylinder (cm/sec)
60	41.6	--
70	45.4	8.9
80	62.5	12.2
100	71.4	14.0

effect on current speeds, which seemed to be regulated primarily by the slope of the trough. The normal inclination of the trough was 10°35', but a variation of ± 1° could have occurred between experiments.

Current speeds were measured in a riffle next to the Rattlesnake Springs laboratory where larvae were numerous and in the trough and plexiglass channel using a TSI Hot-wire Anemometer (Thermo-Systems Inc., Minneapolis, MN). This instrument has a fine probe which can be precisely positioned in the stream so that current speeds directed at the cephalic fans of larvae could be measured. Also since there are no moving parts in the minute probe, it could be used to measure current speeds in dense vegetation. The probe is part of a bridge circuit which supplies electrical current to the wire embedded in the probe in proportion to the speed of the current, which removes heat. A specific voltage reading on the instrument is related to a particular current speed by means of a calibration curve. The probe was calibrated at 24.45°C but for practical purposes current speeds measured near this calibration temperature are still correct due to the temperature compensating probe, which was fixed near the hot-wire probe.

Velocity measurements were made over the gravel in the plastic trough where *Simulium* larvae were attached in an experiment. Current speeds measured in the trough (Table 6) at normal inclination varied from 23.8 to 53.1 cm/sec throughout the trough. Slight variations in inclination could have provided minimum and maximum velocities of 19.5 and 62.2 cm/sec, respectively.

TABLE 6. Measurements of Current Speed in the Plastic Trough Feeding Chamber at 25°C Water Temperature

Inclination	Angle	Current Speed (cm/sec)
Normal	10° 35'	23.8 - 53.1
Reduced	9° 3'	19.5 - 48.8
Elevated	10° 46'	45.2 - 62.2
Increased	14° 25'	62.2 - 74.5
Extreme	16° 22'	62.2 - >90

Current speeds over the large rocks in the plexiglass stream channel also were measured with the hot-wire anemometer at 25.0°C. The arrangement of the four large, smooth-surfaced rocks was identical to that in *Simulium* experiment 12. Measurements were made at various positions in the channel around the second rock downstream in the sequence of four rocks. The ranges of current speeds measured at each position are given (Table 7); rapid changes in current speeds at a single location were detected due to turbulence and normal water flow properties. There was a distinct zonation of current speeds in the plexiglass channel with greatest speeds on the outside of the channel. Only a slight reduction in current speed was observed on approaching the rock surface to within 3 mm (approx. 11%). The water turbulence near the inside edge of the channel seemed to be very low. Larvae exposed to these conditions distributed themselves readily and the majority were observed spreading their fans in the current. Current speeds were similar to those measured in the plastic trough at normal inclination.

Since the rate of filtration of *Simulium* larvae or even the success in feeding depended on whether a suitable current speed was present, measurements of current speed in the feeding chambers were contrasted with measurements made in three riffle habitats where *Simulium* was very abundant. On March 27, 1973, current speed was measured in the riffle section where the digestion time analysis was performed. As determined by the stick float method, the average current speed (n = 3) was 26.9 cm/sec. Measurements made in the same location on April 3, 1973, with a Pigmy current meter

TABLE 7. Measurements of Current Speed in Plexiglass Stream Channel at 25°C Water Temperature

Position	Current Speed (cm/sec)
1 cm over rock, middle of channel	23.8 - 44.5
Fast side of channel, 1 cm over rock	48.8 - 68.3
Fast side, 3 mm over rock	41.5 - 62.2
3 mm above middle front of rock	28.6 - 44.5
Near bottom of channel on fast side, next to rock	26.2 - 48.8
Fast side of channel, in front of rock but sheltered by rock 1	15.2 - 34.4
Slower side of channel (inside)	28.6 - 41.5
Slower side, extreme edge	41.4 - 28.6

yielded an average speed (n = 5) of 37.6 cm/sec. Measurements made on March 27 just upstream in a faster riffle with the stick float method yielded an average speed (n = 3) of 66.6 cm/sec.

Using the hot-wire anemometer it was found that while Simulium larvae can inhabit the fastest sections of the riffles next to the Rattlesnake Springs laboratory where speeds exceed 95 cm/sec, they also are found in current speeds of 19.5 cm/sec but usually not in slower water. It appears (see Appendix B) that current speeds directed at the heads of larvae are occasionally slightly lower than speeds in the water column immediately upstream from the larvae, but current speed measurements with the hot-wire anemometer do not indicate a significant boundary layer effect. Hynes (1970) stated that the boundary layer is 2-3 mm thick on rock surfaces, which would mean that early instars of Simulium would be shielded from the current. If this were true, then filter-feeding by these minute larvae would be inhibited. However, only slight decreases in current speeds could be detected as the probe approached rock surfaces. Current speeds

in the trough and plexiglass chambers corresponded to velocities measured in the stream while those in the glass cylinder were less than any speeds measured in riffles where Simulium was found.

Preparation and Acclimation of Consumer Organisms

While labelled algae were being prepared, animals were being acclimated to experimental conditions. Tricorythodes was maintained on nonlabelled food (natural detritus from the stream) under experimental conditions at least 24 hours prior to feeding time. Because of the need for using membrane filtered (0.45 μm) water in the Simulium and Cheumatopsyche experiments, these animals were acclimated to experimental conditions on filtered water. Acclimation time for Simulium varied from 0 to 21 h but generally was 0.5 to 2.0 h; that for Cheumatopsyche was 20 to 22 h. It was felt that within these time limits the animals could seek out suitable microhabitats, assume normal spatial arrangements, secure themselves to the substratum, and that Cheumatopsyche could establish a net for feeding. Acclimation times were judged to be not long enough to weaken the animal from starvation.

Tricorthodes nymphs were prepared in two different ways. Animals (approx. 100 specimens) sometimes were sorted into 1-mm size groups (1.6-2.5, 2.6-3.5, 3.6-4.5 mm) and held in separate dishes on nonlabelled food. Prior to feeding on labelled food the animals were transferred by pipette into a large pan of filtered water. A second transfer into the feeding dish eliminated extraneous unlabelled food. At 0.5 or 1.0 h time intervals groups of five to ten of the animals were removed from the labelled food and dried. Alternatively, presorting was eliminated and nymphs were randomly distributed into three to six dishes to be maintained on nonlabelled food. These animals were also transferred to feeding dishes with labelled food. At specified times after their introduction all animals from a certain dish were removed and killed in CO_2-saturated water (this prevented distortion in body length due to contraction of the abdominal segments and loss of food by regurgitation or defecation). Dead animals were then sorted into size groups.

In Simulium experiments, animals were washed or brushed off rocks, glass slides, or plexiglass sheets from Rattlesnake Springs into a porcelain pan. Cheumatopsyche larvae were collected individually with forceps from rocks in Snively Creek. Simulium larvae were returned to the laboratory within approximatley 0.5 h of collection; Cheumatopsyche were returned within about 1.0 h. Larvae were collected at a corner of the pan and were transferred to substrata (glass slides, gravel, large stones) in the various feeding chambers with a soft-brush. Experimental temperatures were never more than 5°C greater than natural stream temperature (see

Tables 3 and 4) on the day of each Simulium or Cheumatopsyche experiment (except in Simulium experiment 1 in which experimental temperatures were 24°C). When Simulium was fed in the plexiglass stream channel, the larvae were distributed evenly over the middle two of the four smooth-surfaced rocks, which were then submerged slowly in filtered water in the experimental channel so that the larvae remained attached. The pump was started and current speeds slowly were increased to their maximum level, which provided a range of speeds from 25-65 cm/sec in the channel. Animals seemed to find a suitable location quickly and were evenly spaced on the top and side surfaces of rocks.

Introduction of Labelled Food

Animals for all experiments were acclimated for times fitting their behavioral requirements and then labelled food was added. The food of Tricorythodes was particulate detritus (ground watercress), blue-green algae, or periphyton attached to slides. Food of Simulium and Cheumatopsyche was a fine unicellular suspension of materials (diatoms, bacteria, or suspended particulate watercress) in the filtration experiments and attached periphyton in the grazing experiments. At intervals during the feeding of Simulium and Cheumatopsyche on labelled food, 5 or 10-ml samples of the suspension were filtered on pre-weighed 13-mm 0.45 μm filters to obtain the μg/ml and the activity of the food in μg/cpm. The ^{14}C and ^{51}Cr-labelled food source was pre-washed thoroughly in distilled water and resuspended in filtered creek water. This suspension was diluted to 100 ml in a volumetric flask. Prior to introduction into the feeding chamber three 1-ml samples of this suspension were filtered onto a 13-mm membrane filter (Gelman AN-450 Acropor). The filters were dried at 55°C for at least 1.0 h and then exposed to the air for exactly 10 min to equilibrate, after which time the weights of the filters were stable and were reproducible if redried and weighed. The radioactively tagged material was filtered in a 10-ml plastic syringe with a Swinnex filter holder (Millipore Corp). One drop of 0.2% Triton-X-100 solution (Beckman Co.) was added to the filter to reduce the surface tension of water (see Strickland and Parsons 1968) and speed up the filtration. Tapping the filter holder to free trapped bubbles also was required to initiate even filtration. Filters were weighed to the nearest μg on a Cahn M-10 electrobalance. Two Coleman gasoline lantern mantles (a source of alpha particles) were placed in the weighing compartment to reduce electrostatic attraction (see Eaton and Likens 1969). In addition to sampling food for radioactivity, the particle size and cell counts were determined on food suspensions in Simulium experiments 2-5. Because diatoms were added in filtration experiments as finely dispersed clumps and because the circulation of the food was expected to reduce the size of food particles, the food suspension was examined during feeding. The size of food in suspension in laboratory

experiments was examined by periodically filtering 1-ml portions on a 25-mm 0.45-μm membrane filter. After brief circulation all material was present as single cells. For Simulium experiments 2 through 5, cell counts on membrane filters were made so that the number of cells per ml could be calculated. If the entrapment of diatoms on mesh funnels in the glass cylinder is ignored, then the average weight of cells can be estimated knowing μg/ml and cells/ml. In Simulium experiments 2 through 5, Achnanthes and Synedra were virtually the only species present. Forty fields were counted on high power (430X). A field consisted of a $(130\ \mu m)^2$ grid in the center of the viewing area. The smallest squares within the grid were 676 μm^2. Each cell was counted as one and any clumps or colonies occupying half the area of the smallest square were counted as one; two small pieces of debris, each occupying one-fourth the area of the smallest square, were summed and counted as one; only cells which lay more than half way into the grid were counted as one; only cell fragments comprising more than half of a cell were counted as one. Forty random fields were counted by scanning 20 fields horizontally and 20 fields vertically (Kuznetzov and Romanenko 1964). The diameter of the membrane filter holder was 15.7 mm, yielding a filter area of 194.83 mm^2.

The average number of cells per field in 40 fields in Simulium experiment 2 (1 ml/700 ml sampled at t = 0) equaled 1.40 cells/field. There were 11,528.28 fields on the filter at 430x; therefore, there were 1.614×10^7 cells/ℓ. With a food concentration of 26.9 μg/ml the average cell weighed 0.00167 μg/cell. The ratio of numbers of Synedra to Achnanthes was 0.61. In Simulium experiment 5 grazing was measured. To be sure that material washed off the slide and suspended in the water would not be a significant supply of food for filter feeding animals, 5-ml portions were filtered at t = 50 and 56 min. Cells were counted at 200x; the grid at this power was $(250\ \mu m)^2$.

Measurement of Radioactivity of Food, Feces, and Animal Samples

Sampling Animals and Feces

After a specified time some animals in a feeding experiment were killed and prepared for ingestion rate analysis while others were transferred alive to clean chambers with unlabelled food to allow defecation. Tricorythodes nymphs are very pubescent. They became coated with tagged algae and required careful brushing to remove all algae attached to their surfaces. Nymphs distributed by size into feeding dishes were randomly sampled at varying time intervals (sample size was five to ten animals); when a series of dishes containing nymphs sorted without regard to size were used, consecutive sampling at time intervals of all nymphs in one dish minimized disturbance of feeding beahvior to remaining animals.

When the glass cylinder chambers (Simulium experiments 2-6) were used in parallel, animals of one chamber were isolated by clamping the incurrent tygon tube and were killed by filling the chamber with CO_2-water; animals of the other chamber were removed alive and allowed to defecate labelled food. Animals from the plastic trough could be sampled at regular time intervals. However, the normal routine with Simulium and Cheumatopsyche was to remove the gravel plus animals in three of the five trough sections and kill the larvae in CO_2-water (or 30% ethanol) and allow the animals of the remaining two chambers to defecate in a funnel or a pan. Animals in Simulium experiments 1-8 and Cheumatopsyche experiments 1-5 were killed by anaesthetizing them in CO_2-saturated water. The CO_2-water effectively killed Cheumatopsyche but excessively softened the tissues during prolonged exposure. Larvae from Simulium experiments 9-12 were killed in 30% ethanol which killed larvae without inducing loss of gut contents.

Tricorythodes nymphs removed from feeding chambers were fed on nonlabelled food so they could void their guts. Nonlabelled food plus the associated labelled feces in a petri dish after defecation were filtered on a 5-μm membrane filter for later radioactive counting. Funnels were used for Simulium and Cheumatopsyche as containers for maintaining these animals during defecation. Feces were easily collected in a 50-ml flask by opening a valve at the bottom of the funnel and rinsing out the funnel with distilled water. These feces were filtered on a 5-μm membrane filter for later radioactive counting. Funnels were used for Simulium and Cheumatopsyche as containers for maintaining these animals during defecation. Feces were easily collected in a 50-ml flask by opening a valve at the bottom of the funnel and rinsing out the funnel with distilled water. These feces were filtered on a 5-μm membrane filter.

Since Simulium and Cheumatopsyche require a fast current for feeding, it was not possible to adequately supply nonlabelled food during feces collection. Fortunately Simulium has a relatively constant rate of defecation until the point when its gut is nearly two-thirds void. Cheumatopsyche also eliminated some of its gut contents without ingesting new food as evidenced by the level of radioactivity in the funnel washings (experiments 4 and 5). Contrarily, Tricorythodes seemed to depend on the simultaneous intake of food for feces production. A simpler method used in later experiments to recover Simulium feces was to place the larvae in a pan of filtered creek water and collect pellets with a disposable pipette attached to a syringe. The pellets were filtered on a preweighed 13-mm 0.45-μm filter so that the weight of pellets could be related to activity without admixture of nonlabelled components.

Killed animals were sorted according to 1-mm size classes, placed in groups of 5 to 10 animals and dried at 55°C on half of a 47-mm Whatman #40 filter paper. The filter papers plus animals were stored in scintillation vials, which were sealed with a screw cap when removed from the oven.

After cooling, the animals were removed from their filter papers and weighed to the nearest μg. Membrane filters with tagged food were weighed also so that the weight and the activity of labelled food (in μg/cpm) could be measured. Animal samples were wrapped in their original filter paper (so that body fluids absorbed in drying were included) and were placed in 15 mm x 250 mm test tubes for counting of the ^{51}Cr activity on a Nuclear Chicago gamma ray spectrometer. Samples then were arranged in order of increasing gamma counts and oxidized sequentially in a Packard Tri-Carb Sample Oxidizer. Approximately 1% of the counts from one burn are carried over to the next sample. This sample arrangement prevents a high activity sample from obscuring a subsequent sample of low activity. The $^{14}CO_2$ produced in the combustion was absorbed in ethanolamine and the solution then was homogeneously mixed in a toluene-based scintillation cocktail for counting.

Percent Error in Accumulation Values Due to Statistical Counting Errors

The accuracy of individual accumulation (ingestion and assimilation) values depends on the accuracy with which the net sample counts are known. Because net counts = gross counts - background counts, the standard deviation of the net count rate is equal to the square root of the sum of the squares of the standard deviations of gross and background count rates. The standard deviation in the net count rate R_s for each sample is $R_s = (\sigma^2 R_{S+b} + \sigma^2 R_b)^{1/2}$ (Overman and Clark 1960) where R_{s+b} is the observed gross sample count rate (sample counts plus background) and R_b is the background count rate. Since repeated counts observed from a radioactive source follow a Poisson distribution, the standard deviation of the count rate is $(R/t)^{1/2}$ so that $\sigma R_s = \left(\frac{R_{s+b}}{t} + \frac{R_b}{t}\right)^{1/2}$. When background count rate is large in comparison with the gross count rate the fluctuations observed in background counts widen the confidence limits around the calculated net rate, thus increasing the error of the determination. In order to evaluate the reliability of accumulation values, the error attributable to the background must be known. In addition, the relative standard deviation is directly related to gross counts observed. That is, if 10,000 counts are observed, the standard deviation is $\sqrt{10000} = 100$ which is 1% relative standard deviation regardless of the count rate.

The Selection of Sample Oxidation Methods

The most desirable method of ^{14}C counting requires combustion of samples to $^{14}CO_2$ and counting in a homogeneous solution with as few chemical additives as possible. Many methods of oxidation and gas trapping are available (see Szekielda 1967 and Parmentier and Ten Haaf 1971). Wet and dry combustion methods are available for oxidizing samples. Many acid mixtures are unable to oxidize certain chemical compounds. Completeness of combustion of organic samples generally varies from about 85-100% for a wide range of acid mixtures. After the combustion the $^{14}CO_2$ generated must be trapped or else counted directly in a proportional gas flow counter. If the trap is used, then time (up to 24 h) may be required for diffusion and absorption of the gas by a KOH solution in a well within a sealed vessel. Dry combustion flask methods utilize a potentially explosive pure O_2 atmosphere. The sample, placed in a platinum basket is ignited by spark or infrared heat source. Combustion often is incomplete.

In a Packard Sample Oxidizer ethanolamine absorbent is spread over a baffle with a large surface area so that diffusion and trapping of the CO_2 is complete. In other systems the gas is actively passed through or over an absorbent one or many times to achieve complete absorption. Sorokin (1968) bubbled the gases of combustion through a column filled with glass beads and 0.05 N KOH. A Vigreaux tube was used to the same effect by Fuchs and de Vries (1972). Watson and Williams (1970) suggested using a peristaltic pump to recirculate gases through a mixture of ethanolamine: ethylene glycol monomethyl ether. The use of a 1:2 v/v mixture of this absorbent, as described by Jeffay and Alvarez (1961), is the most effective in trapping the gas and provides counting efficiencies of about 86%. After the CO_2 is trapped it may be precipitated as $BaCO_3$, filtered and counted by Geiger-Mueller counting as a dry solid. However, inaccuracies developed through self-absorption make this method undesirable. Scintillation counting of an aliquot of the ethanolamine trap provides high efficiency counting and uniform quenching characteristics from sample to sample. Within a normal range of samples sizes, the variable amount of ethanolamine-carbonate generated has low quenching properties.

The Wet-Combustion Apparatus

Design of the wet-combustion apparatus used was adapted from that of Watson and Williams (1970), with several improvements (see Fig. 5; refer to index letters on figure for part discussed). The apparatus has been simplified by elimination of one three-way stopcock and the NaOH gas-washing bottle. Two hydroquinone-sulfuric acid gas washing bottles (a and b; 500 and 250 ml, respectively) were employed to improve the efficiency of gas scrubbing (as suggested by Watson, personal communication). The first of these bottles (a) has an open-ended gas dispersion tube instead

A. GAS WASHING BOTTLE WITH OPEN ENDED DISPERSION TUBE

B. GAS WASHING BOTTLE WITH COARSE FRITTED GLASS DISPERSION TUBE

C. VACUUM TRAP

D. PERISTALTIC PUMP

E. CONDENSER

F. COMBUSTION FLASK

G. ACID TRAP

H. 3-WAY STOPCOCK

I. 3-WAY STOPCOCK

J. 50-mℓ-GRADUATED CYLINDER CONTAINING ACID REAGENT

K. SCINTILLATION VIAL

L. DISPERSION TUBE

M. NITROGEN GAS

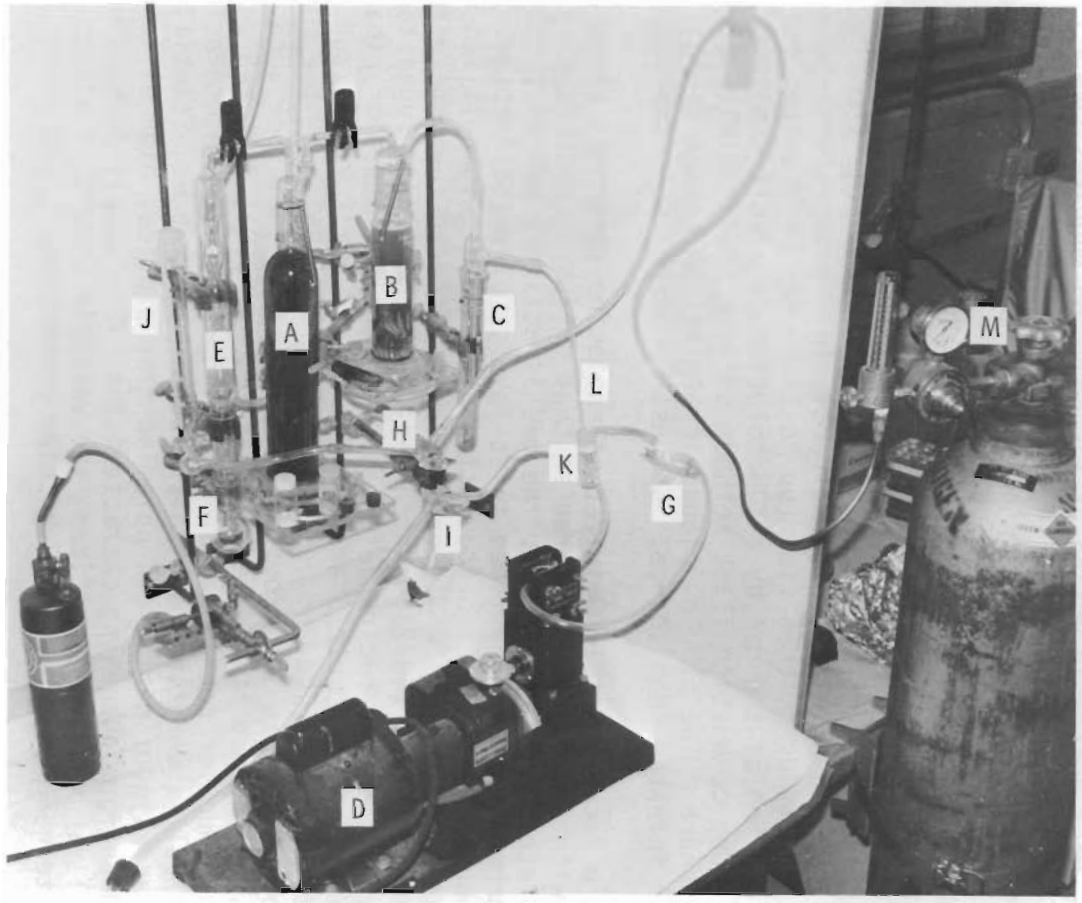


FIGURE 5a. Wet-Combustion System Tested at Battelle-Northwest

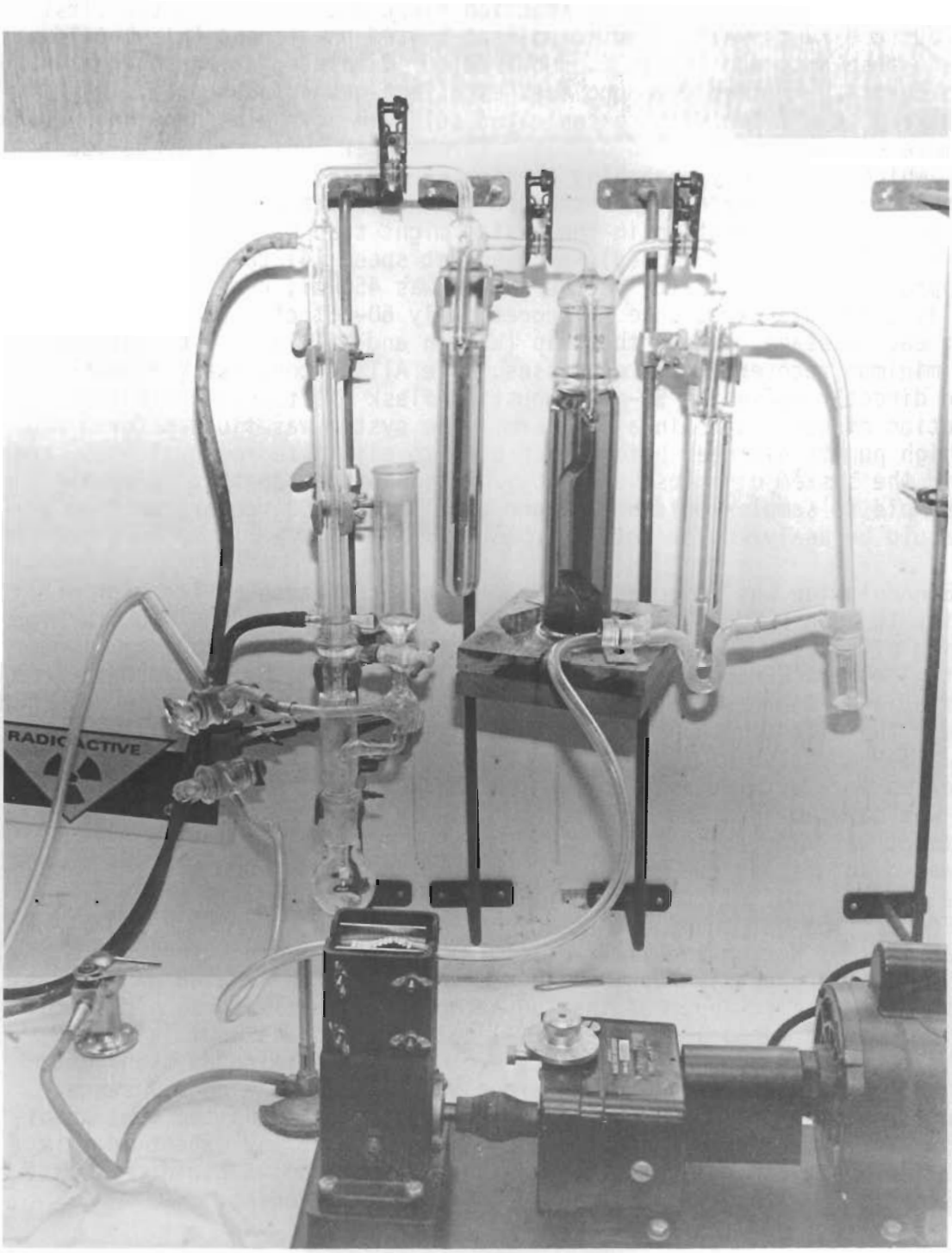


FIGURE 5b. Wet-Combustion System Improved at Idaho State University

of fritted glass so that the small amount of crusty material which accumulates in this bottle does not become clogged in the tube. Most of the iodine and other vapors from the reaction flask are removed in the first tower but the fumes which tend to collect toward the top of this bottle indicate that a second bottle is required for complete gas scrubbing. A coarse porosity fritted disc provides excellent gas scrubbing. A 125-ml vacuum trap (c) filled with concentrated sulfuric acid also has been added to remove nitrogen oxides (Gorbach and Ehrenberger 1961, see Szekeilda 1967), which are highly quenching chemicals, and any water vapor carried through the system (water vapor trapped in the absorbent could increase quenching, and condensation in the system might trap CO_2 in solution). A Sigmamotor peristaltic pump (d) was used (at speed 14) to circulate gases. The approximate gas capacity of the system was 450 ml, requiring 2.25 min to fully circulate gases once. Theoretically 60-65% of the CO_2 is absorbed during each passage through the trap (Watson and Williams 1970), providing 93.6% minimum recovery in three passes. The Allihn condenser (e) was placed directly above the 50-ml combustion flask (f) to eliminate the collection of condensate in a side arm. The system was flushed for 3 min with high purity nitrogen before each burn to eliminate residual $^{14}CO_2$ and to void the system of atmospheric CO_2. This was done so that carry-over from sample to sample would be low and also so that CO_2 generated from a burn could be analyzed for total carbon.

Ethanolamine was selected as trapping agent because of its high capacity to absorb CO_2 (1 ml will absorb 8 millimoles of CO_2) and its high counting efficiency in toluene. In the absorbent mixture ethylene glycol monoethyl ether (EGMEE) was used instead of the monomethyl ether described by Jeffay and Alvarez (1961) to keep the ethanolamine carbonate in solution. Although the mixture ethanolamine: EGMEE is quite low in volatility (cf. Jeffay and Alvarez (1961), some extraordinarily high total carbon recoveries were attributable to the low boiling point of EGMEE (133-135°C) which was carried into the combustion flask as a vapor. For this reason an attempt was made to employ 2 ml of straight ethanolamine as a trap. This was discontinued due to low ^{14}C recoveries and an even greater passage of the vapors into the combustion flask than when ethanolamine was mixed with EGMEE. The gas transport rate in my combustion system was 200 ml/min, whereas that of Watson and Williams (1970) was 740 ml/min. Their rate of gas circulation probably resulted in considerable vaporization of the absorbent because it appeared that an acceptable limit was reached in the gas flow rate in my apparatus. A small acid trap (glass wool saturated with half-strength phosphoric acid (see g, Fig. 5) eliminated transport of ethanolamine and brought total carbon values down to a normal range. Eventually 5 ml of the 1:2 absorbent mixture was chosen as the best combination. It is uncertain whether EGMEE was removed to any extent in this trap. Two-butoxyethanol which has a higher boiling point could be substituted for EGMEE without significantly increasing quenching.

Operation of the Combustion Apparatus. The samples were weighed on a Cahn electrobalance and placed in the combustion flask (or were pipetted into the flask in the case of liquid ^{14}C standards). Liquid samples containing $^{14}CO_2$ in solution may also be acidified in the flask to derive the $^{14}CO_2$ of a 25-50 ml sample. One g of solid reagent (3:1 KIO_3:$K_2Cr_2O_7$) was added to the flask, which was secured to the apparatus with a utility clamp. The joint at the combustion flask was lubricated with syrupy phosphoric acid. This acid was not an ideal lubricant for the ground glass stopcock used to deliver acid to the combustion flask because friction impaired rotation; a teflon stopcock should have been employed instead. Silicone grease, if replaced regularly, makes a fairly nonreactive lubricant at this joint but using only teflon joints or lubricating joints near the combustion flask with phosphoric acid can assure no organic contamination. O-ring joints between gas-washing bottles required no lubrication; 3-way stopcocks were lubricated with silicone grease; ground joints in the gas-washing bottles also were lubricated with silicone as the acid strength (1 N) was mild in the bottles.

In order to operate the combustion apparatus stopcock h (Fig. 5) is rotated to connect the gas supply to the combustion system, and stopcock i is rotated to the exhaust position. The system is flushed with high-purity water-pumped N_2 gas for 3 min before each operation or long enough to assure at least one complete flushing. The tygon tubing running through the peristaltic pump, unconstricted during the N_2 purge, is then pinched as the pump is operated. At this point N_2 gas enters from the tank and gas is forced out the exhaust port by the pump. Stopcocks h and i are then rotated so that the system is cyclic and a check is made to see whether the flow rate through the washing bottles is maximal. Next stopcock i is rotated to the exhaust position for 25 sec to create a vacuum sufficient to accomodate the CO_2 and O_2 generated in the reaction. With the system again cycling, 5 ml of acid reagent (j) is delivered to the combustion flask and the sample is heated with a micro-bunsen burner for 2.5 min, after which the solution turns greenish brown and emits dense white fumes. Combustion is complete at this point and further heating only releases more O_2 from the chromate (Van Slyke and Folch 1940). After an additional 8 min of gas circulation, stopcock i is turned to exhaust, stopcock h is opened to receive more N_2, and the screw cap scintillation vial (k) containing the trapped CO_2 is removed. Six ml of absorbent are used in the vial as a trap. Aliquots of this absorbent then are used in scintillation counting and in total carbon analysis.

Counting the ^{14}C Activity. A 1-ml aliquot of the 6-ml trap solution was added to 7 ml of EGMEE in a scintillation vial. This was followed by 10 ml of a toluene-PPO-M_2POPOP solution (5 g/ℓ PPO; 0.25 g/ℓ M_2 POPOP) and counting for ^{14}C activity in a scintillation counter. Sample counts were multiplied by 6 to obtain the total sample activity. Another aliquot was analyzed for total carbon by methods described below. After each combustion methanol was used to clean the fritted gas dispersion tube (ℓ, Fig. 5) entering the scintillation vial trap. Watson and Williams (1970) washed this tube down with 1.5 ml EGMEE and added the rinsings to the scintillation

vial but personal experience makes it doubtful whether all the trapping solution could easily be rinsed from the fritted disperson disc. Counting an aliquot of the total mixture obviates this task. ^{14}C samples were counted for a preset time of 5 min. Gross sample count rates were nearly always at least five times the average background count rate of approximately 32 cpm.

Measurements of Total Carbon. Total carbon was measured by removing 1 ml of the absorbent mixture with a 1-ml syringe, transferring this to a 50-ml Erlenmeyer flask with minimal exposure to air, and adding 5 ml of a 0.2 N $BaCl_2$ solution. (The $BaCl_2$ solution was prepared using CO_2-free distilled water (American Public Health Association 1971). It was necessary to heat the flask and contents for 5 min in a hot water bath (Balica and Savu 1966) to initiate precipitation of $BaCO_3$. The stoppered flasks were immediately placed in a refrigerator; 0.5 h was allowed for complete precipitation. The solubility of $BaCO_3$ was lowered by cooling and also by the excess of $BaCl_2$ added in the precipitation process. The precipitate was then filtered onto preweighed 13-mm 0.45 μm filters. Precipitate remaining in the flask was rinsed out with 5 ml of 0.2 N $BaCl_2$, which also was filtered. The filters were dried at 55°C for 24 h, exposed to the air 10 min and then weighed on a Cahn electrobalance. The total carbon was calculated from the weight of $BaCO_3$ (mg C = 0.06086 x mg $BaCO_3$). The filter pore size was chosen with the knowledge that $BaCO_3$ particles may be as small as 1 μm (Wood 1970). Two drops of 0.2% Triton-X-100 neutral detergent were added to each flask to reduce the tendency for precipitate to climb the walls (Strickland and Parsons 1968). Several alternative methods of measuring total carbon were abandoned. The complexometric method of Balica and Savu (1966) was found to be adequate but too time-consuming and elaborate. This method required the precipitation of the carbonate of ethanolamine as $BaCO_3$, followed by the addition of a Mg-EDTA solution to the precipitate. Barium has a greater affinity for EDTA than magnesium and so binds with EDTA, releasing Mg, which is then titrated with EDTA using eriochrome Black T as an indicator. The titration of excess ethanolamine in the trap solution with HCl was not possible as a method of measuring total CO_2 because the $BaCO_3$ precipitate began to dissolve as the end point was approached (below pH 5). Using a KOH trap was a possible alternative because the titration of excess KOH is simple. However, the water vapor from this trap was readily carried through the system during gas bubbling. Also, ^{14}C counting efficiencies in an aqueous scintillation medium are not so high as in a toluene medium.

Standardization of Combustion. Standardization of the combustion apparatus was made using Na palmitate-^{14}C and ^{14}C-hexadecane. Samples of 25 or 50 μl of the radioactive standards were combusted in the apparatus with standard Van Slyke wet-combustion reagents (Van Slyke and Folch 1940,

Van Slyke, Plazin, and Weisiger 1951). The percentage of ^{14}C using the Na palmitate standard generally varied between 88 and 90%. The ^{14}C-hexadecane was an unsuitable standard as it vaporized to a large extent while the acid was heated. It was necessary to absorb each standard in a filter paper square to prevent the sample from climbing the walls of the flask and escaping combustion. Ten-mg samples of anhydrous dextrose also were burned and the total carbon recovered in the trap was analyzed gravimetrically as $BaCO_3$ precipitate. The weight of carbon in dextrose equals 40.0% of the total sample weight. Watson and Williams (1970) modified Van Slyke's solid reagent mixtures from 2:1 to 5:1 KIO_3:$K_2Cr_2O_7$. This mixture was found undesirable for most biological samples in my research as it tended to release large quantities of I_2 vapors. The acid mixture (fuming sulfuric: phosphoric acid) could not be heated sufficiently for complete oxidation before the evolution of I_2 commenced. A 2:1 mixture of solid reagents decreased the production of I_2. Iodic acid is presumed to increase the yield of CO_2 by the conversion of small amounts of CO to CO_2. Excess dichromate is necessary to keep the iodine in its oxidized form (IO_3). Five ml of the 2:1 fuming sulfuric:phosphoric acid mixture is sufficient to combust samples with up to 15 mg C. One g of solid reagent is added to samples with 7-15 mg C. A smaller amount of solid reagent is used for samples with less than 7 mg C because of the large amount of O_2 generated from the unreacted chromate.

Additions and Improvements. To improve $^{14}CO_2$ recoveries it is recommended that at least 6 ml of CO_2 absorbent mixture be used in the trap. To insure against leaks from ground joints sealed with syrupy phosphoric acid, a small amount of P_2O_5 and CrO_3 can be added to the phosphoric acid to create a high pressure seal (Buchanan and Nakao 1952). A further reduction in the volume of the combustion system would decrease gas circulation times required for complete recovery. Improved efficiency of operation and recovery was achieved at ISU by remodelling the combustion apparatus tested at Rattlesnake Springs. A 125-ml vacuum trap with open ended dispersion tube and a 250-ml gas-washing bottle with a coarse fritted dispersion disc were substituted for the 500- and 250-ml gas-washing bottles, respectively. A 300-mm straight tube condenser (15 ml) was substituted for the 300-mm Allihn condenser to reduce the system volume, because very little water to be retained is present during digestion and much of the condensate eventually is carried into gas-washing bottles anyway. Chloride in samples is converted to chromyl chloride (CrO_2Cl_2), which deposits on the condenser but then decomposes releasing Cl_2 (Allison 1960). Substitution of butyl cellosolve (higher b.p.) for ethyl cellosolve (EGMEE) in the CO_2 trap mixture could reduce the slight interference of volatile organics in measuring total carbon. A gas-washing bottle containing 4% $KMnO_4$ in 2 N H_2SO_4 could be substituted for the second hydroquinone-sulfuric acid tower to remove SO_2 gases evolved during combustion (Fuchs and de Vries 1972). After combustion of a sample the acid residue in the combustion flask could be rinsed into a 50-ml volumetric flask and diluted to 50 ml. A 5-ml sample could then be neutralized in a micro-Kjeldahl apparatus so that NH_3 could be steam distilled and

analyzed. In this way the C/N ratio of a sample could be determined and the approximate protein content of the sample analyzed. A more rapid determination of total carbon than $BaCO_3$ precipitation would be to release the CO_2 from ethanolamine into a gas chromatograph (see method of Oppenheimer, Corcoran and Van Arman 1963, see Szekielda 1967). An alternative method which promises to be fast and precise requires titration of CO_2 captured in ethanolamine using 0.15 N sodium methanolate in methanol with a thymolphathalein indicator (Vander Laarse and Van Leuven 1966).

Methods for Calculating the Energy Budget

The ultimate goal in energy budget analysis is to be able to account for the total apportionment of the energy of ingestion into the primary components: production, respiration, and feces. The purpose of the feeding experiments was to supply the animal with foods of a type and quality similar to those available naturally so that the ingestion rates and assimilation efficiencies on these foods could be determined. Assimilation efficiencies on single species cultures may provide insight into the ecological importance of healthy or scenescent plants in animal nutrition but are not indicative of the assimilation of the natural mixtures of algae and detritus. The assimilation of organic matter from a mixed culture may be different from the sum of the assimilation of each species individually, and the task of summing these assimilated components from data on the relative abundance of each food type ingested and assimilation efficiency of each is too complex. Gravimetric analyses are especially suited to deal with this problem because a food mixture can be used directly from the natural habitat. However, it is felt that the disadvantages of the gravimetric method outweigh any advantages and that the radiotracer methods are far more sensitive.

When radiotracer methods are used to determine ingestion rate (or assimilation efficiency), the digestion time must first be ascertained. This is especially true when ^{14}C-labelled food is used to determine ingestion rate since even after the gut is filled the body activity continues to increase due to assimilation. This continual absorption also is a problem with so-called nonabsorbed isotopes such as ^{51}Cr and ^{144}Ce. Assimilation, determined radiometrically for all instars or size classes on a natural food source and integrated for the total life cycle, should equal assimilation determined as the sum of production and respiration during the life cycle. Production of exuviae often is disregarded in the energy budget but can account for a substantial energy loss. This component has been measured in the energy budget for Tricorythodes, which has 18-22 molts at regular intervals during the life cycle (R. L. Newell, personal communication). Excretory energy losses, though possibly substantial, were not measured in this research.

Digestion Time

Digestion time is the first energetic variable which must be identified before an energy budget can be computed. Digestion time (gut retention time) is the time required for a particle of food to pass completely through the gut. Digestion time equals gut-filling time for continuously feeding animals but may be considerably longer than gut-filling time with animals which feed periodically (especially carnivores) or are suddenly exposed to excess food. Digestion time depends on food type, season,

temperature, and various other physical and chemical factors, as well as nutritional state and size of the animal. It is under a definite set of such variables that a digestion time is specified for an animal.

One method of digestion time analysis was suggested by Sorokin (1968). This requires pulse feeding an animal on a labelled food source and noting the length of time required for radioactive food to appear in the feces. Similarly an inert marker (e.g. Nigrosin B, powdered charcoal) may be used (Ladle et al. 1972, Cummins et al. 1973a). In this research powdered charcoal was used successfully as a marker on Simulium and Tricorythodes. A 3-min pulse feeding of Simulium larvae in situ with 18-l suspension of charcoal (approx. 50 g) was used to establish a solid black plug in the gut which was easily seen after the animal was dehydrated and cleared in a series of alcohol baths (24 h each in baths of 30, 50, 70, 85, 95, 100% ethanol and finally 100% beechwood creosote). After 3-min of feeding on the charcoal suspension animals were sampled at 5-min intervals, until 35 min had elapsed, from rocks downstream from the point of carbon introduction. The average percentage of total body length travelled was determined for at least 10 animals sampled at each 5-min period. The distance from the base of the cephalic fans to the anterior edge of the carbon plug in the gut was measured using an ocular micrometer. This distance divided by the total body length (from base of cephalic fans to anus) corresponds to the percent of the gut travelled. Also (time at each interval) ÷ (fraction of gut travelled in this time) equals projected gut clearance time. The temperature of Rattlesnake Springs was 15.4°C during the digestion time analysis, corresponding roughly to the experimental laboratory stream temperatures (16-20°C).

This method also was used successfully with Tricorythodes. Tricorythodes nymphs were maintained for 24 hours on partially decomposed Cladophora and detritus at experimental temperatures prior to analysis of digestion time. The texture of this food was similar to the comminuted blue-green algae used in Tricorythodes experiments 4 and 5. Gut-filling times were measured at 12.5, 17.8, and 21°C by transferring nymphs to petri dish filled with the Cladophora-detritus mixture with powdered charcoal marker. Animals were sampled and killed in 30% alcohol after 5, 6 to 7, and 10 min of feeding on the marked food. Entire guts were dissected from the body and the fraction of the gut filled by the marked food was used to estimate gut-filling time.

Animals fed on a radioactive food source can be sampled at intervals with the point where the body activity levels off indicating the time required for gut-filling. The total body accumulation (μg/mg) computed at each hourly interval is divided by the number of hours elapsed since the initiation of feeding. If the gut-filling time is 2.0 h, the μg/mg/h values computed at 1.0 and 2.0 h should be equal since the gut is being filled until the 2.0 h point. Thereafter the μg/mg value of animals sampled increases

more slowly because only assimilation of food increases the activity. When the body activity values (μg/mg) after the gut is full are divided by the sampling time (t = x hours), the values begin to decrease. Assuming a constant rate of ingestion, the time required for gut-filling is located at the point where the μg/mg/h values start to decrease. If ^{14}C-labelled food is used for this purpose, then the results can be difficult to interpret as newly assimilated radioactive material continues to increase the body activity. The use of a nonabsorbed isotope such as ^{51}Cr is helpful in this respect. A small amount of ^{51}Cr can be expected to be absorbed by the gut or absorbed by the body surface but the leveling off of activity should be abrupt after the gut is filled. In this study an attempt was made to determine digestion time of Tricorythodes fed on ^{14}C-labelled food. An indication of Simulium and Tricorythodes digestion time was gained through feeding of ^{51}Cr-labelled food.

Ingestion Rate

Ingestion rates of large aquatic animals such as fish can be estimated by force feeding food pellets over a period of several days. The maintenance level of food intake is that at which the metabolic requirements are met but weight gain is zero. More reliable ingestion rates are gathered if natural feeding is allowed rather than forced feeding. Natural feeding rates can be determined by various gravimetric and radiotracer methods.

A gravimetric method used in the present study to determine ingestion rate was to divide the gut fraction (ratio of gut weight to total body weight) as μg/mg by the digestion time. The total body lengths of larval Simulium collected from the Rattlesnake Springs were measured and guts were removed and placed on tared foil squares. All excess material (fat and Malpighian tubules) was stripped away from the gut which was then dried (55°C) and weighed. Although it was sometimes feasible to remove the gut wall from the gut contents, the additional weight incurred by weighing whole guts probably was negligible. Tared foil squares weighing roughly 0.65 mg were sufficiently large to hold the gut contents of the largest Simulium larva. The guts of eight Tricorythodes nymphs which had fed on Cladophora in the laboratory were removed and the dry weight of gut contents was determined. The total body length of the nymphs was used to ascertain individual dry weights from a log-log plot of dry weight vs. body length (D. McCullough and R. L. Newell, unpublished data). The gut fraction was then calculated for each individual. As an alternative to direct gravimetric analyses the total gut volume of various size animals can be measured with an ocular micrometer (Mecom 1970, Cummins et al. 1973a) and if average weight-to-volume ratios for various combinations of detritus:diatom:animal parts are used, the weight of gut contents can be estimated.

Radiotracers also were used to measure ingestion rate. Animals feeding on radioactive food sources were sampled at regular time intervals. If activity of the food in terms of μg/cpm is known, the amount of food ingested at each time interval can be calculated by multiplying the cpm (animal) x μg/cpm (food) (see Sorokin and Panov 1966, and Sorokin 1968 for explanations of the ^{14}C method).

From the standpoint of time required it is simplest to feed a ^{51}Cr-labelled food source to a large number of experimental animals and to sample them regularly during the first 12 to 24 hours of feeding. Average digestion times and ingestion rates for large numbers of animals of various size classes can be ascertained at the same time.

A true ingestion rate can only be determined with respect to the digestion time. If digestion time is unknown, the ingestion rate can be determined from an accumulation rate derived from a nonabsorbed isotope. The reason for stating an accumulation rate in terms of μg/mg/1.5 h (as in Simulium experiment 6) is that if the digestion time is 0.5 h, the true ingestion rate (μg/mg/h) would be two times this ^{51}Cr-derived accumulation rate; if digestion time is 3.0 h then the true ingestion rate (μg/mg/h) is 0.66 times this accumulation rate. In the case of experiment 6, however, adsorption was probably the major source of ^{51}Cr accumulation and the gut was not being filled even though the digestion time was exceeded. For this reason the accumulation rate was stated in terms of time allowed for accumulation.

Assimilation and Assimilation Efficiency

Provided that both the ingestion and defecation of material are rather continuous, the rate of assimilation of food materials can be determined by subtracting the defecation rate from the ingestion rate. If these rates are measured gravimetrically, it is essential that fecal pellets are discrete and can be retrieved from food or substrate material. Because of the difficulties involved in accurately estimating defecation rate this method was not used. An innovative method for feces recovery which continuously collects and preserves fecal pellets is described by Appelby and Brinkhurst (1970).

The amount of material assimilated in a specific period of time can also be calculated as the sum of material added in growth and lost in respiration within this time. This requires accurate gravimetric analysis of weight gain over a short period of time (instantaneous growth rate) and respirometry. In this and the previous method the total amount ingested during the time interval must be determined so that AE (assimilation efficiency) can be determined as A/I.

Radiotracer methods employing a wide variety of isotopes are also available to determine assimilation efficiency (^{14}C--Sorokin 1968; ^{32}P--Trama 1957; ^{51}Cr--Crossley and Van Hook Jr. 1970; ^{85}Sr--Hubbell et al. 1965). Carbon-14 probably is the best choice of an isotope because of the universal occurrence of carbon in organic compounds which are assimilated. In general if 50 animals with a digestion time of 2.0 h are fed on ^{14}C-labelled food, 25 are killed after 1.0 h and analyzed in five groups of five to determine an average ingestion rate. The remaining 25 animals are allowed to continue feeding on nonlabelled food for an additional 2.5 h, sufficient to completely eliminate the labelled food from the gut but also short enough so that respiratory $^{14}CO_2$ losses are small. If it can be assumed that respiratory losses are negligible, the activity remaining in the body after gut clearance will indicate the amount of material assimilated. If animals are maintained in a small aquarium (pH 8.3) during defecation and the water is sampled and measured for $^{14}CO_2$ by scintillation counting, assimilation can be corrected for this loss (Sorokin 1968).

Since the assimilation efficiency of the ash minerals in the food may not be the same as that of the organic component, assimilation should be specified in terms of ash-free dry weight (AFDW) or calories instead of simply dry weight of food material. In certain cases the results of experiments in this research are given simply in terms of dry weight when further calculations did not necessitate the conversion to organic dry weight. The percent organic matter in the experimental foods is given so that assimilation of organic dry weight can be computed.

Calow and Fletcher (1972) recently introduced a new method for computing assimilation efficiency using dual-labelled (^{51}Cr, ^{14}C) food. This technique is a variation of the indicator method and has the added advantage that the organic material of the food is labelled with ^{14}C, thus distinguishing unassimilated organic material in the feces from organic material secreted into the gut (mucus) or produced by the gut (peritrophic membrane). These organic additions to the gut contents would result in an underestimation of assimilation efficiencies in gravimetric analyses. With Calow and Fletcher's (1972) method,

$$AE = 1 - \frac{cpm\,^{51}Cr\ (food)/cpm\ ^{14}C\ (food)}{cpm\,^{51}Cr\ (feces)/cpm\ ^{14}C\ (feces)} \times 100.$$

Quantitative recovery of feces is not required with the dual-label method nor is complete gut clearance. This implies a dictinct advantage over the ^{14}C radiotracer technique in ease of determination because corrections for loss of assimilated ^{14}C through respiration, excretion, or secretions do not need to be made. Corrections for solubilization of

^{14}C and ^{51}Cr from feces, however, must be made. Four assumptions are inherent in this method (Calow and Fletcher 1972):

1) That ^{14}C and ^{51}Cr are uniformly distributed throughout the food material.
2) That ^{14}C and ^{51}Cr move along the gut at similar rates.
3) That ^{51}Cr is not absorbed to any significant extent.
4) That the nonabsorbed indicator is all present in the feces (i.e., is not readily leached out).

These assumptions seem to be well satisfied in work performed on snails by those authors. The extent of absorption and leaching of the ^{51}Cr from feces should be measured for other animals.

The dual-label method was used for AE determination in the three test species in this research. Leaching of the isotopes from food and ^{51}Cr absorption was examined, but absorption of ^{51}Cr was assumed to be insignificant in AE calculations. Leaching of ^{51}Cr from food is continuous; the activity density of the food should be monitored periodically and losses should not be too severe for accurate ingestion measurements to be made. Losses of ^{51}Cr from feces, though possibly important for these organisms, were not measured.

Conover (1966) described a method for measuring assimilation efficiencies which is similar to Calow and Fletcher's method. In Conover's method the assumption is made that only the organic fraction of the food is absorbed by the animal while the ash passes through unassimilated. Calow and Fletcher (1972), using ^{51}Cr as an essentially nonabsorbed substance could determine to what degree absorption occurred and correct for it. The assumption that no ash (mineral matter) is absorbed is clearly false since in growth the ash component of the animal grows with the animal. However, the rate of addition of ash should be expected to be in proportion to the rate of assimilation of organic matter. Turnover of the ash component may be rapid though and seasonal accumulations or losses may occur. This could be examined by comparing the rate of decrease in body activity of animals with equilibrium concentrations of ^{14}C vs. a labelled mineral material, e.g., ^{85}Sr, ^{60}Co. The rate of assimilation of ash can be analyzed by determining the turnover of these isotopes. Van Hook, Jr., and Crossley (1969) and others have shown the assimilation and retention of different isotopes to vary widely. Conover and Francis (1973) recently cautioned that radiotracer estimates of ingestion and assimilation rates could be in error if intermediate pools at various trophic levels exist where initially high rates of turnover occur.

The computations are made using the following formula:

$$U' = \frac{(F'-E')}{(1-E')(F')} \times 100,$$

where U' is the assimilation efficiency, F' is the ash-free dry weight: dry weight ratio (fraction of organic matter) in the ingested food and E' is the same ratio in a representative sample of feces. Quantitative feces recovery is unnecessary but errors may be involved when secretions into the gut are mixed with feces. Secretions are previously assimilated materials which may have been metabolically altered at some cost in energy. By organically enriching the feces, the added secretions give a deceptively low assimilation efficiency while providing an increased fecal production rate. However, the production of secretions probably is continuous so that in effect a net assimilation is measured by this method. Radiotracer experiments generally are short enough so that the body burden of isotope is low and very little isotope is recycled back to the gut. For this reason isotope methods are more apt to measure true assimilation.

Assimilation efficiencies were measured for Simulium by the ash-ratio technique of Conover. The dry and ash weights of food and feces samples were measured on foil squares and on 13-mm glass fiber filters. In the foil method Simulium larvae (approx. 100-150 larvae) were brushed or washed off rocks from Rattlesnake Springs and were returned to the laboratory within 3 min in a porcelain pan. Fecal pellets produced during the first 0.5 h of defecation were collected with a pipette, concentrated on bolting cloth, and then transferred to tared foil squares (< 5 mg). The natural seston in 2 ℓ of stream water was concentrated on a 5-μm membrane filter, scraped off the filter, and placed on foil squares. Food and feces samples were dried at 55°C for 24 h and ashed at 500°C for 24 h to obtain dry and ash weights.

Percent ash determinations of food and feces were made using glass fiber filters decontaminated either by pre-ashing or by boiling in acid. Filters ashed at 500°C for 24 h were found to lose 0.443% (SD = 0.033, n = 12) of their dry weight. The variation between filters in percent weight lost was minor but after reashing, additional weight losses were observed. Also pre-ashed filters were very fragile and care was required to avoid crumbling of the filter. Further percent ash determinations using glass filters were made on filters boiled 1.5 h in concentrated H_2SO_4 to eliminate organic contamination. These filters were then soaked 24 h in acid and washed seven times by drawing 100-ml volumes of distilled water through them in a vacuum filtration apparatus. Filters were dried 24 h at 55°C and weighed. In glass filter experiments feces were collected with a fine pipette from larvae defecating in a porcelain pan. Fecal pellets were placed on filters by ejecting them from the

pipette with a small amount of water. Excess water passed through the filter and was collected by a filter paper. Natural seston was sampled by filtering 100-ml volumes of stream water collected in sub-surface flow on pre-ashed or acid-boiled filters. Filters were dried 24 h at 55°C, cooled in a desiccator, weighed and then ashed for 24 h at 500°C. Dry weights of filters were corrected for losses due to leaching and ashing.

A method of AE determination developed in this work seems to have some potential when used in conjunction with ingestion rate studies. This method is applicable when ^{14}C-labelled food is used but is more valuable as a cross check of the Calow and Fletcher (1972) method when both ^{51}Cr and ^{14}C are used. If the digestion time can be determined approximately from literature values (most digestion times are 30 min to 24 h) or derived experimentally, an economical experiment incorporating this method would be represented by the following: Digestion time is estimated to be 1.0 h. Sixty animals are fed on ^{14}C-^{51}Cr-labelled food. At 0.25, 0.50, 0.75, 1.00, 2.00, 3.00, 4.00, and 5.00 h periods after feeding commences five animals are collected. The steady increase in ^{14}C and ^{51}Cr activity traces the ingestion and later assimilation of food. Carbon-14 counts continue to increase slowly after 1.0 h due to assimilation of ^{14}C organic material, while ^{51}Cr counts level off at 1.0 h indicating that 1.0 h was the correct digestion time (gut-filling time). Feces are then collected from the remaining 20 animals and Calow and Fletcher's method is used to compute assimilation efficiency. If the feces of these animals can be collected without the admixture of unlabelled food, the feces can be filtered on sterile glass filters and weighed. Provided that the food was labelled uniformly, the ^{14}C activity in terms of μg/cpm of the ash-free fecal material should be the same as that of the food originally. However, if carbohydrates, for example, were strongly labelled and fats were not labelled well, and if carbohydrates were preferentially assimilated, then a falsely high assimilation efficiency would be indicated. Activity levels of ^{14}C and ^{51}Cr can be depicted in the following graph (Fig. 6). If, as in this hypothetical experiment, the digestion time is 1.0 h, assimilation efficiency is 50%, and ingestion rate is 10 μg/mg/h, then during the 1.0 h period 10 μg of food are ingested. This amount of food fills the gut, as is shown by the leveling of ^{51}Cr activity. Additional ingestion is balanced by defecation, with only small increases in ^{51}Cr due to slight absorption of ^{51}Cr. After 1.0 h the ^{14}C activity continues to increase due to assimilation. At an assimilation efficiency of 50%, 5 μg/mg/h are added to tissue activity. A tally of the material accumulated (ingested and assimilated) is the following:

Time (h)	0.5	1.0	2.0	3.0	4.0	5.0
Accumulation (μg/mg)	5	10	15	20	25	30

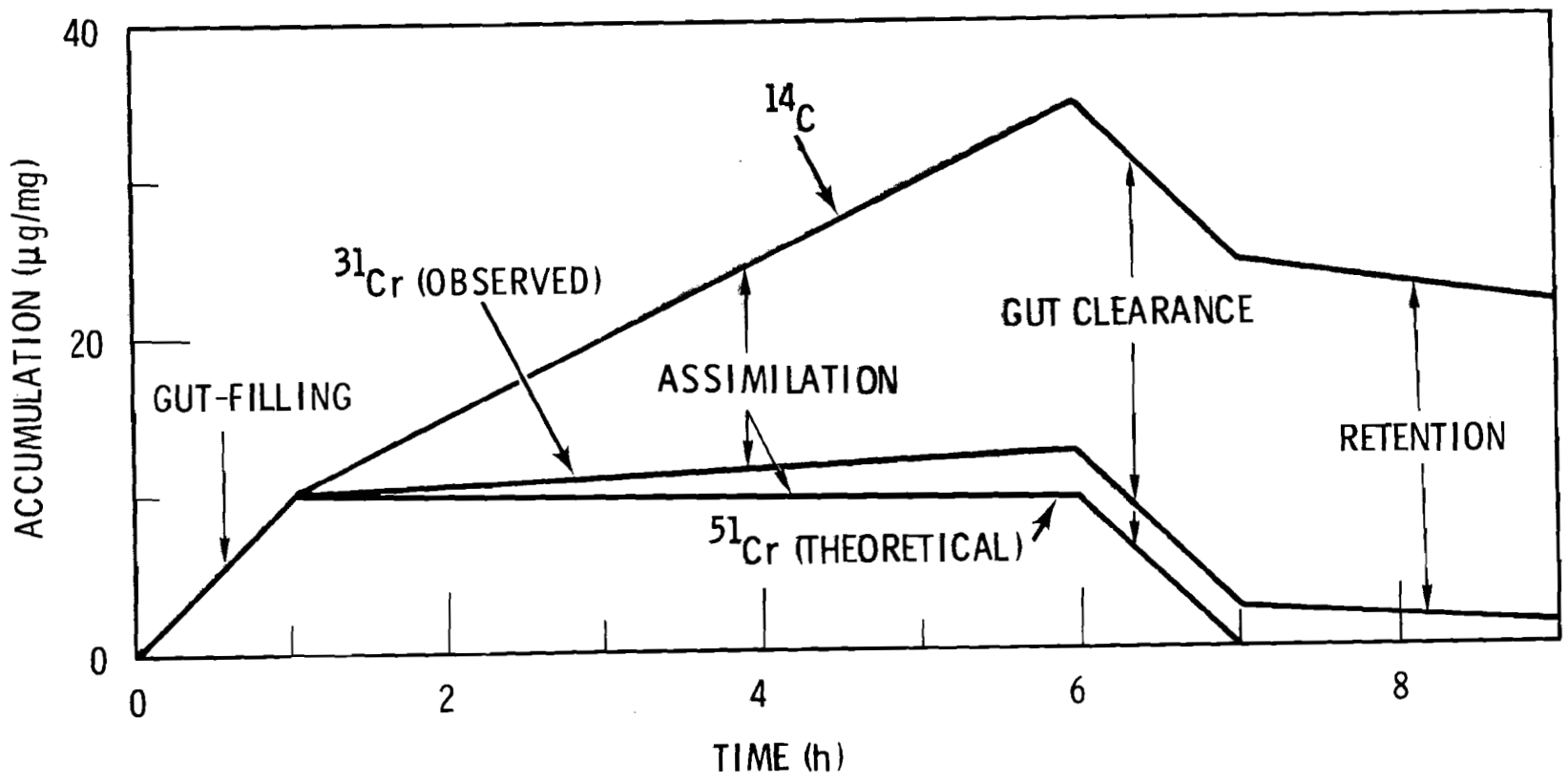


FIGURE 6. Accumulation and Retention of Labelled Food Measured by ^{51}Cr and ^{14}C. Feeding Takes Place for 6 h, After Which Animal is Transferred to Nonlabelled Food. Ingestion Rate = 10 µg/mg/h; AE = 50%; Gut-loading Time = 1 h.

If the accumulation is divided by the time, all values computed up to the time required for digestion (1.0 h) equal the ingestion rate (μg/mg/h). All other values will be called derived ingestion rate values.

Time (h)	0.5	1.0	2.0	3.0	4.0	5.0
Ingestion rate (μg/mg/h)	10.0	10.0	7.5	6.66	6.25	6.0

Decreasing values after 1.0 h are caused by dividing a slowly increasing ^{14}C activity by an increasingly greater time. An asymptote is reached quickly for large assimilation efficiencies and more slowly with small assimilation efficiencies. In the present example the percentage change in this derived value between 4.0 h and 5.0 h is

$$\frac{6.25 - 6.0}{6.0} = 4.0\%. \quad \text{The ratio of } \frac{6.0\ \mu g/mg/h}{10\ \mu g/mg/h} = 0.60$$

A graph (Fig. 7) is made of theoretical assimilation efficiencies vs. the ratios of the derived ingestion values (at the point where the rate of change of values is 5%) to the ingestion rate (e.g., 50% vs. 0.60). An unknown assimilation efficiency can be calculated by dividing the derived ingestion value in the asymptote region (where the change in derived values is < 5%) by the true ingestion rate and consulting the graph. A graph of these ratios vs. assimilation efficiencies is given below where digestion time equals 1.0 h. This graph provides accurate assimilation efficiencies only if respiratory losses of ^{14}C are small and if digestion time is close to 1.0 h. Errors of only a few percent are created if digestion time varies from 0.5 to 1.5 h. Beyond this range a new graph must be created for different digestion times. Provided that the graph is created for the correct digestion time, it is useful under any ingestion rate. It is obvious that if assimilation efficiency is 100%, the line in Fig. 6 continues with the same slope after 1.0 h; at 0% assimilation efficiency the line parallels the X-axis after gut-filling; intermediate efficiencies are indicated by intermediate slopes. Normal biological variations in gut-filling and assimilation for a population can yield irregularly increasing slopes. This new method is intended to simplify an efficiency determination by picking a derived ingestion rate value on the asymptote and relating this to the average ingestion rate. Any derived value on the asymptote near the point of 5% change can be used to calculate the efficiency with good precision.

Growth

Growth, as a component of assimilation, can be obtained by direct measurements or by subtracting the respiratory loss from assimilation. When growth rate is not measured for the experimental animal, published values of growth rates for the same or similar species can be used.

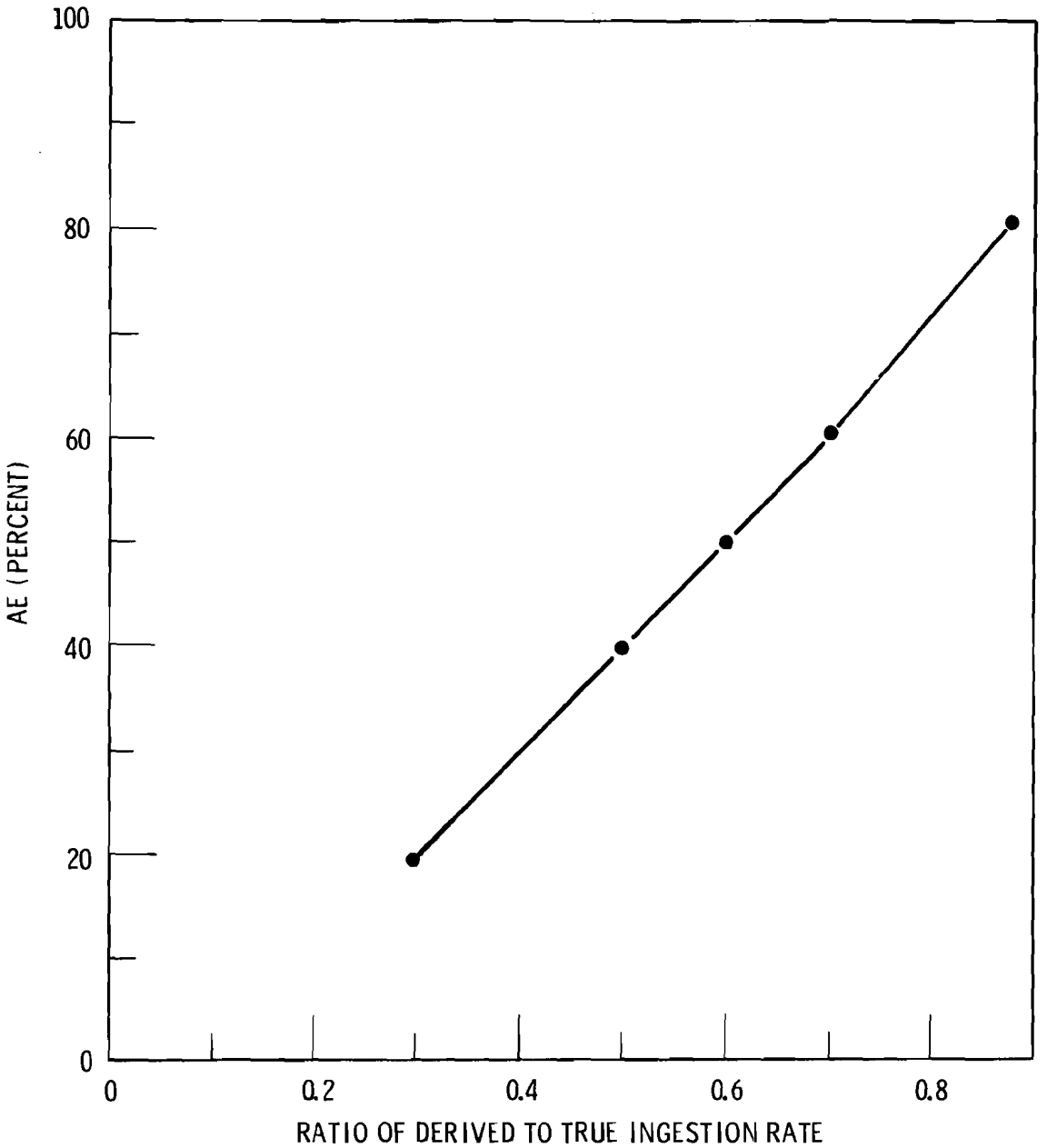


FIGURE 7. Plot of AE's vs. Ratios of Derived Ingestion Values (Where Change < 5%) to True Ingestion Rate. Digestion Time = 1.0 h

Stream type and physical and chemical conditions should be as nearly the same as possible. Especially important is the yearly temperature regime and degree-days per year (Nilsson 1974). Data from Fredeen (1959) and Tarshis (1968) on length of the life cycle in Simulium, combined with measured dry weights for various size classes, were used to calculate growth for blackfly larvae.

Growth rate can also be determined using a regression equation derived between length and weight measurements. If generations are clearly distinguishable in weekly field collections, then average length increases for the population can be transformed to average weight gain under a specific set of variables. Growth rate data on Tricorythodes available from R. L. Newell (unpublished data) in conjunction with a length: dry weight regression (D. McCullough and R. L. Newell, unpublished data) were used in calculation of the energy budget for this species. A minor source of error in determining growth of insects lies in knowing whether the gut is empty or full (see Keister and Buck 1964). There is considerable diurnal and seasonal variation in the percentage of the gut which is filled (Mecom 1970) and certain insects completely defecate before molting or pupation. Gut contents comprise a sizeable percentage of total animal weight and the gut grows with the animal. Actual tissue production of Simulium was calculated by subtracting gut weight from total body weight. If samples are always immediately preserved upon collection, animals have no time to defecate and variations in the degree of gut-filling might be reduced. Alcohol is more desirable than formalin because it reduces regurgitation and defecation, but it can leach soluble fats. In this study a saturated CO_2 solution (Arnold 1971) was used effectively to kill all three test species.

Field measurements of population or individual growth rate are always to be preferred over laboratory measurements although they are not always possible. Certain herbivore-detritivores such as Tricorythodes can be easily confined in screened chambers in the stream. Data of this kind are available from R. L. Newell (unpublished data) for Tricorythodes. He measured weekly increases in total body length. One problem encountered in measuring the lengths of live insects is that they move too quickly, or curl up. An anaesthetic might be used to quiet the animal and eliminate contraction of the abdomen which interferes with length measurements (Guttman 1967, Glwicz 1968). Another method was successfully used to measure field growth rates of Hydropsyche occidentalis in Deep Creek. Approximately 60 larvae which were nearly identical in size were tagged with ^{14}C in the laboratory (3 day feeding) and then were placed in a 25 cm square tray filled with natural stream-bottom materials. The difference in weight measured in 1 week between initial (n = 10) and final weights of tagged larvae was a measure of field growth. Detection of tagged larvae in trays was made with a windowless proportional gas flow counter.

Although no laboratory growth rates were determined for experimental animals in the present research, potential methods for measuring growth arising from preliminary experimentation with gravimetric techniques will be suggested. Laboratory growth rates can be reliable if natural light and temperature conditions are provided, and the food offered makes up the normal diet. In addition, food must be as abundant as in the field and metabolites which could inhibit growth must not be allowed to accumulate. Food should be replaced often, as the nutritive value declines on aging. Animals reared in the laboratory can be weighed fresh at intervals. A fresh weight:dry weight conversion factor is then used to compute dry weight gains. Tricorythodes withstands temporary drying for at least 15 min with no obvious adverse effects. Wet weights can be rapidly obtained on a Cahn electrobalance after blotting the animal for 1 min on absorbent paper. The X range on the Cahn electrobalance can be used to identify the point at which the percentage change in weight of the animal after blotting is minimal. At this point a reproducible wet weight can be achieved. Centrifugation of animals (see Sugden 1967 and Stanford 1973) also has been used effectively to remove water from the insects' bodies.

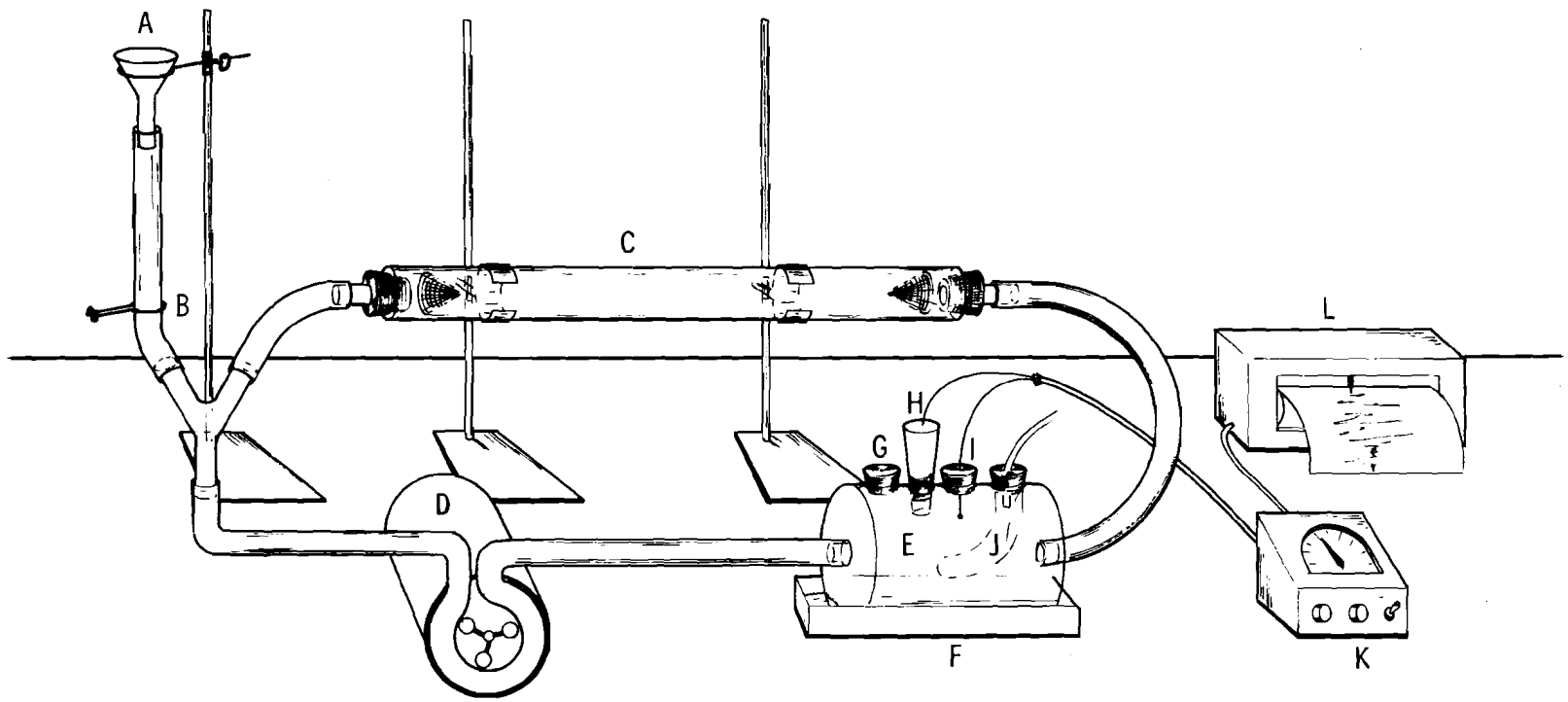
Respiration

Respiration is a major source of energy expenditure. The energy assimilated and stored in the tissues is consumed metabolically, with the resultant production of CO_2 and waste heat. The volume of CO_2 produced is equal to 0.707 to 1.00x the volume of O_2 consumed depending upon the metabolic energy source (lipid, protein, carbohydrate). This ratio of CO_2/O_2 volumes is the respiratory quotient (R.Q.) and may vary seasonally with quality of available food, physiological state of the animal (e.g., molt, dormancy, egg, larva, pupa), and temperature. R.Q. generally is assumed to be approximately 0.86. A specially devised system was used in preliminary experimentation with R.Q. measurements. The system consisted of a 150-cm^3 plexiglass cylinder with a plexiglass lid, which was sealed to its base with silicone grease. Two pH probes were inserted through holes in the lid and and were sealed in place with silicone cement. Micro-Winkler titrations were performed on this water using the 10-ml syringe method (Burke 1962). Hypodermic needles were inserted through a rubber serum cap sealing another hole. Incurrent air filled a small balloon, sealed at the mouth of a small hole in the lid, preventing diffusion of gas into the water. A water current was created with a magnetic stir bar. A sterile gravel substrate and membrane-filtered water were used to limit bacterial respiration. CO_2 production was determined through a pH change using methods of Verduin (1961) and Verduin, Beyers, and Odum (1961).

One difficulty in measuring CO_2 changes with a pH probe is interference by NH_3 (see Trama 1957). Ammonia is a metabolic product of most insects (Wigglesworth 1965) and without a separate analysis for NH_3 the portion of pH change caused by CO_2 alone cannot be determined. Many methods are available for micro-determinations of NH_3. Levanidov (1949) found the calorific equivalent for NH_3 is 5300 cal/g. One way to avoid the NH_3 interference is to precipitate the CO_2 with $Ba(OH)_2$ and to determine CO_2 by gravimetric analysis of the precipitate. Alternately NH_3 can be measured separately by micro-Kjeldahl analysis, and the pH change in the culture water from a measured quantity of NH_3 can be separated from the pH change due to CO_2 production.

A valuable radiotracer technique described by Sorokin (1968) is the determination of the relative rate of respiration. After an animal has been heavily labelled with ^{14}C-labelled food, the production of $^{14}CO_2$ is constant for a time. The amount of $^{14}CO_2$ entering the water can be measured and the effect of different environmental conditions on the animal can be detected by elevated or decreased respiration rates (i.e., $^{14}CO_2$ production). Also the extent to which assimilated foods are stored or metabolized directly can be measured. Animals which have consumed ^{14}C-labelled food could be placed in an open container (500 ml) with a dialysis bag filled with KOH absorbent to trap respired $^{14}CO_2$ over a long period of time (1-2 days) provided the water is buffered at approximately pH 8.3 (Sorokin 1968); loss of CO_2 to the air will be minimal.

Measurements of respiration rate were made on Tricorythodes in a circular flow-through system using a galvanic cell O_2 electrode (Precision Scientific Co., Chicago). The respiration chamber was a glass tube (59.5 cm long, 1.7 cm I.D.) with a monofilament net substratum (see Fig. 8). Membrane filtered (0.45 μm), aerated water was circulated in tygon tubing between the glass tube and a plexiglass water reservoir with a Masterflex peristaltic pump. Tricorythodes in the tube was exposed to a mean current speed of 6.8 cm/sec. Nymphs were acclimated under abundant food supply at 20°C for 24 h prior to the experiment. The reservoir was a plexiglass cylinder with incurrent and excurrent tubes and a removable lid. A circular plexiglass disc in the lid fit tightly inside the open end of the reservoir cylinder. The reservoir contained ports for insertion of the oxygen probe and a temperature probe; a third port accepted a rubber septum through which micro-Winkler oxygen measurements were made with a 10-ml syringe. A balloon made of surgical latex was secured inside the reservoir to a glass tube passing through the lid and filled with air as water samples were withdrawn. Atmospheric gases never contacted the water in the respiration chamber after the system was closed. Triplicate micro-Winkler D.O. measurements were made before starting a respiration run to determine the sensitivity coefficient of the O_2 probe. Then 30 ml of water were returned to the system via a T-connector in the tygon tubing to collapse the balloon and restore the original water volume. The initial D.O. measurement at t = 0 min was then determined from the meter reading on the O_2 analyzer and the sensitivity coefficient. Tricorythodes respiration experiments 1 and 2 were made for 495 and 305 min,



A. FUNNEL FOR FILLING SYSTEM
B. CLAMP
C. FLOW-THROUGH RESPIRATION CHAMBER
D. PERISTALTIC PUMP
E. RESERVOIR
F. ICE BATH
G. SERUM CAP
H. GALVANIC CELL O_2 PROBE
I. TEMPERATURE PROBE
J. LATEX TUBING
K. O_2 METER
L. STRIP CHART RECORDER

FIGURE 8. Flow-Through Respiration Chamber with Continuous Monitoring of O_2 Concentration by Galvanic Probe

respectively. After 305 min the percent saturation of the water was 51%. Respiration measurements were done on 124 and 314 nymphs in the two experiments, respectively (total animal dry weights were 34.9 and 85.6 mg, respectively). Water temperature was 20.0 ± 0.2°C. Temperature and dissolved oxygen in the system were recorded continuously on TOA electronic polyrecorders (Martek Instruments Inc., Newport Beach, CA). Triplicate micro-Winkler titrations were performed midway through each experiment and also at the completion of the run. Mean sensitivity coefficients at the three sampling times were plotted against time. Additional sensitivity coefficients were determined at 0.5 h intervals from the line connecting these points and the values were divided into the meter reading (μ amps) to calculate dissolved oxygen (mg/l) at each interval. The change in oxygen concentration at each interval was figured as a rate of change in the absolute amount of oxygen. This was based on a total initial water volume of 425 ml corrected for withdrawal of water for Winkler titrations. Mean oxygen consumption rate was calculated as μl O_2/mg DW/h.

Organization of Data

Ingestion rates and assimilation efficiencies (AE) for *Simulium* spp., *Cheumatopsyche*, and *Tricorythodes* were derived from feeding experiments on dual-labelled food. AE was calculated using the method of Calow and Fletcher (1972). Results from 12 *Simulium*, 5 *Cheumatopsyche*, and 11 *Tricorythodes* experiments are given.

Escherichia coli was the food source in *Simulium* experiments 1 and 6. Diatoms (primarily *Synedra ulna* and *Achnanthes lanceolata*) were the food source in experiments 2-5. In *Cheumatopsyche* experiments 1-5 and *Simulium* experiments 7 and 8 a mixture of diatoms from Rattlesnake Springs which had been cultured on slides was used. *Synedra* and *Achnanthes* were common but *Melosira*, *Gomphonema*, *Navicula*, and others also were numerous. It should be noted that *Simulium* experiment 7 and *Cheumatopsyche* experiment 4 are paired, as are *Simulium* experiment 8 and *Chematopsyche* experiment 5. Each set of paired experiments was run as one operation in a single feeding chamber. *Simulium* experiments 9 and 10 used Columbia River diatoms which had first been used in *Tricorythodes* experiments 6, 7, 8, and 11. These diatoms, primarily *Melosira*, *Cymbella*, and *Synedra*, with smaller amounts of *Gomphonema*, *Fragilaria*, and *Epithemia*, had not been respiked with ^{51}Cr since the mayfly experiment. Experiments 11 and 12 used the watercress which had been used in *Tricorythodes* experiments 9 and 10. Before *Simulium* experiment 11 was performed, the watercress was divided equally into two suspensions (63 ml each); *Simulium* experiment 11 used half of the supply. The remaining 63-ml suspension was respiked with ^{51}Cr on July 23, 1973, before experiment 12. No obvious fungal or bacterial invasion had taken place during its storage at 3°C in the refrigerator.

Feces for all Cheumatopsyche experiments were collected by emptying funnels (75 ml) in which the larvae had been incubated. Time allowed for defecation varied and is specified in the results. Feces collections for Simulium were performed in the same way. Gravel substrata and aeration were provided these animals, although a natural current was not simulated. In cases where actual weights of feces were obtained and counted for radioactivity, pellets were collected in a porcelain pan by pipette and were filtered on a 13-mm tared filter.

The acclimation time and feeding time vary for each experiment (see Tables 3 and 4). In Tables 3 and 4 experimental temperatures are given first with natural stream temperatures (mean daily temperature or temperatures at the time of collection) given next. The type of feeding chamber (glass cylinder, plastic trough, petri dish, plexiglass channel) is also specified. The total water volume of each of these systems is given so that the total amount of suspended material can be calculated from the μg/ml data provided. In many experiments an initial 100 ml dilution of the food was made and 1 ml samples were filtered and counted. Five, 10, or 15-ml samples of the final dilution were also filtered to determine the activity density of algae available for consumption. This activity generally is 50% or less of the initial activity, indicating a dilution by organic or inorganic contamination of the filtered water. If 200 larvae are acclimated for 1.0 h, there conceivably could be 200 x 45 μg/larvae = 9.0 mg of feces released to the water. Abrasion of the gravel substrate also might add material.

Measurements of in situ digestion time and gut weight on Simulium were used to determine an approximate ingestion rate. Ingestion data derived from tracer feeding experiments were evaluated on the basis of theoretical ingestion rates. Since the ingestion rates of filter feeders such as Simulium and Cheumatopsyche can depend heavily on food concentration and current speed, the experimental and natural food concentrations and current speeds were measured. The percentages of ash of natural seston and Simulium feces are given and AE is calculated by the ash-ratio method of Conover (1966). The percentage ash of experimental food also was determined so that asssimilation could be calculated in terms of ash-free dry weight. Experimental conditions which existed while grazing and filtration feeding rates were being measured in Simulium and Cheumatopsyche experiments (see Tables 3 and 4) are discussed in relation to their possible effects on feeding rates.

Because Simulium and Tricorythodes often were fed for periods longer than the gut-filling time, values of activity in the animals are given in terms of μg/mg per length of feeding (feeding times vary with each experiment). Because ^{14}C calculated uptake rates are expected to be greater than those of ^{51}Cr due to assimilation after the gut-filled time, and because ^{51}Cr activity levels off at the gut-filled time, a

direct conversion to ingestion rate in terms of μg/mg/h cannot always be given. For Simulium and Tricorythodes information on ingestion rates and assimilation efficiencies determined in this study were combined with data on growth and respiration to create a total energy budget. The validity of the energy budgets was checked by comparing absolute assimilation derived as a summation of growth and respiration (G + R) and that derived by multiplying ingestion rate by AE. In cases where feces were sampled, the cpm of food and feces are provided so that calculations of assimilation efficiency can be made using Calow and Fletcher's method.

Data on feeding experiments with Tricorythodes show accumulation rates of food during prolonged feeding (beyond the gut-filling time) on blue-green algae, diatoms, and watercress detritus. Accumulation was determined by the ^{14}C method and by dual tracers (^{14}C, ^{51}Cr) and AE was calculated by the dual-isotope technique. Results of the Tricorythodes experiments were calculated in terms of dry weight (DW). However, experiments using different food sources can be compared only on the basis of AFDW. The mean percent organic matter of the blue-green algae was 86.23%; diatoms--18.47%; watercress detritus--84.61%. Because further calculations in most cases did not require conversion of all individual values to AFDW, tabulated values are given only as dry weight.

RESULTS

Values for accumulation of various radiolabelled food sources in 12 Simulium, 5 Cheumatopsyche, and 11 Tricorythodes feeding experiments are presented. The accuracy of accumulation values in relation to errors of counting and biological variability is discussed. In Simulium experiments values of food accumulated by filtration and grazing are given. Filtration feeding rates are compared according to types of feeding chambers. Grazing rates are compared with filtration rates from a single feeding chamber. Assimilation efficiencies were calculated using the dual-label method. Data are then developed (length-dry weight relationship, gut-fraction and digestion time measurements and theoretical ingestion rate) which are used to create an energy budget. The concentrations of food in experiments and in Rattlesnake Springs are compared in relation to experimental feeding rates to evaluate the suitability of the feeding chambers. The percentage ash values of food and feces of Simulium are given so that the AE can be derived by the ash-ratio method and so that conversion of ingestion rates to calories is possible. Measurements of accumulation by grazing for Cheumatopsyche are given. Filtration rates are presented in relation to the type of feeding chamber. AE values based on the dual-label method were computed. Values of food accumulation after various feeding periods are given for Tricorythodes. Comparisons are made between the uptake curves obtained in feeding experiments and inferences are drawn regarding ^{51}Cr assimilation, digestion time, assimilation rate, and retention of ^{14}C. AE values were derived for Tricorythodes on two food sources based on the dual-label method. Chromium-51 adsorption and assimilation and the leaching of isotopes from food is then discussed regarding effects on the accumulation values.

Simulium

Simulium Feeding Experiments

The accumulation rates of ^{51}Cr- and ^{14}C-labelled food sources by Simulium under a variety of physical conditions such as current speed and food concentration were measured in 12 feeding experiments. The environment created by three different feeding chambers also imposed a set of unique physical conditions upon the feeding process. Results of the Simulium feeding experiments give the amounts of food accumulated within the feeding time. Because the elapsed feeding time sometimes exceeded the gut-filling time, these accumulations were not converted to an equivalent accumulation per hour.

In the 12 Simulium and 5 Cheumatopsyche experiments, 15 different 10-min counts were made of background for ^{51}Cr. The mean count rate (n = 15) was 47.2 cpm. However, applying Chauvenet's criterion (Chase and Rabinowitz 1967) the count rates in Simulium experiment 1 (63 cpm) and Cheumatopsyche experiment 1 (56 cpm) were rejected because the probability of their occurrence was < 1/2 n where n is the number of observations. The mean of the remaining 13 background count rates was 45.3 ± 0.59 (1 S.D.) cpm. The background count rate is known precisely; the standard deviation of the mean is small because the total counting time was long (130 min) and many counts were observed. The variation expected from background within a shorter time span would be much greater. Since samples were counted for 10 min, the standard deviation of the mean background count rate in 10 min (45.3 ± 2.13 cpm) was applied to percent error determinations. Knowing the mean background count rate and the value k (gross counts/background counts), then the standard deviation of the net count rate, the confidence limits (P = 0.05) and percent error of the net rate can be calculated.

For example if $k = 2.8$

and $R_b = 45.3$ cpm

then $R_{s+b} = 126.8$

$R_s = 81.5$

and $\sigma R_s = \left(\frac{126.8}{10} + \frac{45.3}{10}\right)^{1/2} = 4.1$

$R_s = 81.5 \pm 8.1 \ (P = 0.05)$

$\therefore R_s = 81.5 \pm 10\%$

If the net count rate is known within ± 10%, then the percent error of the accumulation values is 10%. The minimum, median and maximum k values were calculated (Tables 8 and 9) for all samples of Simulium and Cheumatopsyche larvae which measured ingestion using ^{51}Cr. In addition, the confidence limits of the net count rate and the percent error were calculated for many values of k (Table 10). The values of percent error of the net count rate change rapidly between k values of 1.2 and 2.3 (from 68.1 to 12.9%, respectively) but range only from 10 to 5% while k values range from 2.8 to 5.8. For k values about 5.8 the percent error of the net count rate is < 5%. Percent error of ^{14}C-derived accumulation values were insignificant compared to ^{51}Cr-derived values.

TABLE 8. Minimum, Median, and Maximum Values of k ($\frac{\text{gross sample count rate}}{\text{background count rate}}$) Calculated From Those Samples of *Simulium* Larvae Using ^{51}Cr. Values based on mean background of 45.3 cpm (n = 13). The minimum, median, or maximum percent error in accumulation values from *Simulium* experiments 2-12 is found by entering Table 10 with the appropriate k value.

Simulium Experiment	k Values		
	Minimum	Median	Maximum
2	1.4	1.8	3.5
3	1.4	3.2	7.1
4	1.6	4.7	10.8
5	1.3	2.7	3.8
6	1.2	1.6	2.1
7	34.7	60.1	262.1
8	3.4	7.0	25.8
9	3.6	10.8	32.1
10	21.8	31.5	74.9
11	5.8	6.0	7.5
12	13.9	58.1	108.4

Filtration Experiments in the Glass Cylinder Chamber Using a Bacterial Food Source

Simulium experiments 1 and 6 (Tables 11-12) measured the filtration rate of *Simulium* on *E. coli*. The food concentration in these experiments was 31.2 and 15.0 mg/ℓ, respectively, which provided a much greater food concentration than available in Rattlesnake Springs (0.97-2.5 mg/ℓ). Accumulation in experiment 1 was measured with both ^{51}Cr and ^{14}C. The mean of four ^{51}Cr-derived measurements was 2.139 μg/mg although this mean would be reduced if zero values were averaged. Because k values (gross count rate/background count rate) are near unity for the samples where accumulation values were derived, the percent error involved in the measurements probably makes these values meaningless. The ^{14}C activity density

TABLE 9. Minimum, Median, and Maximum Values of k Calculated from Those Samples of *Cheumatopsyche* Larvae (Experiments 1-5) Which Estimated Accumulation Rate Using ^{51}Cr. Values are Based on Mean Background of 45.3 cpm (n = 13). The Minimum, Median, or Maximum Percent Error in *Cheumatopsyche* Experiments 1-5 is Found by Entering Table 10 with the Appropriate k Value.

Cheumatopsyche Experiment	k Values		
	Minimum	Median	Maximum
1	1.6	1.8	4.2
2	2.3	4.9	17.1
3	4.4	11.1	15.2
4	27.1	87.2	162.7
5	8.3	30.1	87.5

TABLE 10. Confidence Limits and Percent Error of Net Sample Count Rates (P = 0.05) Where Background is 45.3 cpm and k Varies from 1.2 to 5.8. If the Ratio of Gross Count Rate/Background Count Rate of a Sample is Calculated the Confidence Limits and the Percentage Error of the Net Count Rate can be Derived.

k	Net Count Rate (cpm)	Confidence Limits (P = 0.05) (± cpm)	% Error
1.2	9.1	6.2	68.1
1.3	13.6	6.3	46.3
1.4	18.1	6.5	35.7
1.6	27.2	6.7	24.7
1.8	36.2	7.0	19.3
2.3	58.9	7.6	12.9
2.8	81.5	8.1	10.0
3.1	95.1	8.4	8.9
3.4	108.7	8.8	8.1
4.4	154.0	9.6	6.2
5.5	203.9	10.6	5.2
5.8	217.4	10.9	5.0

TABLE 11. Accumulation of Labelled Food (Bacteria) by *Simulium* (Experiment 1) During 30 Min Feeding

ANIMALS

Animals Size (mm)	Animals No. (n)	Total DW (mg)	µg DW Food Accum/n Indiv ^{51}Cr	µg DW Food Accum/mg ^{51}Cr
4.0 - 5.0	5	1.645	3.254	1.978
4.5 - 5.5	5	1.678	8.430	5.024
4.5 - 5.5	5	1.280	-	-
4.5 - 5.5	5	1.872	-	-
5.5 - 6.5	3	1.781	-	-
5.5 - 6.5	5	2.168	1.035	0.477
6.5 - 7.5	12	9.905	10.649	1.075

FOOD

Aliquot	µg DW Filtered	Corrected cpm ^{51}Cr	Corrected cpm ^{14}C	Food Activity µg/cpm ^{51}Cr	Food Activity µg/cpm ^{14}C
1/100	156	106.2	799.09	1.469	0.195
1/100	156	102.3	810.3	1.525	0.193
1/100	152	102.1	767.7	1.489	0.198
1/100	160	111.2	797.1	1.439	0.201

Background (cpm) ^{51}Cr - 63
^{14}C - 20

of bacteria was nearly 8 times greater than for ^{51}Cr which would increase the precision of accumulation measurements. Animal radioactivity, however, was not significantly different from the background so that zero ingestion was detected.

The high activity density of ^{51}Cr-labelled bacteria in experiment 6 enabled precise measurements of accumulation. Because of k values ranged only from 1.4-1.8 (Table 8) the percent error of the mean ranged from

TABLE 12. Accumulation of Labelled Food (Bacteria) by *Simulium* (Experiment 6) During 1.0 h Feeding

ANIMALS

Animals Size (mm)	Animals No. (n)	Total DW (mg)	µg DW Food Accum/n Indiv ^{51}Cr	µg DW Food Accum/mg ^{51}Cr
4.6 - 5.5	10	5.528	0.360	0.0651
4.6 - 5.5	5	3.278	0.149	0.0455
5.6 - 6.5	10	10.792	0.374	0.0347
5.6 - 6.5	10	10.658	0.362	0.0339
5.6 - 6.5	10	9.216	0.385	0.0418
5.6 - 6.5	10	8.996	0.444	0.0494
5.6 - 6.5	10	11.472	0.444	0.0387
5.6 - 6.5	10	9.594	0.924	0.0963
5.6 - 6.5	10	10.140	0.529	0.0522
5.6 - 6.5	10	10.004	0.325	0.0325
5.6 - 6.5	10	10.978	0.696	0.0634
5.6 - 6.5	5	4.900	0.239	0.0488
6.6 - 7.5	10	13.816	0.376	0.0272
6.6 - 7.5	10	11.796	0.495	0.0420

FOOD

Aliquot	µg DW Filtered	Corrected cpm ^{51}Cr	Food Activity µg/cpm ^{51}Cr
1/100	109	7970.2	0.01368
1/100	86	6838.6	0.01258
1/100	116	7560.4	0.01534
1/100	115	8187.7	0.01405
			0.01391 (Ave)

Background 46

35.7 to 19.3%. In fact, the 95% confidence limits of the mean accumulation (n = 14) were equal to the mean ± 21%. The accumulation of bacteria ($\bar{x}$ = 0.048 μg/mg) is negligible; the calculated values are probably indicative only of ^{51}Cr adsorption.

Filtration Experiments in the Glass Cylinder Chamber Using a Diatom Food Source

Simulium experiments 2-4 measured filtration of diatoms in the glass cylinder feeding chamber (Tables 13-15). The mean ^{51}Cr-derived accumulation during the feeding periods in these experiments was 2.283, 1.407, and 1.020 μg/mg, respectively. Mean ^{14}C derived accumulations in these experiments were less than ^{51}Cr-derived values, being 1.931, 1.307, and 0.920 μg/mg, respectively. There is, however, no significant difference between ^{51}Cr- and ^{14}C-derived values because of the large overlap in confidence limits (Table 23). The accumulation values derived from the lowest gross count rates in the three experiments (Tables 13-15) have k values of 1.4 or greater (Table 8) so that the maximum error of ^{51}Cr-derived mean values is about 35.7% (Table 10). The minimum error in experiments 2-4 was 8% or less. The confidence limits of samples used to estimate accumulation (^{51}Cr) (Table 23) were equal to the mean ± 81, 49, and 65%, respectively, in the three experiments. Variations of this magnitude among samples is largely biological variability because statistical errors of counting are much smaller. The median k values of samples in these experiments (Table 8) indicate that the median errors due to counting were about 19.3, 8.7, and 5.8%, respectively.

The values of food accumulation by Simulium in experiments 2-4 are all similar. In each experiment the highest value was found in the smallest size class. The mean ^{51}Cr values of food retained after defecation are not significantly less than (P = 0.05) the mean values of accumulation, although ^{14}C-derived values after defecation in experiments 2 and 3 are. This probably indicates that either ^{51}Cr adsorption accounts for a large proportion of the ^{51}Cr-derived accumulation or that food in the anterior portion of the gut is not defecated during starvation. In either case little food was ingested in the glass cylinder feeding chamber although the amount was greater than in experiments 1 and 6 when bacteria were offered.

Accumulation of Diatoms by Grazing in the Glass Cylinder Chamber

The ^{51}Cr- and ^{14}C-derived mean accumulation values by grazing (experiment 5, Table 16) were 0.150 and 0.245 μg/mg, respectively. The coefficient of variation among ^{51}Cr values is greater than among ^{14}C values

TABLE 13. Accumulation of Labelled Food (Diatoms) by *Simulium* (Experiment 2) during 45 min Feeding

ANIMALS

Animals Size (mm)	Animals No. (n)	Total DW (mg)	µg DW Food Accum/n Indiv ^{51}Cr	µg DW Food Accum/n Indiv ^{14}C	µg DW Food Accum/mg ^{51}Cr	µg DW Food Accum/mg ^{14}C
4.5 - 5.5	10	5.782	32.11	25.37	5.553	4.388
5.5 - 6.5	10	9.716	9.48	10.74	0.976	1.105
5.5 - 6.5	10	8.104	14.87	12.50	1.835	1.542
6.5 - 7.5	10	14.746	14.38	14.84	0.975	1.006
6.5 - 7.5	10	13.944	19.69	17.78	1.412	1.275
7.5 - 8.6	10	16.286	48.49	37.00	2.977	2.272
After 1 h defecation	23	25.098	23.94	17.74	0.954	0.707

FOOD

Aliquot	µg DW Filtered	Food Activity µg/cpm ^{51}Cr	Food Activity µg/cpm ^{14}C
1/100	165	0.385	0.005207
1/100	192	0.432	0.005863
		0.409(Ave)	0.005535(Ave)

Background (cpm) ^{51}Cr - 40
^{14}C - 49

TABLE 14. Accumulation of Labelled Food (Diatoms) by *Simulium* (Experiment 3) during 1.0 h Feeding

ANIMALS

Animals Size (mm)	No. (n)	Total DW (mg)	µg DW Food Accum/n Indiv ^{51}Cr	µg DW Food Accum/n Indiv ^{14}C	µg DW Food Accum/mg ^{51}Cr	µg DW Food Accum/mg ^{14}C
4.6 - 5.5	10	6.750	16.10	13.87	2.385	2.055
4.6 - 5.5	10	5.324	7.62	7.71	1.431	1.448
4.6 - 5.5	7	4.292	2.04	2.35	0.475	0.548
5.6 - 6.5	10	12.272	8.91	9.18	0.726	0.748
5.6 - 6.5	10	13.356	13.57	13.55	1.016	1.015
5.6 - 6.5	10	10.492	27.21	24.35	2.593	2.321
6.6 - 7.5	10	15.452	31.18	25.99	2.018	1.682
6.6 - 7.5	6	10.574	6.59	6.79	0.623	0.642
After 1.5 h	24	30.360	21.84	18.20	0.719	0.599
defecation	38	36.926	29.84	25.47	0.808	0.690
					0.764 (Ave)	0.645 (Ave)

FOOD

Aliquot	µg DW Filtered	Corrected cpm ^{51}Cr	Corrected cpm ^{14}C	Food Activity µg/cpm ^{51}Cr	Food Activity µg/cpm ^{14}C
1/100	175	1493.0	43268	0.1172	0.004130
1/100	201	1719.0	47030	0.1169	0.004274
1/100	176	1701.3	48319	0.1034	0.003642
				0.1125 (Ave)	0.004015 (Ave)

FECES

Corrected cpm ^{51}Cr	Corrected cpm ^{14}C	Approx. µg DW ^{51}Cr	Approx. µg DW ^{14}C
21.8	1143	2.45	4.59
13.0	313	1.46	1.26

Background (cpm) ^{51}Cr - 46
^{14}C - 49

TABLE 15. Accumulation of Labelled Food (Diatoms) by *Simulium* (Experiment 4) during 45 min Feeding

ANIMALS

Animals Size (mm)	No. (n)	Total DW (mg)	µg DW Food Accum/n Indiv ^{51}Cr	µg DW Food Accum/n Indiv ^{14}C	µg DW Food Accum/mg Animal ^{51}Cr	µg DW Food Accum/mg Animal ^{14}C
4.6 - 5.5	9	4.002	16.020	12.857	4.003	3.213
5.6 - 6.5	10	8.022	11.848	10.430	1.477	1.300
5.6 - 6.5	10	7.480	19.098	14.874	2.553	1.989
5.6 - 6.5	10	7.288	3.409	4.072	0.468	0.559
5.6 - 6.5	7	5.076	3.823	3.772	0.753	0.743
6.6 - 7.5	10	13.034	9.254	8.843	0.710	0.678
6.6 - 7.5	10	13.872	3.435	4.317	0.248	0.311
6.6 - 7.5	10	14.192	6.599	6.848	0.465	0.483
6.6 - 7.5	10	13.388	7.146	7.708	0.534	0.576
6.6 - 7.5	7	8.018	1.129	2.031	0.141	0.253
7.6 - 8.5	10	15.888	14.288	13.449	0.899	0.846
7.6 - 8.5	10	18.190	12.848	12.148	0.706	0.668
7.6 - 8.5	12	19.484	5.793	6.579	0.297	0.338
After 3 h	25	25.086	17.753	15.304	0.708	0.610
Defecation	26	29.376	4.521	6.958	0.154	0.237
	36	36.570	35.630	30.390	0.974	0.831
					0.612 (Ave)	0.559 (Ave)

FOOD

Aliquot	Filtered µg DW	Corrected cpm ^{51}Cr	Corrected cpm ^{14}C	Food Activity µg/cpm ^{51}Cr	Food Activity µg cpm ^{14}C
1/100	139	3038.3	27631.0	0.0457	0.00503
1/100	148	3483.6	33043.6	0.0425	0.00448
1/100	147	3581.9	31888.8	0.0410	0.00461
				0.0431 (Ave)	0.00477 (Ave)

FECES

Corrected cpm ^{51}Cr	Corrected cpm ^{14}C
248.1	4303.0
457.1	4020.4

Background ^{51}Cr - 46 (cpm)

TABLE 16. Accumulation of Labelled Food (Diatoms) by *Simulium* (Experiment 5) during 1.0 h Feeding

ANIMALS

Animals Size (mm)	No. (n)	Total DW (mg)	µg DW Food Accum/n Indiv ^{51}Cr	µg DW Food Accum/n Indiv ^{14}C	µg DW Food Accum/mg Animal ^{51}Cr	µg DW Food Accum/mg Animal ^{14}C
4.6 - 5.5	9	4.452	0.581	1.147	0.131	0.258
5.6 - 6.5	10	10.960	1.682	2.616	0.153	0.239
5.6 - 6.5	10	8.698	1.054	1.830	0.121	0.210
5.6 - 6.5	8	7.302	1.733	2.878	0.237	0.394
6.6 - 7.5	10	14.002	3.327	3.433	0.238	0.245
6.6 - 7.5	6	8.652	0.408	1.356	0.0472	0.157
6.6 - 7.5	10	16.952	2.092	3.758	0.123	0.222
6.6 - 7.5	10	13.604	2.152	3.435	0.158	0.252
6.6 - 7.5	10	13.854	1.984	3.348	0.143	0.242
6.6 - 7.5	10	15.852	2.379	3.064	0.150	0.193
7.6 - 8.5	7	12.484	1.909	3.483	0.153	0.279
After	29	36.462	1.413	6.561	0.0388	0.180
Defecation	9	11.584	0.309	1.892	0.149	0.163
					0.0939 (Ave)	0.1715 (Ave)

FOOD

Dry wt (µg)	Corrected cpm ^{51}Cr (x 104)	Corrected cpm ^{14}C (x 105)	Food Activity µg/cpm ^{51}Cr	Food Activity µg/cpm ^{14}C
1030	3.531	3.696	0.02917	0.002787
3874	15.052	13.130	0.02574	0.002949
1750	77.532	7.284	0.02257	0.002402
			0.02583 (Ave)	0.002713 (Ave)

FECES

Corrected cpm ^{51}Cr	Corrected cpm ^{14}C	Approx. µg DW ^{51}Cr	Approx. µg DW ^{14}C
21.7	300.3	0.561	0.815
10.6	167.9	0.274	0.456

Background (cpm) ^{51}Cr - 45
^{14}C - 36

(Table 23). Chromium-51- and ^{14}C-derived mean values are significantly different ($P < 0.05$). The maximum error due to ^{51}Cr counting was 46% in this experiment while the median error was about 11% ($k = 2.7$). Differential absorption was probably responsible for the greater ^{14}C values. Nonetheless, accumulation was very low and probably entirely due to adsorption; grazing apparently was not significant.

Accumulation of Diatoms by Filtration in Trough Feeding Experiments

Filtration of diatoms in the trough feeding chamber was measured in Simulium experiments 7-10 (Tables 17-20). Feeding times in these experiments varied from 1.0 to 20 h and experimental temperatures varied from 15 to 22.5 C (Table 3). Mean ^{51}Cr-derived accumulation values ranged from 6.723 μg/mg in 1.5 h to 124.24 μg/mg in 20 h (Table 23). Mean accumulation in 3.0 h in experiment 7 (20.560 μg/mg) is nearly three times the 1.0 h accumulation in experiment 8 (7.695 μg/mg). The mean ^{51}Cr- derived accumulation in 1.5 h in experiment 9 is roughly equal to the value in experiment 8. The total ^{51}Cr-derived accumulation after 20 h feeding in experiment 10 (124.24 μg/mg) is much greater than the values from other diatom filtration experiments but the accumulation per h (6.21 μg/mg/h) is similar to values calculated from experiments 7-9. It appears that the low accumulation rates determined in 1.0 to 3.0 h feeding may be sustained for longer feeding periods. Neither the ^{51}Cr- nor ^{14}C-derived accumulation values after 5.0 h of defecation in experiment 7 were significantly different from the mean accumulations after 3.0 h feeding. In experiment 7 it seems clear that there is an inverse correlation between accumulation and size class. Chromium-51-derived accumulations in experiment 10 are very similar to gut fractions for Simulium calculated from work by Ladle et al. (1972) (Table 31). This indicates that under these feeding conditions 20 h may be required for gut-filling. This gut-filling time is much greater than the 1.0 h time derived by in situ measurements.

Samples acting as a control for ^{51}Cr and ^{14}C adsorption in experiments 9 and 10 were similar even though there was a large difference in exposure time (1.5 cf 20 h). The values ranged from 0.65 to 1.32 μg/mg. Accumulations of diatoms by filtration in the glass cylinder feeding experiments (2-4) were not greatly different from the control values in the trough feeding experiments indicating that adsorption was largely responsible for the accumulations in those experiments.

In experiment 10 ^{51}Cr-derived accumulation values are much greater than ^{14}C-derived accumulations (significantly different at $P = 0.01$). Accumulations were calculated based on three food activity density measurements between $t = 165$ and 195 min. Leaching of ^{51}Cr from diatoms during the 20 h feeding period was greater than with ^{14}C. According to these data this should tend to make ^{51}Cr-derived accumulation values

TABLE 17. Accumulation of Labelled Food (Diatoms) by *Simulium* (Experiment 7) during 3.0 h Feeding

ANIMALS

Animals Size (mm)	Animals No. (n)	Total DW (mg)	µg DW Food Accum/mg Animal ^{51}Cr	µg DW Food Accum/mg Animal ^{14}C
3.6 - 4.5	10	1.670	36.74	29.79
3.6 - 4.5	10	1.924	29.15	22.32
4.6 - 5.5	10	3.976	24.56	19.25
4.6 - 5.5	10	3.888	24.22	20.94
4.6 - 5.5	10	4.267	35.87	29.02
4.6 - 5.5	10	3.689	26.10	20.41
5.6 - 6.5	10	8.984	14.14	11.37
5.6 - 6.5	10	8.162	10.69	9.91
5.6 - 6.5	9	9.436	10.48	8.70
5.6 - 6.5	10	8.270	19.98	15.39
5.6 - 6.5	10	8.516	16.26	13.48
5.6 - 6.5	10	8.851	13.99	12.32
6.6 - 7.5	9	10.262	8.44	7.10
Assorted Sizes		25.194	17.22	13.99
After 5.0 h		24.398	15.14	11.62
Defecation		37.703	18.32	13.98
			16.73 (Ave)	12.80 (Ave)

FOOD

Aliquot	µg DW Filtered	Sample Time (t = min)	Corrected cpm ^{51}Cr	Corrected cpm ^{14}C	Food Activity µg/cpm ^{51}Cr	Food Activity µg/cpm ^{14}C
1/100	246	0	6309.0	49051.6	0.03899	0.005015
1/100	239	0	6528.3	51194.0	0.03661	0.004669
1/100	233	0	6772.5	51644.0	0.03440	0.004512
					0.03667 (Ave)	0.004732 (Ave)
5/1095	64	21	564.0	5195.6	0.1135	0.01232
5/1095	41	124	125.9	1619.6	0.3257	0.02531
5/1095	60	131	245.5	3117.2	0.2444	0.01925
					0.2279 (Ave)	0.01896 (Ave)

FECES

Corrected cpm ^{51}Cr	Corrected cpm ^{14}C
240.2	853.0
979.3	3462.8

Background (cpm) ^{51}Cr - 44
^{14}C - 27

TABLE 18. Accumulation of Labelled Food (Diatoms) by *Simulium* (Experiment 8) during 1.0 h Feeding

ANIMALS

Animals Size (mm)	Animals No. (n)	Total DW (mg)	µg DW Food Accum/n Indiv ^{51}Cr	µg DW Food Accum/n Indiv ^{14}C	µg DW Food Accum/mg Animal ^{51}Cr	µg DW Food Accum/mg Animal ^{14}C
3.6 - 4.5	10	1.922	18.464	14.495	9.607	7.542
4.6 - 5.5	10	3.720	26.613	17.902	7.154	4.812
4.6 - 5.5	10	3.875	15.231	15.487	3.931	3.997
4.6 - 5.5	10	3.110	27.939	21.993	8.984	7.072
4.6 - 5.5	10	3.404	18.411	12.191	5.409	3.581
5.6 - 6.5	10	7.880	30.537	22.144	3.875	2.810
5.6 - 6.5	10	6.938	121.253	99.142	17.477	14.290
5.6 - 6.5	10	7.230	58.993	42.909	8.159	5.935
5.6 - 6.5	10	5.989	55.899	45.204	9.336	7.548
6.6 - 7.5	10	10.692	11.512	10.925	1.077	1.022
6.6 - 7.5	10	8.288	71.583	52.293	8.637	6.309
Assorted Sizes		8.087	70.311	53.934	8.694	6.669

FOOD

Aliquot	µg DW Filtered	Sample Time (t = min)	Corrected cpm ^{51}Cr	Corrected cpm ^{14}C	Food Activity µg/cpm ^{51}Cr	Food Activity µg/cpm ^{14}C
1/100	199	0	4800.9	56196.9	0.04145	0.03541
5/1110	77	0	865.6	9214.7	0.08896*	0.008356
5/1105	45	30	406.5	5589.7	0.1107*	0.008051
5/1100	76	45	624.5	8439.1	0.1217	0.01193
5/1090	57	60	460.8	6369.3	0.1237*	0.006867
					0.1078 *(Ave)	0.007758 *(Ave)

FECES

Corrected cpm ^{51}Cr	Corrected cpm ^{14}C
1224.6	8300.7
2082.6	13928.3

Background (cpm) ^{51}Cr - 45
^{14}C - 43

TABLE 19. Accumulation of Labelled Food (Diatoms) by *Simulium* (Experiment 9) during 1.5 h Feeding

ANIMALS

Animals Size (mm)	Animals No. (n)	Total DW (mg)	µg DW Food Accum/n Indiv ^{51}Cr	µg DW Food Accum/mg Animal ^{51}Cr
4.6 - 5.5	11	1.738	9.284	5.342
5.6 - 6.5	9	2.752	8.015	2.912
6.6 - 7.5	25	14.650	97.163	6.632
7.6 - 8.5	6	4.336	52.062	12.007
Control	-	14.248	12.592	0.884

^{51}Cr Dissolved in Water

Sample Volume (ml)	Sample Time (t = min)	Corrected cpm ^{51}Cr	cpm/ml
5	20	96.9	19.4
10	90	132.1	13.2

FOOD

Aliquot	µg DW Filtered	Sample Time (t = min)	Food Activity µg/cpm ^{51}Cr
5/900	218	0	0.06316
5/890	106	40	0.06545
10/885	169	60	0.07486
10/875	156	82	0.07221
			0.06892 (Ave)

FECES

Dry wt (µg)	Correct cpm ^{51}Cr
143	10
54	0.1
197	127.3
91	-1.9

Background ^{51}Cr - 44 (cpm)

TABLE 20. Accumulation of Labelled Food (Diatoms) by *Simulium* (Experiment 10) during 20 h Feeding

ANIMALS

Animals Size (mm)	Animals No. (n)	Dry wt (mg)	µg DW Food Accum/n Indiv ^{51}Cr	µg DW Food Accum/n Indiv ^{14}C	µg DW Food Accum/mg Animal ^{51}Cr	µg DW Food Accum/mg Animal ^{14}C
2.6 - 3.5	27	1.770	127.67	72.88	72.13	41.17
3.6 - 4.5	14	0.858	130.78	64.26	152.42	74.89
4.6 - 5.5	15	2.332	362.05	194.99	155.25	83.61
5.6 - 6.5	11	3.928	452.58	227.69	115.22	57.97
6.6 - 7.5	3	1.482	186.98	115.37	126.17	77.85
Control	-	3.620	3.69	2.35	1.02	0.65
Control	-	2.444	3.23	1.91	1.32	0.78

FOOD

Aliquot	Dry wt (µg)	Sample Time (t = h, min)	Food Activity µg/cpm ^{51}Cr	Food Activity µg/cpm ^{14}C
10/820	130	165 min	0.1201*	0.005145*
10/810	141	180 min	0.1435*	0.006133*
5/800	74	195 min	0.1421*	0.006264*
10/795	102	19 h 15 min	0.7328	0.01110
5/785	56	19 h 35 min	0.9318	0.01351
5/780	263	19 h 45 min	4.052	0.06143
			0.1352* *(Ave)	0.005847* *(Ave)

FECES

Dry wt (µg)	Corrected cpm ^{51}Cr	Corrected cpm ^{14}C	Feces Activity µg/cpm ^{51}Cr	Feces Activity µg/cpm ^{14}C
295	2000.8	19807.6	0.1474	0.01489
136	963.7	10547.8	0.1411	0.01289
108	887.8	9442.8	0.1216	0.01143
231	946.5	9610.0	0.2441	0.02403
267	1686.3	14717.4	0.1583	0.01814
482	2894.8	29914.6	0.1665	0.01611

Background (cpm) ^{51}Cr - 46
^{14}C - 32

smaller than ^{14}C-derived values. However, the relationship between the three initial food activity densities and feces activity densities did not conform precisely to theory as there was virtually no difference between food and feces ^{51}Cr activity densities. It was expected that ^{51}Cr activity density of feces samples would increase from food values. Leaching of ^{51}Cr in the gut must have balanced its concentration as organic matter was assimilated. The ^{14}C activity density of food decreased in the feces as expected, showing preferential uptake of the organic fraction of diatoms.

The errors due to counting are a relatively insignificant source of variation compared to natural biological variability. Median values of k show that the median percent errors due to counting a sample are approximately 4.2% or less in experiments 7-10 while the confidence limits for accumulation values are about ± 25.7, 20.8, 91.2, and 33.6% of the mean values.

Accumulation of Watercress by Filtration in the Trough Feeding Chamber

The means of ^{51}Cr- and ^{14}C-derived accumulation of watercress by filtration during 6.0 h feeding (experiment 11, Table 21) were only 5.84 and 8.23 μg/mg, respectively. These values are comparable to accumulation values from only 1.0 and 1.5 h of feeding in experiments 8 and 9 in which diatoms were the food source. Experimental temperatures in experiment 10 were equivalent to those in experiment 11 but the mean accumulation in the former experiment was far greater. The food concentration in experiment 11 was 8.0 mg/ℓ (Table 33), which is much higher than natural food concentrations in the stream. No other explanation for the low accumulation values can be formulated except that preference of Simulium for finely ground watercress is less than for diatoms.

Leaching of both isotopes accounts for the serial reduction in activity density of food samples with time. It is unlikely that addition of unlabelled feces to the water after 0.5 h acclimation would be great enough to be responsible for the continual reduction. The activity densities of ^{51}Cr and ^{14}C both decreased from the food to the feces by a factor of about 10. It is probable that leaching in the gut is responsible for loss of ^{51}Cr and that a portion of the reduction in ^{14}C activity density was simply a result of assimilation. It appears that isotope leaching in experiments where water circulation is rapid results in some uncertainty in measurements.

TABLE 21. Accumulation of Labelled Food (Watercress) by *Simulium* (Experiment 11) during 6.0 h Feeding

ANIMALS

Animals Size (mm)	Animals No. (n)	Total DW (mg)	µg DW Food Accum/n Indiv ^{51}Cr	µg DW Food Accum/n Indiv ^{14}C	µg DW Food Accum/mg Animal ^{51}Cr	µg DW Food Accum/mg Animal ^{14}C
Assorted		7.067	42.13	60.66	5.96	8.58
Assorted		6.272	40.78	56.33	6.50	8.98
Assorted		11.020	55.62	80.15	5.05	7.27

FOOD

Volume Sampled (ml)	Sample Time (t = min)	Dry wt (µg)	Corrected cpm ^{51}Cr	Corrected cpm ^{14}C	Food Activity µg/cpm ^{51}Cr	Food Activity µg/cpm ^{14}C
5	30	34	266.4	1336.6	0.1276	0.02544
10	35	89	445.8	2740.2	0.1996	0.03248
10	70	84	352.2	2380.0	0.2385	0.03529
					0.1886 (Ave)	0.03107 (Ave)

FECES

Dry wt (µg)	Corrected cpm ^{51}Cr	Corrected cpm ^{14}C	Feces Activity µg/cpm ^{51}Cr	Feces Activity µg/cpm ^{14}C
100	52.5	352.2	1.9048	0.2839
259	77.9	695.7	3.3248	0.3723
416	228.4	1542.1	1.8214	0.2698
396	133.8	1149.1	2.9596	0.3446
318	201.7	1549.8	1.5766	0.2052

Background ^{51}Cr - 46 (cpm)

Accumulation of Watercress by Filtration in the Plexiglass Stream Channel

Mean ^{51}Cr- and ^{14}C-derived accumulation values in experiment 12 (Table 22) in which finely ground watercress was filtered were 32.382 and 41.155 μg/mg, respectively. Since feeding took place for only 45 min it seems clear that physical properties of the plexiglass stream allowed much greater ingestion rates (43.07 and 54.74 μg/mg/h, respectively) than observed in the other feeding chambers. The fact that a very low food accumulation was observed in experiment 11 even after 6.0 h shows that finely ground watercress was a suitable food and that feeding in the glass cylinder and trough chambers was suboptimal. Control values for accumulation due to leaching were much smaller than in other experiments because activity reduction in food samples and appearance of free ^{51}Cr in the water was negligible. Nonetheless, ^{51}Cr activity density in feces was about one-fifth that of food, whereas it should have increased. Leaching of ^{51}Cr in the gut would appear to be responsible.

Adsorption of ^{51}Cr

In *Simulium* experiment 9 the black plastic trough was used to determine feeding rates on diatoms. Five-ml volumes of water were filtered at t = 20 and 90 min during the 1.5 h feeding experiment. These samples yielded an average of 16.3 cpm/ml, which is within the range of activity found in a leaching experiment. The mean ^{51}Cr available in water samples from the leaching experiment and *Simulium* experiment 9 are nearly identical (13.0 vs. 16.3 cpm/ml) even though the total cpm of ^{51}Cr available in labelled diatoms at t = 0 in each case are quite different (8.6 mg dry wt ÷ 0.054 μg/cpm = 1.59×10^5 cpm in the leaching experiment vs. 39.2 mg dry wt ÷ 0.063 μg/cpm = 6.22×10^5 in experiment 9). This is an indication that adsorption should be minor and consistent between experiments. The control animals in experiment 9 (killed before being placed in the system in a mesh bag) showed only a 0.884 μg/mg accumulation in comparison to the average total accumulation of 6.723 μg/mg. The control value is very low and distinguishable from actual ingestion rates even though this value is just within the 95% confidence limits for ingestion rates in experiment 9. The average ingestion rate in experiment 9 is slightly less than that of experiment 8 due to differences in feeding times.

TABLE 22. Accumulation of Labelled Food (Watercress) by *Simulium* (Experiment 12) during 45 min Feeding

ANIMALS

Animals Size (mm)	Animals No. (n)	Total DW (mg)	µg DW Food Accum/n Indiv ^{51}Cr	µg DW Food Accum/n Indiv ^{14}C	µg DW Food Accum/mg Animal ^{51}Cr	µg DW Food Accum/mg Animal ^{14}C
3.6 - 4.5	9	0.920	25.06	34.99	27.24	38.03
4.6 - 5.5	10	2.180	91.53	123.65	41.99	56.72
5.6 - 6.5	12	4.280	169.48	237.89	39.60	55.58
6.6 - 7.5	10	7.125	209.33	241.65	29.38	33.92
6.6 - 7.5	6	3.465	131.135	142.18	37.85	41.03
7.6 - 8.5	3	2.729	49.74	59.08	18.23	21.65
Control	18	6.379	0.499	1.03	0.078	0.161
(Assorted)	12	4.444	0.090	0.358	0.020	0.081
After 80 min	16	11.585	219.21	318.51	18.92	27.49
Defecation	18	12.025	286.66	358.43	23.84	29.81
					21.38 (Ave)	28.65 (Ave)

FOOD

Sample (ml)	Dry wt (µg)	Sample Time (t = min)	Corrected cpm ^{51}Cr	Corrected cpm ^{14}C	Food Activity µg/cpm ^{51}Cr	Food Activity µg/cpm ^{14}C
10	27	2	532.2	783.4	0.05073*	0.03447*
10	16	20	434.5	580.6	0.03682*	0.02756*
10	13	33	312.6	475.2	0.04159*	0.02736*
10	16	79	274.8	393.6	0.05822	0.04065
15	35	85	332.0	548.4	0.1054	0.06382
					0.04305* *(Ave)	0.02980* *(Ave)

FECES

Dry wt (µg)	Corrected cpm ^{51}Cr	Corrected cpm ^{14}C	Feces Activity µg/cpm ^{51}Cr	Feces Activity µg/cpm ^{14}C
301	1175.1	2987.2	0.2561	0.1008
510	2330.9	5145.6	0.2188	0.0991
769	3030.4	6743.8	0.2538	0.1140

WATER (Filtered 0.45 µm)

Sample Volume (ml)	Sample Time (t = min)	cpm ^{51}Cr/5ml
5	9	10.4
5	25	12.6
5	95	19.7

Background (cpm) ^{51}Cr - 46
^{14}C - 30

Comparison of Ingestion Rates Estimated by Use of ^{51}Cr and by ^{14}C

A one-way analysis of variance was conducted on selected experiments (refer to Table 24) to determine whether there was a significant difference in the means of ^{51}Cr- and ^{14}C-estimated ingestion rates. Both ^{51}Cr and ^{14}C were used in an attempt to estimate the same value. Provided that (1) the activity determined for the food was the same as that actually ingested, (2) that adsorption rates of the isotopes were equal and minor or were subtracted from accumulation rates, and (3) that the feeding time was not longer than the gut-filling time (so that ^{14}C counts were not added through assimilation), then the ingestion rates determined with the two isotopes will be equal. The mean of n samples in each experiment was calculated for ^{51}Cr- and ^{14}C-determined values, respectively, and an analysis of variance was carried out to test the null hypothesis $u_1 = u_2$. The alternative hypothesis is that $u_1 \neq u_2$ or that there is a significant difference at the P = 0.01 or P = 0.05 level. If a significant difference in the ingestion rates determined with the two isotopes was found, one of the three factors mentioned above might have been operating. An F test was used to decide whether a significant difference existed. A significant difference between treatments (^{51}Cr and ^{14}C) can be noted easily when there is little or no overlap in the confidence intervals (UL and LL). A few experiments where the differences were not so obvious were examined using analysis of variance (Table 24). Standard error of the mean (S.E.) and the coefficient of variation (C.V.) where C.V. = $s/\bar{x}$ were also calculated. This provides an estimate of the relative variation between two groups and is especially useful if there is a positive relation between the means and standard deviations.

A significant difference in the means (Table 24) shows that different things actually were being measured (e.g. experiments 5 and 10). In those cases where feeding time exceeded gut-filling time, it was expected that the mean of ^{14}C values would exceed the ^{51}Cr values. Absorption of ^{51}Cr by the gut could interfere with this expectation. In all Simulium feeding experiments except experiment 1, feeding time was equal to or greater than the minimum gut-filling time determined for blackfly larvae at Rattlesnake Springs. A lack of significant difference in means for Simulium experiments 1-4 could be related to the relatively short feeding periods but is more likely due to similar external adsorption for both isotopes. The significant difference found in experiment 5, in which feeding time equaled gut-filling time, may indicate a greater adsorption of ^{14}C, since the percent errors in counting for each isotope were small. The median percent error in the net count rate (^{51}Cr) in experiment 5 was about 10% (k = 2.7). This could not account for the difference in mean accumulation rates observed. The significant difference determined between means in Simulium experiment 10 is dramatic and is due to inappropriate values for food activity, caused by leaching over the long feeding period. Chromium-51-derived ingestion values nearly twice as great as ^{14}C values could

TABLE 23. Mean ^{51}Cr- and ^{14}C-derived Accumulations of Dry Weight of Food by *Simulium* (Experiments 1-12) during the Specified Feeding Period (μg/mg/xh). Confidence Limits (UL and LL) and Standard Errors (SE) of Mean ^{51}Cr- and ^{14}C-derived Values Are Given; The Coefficient of Variation (CV) of n Samples per Experiment Are Also Provided

Experiment	Feeding Period (h)	No. Samples (n)	Mean (μg/mg/xh)		SE		CV		UL		LL	
			^{51}Cr	^{14}C	^{51}Cr	^{14}C	^{51}Cr	^{14}C	^{51}Cr	^{14}C	^{51}Cr	^{14}C
1	0.5	4	2.139	0	-	-	-	-	-	-		
2	0.75	6	2.288	1.931	0.720	0.525	77.08	66.60	4.133	3.277	0.443	1.346
3	1.0	8	1.407	1.307	0.294	0.238	59.19	51.43	2.103	1.869	0.711	0.745
4	0.75	13	1.020	0.920	0.305	0.231	107.75	90.41	1.683	1.422	0.356	0.417
5	1.0	11	0.150	0.245	0.0159	0.0180	35.14	24.46	0.186	0.285	0.115	0.204
6	1.5	14	0.0480	-	0.0475	-	37.02	-	0.0582	-	0.0377	-
7	3.0	14	20.560	16.714	2.448	1.908	44.54	42.70	25.847	20.834	15.273	12.593
8	1.0	12	7.695	5.966	1.180	0.959	53.13	55.69	10.300	8.080	5.092	3.851
9	1.5	4	6.723	-	1.923	-	57.19	-	12.856	-	0.591	-
10	20.0	5	124.24	67.096	15.09	7.761	27.17	25.86	166.007	88.572	82.469	45.620
11	6.0	3	5.84	8.23	-	-	-	-	-	-	-	-
12	0.75	6	32.382	41.155	3.697	5.456	27.97	32.47	41.854	55.134	22.909	27.176

TABLE 24. Statistical Evaluation of The Null Hypothesis That The Means of ^{51}Cr- and ^{14}C-derived Ingestion Values Are Equal

Simulium Experiment	DF*	F	Significance Level
4	1, 24	0.682	NS (95% level)
5	1, 20	15.3	$P<0.01$
8	1, 22	1.29	NS (95% level)
10	1, 8	11.3	$P<0.01$

*Among treatments, within treatments

indicate that ^{51}Cr leached twice as fast as ^{14}C from the food during the 3.0 h of feeding before sampling food for radioactivity. The mean ^{14}C ingestion value for Simulium experiment 12 is greater than the ^{51}Cr value but the great overlap in confidence limits indicates no difference, as should be expected with a feeding time equal to the minimum filling time.

Since the ^{14}C activity density of food sources in all Simulium experiments was nearly 10 times greater than the ^{51}Cr activity density, the gross ^{14}C count rates of samples were 10 times greater than the gross ^{51}Cr count rates so that the accuracy of ^{14}C-derived accumulations was much greater. The fact that mean ^{51}Cr- and ^{14}C-derived accumulations were generally not statistically different (when feeding times did not exceed gut-filling time) indicates that percent error in individual ^{51}Cr-derived values is reduced when values from several samples are averaged. The coefficient of variation (C.V.) of ^{51}Cr-derived values was generally greater than that for ^{14}C-derived values (Table 23).

Assimilation Efficiencies Calculated for Simulium by the Calow and Fletcher Equation

Assimilation efficiencies reported for Simulium (Table 25) were calculated on the basis of activity ratios in food and feces of these animals using Calow and Fletcher's equation. Accuracy of these values must be interpreted with regard to measured levels of food consumption

TABLE 25. Assimilation Efficiencies Calculated from *Simulium* Experiments Using Dual-label Method

Simulium Experiment	Assimilation Efficiency	*Simulium* Experiment	Assimilation Efficiency
2	neg.	11	neg.
			neg.
3	neg.		neg.
	14.06%		neg.
			neg.
7	54.33%	12	neg.
	54.53%		neg.
			neg.
8	9.41%		
	neg.		
10	57.21%		
	52.70%		
	54.03%		
	56.12%		
	62.29%		
	65.78%		

in ingestion experiments. The mean activity ratio ($^{51}Cr/^{14}C$) for food samples was used in computing assimilation for each feces sample. Food source activities used to obtain the mean in each experiment are marked by asterisks in Tables 11-22 unless all values were averaged. These values are the activity of food after dilution in the total water volume in most instances which pre- and post-dilution activities were measured. When radioactive feces were obtained in *Simulium* feeding experiments, AE values were calculated using individual $^{51}Cr/^{14}C$ activity ratios for each sample with the mean value determined for food.

In cases where negative results were specified, the equation of Calow and Fletcher yielded a negative number and no assimilation efficiency could be derived. A negative number results from a decrease in ^{51}Cr activity relative to ^{14}C in the feces. Although a positive assimilation efficiency was computed in *Simulium* experiment 3, the low activity in the feces makes it clear that this value is not reliable. The low ingestion rates, compared to the theoretical rates derived later from gut weight and digestion time analysis, also show that since little was consumed,

production of radioactive feces probably did not occur. Assimilation efficiencies calculated in Simulium experiments 7 and 10 are high but consistent and since ingestion and defecation rates were high in each case these values appear reliable. The negative AE calculated for Simulium experiment 12 is unexpected because ingestion and defecation rates were high. Possibly ^{51}Cr binds less well to watercress than it does to diatoms. In Simulium experiment 10 leaching caused the ^{51}Cr and ^{14}C activity of food in the three food samples taken at approximately t = 19 h to differ significantly from those taken near t = 3.0 h. Since fecal pellets collected may be indicative of feeding just prior to the t = 19 h sampling of food, AE values also were calculated for each fecal sample using the mean $^{51}Cr/^{14}C$ activity ratio of food samples taken at t = 19 h. The values range from 83.36 to 88.13%, which is considerably higher than values based on initial food samples. Because of leaching errors it is uncertain which set of values is more appropriate but the similarity of the original estimate to that in experiment 7 favors a mean AE of 57.12%.

Preliminary Calculations for the Simulium Energy Budget

Dry Weights vs. Size of Simulium Larvae

Ladle et al. (1972) measured the increase in weight of larvae of one generation through a growth period. The body weights vs. size calculated for Simuliidae of Rattlesnake Springs were measured at intervals but a generation was not necessarily following through its growth since, in fact, many generations appear to exist during the year. The relationship between length and weight of Simulium based on mean dry weights for all samples from January 29 to July 26, 1973 was linear except for the smallest sizes (Fig. 9). An examination of Table 26 reveals that within each size group but the largest, body weight increased from January 29 to February 20 and then declined through June 1. The largest size class increased in weight until at least March 10. February maximum values for sizes 4.6-5.5, 5.6-6.5, and 6.6-7.5 mm were 3.8, 3.4, and 3.3 times greater than the June minima of 0.155, 0.357, and 0.494 mg/indiv, respectively. This weight difference could simply be a difference between species since Simulium present in March were almost exclusively S. argus while those present in July were almost exclusively S. vittatum. However, Chutter (1972) also noticed a similar decline in size of Simuliidae from spring through summer and believed it to be related to temperature. Results of this study support his interpretation.

Examination of Table 27, which gives the average of mean daily temperatures and the mean of the daily temperature differences (maximum minus minimum) for November 1-August 30, shows that mean daily temperatures and fluctuations were nearly constant during well-defined time periods but

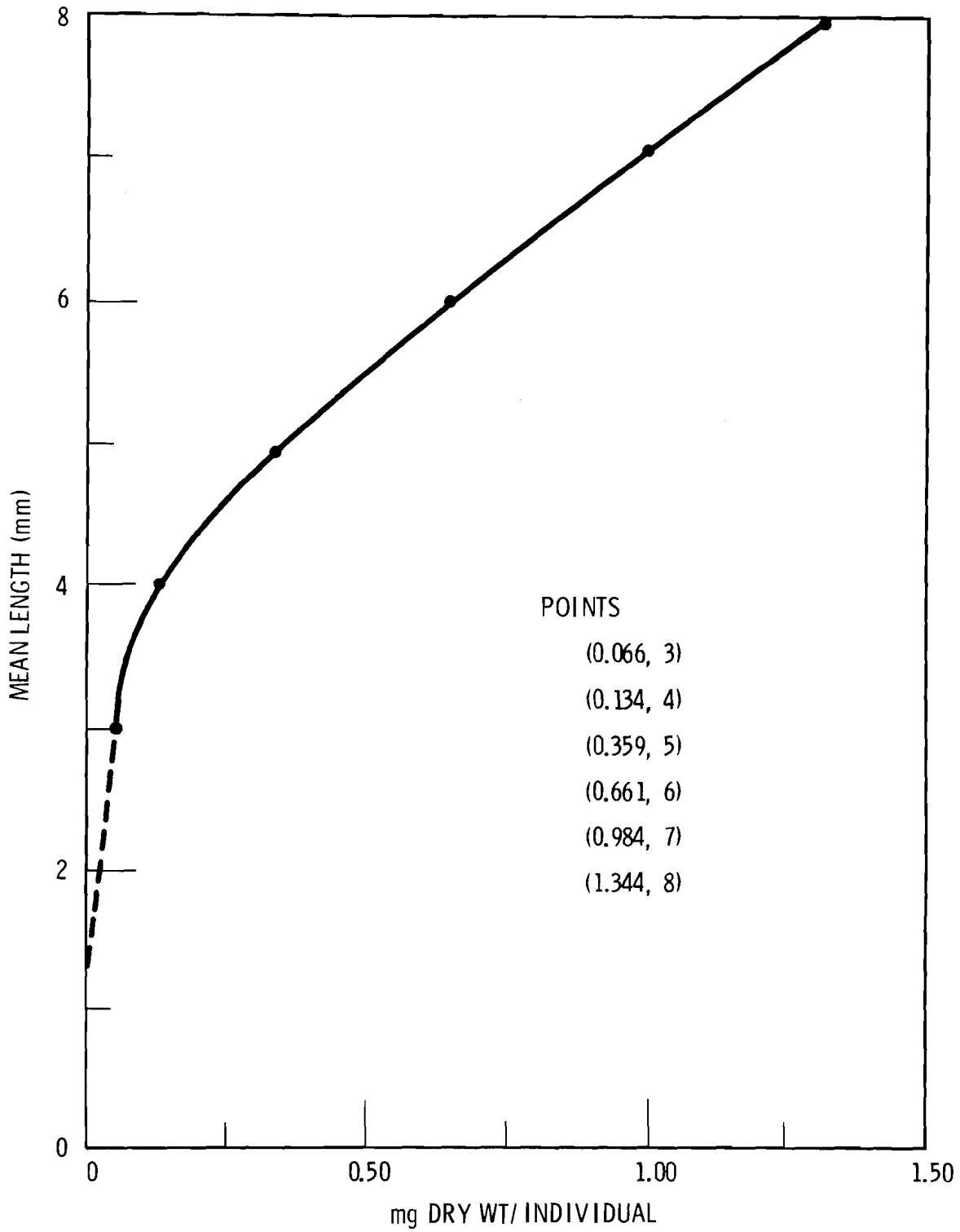


FIGURE 9. Length (mm) vs. Dry Weight (mg) for *Simulium*. Dry Weights for Each Size Class Are Mean Weights from Samples Throughout the Period January 29 to July 26, 1973 (See Table 26).

TABLE 26. Dry Weights (mg) of *Simulium* Larvae Sampled in Ingestion Rate Experiments throughout the Series of 12 *Simulium* Experiments. Values for Mean Dry Weight per Individual Were Based upon Measurements of n Animals (Given on Left Side for Each Size Class) Weighed in Samples of 10 Animals Each. If n = 25, for Example, Then There Were Three Samples with Two Groups of 10 and One Group of 5

		Sizes (mm)											
Simulium Experiment	Date	2.6 - 3.5		3.6 - 4.5		4.6 - 5.5		5.6 - 6.5		6.6 - 7.5		7.6 - 8.5	
		n	wt	n	wt	n	wt	n	wt	n	wt	n	wt
1	Jan 29					15	0.322	8	0.494	12	0.825		
2	Feb 14					10	0.578	20	0.891	20	1.435	10	1.629
3	Feb 20					27	0.606	30	1.204	16	1.627		
4	Feb 27					9	0.445	37	0.753	47	1.330	32	1.674
5	Mar 10					9	0.495	28	0.963	56	1.481	7	1.783
6	Mar 23					15	0.587	95	1.018	20	1.281		
7	Apr 23			20	0.180	40	0.396	59	0.885	9	1.140		
8	May 10			10	0.192	40	0.353	40	0.701	20	0.949		
9	May 26					11	0.158	9	0.306	25	0.586	6	0.723
10	Jun 1	27	0.066	14	0.0613	15	0.155	11	0.357	3	0.494		
12	Jul 26			9	0.102	10	0.218	12	0.357	16	0.662	3	0.910
Grand mean			0.066		0.134		0.359		0.661		0.984		1.344
Total no. animals weighed		27		53		201		349		244		58	

TABLE 27. Means of Average Daily Temperatures and Means of Daily Temperature Fluctuations for Specific Periods during 1973 at Rattlesnake Springs (near Bldg. 646)

Date	Average Daily Temperature					Daily Temperature Maximum - Minimum					Days
	Mean	SE	CV	UL	LL	Mean	SE	CV	UL	LL	(n)
Nov 1 - Dec 3	11.78	0.139	6.45	12.05	11.48	1.02	0.132	70.4	1.29	0.75	30
Dec 4 - 17	5.67	0.363	23.9	6.46	4.89	1.51	0.243	60.24	2.04	0.987	14
Feb 1 - 20	11.13	0.121	4.88	11.38	10.87	1.30	0.244	83.86	1.81	0.78	20
Feb 21 - Mar 20	13.23	0.147	5.31	13.53	12.92	2.87	0.140	23.39	3.16	2.58	23
Mar 21 - Apr 8	14.14	0.186	4.76	14.55	13.73	4.66	0.405	31.29	5.54	3.78	13
Apr 9 - Jun 5	16.62	0.160	7.21	16.94	16.30	4.48	0.193	32.25	4.86	4.09	56
Jun 6 - Jul 2	17.46	0.192	5.68	17.84	17.08	3.44	0.220	33.28	3.87	3.01	27
Jul 13 - Aug 15	18.67	0.110	3.37	18.89	18.45	3.69	0.160	24.82	4.00	3.38	33
Aug 16 - Aug 31	16.51	0.106	2.56	16.72	16.30	2.29	0.115	20.04	2.51	2.07	16

varied suddenly between periods. Between February 1 and 20 average daily temperatures were 11.1°C (mean) with a small daily fluctuation of 1.3°C. February 21 marked a day when temperatures became significantly higher and averaged 13.2°C for the period February 21-March 20. Greater daily fluctuations in temperature also commenced at this point. The decline in body weight of Simulium larvae also started near February 21 and body weights never again reached the high levels record on February 20. Fat reserves in the abdomen were very large in the colder months and these reserves seemed to disappear in the simuliid population as temperatures increased, although a causal relationship is not necessarily implied. The greater respiratory demand at higher temperatures is possibly responsible for the reduction in size. The fat reserves could be a storage device which enable larvae to survive periods of low food abundance. Data obtained by E. Wolf (unpublished) indicate low concentrations of suspended organic matter through winter followed by gradual increases to a maximum in May. As water temperatures rise, the increased respiratory demand for energy is met by the increased food concentrations available, which may also make several summer generations possible.

Rattlesnake Springs Simuliidae reached a greater mean individual dry weight than the species studied by Ladle et al. (Table 29) even though all species reached the same larval length. If it can be assumed that generation times are equivalent for all these species, then the growth rate of larvae in Rattlesnake Springs is much greater, even though the quantity of suspended food available in the chalk stream was 15 to 20 times greater than in Rattlesnake Springs. Lower growth rates in the chalk stream could be a factor of low food quality due to dilution of organic matter by suspended inorganic sediment. Microscopic examination of Rattlesnake Springs seston and gut contents of Simulium revealed that suspended inorganic sediment was only a small fraction of the food supply. It was, however, found that the percent ash of the seston was very large (∿ 85%).

Dry Weights, Gut Weights, and Percent Ash of Gut Contents of Simulium Larvae

The gut weights of Simulium larvae of various sizes from Rattlesnake Springs were measured (Table 28). The mean gut fraction based on dry weights of guts and the mean larval weights of Rattlesnake Springs Simulium (Table 29) was 10.9% (n = 9). It appears that inclusion of the gut wall in gut weight measurements yields high values because gut fractions of larvae sampled in July based on mean July larval weights (Table 26) are much higher than expected. The percent ask of Simulium gut contents varied between 76.1 and 82.3%. The relationship between mean body length and the dry weights of larvae and gut contents of two species of Simulium was derived from data presented by Ladle et al. (1972) and contrasted with

TABLE 28. Gut Weight Measurements and Percent Ash of Gut Contents of *Simulium*

Date	Total Length (mm)	Dry wt (μg)	Ash wt (μg)	% Ash	Remarks
Mar 21, 1973	9.0	79			+
	8.0	67			-
Mar 22, 1973	7.0	71.6			-
	8.3	92.0			+
	7.0	74.0			-
Jul 23, 1973	5.5	79.8	60.7	76.1	+
	6.0	84.8	69.8	82.3	+
	5.5	84.2	64.9	77.1	+
	6.2	132.8	101.1	76.1	+

(+) including gut wall
(-) not including gut wall

TABLE 29. Dry Weights (μg) and Gut Weights of *Simulium ornatum* and *S. equinum* Studied by Ladle et al. (1972) and *Simulium* spp. Studied at Rattlesnake Springs

Length (mm)	Ladle's Work *S. ornatum* (μg DW)	Ladle's Work *S. equinum* (μg DW)	Gut wt of *S. ornatum* and *S. equinum* (μg DW)	*Simulium* spp. Rattlesnake Springs (μg DW)
2	30	30	3	
3	80	85	7	66
4	140	175	15	134
5	245	280	26	359
6	380	405	42	661
7	550	555	59	984
8	720	-	80	1344

similar data from Rattlesnake Springs (Table 29). Rattlesnake Springs larvae reached a larger maximum weight even though the small size classes were similar in weight. Data from Rattlesnake Springs are, however, means of all species of Simulium present on sampling dates (probably only two species).

Digestion Time for Simulium

In situ digestion time analysis of Simulium was conducted to derive a theoretical ingestion rate which could be compared with experimentally determined rates. Given the larval gut fraction (μg/mg) and the length of time (h) required for food passage, an approximate ingestion rate can be derived.

The continual increase in projected gut clearance time (Table 30) indicates differential rates of passage along the gut. The rate of movement of a volume of food is slowest in the central large diameter portion. Fredeen (1964) and Ladle et al. (1972) indicated that digestion times for Simulium larvae were 20-30 min. However, in this experiment the digestion time was considerably longer, since less than half the gut length was travelled in 35 min. On the basis of these data it appears that 50-75 min is a better estimate of digestion time. Subsequent experiments performed as water temperatures increased verified that approximately 50-66% of the large central gut section was travelled during 30 min.

TABLE 30. Digestion Time Analysis of Simulium (Feb. 28, 1973) Stream Temperature 15.4°C

Time Since Feeding Ended (min)	Average % Gut Travelled	Number of Larvae Measured	Projected Time Gut Clearance (min)
5	24.8	11	20.2
10	27.1	8	36.9
15	26.7	12	56.2
20	39.8	5	50.3
25	39.4	5	63.5
30	42.8	12	70.1
35	44.9	6	77.0

On June 19, 1973 a check was made to see whether digestion times were still nearly 1.0 h. After a carbon slurry was run into the stream for 3 min and a 30-min period was allowed for digestion, it was found that approximately half of the 16 animals sampled had a carbon plug. The plugs had travelled an average of 50-66% of the way through the gut, indicating that the digestion time was 45-60 min. The lack of a plug in some animals could have been due to non-uniform dispersion of carbon in the stream. On June 22, 1973 another carbon slurry experiment was performed to determine whether removing the rock with larvae from the water for 45 sec and replacing the rock in a new orientation would inhibit feeding. If manipulation inhibited feeding, acclimation times in laboratory experiments needed to be lengthened. After 3 min of feeding on a carbon slurry followed by 30 min feeding on seston, only 3 of 16 animals dissected had a carbon plug which had nearly passed through the gut. The experiment was immediately repeated except that one rock covered with blackfly larvae was untouched and served as a control. A second rock located downstream was the experimental disturbed rock. Each rock was allowed 30 min acclimation after the experimental disturbance. Again 3 min were allowed for feeding on the carbon slurry, followed by 15 min feeding on natural seston. Fourteen of 15 experimental animals had carbon plugs 66-75% of the way through the gut; 6 out of 6 control animals had carbon plugs equally far advanced. The mean temperature for this day was 18.7°C. It seems that water temperature could influence digestion time in Simulium (also described for Pyrrhosoma by Lawton 1971). The digestion time estimates at 18.6°C in Rattlesnake Springs (although less precisely done than in February) are very similar to values reported by Ladle and Fredeen. It appears that a temperature decrease of about 5.5°C could cause a 50% reduction in digestion time. Lawton (1971) found that digestion time at 15°C was twice as great at 5°C.

Digestion time was measured in the laboratory in the plexiglass channel at 21.5°C on July 25, 1973. A large excess of carbon was mixed into membrane filtered water (5 μm) after larvae had been acclimated 1.0 h. After feeding for 30 min on the carbon, larvae were killed in dilute alcohol and guts were examined. Again plugs were 50-66% of the way through the gut, indicating 45-60 min digestion time. Starvation for 1.0 h apparently had no effect on feeding and an excess of food did not seem to cause extraordinary increases in feeding rate. Filter feeders in general have a maximum feeding rate which is not increased by further increases in food concentration (Blazka 1971).

The carbon experiment was repeated on June 20 and again a 45-60 min digestion time was indicated from dissection of 10 guts. In digestion time experiments the effect of time of day seemed to be negligible and gut-filling seemed to occur within 45-60 min with the exception of the one experiment where digestion times were half of that usually found.

Theoretical Ingestion Rates

Gut fractions (gut wt/larval wt) obtained from Ladle et al. (1972) and in this research are similar. Ladle does not specify whether the gut lining was removed in his determinations but from the few weighings made in this research, gut fractions appear to be overestimated when gut linings are included. If the larval weights decrease through the summer without similar reductions in the gut size (which is probable), then ingestion rates in terms of μg/mg should increase slightly as gut fractions increase. Based on Ladle's gut and larval weights and digestion times measured in Rattlesnake Springs (45-60 min) one can expect ingestion rates ranging from 82 to 155 μg/mg/h (Table 31).

TABLE 31. Theoretical Ingestion Rates for *Simulium* Larvae Based on 45-60 min Digestion Time and Gut Fractions (Gut wt/larval wt) Derived from Ladle et al. (1972)

Length (mm)	Ingestion Rate (μg/mg/h)
3	82-110
4	86-114
5	93-124
6	110-147
7	116-155
8	111-147

Concentration of Suspended Matter per Liter in Rattlesnake Springs

Water samples were collected periodically throughout the study for determination of the concentration of suspended matter which was the food source of *Simulium* larvae. Water samples were filtered on membrane or glass filters and were sometimes prescreened to remove large debris. The results (Table 32) indicate that suspended particulate food in Rattlesnake Springs varied little during the three-month-period. The range of individual values was 0.97-2.5 mg/ℓ (mean values ranged from 0.97 to 2.4 mg/ℓ).

TABLE 32. Concentration of Suspended Matter per Liter in Rattlesnake Springs

Date	Mean Concentration (mg/ℓ)	n	Filter Type	Prescreened
Mar 28, 1973	1.9	2	5 μm, M	-
Apr 13, 1973	2.4	2	5 μm, M	+ (250 μm)
May 2, 1973	1.25	3	G	+ (417 μm)
Jun 20, 1973	0.97	3	5 μm, M	-

(M) membrane filter
(G) glass fiber filter

Concentration of Food in Simulium Filtration Experiments

In *Simulium* experiments 1-6 only the initial algal dilution was sampled by filtering 1 ml of a 100-ml suspension. These experiments were done in glass cylinder feeding chambers which could accommodate fewer animals than the plastic trough. Water volumes used in trough experiments were only slightly greater than in glass cylinder experiments. For this reason dilution of labelled food by untagged feces probably created less of a problem than that encountered in trough experiments (7-11). In addition, gravel, which could yield material to the water, was not present in experiments 1-6. Mechanical entrapment of labelled food on monofilament screens (390 μm), which were part of all feeding systems except *Simulium* experiment 12, caused a reduction in the final food concentration in these experiments. Judging from the decrease in food concentration in experiments 7 and 8 (Table 32), this reduction could have been nearly 50% in *Simulium* experiments 1-6 but probably was less because only one small mesh cone was placed in each glass cylinder to prevent animals from being carried through the pump. The six mesh dividers and the gravel substratum in the plastic trough feeding chamber (experiments 7-11) would have screened out diatoms more effectively. It is significant that with experimental food concentrations 6-15 times greater than Rattlesnake Springs water (cf. Table 32 and 33), feeding rates determined in many of these experiments were lower than theoretical rates. Moreover, where food concentration were identical to stream concentrations (experiment 12) high ingestion rates were found.

TABLE 33. Food Concentration in *Simulium* Filtration Experiments

Simulium Experiment	Concentration[a] (mg/ℓ)	Concentration[b] (mg/ℓ)
1	31.2	
2	26.9	
3	26.3	
4	20.7	
6	15.0	
7	21.2	11.0
8	17.9	9.4
9		15.7
10		14.1
11		8.0
12		1.9

(a) Based on initial 1/100 dilution.
(b) Based on final dilution.

In *Simulium* experiments 2-4 the initial food concentration was 20.7 to 26.9 mg/ℓ (Table 16) which was much greater than the natural concentrations of 0.97-2.5 mg/ℓ in Rattlesnake Springs. Fredeen (1964) found the macroplanktonic concentrations (algae plus protozoans) in the Saskatchewan River to range from 13-14 x 10^6 cells/ℓ during one year, while mean suspended solids were 427 mg/ℓ. The diatom cell counts in *Simulium* experiments 2-4 (Table 34) were very similar to those found in nature by Fredeen, ranging from 9.22 to 28.01 x 10^6 cells/ℓ. An average of 1.076 x 10^6 cells/ℓ was found in suspension in experiment 5, which measured grazing. This was 15 times less than in experiment 2 in which the initial food concentration was 26.9 mg/ℓ.

TABLE 34. Cell Counts in *Simulium* Experiments 2-5

Simulium Experiment	Time (t = min)	Cell Counts/Field	Field Size $(\mu m)^2$	Cells/ℓ x 10^6
2	0	1.40	130	16.14
3	0	2.43	130	28.01
	30	1.80	130	20.75
	60	1.73	130	19.94
4	6	1.10	130	12.68
	30	1.88	130	21.67
	45	0.80	130	9.22
5	50, 56		250	1.076

Determination of Percent Ash in Feces of Simulium Larvae and in its Food (Suspended Organic Matter of Rattlesnake Springs)

Data on percent ash of food and feces of *Simulium* calculated by various techniques were used to compute AE values by the ash-ratio method. The ash percentages of food and feces of *Simulium* larvae derived by weighing samples on foil squares are given in Table 35.

TABLE 35. Percent Ash of *Simulium* Food and Feces (Samples Weighed on Foil Squares)

Date	Sample	% Ash	Sample	% Ash
Jun 20, 1973	Food	84.00	Feces	87.17
		84.57		85.19
				79.79
		84.29 (Ave)		
				84.05 (Ave)
Jun 25, 1973		78.83		84.76
		78.47		83.98
		78.65 (Ave)		84.37 (Ave)
Jun 28, 1973		80.99		83.16
		82.82		82.69
				83.29
		81.91 (Ave)		
				83.05 (Ave)

The percent ash of Simulium feces collected in the stream measured by the foil method varied from 79.79 to 87.17%. The natural food available varied in percent ash from 78.83 to 84.57%. In general the percent ash content of feces only slightly exceeded that of the food, indicating a low AE.

In Simulium experiments 9 and 10, Columbia River diatoms were fed in suspension to the larvae. Feces and food were sampled in these experiments and percent ash was determined. The data on radioactivity in the feces of larvae from Simulium experiment 9 (Table 19) indicate that little if any of the labelled food was defecated. The feces collected undoubtedly were of stream origin. Nearly 0.5 h elapsed from the time the larvae were collected until they were placed in the laboratory stream; 2.0 h were allowed for acclimation and 1.5 h for feeding. Therefore, the feces produced had a gut residence time of at least 4.0 h. Feces of larvae from Simulium experiment 10 clearly were radioactive and were derived from the experimental food. Data on percent ash of Columbia River diatoms (the labelled food source) and of Simulium feces are given in Table 36. The mean percentages of ash of food and feces in experiment 10 were 80.11 and 65.94%, respectively. One would not theoretically expect the percent ash values of the food to be greater than the feces. This situation could be possible if mineral absorption is high or if secretions were added to the feces but the differences may not be significant based on such a small sample size.

TABLE 36. Percent Ash Determination of Columbia River Diatoms and Simulium Feces in Simulium Experiments 9 and 10 (Samples Weighed on Foil Squares)

Simulium Experiment	Sample	% Ash	Sample	% Ash
9	Feces	86.51		
		88.97		
		85.01		
		86.83(Ave)		
10	Feces	65.94	Food	76.01
				80.39
				83.94
				80.11(Ave)

Another experiment was conducted to determine the percent ash of Simulium food and feces (Table 37). Several glass fiber filters were preashed at 500°C for 24 h. Previously it was found that filters lost an

TABLE 37. Determination of Percent Ash of *Simulium* Food and Feces, Using Pre-ashed (500°C) Glass Fiber Filters, May 9, 1973

Sample	Dry Wt[a] (55°C, 24 h) (μg)	Ash Wt[b] (500°C, 24 h) (μg)	% Ash
Feces	223	209.6[c]	93.99
	314	269.6[c]	85.86
	479	410.6[c]	85.72
	243	200.6[c]	82.55
			87.03 (Ave)
Food (100 ml Stream Water)	215[d]	160.6[c]	74.69
	110[d]	50.6[c]	46.00
100 ml Distilled Water	-27[e]	-27[f]	
	-3[e]	-21[f]	
Blank Filter	+2	-11	

(a) Final dry weight filter plus contents - initial dry weight filter.
(b) Ash weight filter plus contents - initial filter weight.
(c) Average loss due to re-ashing is 19.6; value corrected for this loss.
(d) Average amount leached out of filter by 100 ml distilled water is 15 μg; value corrected for this loss.
(e) Dry weight filter after leaching - initial dry weight filter.
(f) Ashed weight - dry weight filter after leaching.

average of 0.443% (S.D. = 0.033, n = 12) of their dry weight upon ashing. Filter weights on which food was collected were corrected for leaching and ashing losses. Filters used to collect feces were corrected only for ashing losses because only a few ml of water were filtered. Significant losses in filter weights were observed after filtration of 100-ml water samples and after re-ashing. The concentration of stream seston observed was 1.10 to 2.15 mg/ℓ, corresponding to previous estimates. The mean ash percentages of feces and food were 87.03 and 63.87%, respectively.

Percent ash measurements were repeated with glass fiber filters which were decontaminated in boiling sulfuric acid (Table 38). The mean weight loss on ashing of filters boiled in acid was 128 μg/filter. Such a large loss probably was caused by incomplete washing of filters after soaking in

TABLE 38. Determination of the Concentration and Percent Ash of Food of *Simulium*, Using Glass Fiber Filters Decontaminated in Boiling Sulfuric Acid. (May 30, 1973)

Sample	Net Dry Wt (μg)	Ash Wt (Net)* (μg)	% Ash
100 ml Filtered Stream Water	233	189*	81.11
	215	175*	81.40
	215	159*	73.95
			78.82 (Ave)
100 ml Distilled Water	+10	-120	
	-5	-124	
Blank Filters	-2	-131	
	+3	-133	
	+4	-121	

*The average weight loss on burning of the blank filters was 128 μg. This value is used to correct the ash weights.

acid. The use of nitric acid instead would have been desirable (see Ganf and Milburn 1971). The concentration of suspended material in the stream determined with this method was 2.15-2.33 mg/ℓ with a mean percent ash of 78.82%.

So that assimilation of ash-free organic matter could be determined, the percent ash of other food materials (watercress and blue-green algae) presented in feeding experiments was determined. The mean ash percentages of watercress and blue-green algae were 15.39 (n = 2) and 13.77 ± 1.50 S.D. (n = 6). Percent ash values of cultured diatoms and natural seston were similar (see Table 35) and much higher than for watercress and *Lyngbya*. The fairly low percent ash of *Simulium* larvae ($\bar{x}$ 18.69% ± 2.03; n = 9) indicates that their assimilation or retention of minerals from natural sestonic food (approx. 80-86% ash) is low.

Cheumatopsyche

Cheumatopsyche Feeding Experiments

Grazing Experiments

The accumulation of ^{14}C-labelled diatoms by grazing on slides was studied in Cheumatopsyche experiments 2 and 3. The mean accumulation rates derived in these feeding experiments (Table 44) ranged from 0.380 to 0.735 μg/mg/h. Individual values within these experiments ranged from 0.089 to 3.405 μg/mg/h (Tables 39-40). Feeding times in experiments 2 and 3 were 1.5 and 3.0 h, respectively; experimental temperatures were 14-15 and 19°C. Chematopsyche larvae ranging in size from 5.0 to 11.5 mm were used. The maximum accumulation rate was obtained in the smallest size class in each experiment. The median k values in experiments 2 and 3 were 4.9 and 11.1 (Table 9), respectively, meaning that the median percent error due to counting was about 5.7% and 3.1%. The ^{14}C activity density of diatoms in the Cheumatopsyche feeding experiments was generally 6 to 10 times greater than the ^{51}Cr activity density, giving a much greater statistical accuracy in counting. Even so, the fact that there is no statistical difference ($P < 0.01$) between means of ^{51}Cr- and ^{14}C-derived accumulation rates (Table 45) in grazing experiments shows that ^{51}Cr provided as good a measure of accumulation as ^{14}C. In fact, in experiment 3 the S.E. of the mean for ^{51}Cr values (Table 45) was slightly less than for ^{14}C values. The very low accumulation values obtained in grazing experiments may be more a result of adsorption than ingestion. If ingestion did occur it may only be significant for the smallest size classes and could not supply energy rapidly enough for larger larvae.

Filtration Experiments

Feeding by filtration in Cheumatopsyche was studied in experiments 1, 4, and 5 (Tables 41-43). In experiment 1 the glass cylinder feeding chamber was used and in experiments 4 and 5 the trough was used. Acclimation for 21-24 h was allowed in all three experiments to provide time for construction of nets. Animals ranging in size from 6.6 to 10.2 mm were used in these filtration experiments. The number of animals per sample ranged from 3 to 10 in experiments 4 and 5 while individual animals were used in experiment 1. In experiments 1, 4, and 5 feeding times were 1.5, 3.0, and 1.0 h, respectively; experimental temperatures were all nearly 17°C (Table 4). The median k value in Cheumatopsyche experiment 1 was 1.8 (Table 9), giving a median percent counting error of 19.3% (Table 10). The confidence limits about the mean ^{51}Cr-derived accumulation rate are equal to the mean ± 36% of the mean. Much of the variation in rates

TABLE 39. Accumulation of Labelled Food (Diatoms) by *Cheumatopsyche* (Experiment 2) during 1.5 h Feeding

ANIMALS

Animals Size (mm)	Animals No. (n)	Total DW (mg)	µg DW Food Accum/n Indiv ^{51}Cr	µg DW Food Accum/mg Animal ^{51}Cr
5.0 - 7.0 (Ave 5.9)	6	3.766	19.240	3.405
7.0 - 8.0 (Ave 7.6)	4	5.359	2.545	0.317
9.5	3	8.351	5.808	0.463
9.5 - 10 (Ave 9.6)	3	8.091	4.583	0.377
9.5 - 10 (Ave 9.8)	3	8.916	3.733	0.279
10.0	3	8.631	5.108	0.395
10.5	1	4.278	9.702	1.512
11.0	1	4.926	1.597	0.216
11.0	1	4.513	2.231	0.329
11.0	1	4.508	1.455	0.215
11.5	1	6.146	5.306	0.575

FOOD

DW (µg)	Food Activity (µg/cpm ^{51}Cr)
446	0.0179
334	0.0351
374	0.0264
	0.0264 (Ave)

Background ^{51}Cr - 48

TABLE 40. Accumulation of Labelled Food (Diatoms) by *Cheumatopsyche* (Experiment 3) during 3.0 h Feeding

ANIMALS

Animals Size (mm)	Animals No. (n)	Total DW (mg)	µg DW Food Accum/n Indiv ^{51}Cr	µg DW Food Accum/n Indiv ^{14}C	µg DW Food Accum/mg Animal ^{51}Cr	µg DW Food Accum/mg Animal ^{14}C
8.0-9.0 (Ave 8.6)	4	9.460	20.576	15.953	0.725	0.562
8.0-9.5 (Ave 8.8)	5	13.008	16.187	10.195	0.415	0.261
9.3-10.2 (Ave 9.8)	5	16.684	7.571	9.954	0.151	0.199
9.8-10.0 (Ave 9.9)	5	18.032	4.816	6.135	0.089	0.113
10.6-11.0 (Ave 10.8)	2	8.988	14.652	20.570	1.543	0.763
11.0-11.5 (Ave 11.1)	5	13.512	14.446	18.057	0.357	0.445

FOOD

DW (µg)	Food Activity (µg/cpm ^{51}Cr)	Food Activity (µg/cpm ^{14}C)
1039	0.03287	0.005496
966	0.03492	0.003245
798	0.02833	0.003150
	0.03204 (Ave)	0.003964 (Ave)

Background ^{51}Cr - 47
^{14}C - 38

TABLE 41. Accumulation of Labelled Food (Diatoms) by *Cheumatopsyche* (Experiment 1) during 1.5 h Feeding

ANIMALS

Animals Size (mm)	Animals No. (n)	Total DW (mg)	µg DW Food Accum/n Indiv ^{51}Cr	µg DW Food Accum/n Indiv ^{14}C	µg DW Food Accum/mg Animal ^{51}Cr	µg DW Food Accum/mg Animal ^{14}C
8.5	1	2.106	1.238	1.466	0.392	0.464
8.5	1	1.930	5.807	4.572	2.006	1.579
9.0	1	2.904	1.564	1.341	0.359	0.308
9.0	1	1.942	0.985	1.312	0.338	0.451
9.0	1	2.335	0.985	1.303	0.281	0.372
9.2	1	3.324	4.919	5.088	0.987	1.021
9.5	1	2.924	1.238	1.535	0.282	0.350
10.0	1	3.831	2.468	2.977	0.429	0.518
10.0	1	4.735	1.384	2.246	0.195	0.316
10.0	1	3.384	4.219	4.559	0.831	0.898
10.0	1	2.699	6.222	5.811	1.537	1.435
10.2	1	4.352	1.238	1.801	0.189	0.276

FOOD

Aliquot	µg DW Filtered	Sample Time (t = min)	Food Activity µg/cpm ^{51}Cr	Food Activity µg/cpm ^{14}C
1/100	159	0	0.0238	-
1/100	168	0	0.0222	-
1/100	200	0	0.0248	0.00525
1/100	174	0	0.0216	0.00489
10/760	99	30	0.04068*	0.006862*
10/760	90	40	0.04076*	0.007287*
			0.04072* *(Ave)	0.007075* *(Ave)

Background (cpm) ^{51}Cr - 56
^{14}C - 36

TABLE 42. Accumulation of Labelled Food (Diatoms) by *Cheumatopsyche* (Experiment 4) during 3.0 h Feeding

ANIMALS

Animals Size (mm)	No. (n)	Total DW (mg)	µg DW Food Accum/n Indiv ^{51}Cr	µg DW Food Accum/n Indiv ^{14}C	µg DW Food Accum/mg Animal ^{51}Cr	µg DW Food Accum/mg Animal ^{14}C
7.0 - 7.5 (7.3 Ave)	5	6.070	68.01	71.16	11.20	11.72
8.0 - 8.5 (8.2 Ave)	5	7.988	131.73	120.79	16.49	15.12
7.7 - 8.4 (8.2 Ave)	5	8.000	172.73	157.40	21.59	19.68
8.7 - 9.3 (9.0 Ave)	5	13.248	241.12	189.87	18.20	14.33
9.0 - 9.5 (9.1 Ave)	5	16.694	43.44	39.80	2.60	2.38
8.7 - 9.4 (9.1 Ave)	5	11.592	85.87	87.53	7.41	7.55
9.7 - 10 (9.9 Ave)	5	19.102	154.62	122.90	8.09	6.43
9.5 - 10 (10.0 Ave)	5	17.522	79.23	91.01	4.52	5.19
Assorted (9.0 Ave)	10	27.948	268.71	291.83	9.61	10.44

FOOD (Same as in *Simulium* Experiment 7)

FECES

Corrected cpm ^{51}Cr	Corrected cpm ^{14}C
1785.2	7063.0

Background (cpm) ^{51}Cr - 44
^{14}C - 27

TABLE 43. Accumulation of Labelled Food (Diatoms) by *Cheumatopsyche* (Experiment 5) during 1.0 h Feeding

ANIMALS

Animals Size (mm)	No. (n)	Total DW (mg)	µg DW Food Accum/n Indiv ^{51}Cr	µg DW Food Accum/n Indiv ^{14}C	µg DW Food Accum/mg Animal ^{51}Cr	µg DW Food Accum/mg Animal ^{14}C
6.6 - 7.5	3	3.340	107.035	101.041	32.046	30.252
7.6 - 8.5	5	8.838	184.159	144.560	20.837	16.357
7.6 - 8.5	3	3.328	87.773	63.339	26.374	19.032
8.6 - 9.5	5	13.540	377.858	270.620	27.907	19.987
8.6 - 9.5	5	11.756	141.927	121.105	12.073	10.302
Assorted	9	12.244	422.515	296.646	34.508	24.228
After 24 Hour Defecation		14.758	35.743	81.557	2.422	5.526

FOOD (Same as in *Simulium* Experiment 8)

FECES

Corrected cpm ^{51}Cr	Corrected cpm ^{14}C
1224.6	8300.7
2082.6	13928.3

Background (cpm) ^{51}Cr - 45
^{14}C - 43

observed in this experiment was due to counting errors. However, the high median k values in experiments 4 and 5 make the errors to counting inconsequential. Even though the counting accuracy was lower in experiment 1 than in the other two experiments the mean ^{51}Cr- and ^{14}C-derived accumulation rates were nearly identical and no statistical difference between the sample means was found at P = 0.01 (Table 44). In addition, no significant difference (P < 0.01) was found between means of ^{51}Cr- and ^{14}C-derived accumulation rates in experiments 4 and 5 (Table 44). With experiment 4 the ^{51}Cr and ^{14}C activities found in the initial 100-ml dilution were used to calculate the accumulation rate because considerable leaching of

isotopes occurred in the final dilution. This resulted in minimum accumulation rate values. If the ^{51}Cr and ^{14}C activity densities of the first and third food samples from the total volume are averaged, correction factors of 4.878 x and 3.334 x can be applied to ^{51}Cr- and ^{14}C-derived minimum values, respectively, resulting in mean ^{51}Cr- and ^{14}C-derived rates of 18.00 and 11.47 μg/mg/h, respectively. These corrected values correspond well to values derived in experiment 5 (25.62 and 20.03 μg/mg, respectively). The coefficient of variation is much less among samples in experiment 5 than in experiment 4 (Table 45). Corrected ^{51}Cr-derived accumulation rates in experiment 4 range between 4.24 and 35.12 μg/mg/h but these rates in experiment 5 range only between 12.07 and 32.05 μg/mg/h. The similarity between mean rates of experiment 4 and 5 when the correction is applied to values of experiment 4 tends to support the validity of the correction. Accumulation rates by filtration in the trough experiments are much greater than rates derived from the glass cylinder chamber.

In experiment 5 an accumulation of 2.422 μg/mg due to ^{51}Cr remained in larvae after 24 h of defecation. The accumulation derived from ^{14}C is approximately 2 times greater than that derived by ^{51}Cr after defecation. The small amount of accumulated food remaining (^{51}Cr-derived) after defecation indicates that the majority of ingested food was egested. The measured accumulation value is due to absorption of ^{51}Cr and partial retention during starvation. Assuming defecation was complete after 24 h, absorption of ^{51}Cr ingested during 1.0 h is 9.45%/h.

In experiment 4 the four largest accumulation rates were obtained in the four smallest size classes, and in experiment 5 the smallest size class showed the greatest accumulation and one sample of the largest size class showed the smallest accumulation. It is probable that the small size classes are more strictly herbivorous while carnivory may supplement the diet of a certain percentage of large animals. It is also possible that the somewhat lower accumulation rates of large larvae could be caused by a smaller percentage being able to construct nets within the 21-24 h acclimation time or by the lack of habitat for large larvae.

Determination of Assimilation Efficiency and Assimilation Rate for *Cheumatopsyche*

In *Cheumatopsyche* experiments 4 and 5, ingestion rates were high enough that substantial amounts of feces were recovered in the defecation chambers to allow computation of AE with the Calow and Fletcher technique. The mean value for activity of food in each experiment was used with individual feces samples to calculate AE.

Based on a single feces sample in *Cheumatopsyche* experiment 4 an AE of 49.13% was calculated. Values of 45.97 and 46.70% were derived from experiment 5. Using the mean AE (47.26%) and the ^{51}Cr-derived ingestion

TABLE 44. Mean ^{51}Cr- and ^{14}C-derived Accumulations of Dry Weight of Food by *Cheumatopsyche* (Experiments 1-5) during the Specified Feeding Period (μg/mg/xh). Confidence Limits (UL and LL) and Standard Errors (SE) of Mean ^{51}Cr- and ^{14}C-derived Values Are Given; The Coefficient of Variation (CV) of n Samples per Experiment Are Also Provided

Experiment	Feeding Period (h)	No. Samples (n)	Mean (μg/mg/xh)		SE		CV		UL		LL	
			^{51}Cr	^{14}C	^{51}Cr	^{14}C	^{51}Cr	^{14}C	^{51}Cr	^{14}C	^{51}Cr	^{14}C
1	1.5	12	0.652	0.666	0.169	0.132	89.65	68.72	1.024	0.956	0.281	0.375
2	1.5	11	0.735	-	0.289	-	130.27	-	1.379	0.647	0.0906	-
3	3.0	6	0.380	0.391	0.0974	0.100	62.77	62.89	0.630	14.538	0.130	0.136
4	3.0	9	11.079	10.316	2.141	1.829	57.97	53.19	16.020	27.140	6.138	6.094
5	1.0	6	25.624	20.026	3.329	2.776	31.82	33.96	34.154	50.594	17.094	12.913

TABLE 45. Statistical Evaluation of the Difference of Mean ^{51}Cr- and ^{14}C-derived Accumulation Rates (μg/mg/h) of *Cheumatopsyche* Using the Group Comparison Test (t).

Cheumatopsyche Experiment	Mean Accum ^{51}Cr	Mean Accum ^{14}C	Test (t)	DF	Significance
Grazing 2	0.490	-			
3	0.127	0.130	0.060	4	NS: P<0.01
Filtration 1	0.435	0.444	0.063	10	NS: P<0.01
4	3.69	3.44	0.271	7	NS: P<0.01
5	25.62	20.03	1.29	4	NS: P<0.01

rate from experiment 5 (25.62 μg/mg/h) the assimilation rate on Rattlesnake Springs diatoms (18.47% organic) is 2.237 μg AFDW/mg/h or 0.01011 cal/mg/h based on a caloric equivalent of 4.520 cal/mg AFDW. This is similar to the assimilation rate of 0.00840 cal/mg/h determined for *Hydropsyche fulvipes*, which lives in streams with summer temperature maxima > 15°C (Edington and Hildrew 1973).

Tricorythodes

Tricorythodes Feeding Experiments

Tricorythodes experiment 1 was a preliminary test conducted (Table 46a) to determine whether *Tricorythodes* would be able to ingest a significant amount of food within 15 h at 19°C. Animals were allowed to graze on labelled blue-green algae which was covering a styrofoam substratum.

Samples in this experiment and experiments 2 and 3 were oxidized in the Van Slyke combustion apparatus, which was still in the experimental stage at the time. The reliability of the apparatus at that time was uncertain due to difficulties in the standardization procedure but the similarity between results obtained in these and later experiments indicate that these values are meaningful. The reproducibility of activity determined for food throughout these first three experiments indicates comparable combustion and recovery of sample activity. The values of μg DW/mg accumulated after 15 h of feeding in experiment 1 correspond

TABLE 46a. Accumulation of Labelled Food (Blue-green Algae) at 19°C by Tricorythodes (Experiment 1) - June 8, 1972

ANIMALS

Animals Size (mm)	Animals No. (n)	µg DW Accum/mg Animal/15 h
4.5 (Ave)	4	141.73
3.7 (Ave)	3	209.97

FOOD

Food Activity µg/cpm ^{14}C
0.1892
0.2596
0.2244 (Ave)

closely to accumulation of blue-green algae in 1.0 and 2.0 h in experiment 4 (Table 47). It was concluded that ingestion rates for Tricorythodes are sufficiently great that shorter feeding experiments could be conducted.

An ingestion rate study (experiment 2) was performed to determine whether the activity per mg animal would actually increase with time (Table 46b). Animals of two size classes were removed from the food source at intervals during feeding until t = 5.0 h. The Lyngbya food source, of a tough, leathery consistency, was peeled off styrofoam sheets and washed for 10 min in water (pH 3.5) to eliminate any possible ^{14}C-carbonate precipitate. Samples were oxidized by Van Slyke combustion; percent carbon of each sample was also measured by the $BaCO_3$ precipitate method. The average percent carbon of Tricorythodes was 36.8% excluding the high (65.7%) value obtained. Sample counts used were the average of three 10-min counts. Five animals were sampled at each period. Although the values of food accumulated during feeding do not increase smoothly, an increase with time is evident. Abrupt declines in food accumulated could be a factor of biological variability.

TABLE 46b. Accumulation of Labelled Food Material (Lyngbya) at 20°C by Tricorythodes (Experiment 2) during a 5.0 h Feeding Experiment

ANIMALS

Feeding Time (h)	Accum for 2.6 - 3.5 mm Size (μg/mg)	Accum for 3.6 - 4.5 mm Size (μg/mg)	% Carbon
0.5	27.58	50.51	26.4
1.0	74.76	71.14	39.1
1.5	88.52	61.25	41.4
2.0	107.42	62.09	29.1
3.0	50.03	136.59	41.0 (Ave excluding
4.0	93.30	103.63	43.5 high value
5.0	108.56	44.66	65.7 36.8)

FOOD

Food Activity μg DW/cpm ^{14}C
0.3555
0.3309
0.3432 (Ave)

Lyngbya was used as a food source in experiment 3 (Table 46c). Tricorythodes were sampled hourly until t = 7.0 h and again at t = 20 h. The accumulation rate appeared to reach a plateau at t = 2.0 to 5.0 h in the 2.6-3.5-mm size class and at t = 2.0 to 4.0 h in the 4.6-5.5-mm size class at approximately 50 to 60 μg/mg. This seems to be evidence that Tricorythodes is not a continuous feeder. The total accumulations in 20 h in the two smaller size classes (430.22 and 429.12 μg/mg) are nearly identical. Accumulations at t = 7.0 h are very similar for all size classes indicating a rather uniform rate of assimilation. The grand mean of all percent carbon determinations for Tricorythodes in experiment 3 is 33.99% (n = 24) which compared favorably to the mean percent carbon estimate of 36.8% from Table 45.

TABLE 46c. Accumulation of Food Material (*Lyngbya*) by *Tricorythodes* (Experiment 3) at 20°C - July 29, 1972. Sample size for Each Feeding Period Was 9 Animals. Percentage Carbon Values Were Derived for Each Sample

ANIMALS

Feeding Period	Accum for 2.6 - 3.5 mm Size µg DW/mg	% C	Accum for 3.6 - 4.5 mm Size µg DW/mg	% C	Accum for 4.6 - 5.5 mm Size µg DW/mg	% C
1	40.70	54.92	30.26	26.21	12.30	49.98
2	63.08	36.44	48.09	23.27	52.13	38.80
3	50.62	29.52	103.50	23.80	54.42	36.26
4	61.98	37.17	91.97	20.84	56.30	40.28
5	59.58	27.48	138.25	25.12	28.83	32.70
6	86.24	38.44	114.88	28.31	81.59	32.15
7	168.21	37.99	145.85	25.14	140.82	38.66
20	430.22	42.59	429.12	34.66	178.41	35.03
		38.07 ±8.39 (SD)		25.92 ±4.15 (SD)		37.99 ±5.74 (SD)

FOOD

Food Activity (µg DW/cpm ^{14}C)
0.2413
0.2205
0.2309 (Ave)

Feeding experiment 4 was conducted on *Tricorythodes* of 2.6-3.5-mm and 3.6-4.5-mm size classes with a *Lyngbya* food source (Table 47). Feeding was conducted in petri dish chambers for 1.0 h with the 2.6-3.5-mm size and for 2.0 h with the 3.6-4.5-mm size. Some animals were sampled after feeding; the remainder were fed on nonlabelled food for 3.0 h (the 2.6-3.5-mm class) and 6 and 9.0 h (the 3.6-4.5-mm class) to allow complete defecation before sampling. The values of µg DW accumulated/mg are greater for the large size class because feeding time was 2.0 h for it and 1.0 h for the small size class. The material remaining after the defecation period is food assimilated. It appears that the magnitude of loss of accumulated material in an additional 3.0 h of defecation (Table 47) after the 6.0 h period was small. For this reason the amount remaining at the 6.0 h period is a good measure

TABLE 48. Accumulation of Food (*Anabaena* with Minor Amounts of *Achnanthes lanceolata*) by *Tricorythodes* (Experiment 5) - September 5, 1972. Ten Animals Were Sampled at Each Feeding Period for Each Size Class

ANIMALS

Size (mm)	Total DW (mg)	Feeding Period (h)	µg DW/mg	Average
1.6 - 2.5	1.013	1	78.98	
1.6 - 2.5	0.745	2	113.05	
1.6 - 2.5	0.724	4	158.70	
2.6 - 3.5	2.316	1	65.32	
2.6 - 3.5	1.607	2	75.97	
2.6 - 3.5	2.136	3	103.06	93.37
2.6 - 3.5	1.944	3	83.67	
2.6 - 3.5	2.259	4	115.90	
2.6 - 3.5	2.513	5	135.89	
2.6 - 3.5	2.713	6	221.79	
2.6 - 3.5	2.653	22.5	383.16	
3.6 - 4.5	4.672	1	53.01	48.89
3.6 - 4.5	4.489	1	44.77	
3.6 - 4.5	4.445	2	76.36	
3.6 - 4.5	5.244	3	74.98	83.70
3.6 - 4.5	4.792	3	92.42	
3.6 - 4.5	4.690	4	123.08	
3.6 - 4.5	5.119	5	158.97	
3.6 - 4.5	4.905	6	180.76	
3.6 - 4.5	5.579	22.5	332.40	

FOOD

DW (µg)	Food Activity µg DW/cpm ^{14}C
3914	0.01237
5730	0.01257
	0.01247 (Ave)

TABLE 49. Accumulation of Food Material (Diatoms) by *Tricorythodes* (Experiment 6) at 18°C - May 17, 1973

ANIMALS

ANIMALS

Animals Size (mm)	Animals No. (n)	Total DW (mg)	Feeding Time (h)	µg DW Accum/n Indiv ^{51}Cr	µg DW Accum/n Indiv ^{14}C	µg DW Accum/mg Animal ^{51}Cr	µg DW Accum/mg Animal ^{14}C
2.6 - 3.5	4	0.448	0.5	13.56	11.90	30.26	26.56
3.6 - 4.5	5	2.011	0.5	78.16	66.92	48.81	33.28
3.6 - 4.5	6	1.979	0.5	32.75	38.48	16.55	19.44
3.6 - 4.5	3	1.268	0.5	41.96	62.34	33.09	49.16
4.6 - 5.5	5	4.062	0.5	191.99	183.03	47.26	45.06
4.6 - 5.5	2	0.959	0.5	66.59	44.89	69.44	46.80
4.6 - 5.5	5	3.270	0.5	109.15	171.52	33.38	52.48
						39.83 (Ave)	38.97 (Ave)

FOOD

DW (µg)	Corrected cpm ^{51}Cr	Corrected cpm ^{14}C	Food Activity µg/cpm ^{51}Cr	Food Activity µg/cpm ^{14}C
885	21422.7	234140.4	0.04131	0.003780
374	13299.4	131948.4	0.02812	0.002834
472	18858.8	219152.4	0.02503	0.002154
			0.03149 (Ave)	0.002923 (Ave)

FECES

Corrected cpm ^{51}Cr	Corrected cpm ^{14}C
4637.5	30328.1
12881.2	76158.4

TABLE 50. Accumulation of Food Material (Diatoms) by *Tricorythodes* (Experiment 7) at 18°C - May 20, 1973

ANIMALS

ANIMALS

Animals Size (mm)	Animals No. (n)	Total DW (mg)	Feeding Time (h)	µg DW Accum/n Indiv ^{51}Cr	µg DW Accum/n Indiv ^{14}C	µg DW Accum/mg Animal ^{51}Cr	µg DW Accum/mg Animal ^{14}C
2.6 - 3.5	5	1.151	1.0	21.64	42.06	18.80	36.54
3.6 - 4.5	4	1.593	1.0	49.28	69.22	30.94	43.45
4.6 - 5.5	5	6.456	1.0	131.62	143.81	20.39	22.28
						23.38 (Ave)	34.09 (Ave)
2.6 - 3.5	3	0.658	5.0	20.99	64.02	31.89	97.30
3.6 - 4.5	6	2.614	5.0	91.19	160.64	34.88	61.45
4.6 - 5.5	5	6.035	5.0	238.99	422.71	39.60	70.04
						35.46 (Ave)	76.26 (Ave)

FOOD

DW (µg)	Corrected cpm ^{51}Cr	Corrected cpm ^{14}C	Food Activity µg/cpm ^{51}Cr	Food Activity µg/cpm ^{14}C
763	25576.0	242034.4	0.02983	0.003152

TABLE 51. Data for Computation of AE for *Tricorythodes* (Experiment 8) Feeding on Diatoms at 18°C - May 18, 1973

FOOD

DW (μg)	Corrected cpm ^{51}Cr	Corrected cpm ^{14}C	Food Activity μg/cpm ^{51}Cr	Food Activity μg/cpm ^{14}C
338	9451.6	107597.4	0.03576	0.003141
885	26133.7	240698.4	0.03386	0.003678

FECES

Corrected cpm ^{51}Cr	Corrected cpm ^{14}C
4536.4	25877.9
10675.1	65290.3

TABLE 52. Accumulation of Food Material by Tricorythodes (Experiment 9) Feeding on Watercress at 18°C - May 20, 1973

ANIMALS

Animals Size (mm)	Animals No. (n)	Total DW (mg)	Feeding Time (h)	µg DW Accum/n Indiv ^{51}Cr	µg DW Accum/n Indiv ^{14}C	µg DW Accum/mg Animal ^{51}Cr	µg DW Accum/mg Animal ^{14}C
2.6 - 3.5	4	0.823	1.0	24.83	9.65	30.16	11.72
3.6 - 4.5	6	2.536	1.0	34.28	16.71	13.52	6.59
4.5 - 5.5	4	4.027	1.0	27.76	14.00	6.89	3.48
						16.86 (Ave)	7.26 (Ave)

FOOD

DW (µg)	Corrected cpm ^{51}Cr	Corrected cpm ^{14}C	Food Activity µg/cpm ^{51}Cr	Food Activity µg/cpm ^{14}C
490	1036.2	24833.8	0.4729	0.01973
247	522.5	11967.4	0.4727	0.02064
229	540.1	10063.2	0.4240	0.02276
			0.4565 (Ave)	0.02104 (Ave)

TABLE 53. Data for Computation of AE for *Tricorythodes* (Experiment 10) Feeding on Watercress at 18°C - May 21, 1973

FOOD

DW (μg)	Corrected cpm ^{51}Cr	Corrected cpm ^{14}C	Food Activity μg/cpm ^{51}Cr	Food Activity μg/cpm ^{14}C
467.98	8768.6	21337.3	0.05337	0.02193
451.02	8581.1	16825.4	0.05256	0.02681
524.99	9386.6	23720.3	0.05587	0.02213
			0.05393 (Ave)	0.02362 (Ave)

FECES

Ingestion Time (h)	Defecation Time (h)	Corrected cpm ^{51}Cr	Corrected cpm ^{14}C
2	1.5	5145.8	7549.2
19	1.5	3633.8	2311.8

TABLE 54. Accumulation of Food Material from Feeding of *Tricorythodes* (Experiment 11) on Diatoms at 18°C - May 22, 1973

ANIMALS

Animals Size (mm)	No. (n)	Total DW (mg)	Feeding Time (h)	µg DW Accum/n Indiv ^{51}Cr	µg DW Accum/n Indiv ^{14}C	µg DW Accum/mg Animal ^{51}Cr	µg DW Accum/mg Animal ^{14}C
2.6 - 3.5	2	0.328	0.75	5.99	8.71	18.26	26.55
3.6 - 4.5	4	1.467	0.75	6.33	10.94	4.31	7.46
4.6 - 5.5	5	5.881	0.75	216.54	242.87	36.76	41.23
						19.78 (Ave)	25.08 (Ave)
2.6 - 3.5	6	1.206	1.0	13.64	17.05	11.31	14.14
3.6 - 4.5	4	1.953	1.0	24.17	31.87	12.38	16.32
4.6 - 5.5	5	6.291	1.0	118.73	155.68	18.87	24.75
						14.19 (Ave)	18.40 (Ave)
2.6 - 3.5	12	2.248	5.0	41.71	110.42	18.56	49.12
3.6 - 4.5	2	1.086	5.0	19.54	38.29	17.99	35.26
4.6 - 5.5	4	3.770	5.0	150.54	491.75	39.93	130.44
						25.49 (Ave)	71.61 (Ave)
Assorted (After 1.0 h ingestion 1.5 h defecation Feeding on non-labelled food)	14	9.608		19.40	98.24	2.02	10.23

FOOD

DW (µg)	Corrected cpm ^{51}Cr	Corrected cpm ^{14}C	Food Activity µg/cpm ^{51}Cr	Food Activity µg/cpm ^{14}C
412	12018.9	88890.9	0.03428	0.004635
846	24288.2	214192.4	0.03483	0.003950
470	12079.6	119823.9	0.03891	0.003922
820	22311.8	182533.4	0.03675	0.004492
			0.03619 (Ave)	0.004250 (Ave)

FECES

Ingestion Time (h)	Defecation Time (h)	Corrected cpm ^{51}Cr	Corrected cpm ^{14}C
2	1.5	25126.8	135362.2
1	1.5	2521.8	16945.0

The mean ^{51}Cr-derived accumulation values in Tables 49-54 for 0.5 to 5.0 h of feeding range from 14.19 to 39.83 μg/mg. The high accumulation value (39.83 μg/mg) measured in 0.5 h of feeding indicates that the gut-filling time may well be 0.5 h or less since even after 5.0 h of feeding (Table 50) the mean ^{51}Cr-derived value was still 35.46 μg/mg.

Among ^{51}Cr-derived accumulation values, adsorption of ^{51}Cr is measurable. The difference between mean 5.0 h and 1.0 h values in experiments 7 and 11 (Tables 50 and 54) yields the ^{51}Cr absorption during 4.0 h of feeding. These accumulation rates were 3.02 and 2.38 μg/mg/h, respectively. In experiment 11, 14 Tricorythodes, after feeding for 1.0 h on labelled diatoms, were allowed to clear their guts with unlabelled food. After 1.5 h of gut clearance only 2.02 μg/mg accumulation (^{51}Cr-derived) remained, presumably due to absorption and adsorption of the isotope. This uptake rate was used to correct for ^{51}Cr uptake in certain calculations. Animals sampled after gut clearance had assimilated 10.23 μg/mg/h (^{14}C-derived value). Using the ^{51}Cr-derived weight of a full gut at 1.0 h (14.19 μg/mg) and an assimilation rate of 10.23 μg/mg/h one would calculate that at least 14.19 + 4(10.23) or 55.11 μg/mg should be accumulated after 5.0 h of feeding (a mean ^{14}C-derived accumulation of 71.61 μg/mg was observed). There was a 5.30 and 4.21 μg/mg difference between ^{14}C- and ^{51}Cr-derived accumulations at 0.75 and 1.0 h, respectively. This difference is probably due to assimilation. If the assimilation rate were 10.23 μg/mg/h, one would expect 2.56 μg/mg to be assimilated between t = 0.75 and 1.0 h.

The mean ^{14}C-derived accumulations after 5.0 h feeding were 76.26 and 71.61, respectively (Tables 50 and 54). This seems to indicate that mean ingestion and assimilation rates on a food source were similar between experiments. Subtracting the mean ^{14}C-derived accumulations at t = 1.0 h in each experiment from the values at t = 5.0 h yields the amount accumulated during 4.0 h of feeding. The assimilation rates calculated from these data are:

	from Table 50	from Table 54
μg/mg/h	10.543	13.30
[1]μg AFDW/mg/h	1.947	2.457
[2]cal/mg/h	0.008800	0.01110

1) Based on 18.47% organic fraction for diatoms of Rattlesnake Springs
2) Based on equivalent of 4.520 cal/mg AFDW

In addition, the assimilation rate of animals which cleared their guts in experiment 11 (Table 54) was 10.23 μg/mg/h which is equivalent to 1.889 μg AFDW/mg/h or 0.01043 cal/mg/h. The fact that the assimilation

rates calculated from the data of Table 53 correspond indicates that nymphs completely cleared their guts of labelled food in 1.5 h.

In Table 50 there is a 10.71 μg/mg difference in accumulation between mean ^{14}C and ^{51}Cr accumulations at 1.0 h. If the gut was filled at 0.5 h (as suggested from data in Table 49), the implied assimilation rate is 21.42 μg/mg/h, but if the gut-filling time is 7 min then the implied assimilation rate is 12.12 μg/mg/h.

In the feeding experiment with watercress (Table 52) the ^{14}C-derived accumulation values are lower than the ^{51}Cr-derived values. It is unlikely that ^{51}Cr absorption would exceed absorption of organic matter. An explanation is lacking for these results.

The relationship between the length of feeding and the ratio of ^{14}C- vs. ^{51}Cr-determined accumulation in the mayfly Tricorythodes is given in Table 55 for experiments 6, 7, 9, and 11. If respiratory ^{14}C loss is small and ^{51}Cr uptake is low, then this is a good way to note

TABLE 55. A Comparison of the Ratios

$$\frac{\mu g/mg\ (^{14}C\text{-derived})}{\mu g/mg\ (^{51}Cr\text{-derived})}$$

Determined at Several Time Intervals during the Feeding of Tricorythodes with Various Foods

Experiment	Food Type	Feeding Time (h)	Size (mm) 2.6 - 3.5	3.6 - 4.5	4.6 - 5.5
6	Diatoms	0.5	0.878	0.682	0.952
				1.175	0.674
				1.486	1.571
				1.114 (Ave)	1.066 (Ave)
7	Diatoms	1.0	1.944	1.404	1.093
		5.0	3.051	1.762	1.769
9	Watercress	1.0	0.389	0.487	0.505
11	Diatoms	0.75	1.455	1.731	1.122
		1.0	1.250	1.318	1.312
		5.0	2.647	1.960	3.267

the effect of size on assimilation rate. A small size class showing a greater $^{14}C/^{51}Cr$ ratio at a certain time period than a larger size class has a greater AE, provided that digestion times are equal. After the gut is full, further feeding will increase the ^{14}C-derived accumulation relative to ^{51}Cr-derived accumulation. Since the activity density of ^{14}C is greater than ^{51}Cr in the food, the activities must be compared in terms of μg DW food/mg calculated with each isotope. It appears (Table 55) that gut filling with watercress may not be as rapid as with diatoms due to the low ratio calculated. There does not appear to be a consistent relationship of ratios with size classes, but it does appear that the ratio is greater than 1 after 0.5 h of feeding, indicating that the digestion time is 0.5 h or less. The ratios calculated at 0.5, 1.0, and 0.75 h for experiments 6, 7, and 11, respectively, are similar, ranging from 1.066 to 1.944. Ratios derived at the 5.0 h period in these experiments range from 1.762 to 3.267, showing a considerable increase in assimilated material.

There does not appear to be a consistent relationship between size and the ratios obtained at the various sampling times. Therefore, it cannot be concluded that AE values are greater for one size than another. Within a single experiment there is often much variability in ratios among size classes (e.g., experiment 11, 5.0 h). However, there does seem to be a similarity in ratios obtained with the two larger size classes (e.g. experiment 6, 0.5 h mean; experiment 7, 5.0 h; experiment 9, 1.0 h; experiment 11, 1.0 h).

Assimilation Efficiency Calculations for Tricorythodes

Assimilation efficiencies for *Tricorythodes minutus* were calculated from data in Table 49-54 using the equation of Calow and Fletcher,

where $$AE = 1 - \frac{\text{cpm } ^{51}Cr \text{ (food)}/\text{cpm } ^{14}C \text{ (food)}}{\text{cpm } ^{51}Cr \text{ (feces)}/\text{cpm } ^{14}C \text{ (feces)}} .$$

The digestion time was not known at the time these experiments were conducted. Experiments were performed under the assumption that the residence time was approximately 1.0 h. This seemed to be a reasonable estimate based on the shape of the accumulation curves derived previously for *Tricorythodes* fed for up to 22.5 h (Table 48) on ^{14}C-labelled food sources. That is, the ^{14}C activity incorporated into the animal per hour declined after the initial value at 1.0 h so the gut-filling time must be 1.0 h ± a maximum of 59 min. Later gut-filling rate experiments showed the rate to be very fast (7 min) at 18°C. In the assimilation experiments to be reported on, 15-20 organisms were fed on a labelled food source for 1.0 h or more and then placed in a separate container and allowed to clear their guts by feeding on nonlabelled food. The length of time allowed for

gut clearance must be at least equal to the gut-filling time. Since gut-filling was assumed to occur within 1.0 h, 1.5 h was allowed for complete gut clearance. Assimilation efficiencies calculated here by the dual-label method were not corrected for ^{51}Cr assimilation or ^{14}C respiratory loss. The significance of these corrections is discussed later.

In experiments 6 (Table 49) animals were fed in two groups at 18°C; one group was fed for 2.25 h, the other group for 2.75 h. Defecation took place for 2.0 h in filtered creek water and for an additional 1.0 h in the presence of labelled food. The respective assimilation efficiencies on diatoms computed for these two groups were 39.32 and 45.13%, respectively. The $^{51}Cr/^{14}C$ activity ratios of the food samples were averaged and this average value was used with individual ratios for the feces to compute AE.

In experiment 8 (Table 51) animals were fed on labelled food for 2.75 h at 18°C. Defecation took place for 1.5 h after feeding. Food was sampled after feeding stopped. The mean $^{51}Cr/^{14}C$ activity ratios for diatoms equaled 0.09822. AE values were 43.97 and 39.93%, respectively.

The efficiency of assimilation of watercress was determined from experiment 10 (Table 53). The watercress had been labelled with ^{14}C and ^{51}Cr two days prior to use. It had been ground to a coarse powder and had soaked in water for two days. The soaking watercress was stored at 3°C in the refrigerator so that bacterial action was kept to a minimum. Anything assimilated is considered to be assimilation of pure watercress. Feeding was allowed for 2.0 h and defecation for 1.5 h, with a nonlabelled food source used to clear the gut. AE for this experiment was 35.59%. A second group of nymphs began feeding at 3:30 p.m. and since they could not be seen consuming any watercress, the animals were allowed to feed until 10:30 a.m. the next morning, at which time they were transferred to non-labelled food (fresh detritus from the stream). Rapid ingestion of this natural stream detritus was observed. Fecal pellets were produced copiously and continually and the nymphs appeared at ease. They ate without interacting significantly with other nymphs and made few large shifts in position. However, when feeding on watercress the nymphs appeared to be less well satisfied with the food and moved a great deal. The radioactivity measured in nymphs, however, shows that watercress was eaten. The smaller size of watercress particles in relation to the size of diatom clumps probably made the watercress an unsuitable substratum and increased animal activity. The total feeding time was 19 h; defecation time, 1.5 h. AE was 72.07%, an unexpectedly high value.

Assimilation experiment 11 (Table 54) used diatoms as a food supply. Two groups of animals were used. Group 1 consisted of 35 animals, half of which had been used in previous assimilation studies on diatoms and the other half had just been used in the previous watercress assimilation experiment 10; these were fed on nonlabelled food for 2.5 h to assure

complete gut clearance of labelled food. Group 2 consisted of fresh animals maintained on nonlabelled food and not previously used in any experiment. AE values for the two groups were 36.48 and 20.77%, respectively.

Adsorption, Assimilation, and Leaching of Isotopes

Control for Adsorption of ^{51}Cr by Tricorythodes

A 0.5-ml volume of the stock $^{51}CrCl_3$ solution was added to a volumetric flask and diluted to 100 ml with filtered stream water. Two 5-ml aliquots of this solution were sampled and counted. Two petri dishes were each filled with 30 ml of the solution. In dish 1 were placed six live Tricorythodes; in dish 2 were placed five dead Tricorythodes. In addition, three dead nymphs were removed from the aquarium for determining a background level. However, these animals had been used in previous Tricorythodes assimilation experiments. They had fed on labelled food 3 weeks earlier and were then maintained on nonlabelled food. Their exact time of death was unknown. This experiment (Table 56) shows that the concentration of ^{51}Cr activity from solution is only slightly greater for living than dead animals and is roughly 12 times greater than for the background animals. The mean concentration factor of ^{51}Cr in alive and dead Tricorythodes is 317.8 times after 2.5 h of adsorption.

In order to draw any conclusions from the food accumulation data presented here, the magnitude of ^{51}Cr adsorption must be known. As shown previously in the Simulium and Cheumatopsyche experiments, dead control animals adsorb ^{51}Cr activity equivalent to about 1.0 μg DW algae/mg animal when held in a water volume of 700-1000 ml containing 15-30 mg dry wt algae, with a ^{51}Cr activity of roughly 0.05 μg/cpm. In the ^{51}Cr-^{14}C leaching experiment an average of 13.7 cpm ^{51}Cr solution was leached from the algae in 3.0 h of circulation in the system. This degree of leaching was found in all Simulium experiments in which it was measured. In the Tricorythodes ^{51}Cr adsorption experiment there was very little difference in activity between live and dead nymphs. The mean activity of Simulium control animals in experiment 10 was 8.7 cpm/mg. The activity of Tricorythodes nymphs after soaking in water with 113 times greater cpm ^{51}Cr was 450.9 cpm/mg, an increase in concentration of only 52 times. The concentration factor (cpm/mg animals vs. cpm/mg water) was 632 in the low activity solution (feeding experiments) and 290.3 in the high activity solution (^{51}Cr adsorption experiment). These facts indicate that adsorption of ^{51}Cr is not linearly related to ^{51}Cr activity and that relative adsorption is greater at low ^{51}Cr activity levels. For this reason control values do not necessarily double with a doubling of ^{51}Cr in solution.

Assimilation of ^{51}Cr

Assimilation of ^{51}Cr is another potential source of error. From the data of Tricorythodes experiment 11 (Table 54) it appears that the µg/mg (^{51}Cr-derived values) values of food accumulated remain nearly constant through the 5.0 h feeding period. Data from experiment 7 (Table 50) indicate a small increase in food accumulated from the 5.0 h sampling periods. The control animals in experiment 11 assimilated ^{51}Cr equivalent to 2.02 µg DW algae/mg. The average ^{51}Cr-determined accumulation (gut-plus-tissue) at 1.0 h is 14.18. Subtracting the control value leaves 12.16 µg/mg. This value should represent a full gut although it is equivalent to a gut fraction of only 1.216% of total body weight. With a gut-filling time of 7 min, the ingestion rate would be 104.21 µg/mg/h. Consequently the assimilation efficiency of Tricorythodes for ^{51}Cr is 2.02/104.21 = 1.938%.

Van Hook and Crossley (1969) calculated the assimilation efficiency of ^{51}Cr by Acheta domesticus (Orthoptera) to be 5.7% of the total body activity in 24 hr of feeding. Since gut-filling time for this cricket was 6.7 h, gut contents were turned over 3.58 times per day. Assimilation efficiency should be calculated on the basis of the total quantity of food ingested during the feeding period. Actual assimilation of the ^{51}Cr can be calculated as:

$$\frac{3.58\ X}{(1-X) + (3.58\ X)} = 0.057\%$$

$$X = 0.01529 \text{ or } 1.529\%$$

where X corresponds to the decimal fraction assimilated of a gut load, 3.58 is the number of gut-fillings per day , 3.58 X is the relative amount assimilated during 24 h and (1-X) is the relative amount in the gut after assimilation. The AE of ^{51}Cr for Acheta is nearly the same as that calculated for Tricorythodes. The slightly higher levels found in Tricorythodes may be due to surface adsorption. In any case assimilation of ^{51}Cr is quite small.

Leaching of ^{51}Cr and ^{14}C from the Algae

To assess the extent of ^{51}Cr and ^{14}C leaching from algae the contents of one slide (6.5 mg dry wt diatoms) were circulated in a simulated feeding experiment in 500 ml of filtered stream water in the black plastic trough. The water temperature was allowed to reach room temperature (26°C) to obtain the maximum rate of loss which could be expected. Circulation time of the suspended food was 3.0 h at a pump speed of 62.5 ml/sec. At specified intervals (Table 56) 5-ml samples were filtered on tared 0.45-µm membrane filters. The filtered water was placed in a test tube for counting ^{51}Cr activity. The dry weight of algae was obtained for each tared filter and µg/cpm was calculated for each isotope at each interval. There was a gradual leaching of

TABLE 56. Leaching of ^{51}Cr and ^{14}C During 3.0 h Circulation in 500-ml Water Volume in Black Plastic Trough (Increase in ^{51}Cr in 5-ml Water Samples and Loss of ^{51}Cr and ^{14}C from the Algae of That Volume)

Water Volume (mℓ)	Time (min)	cpm ^{51}Cr
5	0	51.2
	23	90.7
	42	65.8
	60	69.7
	72	66.8
	120	71.6
	180	65.3

Algae*	DW (μg)	Time (min)	μg/cpm ^{51}Cr	μg/cpm ^{14}C
0.45-μm Filter	86	0	0.05434	0.007184
	91	23	0.06111	0.007722
	82	42	0.05606	0.007617
	44	60	0.06491	0.008029
	91	72	0.06951	0.008661
	89	120	0.07826	0.008714
	75	180	0.06911	0.008211
		(h)		
5-μm Filter	6200	24	0.07434	0.01004
0.45-μm Filter	300	24	0.3330	0.007486

*From filtration of 5-ml volume.

activity from algae with each isotope, although the effect did not seem to be as great as that seen in Simulium experiments 7 or 10. The diatoms were thoroughly rinsed, having been used previously in Cheumatopsyche grazing experiments where a large volume of water bathed the slides. Nonetheless, the initial ^{51}Cr activity in filtered water was 10.2 cpm/ml. The increase in activity in the water was very slight during the 3.0 h period and seemed to stabilize at around 13.0 cpm/ml. Leaching after addition of the algal suspension to the system was 5.1% of the total initial activity of the algae. The maximum amount leached was 8.6% of the initial activity. Since Simulium larvae were absent in this experiment, leaching effects which might have been due to partial digestion of cells were not measured.

Very little ^{51}Cr was released to the water after the t = 0 sampling period. After the algal suspension was stored 24 h at 26°C in a flask exposed to light, the isotope activity (of material > 5 μm in size) decreased to some extent but not as much as expected.

Decline of ^{51}Cr Activity in Algae Used as a Food Source

The ^{51}Cr activity of algae used in Cheumatopsyche experiments 1-5 can be compared to note the effect of radioactive decay and ^{51}Cr leaching (Table 57). The algae were grown on 10 slides for these experiments and had been labelled with ^{14}C and ^{51}Cr. In Cheumatopsyche experiment 1 the diatoms on two slides were washed and then scraped off to make a suspension. The remaining eight slides were returned to a solution of ^{51}Cr in which they had been soaking for ^{51}Cr adsorption. For Cheumatopsyche experiment 2 the slides were all washed lightly with distilled water and placed in petri dishes in the plexiglass stream channel for grazing studies (1.5-h soaking in 10 ℓ of stream water). The slides were not returned to a ^{51}Cr solution for the remainder of the experiments. In Cheumatopsyche experiment 3 grazing was also tested. The slides received another 3.0 h soaking in 10 ℓ of stream water. The activity density of algae from grazing experiments was calculated from the counts and dry weight of algae samples scraped directly from slides. For the filtration-type experiments (1,4,5) the activity density of a 1-ml aliquot of an initial 100-ml suspension of the algae is given (Table 58). The ^{51}Cr leaching experiment was carried out on this algae on May 3, 1973, but the activity measured was from 5 ml of the final suspension in the trough immediately after its addition. The average activity of diatom samples in μg/cpm decreased with time (i.e., each cpm ^{51}Cr represents an increasingly larger dry weight from the first to last experiments of the series), which may be due to a combination of radioactive decay and leaching. If these activities are all corrected for radioactive decay to the t = 0 sampling date, the mean activities of all but the last experiment (Cheumatopsyche experiment 5) are nearly identical. If a decrease in activity were occurring due to leaching, this should be noted in experiments 2 and 3 because in these experiments all slides were repeatedly soaked in 10 ℓ of stream water and

sampled at the end of feeding. Contrary to what might be expected, a slight increase in activity was measured. Prolonged exposure of slides to ^{51}Cr solutions resulted in only a slight increase in corrected activities from experiment 1 to 2. Suspending labelled diatoms in 100 ml of water resulted in a small unexpected increase in activity in the initial dilution of experiment 4. It appears that ^{51}Cr leaching is not significant when diatoms are merely in contact with a volume of water but is important when diatoms are circulated rapidly as in filtration type feeding experiments.

TABLE 57. Concentration of ^{51}Cr adsorbed by living and dead *Tricorythodes* in 2.5 h from $^{51}CrCl_3$ solution - June 11, 1973

Sample	Corrected cpm ^{51}Cr	μg/cpm ^{51}Cr	
5 ml Water	8287.2	603	
	7242.5	690	
	Animals No.	DW (mg)	μg/cpm ^{51}Cr
Tricorythodes	6 (alive)	2.930	1.811
	5 (dead)	2.427	2.259
	3 (dead, bkgd)	1.631	21.461

TABLE 58. Decline of ^{51}Cr activity of diatoms throughout *Cheumatopsyche* experiments

Cheumatopsyche Experiment	Date	Days (t =)	Mean Activity (μg DW/cpm)	Decay* Correction Factor	Activity Corrected to t = 0
1	Mar 29	0	0.0231	1.0	0.0231
2	Apr 6	8	0.0264	0.8191	0.0216
3	Apr 16	18	0.03204	0.6383	0.02045
4	Apr 25	27	0.03667	0.5100	0.01870
5	May 14	46	0.04145	0.3176	0.01316
^{51}Cr Leaching Experiment	May 3	35	0.05434	0.4178	0.02231

*The half-life of ^{51}Cr is 27.8 days.

DISCUSSION

In an effort to interpret the present findings in relation to published studies, the various experiments will be discussed with respect to species of animal, the type and amount of food presented, and the adequacy of the various experimental feeding situations in simulating normal feeding conditions.

Simulium

The Use of Bacterial Food and Particle Size Selection

The study by Fredeen (1964) of the growth of blackfly larvae on pure cultures of bacteria prompted an investigation of the assimilation efficiency and ingestion rate of the blackflies on *Esherichia coli*. Fredeen found that of 135 streams in which *Simulium* occurred, bacterial densities ranged from 1×10^5 to 25×10^6 (average 2.2×10^6) cells/ml. Best survival and growth in the laboratory occurred at bacterial densities higher than those encountered naturally although some emergence did occur with feeding at densities as low as 6×10^5 cells/ml. It is doubtful that a population could be sustained at the low growth and survival rates offered by such low food densities. The gram positive bacterium which Fredeen used as food was less satisfactory nutritionally than the gram negative species and resulted in greater mortality. Gram negative species (e.g., *E. coli*) are more complete nutritionally and should be able to supply all essential dietary requirements (Fredeen 1964). For this reason *E. coli* was selected as a food source in the present study. Fredeen found that for *Bacillus subtilis* (a gram positive cell which is larger than gram negative species) 0.05 ml of packed cells provided 2.7×10^6 cells/ml. In *Simulium* experiment 6, 0.1 ml (84 mg wet weight) of packed *E. coli* was offered as food. This should have provided a greater bacterial density than the average (2.2×10^6 cells/ml) found for blackfly streams by Fredeen. Fifteen μg/ml dry weight of cells were present in *Simulium* experiment 6 and twice this amount in *Simulium* experiment 1 (see Table 32) so that food supply was more than adequate. In *Simulium* experiment 1 bacterial cells were not labelled well with ^{51}Cr. More efficient labelling with ^{51}Cr was possible after cells from the growth medium were concentrated, washed, and suspended in a small volume of stream water. The addition of 65 μCi to a 5 ml suspension of 10 mg dry weight of bacterial cells provided good labelling. It is desirable to label cells to 0.01-0.001 μg/cpm (Sorokin 1968). The average accumulation rate in *Simulium* experiment 1 of 2.139 μg/mg in 0.5 h of feeding compares well with values calculated in experiments 2-5 but is highly variable because of poor labelling and consequently poor counting statistics. Many values could not be computed in *Simulium* experiment 1

because counts did not exceed background. In Simulium experiment 6 labelling was sufficient to yield precise values for uptake; net sample counts ranged from 10-70 cpm. The accumulation rate averaged 0.0480 μg/mg/1.5 h with a low coefficient of variation.

Fredeen (1964, and personal communication) indicated that most cells in his experiments were present as single cells but that some clumps and chains possibly were present. Fredeen's intention was to show that bacteria were a possible source of food for Simulium, but the possibility that blackflies could filter only clumped cells or chains seemed likely after these experiments. For this reason a brief experiment was conducted to determine the smallest size particle that could be filtered by blackflies in the stream. A powdered charcoal suspension was drained into the stream above a dense colony of larvae. After time was allowed for filtration, the larvae were sampled and the guts removed. The carbon plug in the gut was smeared on a slide and mounted in a glycerin-gelatin medium. Photographs were taken of the carbon particles at 550x and the photographed particles were measured with calipers. Particles smaller than 1 μm were ingested. It is not likely that these particles could be clumped after drifting in the stream before filtration. Williams and Obeng (1961) indicated that blackflies selectively filter particles in the range 6-14 μm. A much broader selection was observed in this experiment. Simulium ingested particles from < 1 μm to about 140 μm for a 6 mm animal and 150 μm for a 7 mm animal.

The reason for the low ingestion rate on bacteria was puzzling since it was observed that blackfly larvae could filter very small particles. In a stream, larvae could survive on bacteria which were clumped or adhered to suspended sediment, and also apparently which were free floating.

Current Speeds

In Simulium experiment 1 a current speed of 12.2 cm/sec was provided and due to the difficulty that some animals seemed to experience in this current, the current speed was reduced to 6.1 cm/sec in all remaining experiments using the glass cylinder feeding chamber. The feeding rates of stream filter feeders probably are a function of both current speed and food concentration. Current speeds in stream sections where blackflies were located, measured with the stick flotation method and a pigmy current meter, were 27-67 cm/sec. Speeds measured with the more precise hot-wire anemometer ranged from 13.1- > 95 cm/sec in various locations in the riffles where larvae were studied. Minimum speeds measured directly next to the heads of larvae were found to be 13.1-19.5 cm/sec, although larvae usually occupied positions in all the faster areas of the riffle. Current

speeds in the trough feeding chamber ranged from 23.8 to 53.1 cm/sec; the plexiglass channel, 15.2-68.3 cm/sec. Whether the density of larvae is related to the microdistribution of current speeds remains to be studied and the hot-wire anemometer would be a perfect tool for this. Tarshis (1968) suspected that differential growth rates in laboratory blackfly cultures were caused by some larvae having a greater supply of food. If this were true then the greater range of current velocities found in a stream could easily cause the wide range of sizes of larvae found.

Food Concentrations in Simulium Experiments

In order to compensate for the possibility of inadequate food supplies due to suboptimal current speeds, an excess of food was provided in *Simulium* experiments. Natural food concentrations were measured at various times during the study and were found to range between 0.97 to 2.5 mg/ℓ. Data from E. Wolf (unpublished) indicate that these values are typical for Rattlesnake Springs over the whole year. Using Maciolek's (1962) wet oxidation method he found low particulate matter (raw stream water filtered on GF/C glass fiber filter) from October through December (0.0035 mg organic matter/ℓ) with a gradual increase to a maximum of 0.7-1.0 mg organic matter/ℓ in May, followed by a gradual decline to August with stable values (0.0056 mg organic matter/ℓ) through December. Stream discharge (E. Wolf, unpublished data) fluctuated between a minimum of 87 m^3/day in August and a maximum of 140 m^3/day in December with a mean flow of 105-110 m^3/day. Thus food availability for filterfeeders in the stream during the year would seem to be mainly dictated by the seasonal changes in production rather than current speed although animals are always expected to be found in current speeds which are adequate to meet their needs for food.

Food concentrations in *Simulium* experiments 1-11 (Table 32) were 4 to 15 times greater than natural food concentrations in Rattlesnake Springs. There is a slight difference between the concentrations calculated on the basis of the amount of food added to a culture and the final measured concentration due to some food being caught on the plastic screens or rocks which were a part of all glass cylinder or trough experiments. However, the magnitude of the reduction in concentration was probably never more than 50% as seen in the two experiments where both initial and working concentrations were measured.

Simulium Feeding Experiments

Grazing Experiments

Wu (1930) and Peterson (1956) indicated that some species of blackflies can graze on attached materials in the stream to supplement their diet. Peterson's evidence for this behavior was that some material collected from the substratum corresponded to material in the guts. In Rattlesnake Springs this mode of feeding seems unlikely because of the very small amount of periphyton which covered the rocks. Unless very active scraping of the rock surfaces was possible by Simulium, very little food could have been gained in this manner. All studies of Simulium in which gut analyses have been compared to the sestonic food available indicate that the relative proportions of all food components (diatoms, filamentous algae, detritus, sand, etc.) are comparable, thus indicating nonselective feeding. Simulium argus larvae in Deep Creek ate both detritus and diatoms indiscriminately and the proportions of materials were determined by local conditions (Koslucher and Minshall 1973). Simulium experiment 5 was conducted to determine the extent of grazing. If it were true that the current speed was inadequate for normal filtration in the glass cylinder and if grazing were a means of feeding by these larvae, feeding would be apt to occur under such conditions provided no respiratory stress was encountered (Wu 1930). Such stress was unlikely because water was maintained at a constant temperature near the natural stream temperature and was vigorously aerated.

In these grazing experiments larvae were placed on glass slides inside the glass cylinder chamber. Slides were covered with a layer of periphyton (diatoms) which was much heavier than any growth found on rocks in the stream. In order to examine the possibility that cells which washed off the slide during water circulation could be filtered out by larvae, two 5-ml aliquots were filtered onto 25-mm membrane filters and the cells were counted at 200x. The average of 40 fields on each of the two filters indicated 1085 cells/ml were present at the end of the grazing experiment. In Simulium experiment 2 where filtration of suspended diatoms was measured there were 16,139 cells/ml and 26.9 μg/ml initially. Thus the 1085 cells/ml present in the grazing experiment (approx. 1/15 x 16,139 cells/ml) probably represented a maximum of about 1.8 mg/ℓ dry weight of cells (1/15 x 26.9 mg/ℓ) which is comparable to normal stream concentrations. However, the mean "accumulation" rate by grazing was 6.8 to 15 times less than rates calculated in Simulium experiments 2-4 where filtration rate was studied. Although no controls were used in these first Simulium experiments to determine the degree of adsorption of ^{51}Cr or ^{14}C, the very low values obtained in the grazing experiment (Simulium experiment 5) probably indicate that no grazing, but a small amount of adsorption occurred. Values for food accumulation under these conditions indicate the magnitude of adsorption corrections which could be applied. In comparison

with experiment 5, the accumulation rate is even less in experiment 6 where bacteria were filtered. The very low standard error for accumulation rates in Simulium experiment 5 indicates that the μg/mg of ^{51}Cr and ^{14}C adsorbed are nearly uniform for larvae regardless of size. More individual variation would be expected if feeding were actually taking place than if only absorption occurred. Adsorption of ^{14}C was significantly greater ($P < 0.05$) than that of ^{51}Cr.

Filtration Experiments in Glass Cylinder Chambers

Simulium experiments 2-4 were conducted to determine feeding rates on diatoms in suspension. The mean accumulation rates for diatoms obtained by filtration in glass cylinder feeding chambers were 6.8-15 times greater than those in the grazing experiment (the "control" experiment 5). Mean accumulation rates calculated using ^{14}C are smaller in each experiment than the corresponding rate calculated with ^{51}Cr. However, the difference is not statistically significant. There also appears to be a downward trend in mean accumulation rates from experiments 2-4 although its significance is uncertain because of poor labelling of diatoms with ^{51}Cr in experiment 2 (leading to reduced counting accuracy) and because of differences in feeding time in experiments 2-4.

Accumulation of Food by Filtration in Simulium: Trough Experiments Compared to Glass Cylinder Experiments

It is obvious that ingestion rates in Simulium experiments 1-6 (glass cylinder experiments) were much less than those expected to occur in nature (i.e., those derived in Table 30 from gut weight and digestion time studies). The increase in food accumulated in experiment 7 (trough experiment) was caused by the lengthened feeding time but it is doubtful that guts were filled after the 3.0 h feeding. Radioactive feces were produced in experiment 7 but the amounts produced in experiments 8 and 9 were very small and made determination of assimilation efficiency by the dual-label technique imprecise if not impossible.

The feeding time was extended to 20 h in experiment 10 to allow for total acclimation to laboratory conditions while feeding on labelled food. This procedure eliminated starvation during acclimation and contamination of the system with nonlabelled food during a prolonged acclimation. Difficulty with loss of activity from food was encountered in experiment 10 also. However, since the algae were first sampled in experiment 10 after larvae had 4.0 h to defecate (0.5 h during collection, 0.5 h during acclimation, plus 3.0 h of feeding), dilution of algae by feces should have ceased. It was previously observed that defecation in freshly collected

Simulium continues steadily for about 45 min and that possibly 30% of the initial gut load is retained during further starvation. Peterson (1956) found that larvae require a day or more to completely void their guts. In experiment 10, however, significant amounts of radioactive feces were produced. There was close similarity between μg/cpm ^{51}Cr in food and feces. If no leaching were to occur, one would expect the value μg/cpm ^{51}Cr to diminish from food to feces (i.e., ^{51}Cr activity increases in the feces) as organic material is assimilated. When ^{51}Cr absorption is negligible, ^{51}Cr activity values of food and feces would remain the same if no organic matter is assimilated. Assuming ^{14}C assimilation did occur, the food/feces ^{51}Cr activity ratio would approach unity if ^{51}Cr leached from feces or from food in the gut. In fact, ^{51}Cr did leach faster than ^{14}C in the food. The mean initial activity at t = 3.0 h for ^{51}Cr and ^{14}C was 5.3 and 1.9 times greater, respectively, than the mean activity at t = 19.25 h.

The mean initial food activity value (n = 3) was used to calculate ingestion rate in experiment 10. If absorption of ^{51}Cr is assumed to be small during the 20 h feeding period, the amount of ^{51}Cr in the body should indicate the quantity of food required to fill the gut. Therefore, if the Simulium gut-filling time is 1.0 h, the ^{51}Cr-derived food accumulation after 20 h feeding should equal the ingestion rate (μg/mg/h). These values derived in experiment 10 correspond very well to theoretical ingestion rate (and gut fraction) values presented in Table 31. The reason that body activity calculated with ^{14}C is approximately 50% lower than values derived by ^{51}Cr is not known. It is expected that assimilation of ^{14}C-labelled material would continue to increase ^{14}C body activity. Assimilation of ^{14}C should be much greater than that for ^{51}Cr. Respiratory loss of $^{14}CO_2$ could account for some reduction in accumulated activity although Monakov and Sorokin (1972) reported only a 14-16% loss per day of newly assimilated ^{14}C in Daphnia pulex. Adsorption was not an important factor as only minor activity was measured in dead animals in mesh bags. Adsorption values in experiment 10 were similar to those in Simulium experiment 9.

In Simulium experiment 11 the feeding time was reduced to 6.0 h in an attempt to reduce the effect of leaching due to cell damage in the plastic trough. It is obvious that loss of activity in food still occurred. Ingestion rates calculated for animals of assorted sizes were small although the absolute values in μg/mg are very similar to values obtained in experiments 8 and 9. Judging from the low counts measured in the feces, only minor amounts of labelled feces could have been egested and these were greatly diluted by nonlabelled feces. Possibly ingestion did take place under the seemingly suboptimal feeding conditions provided by the trough but occurred at a reduced rate.

In experiments 1-6 in which the cylindrical glass feeding chambers were used, the measured accumulation rates are barely above control values

(approx. 0.15 μg/mg). This indicates that filtration of diatoms at 6 cm/sec was minimal. The trough feeding chamber yielded higher ingestion rates (experiments 7-11) but these were variable due to inaccuracies caused by leaching and other factors to be discussed. Data from experiment 10 indicate that, given enough time, the larvae can fill their guts. However, the low ingestion rates of experiments 8 and 9 show that the rate of gut-filling is slower than theoretical rates in the stream but faster than rates derived in experiments 1-6 where the glass cylinders were used. It is felt that inadequacies related to suboptimal current velocities in the physical environment provided by both feeding chambers inhibited normal feeding.

Feeding Rates in Relation to Acclimation Time, Leaching, and Feeding Conditions

Simulium experiment 7 was the first in the series to use the plastic trough as a simulated stream environment. In this experiment 19 h was allowed for acclimation of the larvae. The effect of starvation during this period is unknown. Although a certain amount of organic matter was added to the water as feces, the digestible organic portion would rapidly be exhausted. Tarshis (1968) states that Simuliidae cannot live in stagnant water for more than 6.0 h. This might indicate that either the current related respiratory demand is not met in stagnant water or that the natural metabolic rate of the larvae is so high that energy reserves are soon spent under starvation. The toleration of starvation for 19 h when a current is present seems to indicate that the current is needed to meet the respiratory demand. Phillipson (1956), however, found that *S. ornatum* could live up to 14 days in still water and that respiration rate was reduced at lower velocities.

The diatoms contained on three slides (total 23.9 mg DW) were added to the membrane filtered stream water. The feeding time in *Simulium* experiment 7 was 3.0 h. A substantially higher feeding rate was achieved under these conditions than in the glass cylinder. In this experiment it became clear that dilution of counts by addition of feces or inorganic material from sterile rocks to the water was an important factor. It also appeared that activity was being leached out of the algae. Since the algae had not been killed before use, respiratory ^{14}C loss was possible during the 3.0 h feeding period. Also excretion of ^{14}C-labelled organic products was likely (Fogg 1971) and due to the continuous rapid circulation of the cells in the system, physical damage and loss of cell contents was possible (see Maciolek and Tunzi 1968). From *Simulium* experiment 7 it appeared that the loss of ^{14}C products from food was less than the loss of ^{51}Cr. Feeding rates were calculated from the initial 100-ml dilution of food so that minimum rates were obtained. If the t = 21 and 131 min values for "working" activity of food are averaged, it is evident that the calculated feeding rates might actually need to be increased by a factor of 4.88 for ^{51}Cr and 3.33 for ^{14}C in experiment 7.

Feeding rates often seemed to decrease with increasing size of the larvae. This situation is noticed in Simulium experiments 2,3,4, and 7 as the accumulation rate for the small size class in each case was greater than the rate for any other size class.

In Simulium experiment 8 feeding was allowed for only 1.0 h. It is noteworthy that the mean accumulation value (μg/mg) for this experiment is about one-third of that in experiment 7, where feeding time was 3.0 h. In experiment 8 there was also the initial reduction in activity at t = 0 due to dilution by feces and suspended inorganic matter. However, the subsequent reduction in activity in ^{51}Cr and ^{14}C was not so great as in experiment 7. It appears that nonrandom sampling of algae in experiment 7 or errors due to inadequate counting statistics could have caused the variations in "working" activity of food with time.

Accumulation of Food by Filtration in the Plexiglass Channel

Although values obtained with the plastic trough occasionally approached theoretical values, the trough was replaced by a circular plexiglass channel which had current speeds ranging from 28 to 60 cm/sec. The feeding time in experiment 12 was 45 min (the calculated minimal gut-filling time) and watercress was used as food. During 79 min of water circulation, activity in the food stayed fairly constant but at t = 85 min the measured activity was half of the initial activity for both isotopes. It seems unlikely that leaching could be entirely responsible for the large loss in activity between t = 79 and 85 min samples. It appears that the reduced accuracy of weighing food samples of approximately 10 μg and normal biological variation account for much of the fluctuation in food activity. Random inspection of guts revealed that larvae were filled on watercress. From isotope-determined ingestion rates the weight of a watercress-filled gut was less than the gut weight of larvae filled with the usual stream seston. The texture of the watercress in the guts was cottony and did not seem so densely packed as the usual mixture of diatoms and grit. Very little ^{51}Cr was present in filtered water in this experiment, with cpm/ml falling in the same range as in previous experiments. This again accounts for the very low adsorption values calculated. The magnitude of food accumulation within 45 min by Simulium in experiment 12 shows that the plexiglass stream simulates the physical environment of Rattlesnake Springs more effectively than the other devices tested.

Feeding in Glass Cylinder, Trough, and Plexiglass Channel Experiments in Relation to Temperature and Current Speed

Depner (1971) studied 13 species of blackflies in southern Alberta and found distribution related to temperature and current speed. Some

species were not found in regions where water was colder than 9°C. Williams and Hynes (1971) found similar temperature and current related distribution patterns in Africa. Ladle et al. (1972) found that feeding by Simuliidae stopped between 5-9°C. This may restrict species which are not able to store lipids before winter. Ulfstrand (1968) found wintering Simulium larvae to be very thin. This evidence contradicts the findings of Chutter (1972) and this research (see Table 26).

The lower stream temperatures in Lapland may greatly reduce the energy demand and therefore allow for overwintering of larvae with low food reserves or the species Ulfstrand studied may be adapted to feed at very low temperatures. S. vittatum is reported to tolerate stream temperatures > 27°C (see Becker 1973) but was not found above a dam where maximum summer temperatures reached 29°C (Spence and Hynes 1971). This species was extremely abundant below the dam, where maximum temperatures were 22°C. The food supply below the dam was uniformly high throughout the year while above the dam winter food supply dropped to 1-2 mg/ℓ. High summer temperatures may have limited distribution here, but the low winter food supply or food quality must have been more important. The range of experimental temperatures in feeding experiments in this study (15-24°C) (Table 3) was well within the limits for normal feeding. Furthermore, experimental temperatures varied little from the natural stream temperatures on the days of feeding.

Depner (1971) states that current speeds measured where 13 Simulium species occurred range from 27.4-137 cm/sec. Ulfstrand (1967) found the distribution of two species of blackflies to be confined principally to shallow water in moderate to fast (25 - > 75 cm/sec) current on bare rocks. Most investigators find Simulium restricted to areas where current speed is swift (Phillipson 1957, Fredeen 1964, Maitland and Penny 1967, Chutter 1968, Tarshis 1968, Hynes 1970, Depner 1971, Williams and Hynes 1971, Minshall and Andrews 1973). Fredeen (1959) states that S. arcticum grew through three instars in water flowing at only 9 cm/sec but was not able to emerge. S. venustum and S. vittatum also grew in water as slow as 6-9 cm/sec but growth and survival were greatly increased at fast current speeds (up to 137 cm/sec). Wu (1930) reported that the minimum current speed tolerated in nature by S. venustum and S. vittatum is 17.1 cm/sec. Harrod (1965) found that larvae in nature occurred in velocities of 20-90 cm/sec, with the greatest number occurring at 50-60 cm/sec. Fifty percent of the larvae held in water at speeds of 8-15 cm/sec died within 7 days; 14% mortality was found at 20-27 cm/sec. At 20-27 cm/sec only 78% of the larvae held their fans open at any one time. She found that 19 cm/sec was the critical speed for opening fans and below this speed fans were opened only intermittently for brief periods, providing substandard feeding rates. Apparently normal feeding is passive, requiring a strong current to keep fans open.

Carlsson (1967) (see Williams and Hynes 1973) felt that a good food supply could compensate for unfavorable current. This does not appear to be the case as the slow (6.1 cm/sec) current speed in glass cylinder feeding chambers was responsible for the small amount of food ingested even in the presence of a food concentration 10 times greater than in the stream. The small amount of food which was ingested probably came from active opening of fans. This small intake of energy would be insufficient to meet the long-term larval food requirements (with regard to gut weight and digestion time data). Larvae of S. ornatum are not adversely affected by O_2 concentrations approaching 25% saturation and their O_2 consumption increases with current speed (Phillipson 1956). Although fast current speed may not be required for adequate respiration, it does seem to be required for normal feeding.

When current speeds measured in the trough at larval attachment sites (Table 6) are compared with current speeds in the stream it is apparent that experimental current speeds were adequate. A wide range of speeds occurred but larvae were always allowed time to select a suitable site for attachment. Larvae in Rattlesnake Springs were found primarily where current speeds were 19.1 cm/sec or greater (Appendix B) and this exceeds the minimum speeds required for feeding (Harrod 1965). Since current speed was more than adequate in the plastic trough, the effect of turbulence is a potential clue to the low ingestion rates. On coarser substrata, less current speed is required to convert laminar flow to turbulent flow. Egglishaw (1969) found an association of Simulium with deep water where flow was less turbulent.

Though larvae are found in currents between 30-150 cm/sec (Maitland and Penney 1967), an increase in speed may create unfavorable turbulence. Maitland and Penney (1967) state that larvae are found on the top surface of rocks where the current is strong and laminar. Ulfstrand (1967) notes that the extreme turbulence of a rushing rather than streaming type flow combined with a coarse substratum will reduce the boundary layer which protects the animal from the full force of the current. The large, smooth rocks in the plexiglass channel offered streaming flow and animals were able to fill their guts (visual gut analysis) and acquire a gut load (radiotracer measurement) approaching theoretical values within 45 min. Although the water volume and amount of labelled food necessary in this system were inconveniently greater than for the glass cylinder or plastic trough, results obtained in this system were superior to those obtained in the other two devices because current speeds and water flow characteristics more accurately duplicated those found in the natural stream.

The physical properties of the trough and plexiglass channels created two different types of water flow. Normal water flow in streams is turbulent. However, relationships between the current velocity, water depth, channel width and substrate particle size determine whether the turbulent flow will be of the streaming or shooting type (Morisawa 1968). Knowing the mean current speed and water depth, the Froude number can be calculated to describe the type of water flow in a channel (Morisawa 1968).

The Froude number is $F = V/(gD)^{1/2}$ where V is mean velocity, g is the acceleration due to gravity, and D is water depth. Values of $F > 1$ indicate shooting flow, characteristic of turbulence as in rapids, whereas F values < 1 indicate streaming flow, characteristic of normal turbulent flow in streams. In the trough feeding experiments Simulium larvae were placed on irregularly shaped gravel which was covered by approximately 5 to 10 mm of flowing water. Current speeds varied from 30 to 50 cm/sec. Using all combinations of current speeds and depths, F values range from 0.96 to 2.3, indicative of shooting flow. Froude numbers calculated for similar speeds in the plexiglass channel in which the water flowing over the smooth rock surfaces was much deeper are all much less than 1. Although mathematical descriptions of water flow are crude tools for calculating the physical environment near a rock surface inhabited by insects, they are a means of selecting experimental streams with water flow properties suitable for measuring realistic rates of ingestion by filter-feeders.

Assimilation Efficiency

Assimilation efficiency was calculated for Simulium by two methods--Calow and Fletcher's (1972) dual-label method and the ash-ratio method of Conover (1966).

Dual Label Method

From the previous discussion it is evident that although some radioactive feces were collected in most of these experiments, the amounts usually were too small to compute a realistic assimilation efficiency. Only in Simulium experiments 7, 10, and 12 was enough food ingested so that sufficiently labelled feces were produced. Due to the form of the equation used to compute assimilation efficiency, negative values (meaningless) were often obtained when the ratio of $^{51}Cr/^{14}C$ in the food divided by that ratio in the feces was greater than 1.0.

Several factors operating in these experiments could have created ambiguous results. Surface adsorption of ^{51}Cr or ^{14}C was not controlled for in each experiment, although sufficient evidence has been presented to show that it was of little importance. Leaching was an important factor in certain experiments, especially when algae were circulated for an extended period. In addition, leaching of one isotope can be greater than for another as evidenced by the 2.85 times greater leaching of ^{51}Cr than of ^{14}C in experiment 10. The plastic trough system produced greater leaching and damage to cells than the plexiglass channel because the trough had 1/10 the water volume of the channel and had a greater turnover of the water. Rapid flow through constricted joints in the trough system also could have contributed to cell damage and leaching.

Continued leaching made accurate estimates of ingestion rates difficult. If absorption of ^{51}Cr was negligible, then leaching alone could cause underestimates of the feeding rate (or gut-full amount). Generally feeding rates were calculated using the average initial activity values for algae (yielding minimum feeding rates).

No correction was made for absorption of ^{51}Cr in the computation of assimilation efficiency. Even though ^{51}Cr is nonessential in insect nutrition, it has been found to be absorbed to some extent. Assimilation of ^{51}Cr was estimated to be 10% in 24 h in snails (Calow and Fletcher 1972) and 5.7% in 24 h for crickets (Van Hook, Jr. and Crossley, Jr. 1969). Correcting for a 10% loss of ^{51}Cr cpm from the feces by absorption does not change the calculated AE appreciably.

In experiment 12 a peculiar situation was encountered. Controls for surface adsorption showed this factor was negligible and leaching was not a problem within the 45-min feeding time. Yet, assimilation efficiency values calculated from the three feces samples were all negative. Even applying a 10% correction factor to the ^{51}Cr activity in the feces did not give positive results (see Calow and Fletcher [1972] for method of correcting AE). Adsorption of ^{51}Cr would need to be at least 34.9-43.4% before a positive efficiency could be calculated if this were the only factor leading to low results. If feces had been contaminated with labelled food during defecation (precautions were taken against this possibility), then AE values would become even more negative. Leaching was not greater for one isotope than another in the food in experiment 12 although the possibility of differential leaching in the feces exists. For negative results to occur, ^{51}Cr must be reduced relative to ^{14}C in the feces. In addition to absorption of ^{51}Cr this could be accomplished by leaching of ^{51}Cr from feces (which Calow and Fletcher claim is negligible for snail feces), or excretion of labelled ^{14}C products into the gut (which is unlikely because the ^{14}C body burden is low and the feeding period short). Another cause could be release of ^{51}Cr into solution in a low pH gut. Measuring the ^{51}Cr activity in solution in the defecation chamber would account for losses by these means.

In experiment 12 it also is seen that the value μg/cpm ^{14}C in the feces is greater than in the food as would be expected if labelled organic material were being removed from the food. The average activity of the food was 0.027 μg/cpm ^{14}C and of feces was 0.101 μg/cpm, meaning that the watercress was 3.74 times more active than the feces. With an average percent ash of 15.39% in watercress the organic fraction is 84.61%. If no ash is assimilated, *Simulium* ingesting watercress would require a 90% AE to create even a 2.4 fold difference in the activities of food and feces (the 3.74 fold difference gave negative results); 90% of the organic matter would be assimilated as would 90% of the ^{14}C cpm. The 3.74 fold difference in activities between food and feces must then be caused largely by leaching.

The magnitude of the difference between μg/cpm ^{14}C activity values of food and feces when (a) AE is 50% (AFDW), (b) the food has 84.61% organic matter, and (c) when it is assumed that inorganic matter is nonabsorbed can be calculated as 100% μg dry wt/100% cpm (food) divided into 57.7% μg dry wt/50% cpm (feces) or 1.16. The μg/cpm ^{14}C ratio in feces was obtained because when AE = 50%, 57.7% of the μg dry wt and 50% of the cpm ^{14}C remain after digestion.

Ash-Ratio Method

House (1961) stated that the mineral requirements of insects remain the most neglected area of insect nutrition. The controversy over the use of the ash-ratio technique (Conover 1966) supports this point. The principal assumption in the use of this technique is that minerals are not assimilated. Conover states that this is true of zooplankton. Certainly some minerals are assimilated and released by zooplankton (see Edmondson 1961) but the extent of assimilation may vary with species. Pavlyutin (1970) criticized the ash-ratio method on the grounds that significant mineral absorption does occur.

Assimilation efficiencies based on the ash-ratio method can be calculated using Stockner's (1971) and Lawton's (1970) data. An assimilation efficiency of 53.9% was calculated for Hedriodiscus in Spring 6 using percent ash values of food and feces provided by Stockner. The assimilation efficiencies calculated by Stockner using the gravimetric method are 53-70% depending on instar. The ash-ratio method, therefore, provides a reasonable estimate in this case. With Lawton's data on percent ash, AE's for Pyrrhosoma with chironomids and with Asellus as prey were calculated to be 38.9 and 63.7%, respectively. Values calculated gravimetrically by Lawton were 84.0% and 76.9%, respectively. Hargrave (1971) found that the assimilation efficiency for Hyalella computed by summing P+R+F+U agreed closely with the 15% value derived by the ash-ratio method. However, Prus (1971) found the ash-ratio method to be unreliable for Asellus, which lost minerals during the winter and accumulated minerals in the summer. Kerambrun (1972) found sexual dimorphism in mineral accumulation in Sphaeroma hookeri (Isopoda). Assimilation efficiencies calculated by Mason (1970a) using the ash-ratio method were much more variable than those calculated by the gravimetric technique although the mean values were comparable. The range of AE for Helix aspersa (Pulmonata) determined by Mason using the ash-ratio method was 28.9-83.3% (mean 53.0%). In the present study AE could not be calculated in several cases with Simulium where the cpm of the inorganic isotope (^{51}Cr) in the feces became smaller in relation to the organic isotope (^{14}C) instead of the reverse. Aside from leaching from the feces, an accumulation of the mineral component in preference to the organic component or release of ^{14}C into the gut is the only logical explanation for this. Mason (1970a) noted that the mineral component of food is sometimes assimilated to a greater degree than the organic fraction.

Mineral assimilation is important in mollusks (Mason 1970a) as evidenced by the composition of their shells. The mean percent ash of Simulium in Rattlesnake Springs was 18.7%. Nelson and Scott (1962) found Simulium larvae contain 21% ash and pupae 9%. The importance of minerals in Simulium may be great if turnover is rapid. Studies on the rate of uptake and release of certain minerals (especially ^{51}Cr) would be valuable.

The assimilation efficiency for Simulium has been calculated using the ash-ratio technique. Since it had been learned that larvae could filter any size particle from the water from < 1 μm to long filaments, the percent ash of food could be found by filtering a water sample from the steam on a glass fiber filter which could be accurately weighed on a Cahn electrobalance. If selective feeding had occurred, then selective sampling of food for percent organic matter analysis would have been required. Preashing filters before use (Maciolek and Tunzi 1968) weakened the filter, causing loss of weight by crumbling. Boiling glass filters in acid and thoroughly rinsing in distilled water before use (Ganf and Milburn 1971) failed to work because some acid was not washed out of the filters (Table 38). Because the weight losses after ashing control filters showed little deviation about the mean, it was possible to correct the calculated values of percent ash. However, due to these difficulties it was simpler to scrape the seston off 5-μm membrane filters and determine percent ash on foil squares. Percent ash of stream seston ranged from 73.95 to 93.99%. Results obtained using acid-cleaned glass filters fell in the range of values determined with foil squares.

Percent ash of diatoms used in Simulium experiments 9 and 10 ranged from 76.01-83.94%. Gut analysis showed diatoms to be the major food; this is emphasized by the fact that the percentage ash values in cultured diatoms and in the gut material and stream seston were all nearly identical. Nalewajko (1966) gives a range for percentage ash of common diatoms of 28-57%, which is low in comparison. Data from J. Gurtisen (unpublished data) show ash content of Columbia River diatoms to be 85.9% (n = 9). An elemental analysis of the algae (primarily diatoms) colonized on slides held in Rattlesnake Springs (F. Rose, unpublished data) for approximately 1 week showed that the mean percentage of carbon (n = 2) was 9.20% of dry weight and the mean C-N-P ratio was 43:8.1. The mean percentage organic matter that I measured for diatoms from Rattlesnake Springs was 18.47%. This indicates that carbon comprises 49.8% of the ash-free dry weight of organic matter.

Assimilation efficiency values obtained in this research by the ash-ratio method (7.59, 27.32, 56.05%) have a wide range. The high value corresponds well to values computed by the dual-label method. Low values were not unexpected as this occurred in the data of Stockner (1971) and Lawton (1970). Due to the great variability of AE values determined by the ash-ratio method and the improbable assumption of negligible assimilation of ash, data derived by this method are only circumstantial without knowledge of insect mineral requirements.

Energetics

In the research on Simulium of Rattlesnake Springs ingestion rates for three food types were measured in relation to current speeds, feeding time, and substratum type. Assimilation efficiency was calculated using the dual-label and ash-ratio techniques. Dry weights of larvae and percent ash of larvae, food and feces were measured from January through July 1973. Estimates of the time required for digestion were made in February and periodically during the remainder of the study period. Gut fractions, measured for larvae of various sizes, were similar to those determined by Ladle et al. (1972). Ladle's data were used to compute the fraction of larval weight due to a full gut (Table 29) and, with data on digestion time, were used to compute a theoretical ingestion rate (Table 31).

Growth rate of larvae was not measured but it is known that less than 28 days are required for development from egg to pupa. On September 7, 1973, watercress completely choked the stream section where Simulium were obtained for experiments. Larvae were completely eliminated in this section but larvae and pupae were very abundant in 28 days after watercress was cleared from the channel. Colonization from upstream by larvae was impossible because extensive shallow pools choked with emergent vegetation blocked immigration. Sandy reaches below the channel prevented colonization from downstream.

Respiration rates for Simulium larvae were reported by Phillipson (1956). An energy budget for Simulium was constructed using information from this research and from the literature so the reliability of AE estimates could be ascertained.

Growth Rates

Most Simuliidae follow a simple one-year life cycle (Ulfstrand 1968). Some species are obligately univoltine (Spence and Hynes 1971) and others are found to be multivoltine, with two or three generations per year (Carlsson 1962, see Ulfstrand 1968; Spence and Hynes 1971, Becker 1973). Eggs of multivoltine species hatch in 3-7 days in warm weather and those of univoltine species laid in late spring hatch the following fall or winter. Eggs laid in late summer or early fall do not hatch until the following spring (Jamnback 1973). Zahar (1951) (see Maitland and Penney 1967) stresses the importance of considering stream sections individually when determining the number of generations per year because growth rate is affected by local variations in temperature and current speed. Often the first generation is very distinct but subsequent generations become more and more overlapped (Chutter 1972).

Becker (1973) found that a 10°C rise in temperature reduced the mean development time from larval to pupal stage of 5-day old S. vittatum larvae by half, from 8 days at 17°C to 4 days at 27°C. Delayed hatching (see Maitland and Penney 1967) of eggs and larval growth stimulated by local temperature variations causes the overlap of generations. Usually only one or two species predominate in any one stream section. In five studies of Simuliidae in large rivers a total of 2-10 species were reported (Maitland and Penney 1967). Tarshis (1968) reared five species of Simulium from eggs in aquaria at 15.6-19.4°C with current speeds of 7.6-16.5 cm/sec (slower than the critical speed stated by Harrod) and found that most eggs hatched within 5 days, pupation usually occurred 11-20 days after hatching, and the pupal stage usually lasted 1-5 days. Becker (1973) found that the maximum duration of the larval stage of S. vittatum was 13 days at 17°C. The minimum times required for Simulium development under laboratory conditions reported by other workers are:

Species	Larval Stage	Pupa	Author
S. vittatum	11 (egg to pupa)	3	Fredeen (1959)
	13-17 days		Wu (1930) from Tarshis (1968)
S. venustum	8	5	Fredeen (1959)
S. meridionale	14	5	Fredeen (1959)
S. decorum	16	4	Fredeen (1959)
S. luggeri	21	4	Fredeen (1959)

Development of the Budget

These data make it clear that under conditions of normal stream temperatures (16-20°C) and abundant food of good quality, the larval stage can be expected to last 11 to 20 days (Tarshis 1968). For purposes of computing an energy budget for Simulium in Rattlesnake Springs, the larval period was assumed to be 16 days. The average dry weights of larvae (Table 26) from January to July were used to compute growth rate in this 16-day period.

There are six or seven instars in Simulium and in this study larvae were weighed in six size classes measured in 1-mm increments from 3 to 8 mm. First instar larvae might be about 1.5 mm long so that the six largest size classes measured represent approximately growth between instars. It is noteworthy that the dry weight of the smallest measured size class ("instar") is nearly doubled successively in all larger size classes except the terminal one (Fig. 9), illustrating Przibram's rule (see McDiffett 1970).

Gut fractions determined for 3- to 8-mm larvae (Table 29) were calculated from data of Ladle et al. (1972); gut-filling time determined in Rattlesnake Springs was taken as 1.0 h. An approximate ingestion rate was calculated from these data.

The mean of eight determinations of percent ash of Rattlesnake Springs seston was 81.53%. The remaining 18.47% organic fraction represents a caloric value of about 1030 kcal/g DW (see regression equation, Winberg 1971). Simulium larvae have an average 81.31% organic fraction, representing 4.55 kcal/g DW (or 5.60 cal/g AFDW) which is equivalent to the caloric values of Simulium (5.52 cal/g AFDW) given by Cummins and Wuycheck (1971). Larval growth was assumed to be linear over the 16-day larval period. Ulfstrand (1965), however, describes growth of Simuliidae as either "fast initial" or "slow initial". The ingestion by larvae smaller than 3 mm was disregarded.

If Simulium larvae grow from the 3-mm to 8-mm size class in 16 days, then 2.66 days are spent in each mm-size class. Total ingestion (AFDW) during the larval stage (see Table 59) was calculated as:

Total ingestion (μg AFDW/indiv/16 days) =
Σ (mg DW of individuals of each size class x μg/mg/h ingestion rate x number of h spent in each size class x 0.1847)

Total calories ingested (Table 53) based on 4.52 cal/mg AFDW caloric value of periphyton (Cummins and Wuycheck 1971) were calculated as:

Total calories ingested (cal/indiv/16 days = total ingestion (mg AFDW/indiv/16 days) x 4.52 cal/mg AFDW

Total calories assimilated, based on an average AE of 57.12% determined by the dual-label method, are:

Total calories assimilated = total calories ingested x 0.5712

Total oxygen consumed in respiration (Table 59) was calculated as:

Total O_2 consumed (μl/indiv/16 days) = Σ (μg DW of individuals of each size class x 5.4 μg O_2/mg/h x 1 mg/1000 μg x number of h spent in each size class. Total energy consumed in respiration (cal/indiv/16 days) = total O_2 consumed (μl/indiv/16 days) x 4.9 cal/ml O_2 x 1 ml/1000 μl.

TABLE 59. Ingestion and Respiration Rates During the Life Cycle (16 d) of *Simulium* Larvae. Estimates are Based on Growth Between 3.0 and 8.0 mm Size Classes; Respiration Rate of 5.4 μℓ O_2/mgDW/h (from Phillipson 1956); Ingestion Rate Based on 1.0 h Digestion Time and Gut Fractions from Ladle et al. (1972) (see Table 31).

Size (mm)	DW (μg)	Ingestion Rate μg/mg/h	μg DW/indiv/h	μg AFDW/indiv/2.66 d
3	66	82	5.412	63.81
4	134	86	11.524	135.88
5	359	93	33.387	393.68
6	661	110	72.710	857.34
7	984	116	114.144	1345.90
8	1344	111	149.180	1759.06

Total ingestion during 16 d period is 4555.67 μg AFDW/indiv

Size (mm)	Respiration Rate μℓO_2/indiv/h	μℓO_2/indiv/2.66 d
3	0.356	22.727
4	0.724	46.220
5	1.939	123.786
6	3.569	227.845
7	5.314	339.246
8	7.258	463.351

Total respiration during 16 d period is 1223.175 μℓ O_2/indiv

Phillipson (1956) found a range of respiration rates for *Simulium ornatum* at 80-87% saturation to be 4.94-6.42 μl/mg DW/h (recalculated from wet weight assuming dry wt/wet wt = 0.20). A caloric conversion for oxygen consumption of 4.9 cal/ml O_2 was selected, assuming an R.Q. of 0.88 (McDiffett 1970). Total production (G + Ex) was not measured, but loss of exuviae probably was minor since there are only 6-7 instars and only the head capsule and part of the intestinal tract are shed. The energy stored in larval growth (Table 53) was calculated as:

Calories stored in growth = [max. larval wt (1344 μg) x mean organic fraction of larvae (0.8131)] - [gut wt (149.2 μg) x mean organic fraction of seston (0.1847)] x 1 mg/1000 μg x 5.521 cal/mg AFDW

Total calories assimilated per individual during the 16 day larval period calculated as I x AE was 11.762 cal (Table 60). Ingestion was calculated by summing ingestion (μg AFDW/mg) during each 2.66 day growth period for each size class (Table 59). Total calories assimilated by summing growth (Table 60) and respiration (Table 59) were 11.875 cal (Table 60). The high degree of similarity between the two estimates tends to confirm the analysis of the bioenergetics of Simulium. The mean assimilation rate for the life cycle based on a mean individual weight of 705 μg was 0.04386 cal/mg/h. The net growth efficiency for Simulium was 49.52%.

Cheumatopsyche

Five feeding experiments with Cheumatopsyche were conducted using diatoms as food. Ingestion rates due to grazing and filtration were studied (see Tables 39-43). Two different feeding chambers were used in measuring filtration rates (glass cylinder and plastic trough) and one system was used in measuring ingestion by grazing (mesh-covered petri plates immersed in the plexiglass channel).

Cheumatopsyche Feeding Experiments

Determination of Grazing Activity

Cheumatopsyche experiments 2 and 3 were attempts to measure grazing activity. Experimental water temperature in the plexiglass channel was comparable to natural stream temperature but the reduced current flow reaching larvae inside the petri dish caused occasional abdominal undulations. It is doubtful that this presented a serious stress to the animals as they were able to live in stagnant water at room temperature for more than one week. Mean accumulation of labelled food was low in both experiments showing that little if anything was ingested. Since no controls were run it is difficult to know whether the activity levels measured represent feeding or simply adsorption. However, the similarity in mean levels of accumulation by larvae in experiments 1-3 in which presumably little or no feeding occurred indicates that the values are in the range of "control" values which would be obtained if all activity resulted from adsorption. The slightly higher "control" values for Cheumatopsyche compared to Simulium is probably due to the greater pubescence of Cheumatopsyche which could trap seston and increase levels of adsorption of the isotope.

Gut contents of hydropsychids generally reflect the seasonal changes in food abundance in the stream (Mecom 1972). Williams and Hynes (1973)

TABLE 60. Energy Budget Computed for *Simulium*. The Assimilation Rate Determined from Ingestion Rate (Table 59) and the AE Derived by the Dual-Label Method are Compared to the Assimilation Rate Derived as a Sum of Growth (16 d) and Respiration (Table 59). Calculations are Based on Diatoms from Rattlesnake Springs (18.47% Organic) of Caloric Value 4.520 cal/mg AFDW; Caloric Value for *Simulium* Larvae (81.31% Organic) of 5.521 cal/mg AFDW.

Assimilation rate determined as (I x AE) where mean AE was 57.12%

I 4555.67 μg AFDW/16 d

A 2602.20 μg AFDW/ 16 d or
11.762 cal/indiv/16 d

Assimilation rate determined as (G + R)

G Maximum larval DW 1344 μg
Maximum larval AFDW 1093 μg

Larval AFDW minus AFDW of gut contents calculated as 1065 μg AFDW

111 μg x 1.344 mg = 149.18 μg DW
149.18 μg x 0.1847 mg = 27.55 μg AFDW

Maximum larval growth 5.881 cal/indiv

R Total respiration in 16 d (Table 59) 1223.175 μℓ O_2/indiv

Respiration converted to calories based on R.Q. = 0.88, 4.90 cal/ml (McDiffett 1970) 5.994 cal/indiv

A Assimilation rate 5.881 cal/indiv growth in 16 d
5.994 cal/indiv respiration in 16 d

11.875 cal/indiv assimilation or

0.04386 cal/mg/h based on mean weight of 705 μg. $\left(\frac{\text{min + max wt}}{2}\right)$

also found this to be true except in certain months when they felt that gut contents of H. sparna were significantly different from the composition of suspended matter, indicating that grazing occurred. There seems to be no obvious explanation why grazing could not occur except for behavioral reasons. Cheumatopsyche did not exhibit significant ingestion of labelled food in these experiments by grazing. One might guess that food rolling along the bottom and not necessarily in suspension could be filtered by these caddis larvae so the Williams and Hynes' conclusion might be unwarranted.

Measurement of Filtration Rate

Cheumatopsyche experiment 1 was an attempt to measure filtration rate of the larvae using the glass cylinder feeding chamber. Gravel cemented to the inside of a plastic mesh sleeve provided a substratum in the glass cylinder. The larvae were allowed a 21 h acclimation period to establish nets. Even though the current speed in the chamber was low (6.1 cm/sec), it appeared to hinder the easy movement of larvae which soon sought shelter between the mesh sleeve and the glass wall. Although Cheumatopsyche is found in fast flowing water, it probably is located only in sheltered areas such as under stones. Only one animal out of 13 attached silk to the gravel while the others laid silk out on the mesh sleeve. However, in no case were normal nets constructed. The low values of accumulated radioactivity indicate that little if any material was filtered. Recently, a digestion time of approximately 2.0 h was found for Hydropsyche occidentalis at Deep Creek using the carbon dust method of Ladle et al. (1972). Assuming that a full gut weighs 8% of the total body weight (80 μg/mg), a theoretical ingestion rate of 40 μg/mg/h is obtained. The values derived in this filtration experiment are far below the theoretical rate and probably represent adsorption. The apparent explanation for low feeding rates is that larvae need building materials and a more complex substratum for construction of nets and this feeding chamber did not adequately provide them.

Experiments 4 and 5 measured filtration rates of larvae which had been established 21 and 24 h, respectively, in the gravel in the plastic trough feeding chamber. Gravel was coarse and allowed interstitial flow. Larvae easily penetrated within the substrate. When the gravel was removed from the chambers at the end of feeding, it was apparent that much of the gravel was cemented together with silt, and fine gravel (2 mm) often was stuck to the larger pieces. Larval interaction was frequent in a still water container but ceased as animals entered the artificial stream. Gut-filling time for the larvae was assumed to be 4.0 to 8.0 h, the value determined for the closely related species Hydropsyche betteni (Cummins 1973). Feeding times in experiments 4 and 5 were 3.0 and 1.0 h, respectively. Since feeding times were well within the estimated gut-filling time, ^{14}C-derived ingestion rates should equal ^{51}Cr-derived rates. The mean ingestion rate 3.4-3.7 μg/mg/h (calculated by ^{14}C and ^{51}Cr) in Cheumatopsyche experiment 4 is a

minimum value and may need to be corrected by a factor of 3 to 4 as in Simulium experiment 7, which was run simultaneously in the plastic trough.

Cheumatopsyche experiment 5 (run simultaneously with Simulium experiment 8) provided high, consistent ingestion rates of 20.0-25.6 μg/mg/h. Since diatom radioactivity remained constant during feeding, this is taken as a reliable estimate of filtration rate. In addition, these feeding rates are near the theoretical ingestion rates (40 μg/mg/h) calculated for Hydropsyche occidentalis. There is, however, one consideration which must be dealt with. One Cheumatopsyche larvae was found to have a blackfly larva in its mouth and two more larvae in its gut. Since no Simulium were found in Snively Creek where Cheumatopsyche was collected, the larvae must have come from the plastic trough. In Simulium experiment 8, 2.0 h were allowed for acclimation of Simulium, during which time unlabelled larvae could have been preyed upon by Cheumatopsyche. After 1.0 h of feeding the average blackfly larva had 2-7 μg (av. approx. 5 μg) of labelled algae in its gut; therefore, after 0.5 h of feeding only 2.5 μg would have been present in each larva. Calculations of average tracer intake due to ingestion of Simulium must be based on ingestion at the 0.5 h period because Simulium can be preyed on from t = 0 to 1.0 h. After discovering the caddis with three blackfly larvae, four more Cheumatopsyche were dissected and no blackfly larvae were observed in their guts. Also only one of three chambers in the plastic trough sampled for ingestion analysis had both Simulium and Cheumatopsyche present together. So if each Cheumatopsyche larva ate three blackfly larvae this would provide a maximum ingestion of 3 x 2.5 = 7.5 μg of labelled algae per larva. Gut contents of individual caddis larvae weighed between 29.2-75.5 μg (based on ^{51}Cr). Since few Cheumatopsyche larvae contained Simulium and the maximum intake of tracer due to predation could comprise no more than 26% of the total gut contents of an individual, the significance of predation in supplying labelled food for Cheumatopsyche was small. Clearly filtration was the major factor being measured.

In Cheumatopsyche experiment 5 the assimilation of ^{51}Cr during 1.0 h of feeding was equivalent to 2.422 μg/mg. Although it is uncertain whether this accumulation is due more to adsorption of ^{51}Cr or incomplete defecation, the fact that this absorption rate (2.422 μg/mg/h) is similar to those rates obtained in Tricorythodes experiments (range 2.02-3.02 μg/mg/h) where only ^{51}Cr uptake with time was measured, it is likely that defecation was complete and that ^{51}Cr absorption caused this apparent accumulation. With this assumption a 27.6% AE can be calculated from the ^{14}C-derived accumulation present after 24 h defecation. That this value is less than the 45.97-49.13% AE's derived using the dual-label method shows that respiratory losses of ^{14}C may be important during 24 h of starvation.

Within minutes of the first circulation of the food all the small clumps of diatoms were reduced to individual cells. In experiments 4 and 5 no large particles were observed in nets which were visible above the gravel

and it seems unlikely that the filtering efficiency of nets would have been so great that the large total quantities of food could have been ingested only during the initial few minutes, when larger particles were present. Strands of the nets were colored green indicating that diatoms were stuck to them. Maciolek and Tunzi (1968) questioned whether microseston is actually ingested by hydropsychid larvae because the mesh dimensions of the net are comparatively large. They noted that gut contents of the larvae contained filamentous algae and entire or fragmented insects. Mesh dimensions for nets of 5th instar Hydropsyche sparna larvae are 190 x 300 μm (Williams and Hynes 1973) and apparently only large particles held crosswise on strands of the net are apt to be caught. The net is sticky and will occasionally catch diatoms on individual strands but presumably this is not the main means of food gathering.

Requirements of Hydropsychidae and Simuliidae

It seems unlikely that turbulence within the gravel substratum colonized by Cheumatopsyche in the plastic trough feeding experiment would be significant factor influencing its feeding rate. The presence of a suitable substratum with appropriate building materials probably is of greatest importance because feeding depends on successful net building.

Just as the percent of Simulium larvae filtering at any one time varies with current speed, the percent of Hydropsyche which have constructed nets after 24 h varies at different flow rates (Edington 1968). H. instabilis builds few nets at current speeds below 10 cm/sec; at 10, 15 and 20 cm/sec in the laboratory the proportions of individuals building nets within 24 h were 20, 48, and 73%, respectively. Philipson (1969) also found that nets of H. instabilis larvae were confined to current speeds above 15 cm/sec and that the percentage of net-spinners increased with current speed to a maximum of 80% in 24 h at 40 cm/sec. In addition, he found that the percent of net-spinners increased with temperature up to 12°C but decreased when the temperature was raised to 18°C. Early workers claimed that Hydropsyche do not spin nets in winter (see Williams and Hynes 1973). Although Philipson found a reduction in percent net-spinners at lower temperatures, he found that instars 3-5 did spin nets in winter. Possibly just the rate of net spinning is influenced by temperature. Current speed must be adequate for normal net building. After the net is built, however, reduced flow is more easily tolerated (Philipson 1969). From current speed measurements in the plastic trough, speeds should have been sufficient throughout the experiment. However, since acclimation time was only 24 h in these experiments, the ingestion rates may represent feeding by only 70-80% of the population. Current speeds in the trough feeding experiments exceeded the minimum requirements for net construction. Since only 21 to 24 h were allowed for acclimation (net building) to avoid prolonged starvation, ingestion rates may represent feeding by only 70-80% of the population.

Studies on invertebrate distribution invariably show the strong correlation between the Simuliidae and Hydropsychidae. Hydropsyche are found where current speeds are 10 to 50-140 cm/sec (Edington 1968, Petr 1970, Williams and Hynes 1973). Simulium is found in the same range of current speeds. Simulium and Hydropsyche both abandon their habitat when flow or water level decrease (Edington 1968, Minshall and Winger 1968, Tarshis 1969) but the Hydropsychidae migrate less easily than the Simuliidae and cannot colonize new areas so easily under favorable conditions (Chutter 1968). Both families depend on current for respiratory requirements, survival often being difficult under low O_2 conditions (see Edington 1968 and Philipson 1969). Filtration rate is said to depend on current speed and food concentration for both families and each family has been cited for supplementing its food supply by grazing, although this could not be demonstrated in the present study. Also from experiments here it seems that feeding rate and digestion time in Simuliidae reach a plateau at a fairly low food concentration. The Hydropsychidae are able to vary filtration capacity by changes in net size with age (Williams and Hynes 1973) and temperature (Philipson 1969). Filtrating species from pools (slow water) build larger nets than those in riffles. This behavior probably minimizes the variations in feeding rate between individuals in stream sections with different current speeds. In a recent experiment with Hydropsyche in which a high concentration of short strands of Vaucheria was offered as food, larvae were observed casting strands out of the net. This behavior may indicate that ingestion rates could decrease after an optimum food concentration is reached due to increased time required to keep nets clean.

Hydropsyche and Simulium are also closely associated because of the predator-prey relationship (Chutter 1968). Although Cheumatopsyche in the laboratory filtration experiments were not observed above the stones, limited predation upon Simulium did occur. Mecom (1972) states that the diel behavior pattern of Trichoptera is very poorly known. If active predation on Simulium occurs mainly at night and if digestion time is 4.0 to 8.0 h, then daytime sampling will miss the importance of carnivory in the diet of this omnivore. The migratory behavior of Simulium exposes the larvae to predation (Chutter 1968). After hatching, first instar simuliids enter the drift to seek a suitable site for colonization (Chutter 1972) and may be heavily preyed upon. Kaiser (1965) (see Chutter 1968) found that young Hydropsyche are plankton feeders while older larvae prefer larger prey. This difference in food selection with age may be due to the decreasing filtering efficiency of nets with an increase in size of the mesh. Cheumatopsyche in all feeding experiments were sampled by size class. It appears in experiments 4 and 5, in which filtration was measured, that small larvae have higher ingestion rates than the larger larvae.

Chutter 1963 (see Petr 1970) noticed that an increase in the number of hydropsychids was correlated with the increase in plankton below a dam and that after 8 km the hydropsychids exhausted the food supply and population

densities decreased. Maciolek and Tunzi (1968) noticed a similar reduction in seston concentration caused by Simulium. Over 60% of the stream plankton was removed within a stretch of 400 m below a lake. Both the Hydropsychidae and Simuliidae are self-limiting in extent of distribution and depend on algal or detrital drift, fragmentation of leaves by shredders, and other decomposition processes to maintain their food supply and quality. Both families seem to prefer the largest substrate, fastest current and shallowest water (Chutter 1968, Williams and Hynes 1973) but food supply is the primary requirement. In Rattlesnake Springs Cheumatopsyche have a wider distribution than Simulium and are not reduced in abundance as greatly by reduction in current speed due to growth of watercress. In fact, the extra detritus trapped in watercress might benefit these caddis. Silt deposition adversely affects Simulium but not Hydropsyche (Nuttall 1972). Simulium seemed to tolerate fast current speeds much more readily than Cheumatopsyche in all feeding chambers. Cheumatopsyche had difficulty moving against a 6.1 cm/sec current in the glass cylinder chamber and in the trough sought shelter in the gravel substratum. Even though Simulium tolerated the high current speeds in the trough, feeding ability was noticeably impaired by the unnatural physical environment. Feeding rates of Cheumatopsyche in the trough approached theoretically natural rates because turbulence had little effect on larvae in the gravel.

Tricorythodes

Tricorythodes Feeding Experiments

A series of experiments was performed with Tricorythodes minutus using ^{14}C-labelled blue-green algae and dual-labelled (^{14}C, ^{51}Cr) diatoms and watercress. The objective of these experiments was to measure the ingestion rates and assimilation efficiencies of animals consuming these food sources and to compare the results obtained by the standard ^{14}C method with those of the new dual-label method. Initial experiments (1, 2, and 3) using a blue-green algal food source utilized the Van Slyke wet-combustion method of sample oxidation which was in developmental stages at the time. For this reason a discussion of these early trials (Tables 26 a-c) will be deferred until other experiments are examined. Samples from these latter experiments were oxidized with a Packard Sample Oxidizer and are considered to be more reliable.

Experiments 4 and 5 (Tables 47 and 48) were designed to test the ^{14}C method of determining ingestion rates and assimilation efficiency. In these experiments utilization of blue-green algae (Lyngbya and Anabaena, respectively) was examined. All the feeding experiments with T. minutus were conducted under the assumption of a gut-filling time of 1.0 h or more,

indicated from the preliminary experiments 1-3. Later experimentation in October and November 1973 indicated that gut-filling on partially decomposed Cladophora occurred within 7 min. The gut weight averaged 8.70% (SD = 2.49, n = 8) of the body weight (including gut contents). This is equivalent to stating that an animal with a full gut load contains 87 μg/mg. If the digestion time, in addition, is 7 min then the approximate ingestion rate can be calculated by multiplying the number of gut loads per hour (8.57) by the gut weight (87 μg/mg) to give 745.59 μg/mg/h. With the low assimilation efficiencies of most herbivores this method of calculating ingestion rates provides useful estimates.

The method improves in accuracy with decreasing AE. This point can be simply illustrated: If ingestion rate is 10 μg/h, digestion time is 1.0 h, and AE is 50% so that defecation rate is 5 μg/h and assimilation rate is 5 μg/h, the amount in storage in the gut at any instant varies from 5 μg to 10 μg depending on whether the amount assimilated is removed from the gut immediately upon entering storage or immediately before leaving storage. If the assimilation of food occurs uniformly throughout the gut length, then 7.5 μg would be found in storage at any instant assuming ingestion proceeds at a constant rate throughout the 1.0 h digestion time period. However, with any slug of food passing through the gut the posterior portion is more fully digested than the anterior portion because most absorption occurs in the posterior region. For this reason the gut weight would be intermediate between 7.5 and 10 μg and would shift toward 10 μg as the AE decreases.

Determination of Gut Fractions, Assimilation Rates and Assimilation Efficiency Using ^{14}C-Labelled Blue-Green Algae

Using data derived from ingestion of ^{14}C-labelled blue-green algae and the digestion time and gut fraction data derived independently, Table 61 was constructed to compare theoretically and experimentally determined gut fractions and assimilation efficiencies from experiment 4 (Table 47). Dividing the quantity of assimilated matter (in terms of dry or ash free dry μg/mg/h) by the amount ingested during the same time period provides an estimate of the assimilation efficiency. Also subtracting the quantity of assimilated matter (activity remaining after defecation assuming zero respiratory loss) from the total quantity accumulated in the animal body by the termination of feeding on labelled food (combined gut weight plus assimilated matter) yields the gut weight in terms of μg/mg. To convert the dry weight of blue-green algae assimilated or ingested into ash-free dry weight, the dry weight was multiplied by 0.8623 (the percent ash in Lyngbya was 13.77%). It is important to note that the gut fractions calculated for the 2.6-3.5 and 3.6-4.5-mm size classes by the ^{14}C method are very close to the average value derived from gut weight measurements (8.7%). The probable range of theoretical assimilation efficiencies was calculated using AE values derived using 8.7 and 11.1% gut fraction values. A value of 11.1% is the largest

TABLE 61. Theoretical and ^{14}C-derived AE's, Gut Fractions, and Assimilation Rates from *Tricorythodes* (Experiment 4) Using Blue-green Algae. Size Class 2.6 - 3.5 mm was Fed for 1.0 h; 3.6 - 4.5 mm size class was fed for 2.0 h. Values Given in 1 - 7 Below are as µg DW Algae/mg; Values in Parentheses are AFDW; All Values are Uncorrected for Time Required for Gut-Filling.

	Size Class 2.6 - 3.5 mm µg DW Algae/mg Animal	Size Class 3.6 - 4.5 mm µg DW Algae/mg Animal
(1) Average DW Accumulated (gut plus tissue)	142.22	205.93
(2) Average Tissue Accumulation	77.37	127.79
(3) Average Tissue Accumulation per h	77.37 (66.72)	63.89 (55.09)
(4) Gut Fraction (1) - (2)	64.85	78.14
(5) (a) Theoretical Ingestion Rate based on 8.57 gut-fillings/h and 111 µg/mg gut fraction	951.27	951.27
(b) 87 µg/mg gut fraction	745.59	745.59
(c) 80 µg/mg gut fraction	685.60	685.60
(6) Theoretical Ingestion Rate based on 8.57 gut fillings/h and gut fraction derived in (4)	555.76	669.66
(7) (a) Theoretical Tissue Accumulation [(1)-Theoretical gut fractions as in (5)(a), (b) or (c)]	31.22	94.93 (or 47.47/h)
(b)	66 22	125.93 (or 62.97/h)
(c)	55.22	118.93 (or 59.47/h)
(8) (a) Theoretical AE (c) (7) : (5)(a) - (c)	3.28%	4.99%
(b)	7.41%	7.98%
(c)	9.08%	9.18%
(9) Derived AE (3) : (6)	13.92%	9.54%

Simulium gut fraction calculated from Ladle's data and is within the range of Tricorythodes measurements. To calculate AE, the theoretical gut fraction (87 or 111 μg/mg) was subtracted from the combined dry weight accumulation of gut plus tissue components to yield a theoretical tissue accumulation. The rationale behind this procedure was that during the 3.0 h provided for defecation of labelled food, some respiratory loss of assimilated material could occur so that the tissue component after 3.0 h would not be equal to that amount remaining immediately after defecation. If this is true then the derived gut fraction will be an overestimate. The similarity between derived and measured gut fractions, however, supports the contention that respiratory loss is small. The fact that the ^{14}C-derived tissue accumulation is greater than the theoretical could indicate that (a) defecation was incomplete (unlikely), (b) a slight amount of surface adsorption of ^{14}C occurs, (c) the gut fraction is actually slightly less than 8.7%, or (d) respiratory loss was small.

In Tricorythodes experiment 4 two size classes were used (2.6-3.5 and 3.6-4.5 mm). Samples consisted of 10 animals per size class. Nymphs from larger size classes were not studied because the gut begins to atrophy in nymphs > 5 mm long. On the basis of preliminary estimates of gut-filling time in experiments 1-3, nymphs of each size class were fed for the prescribed time (less than the assumed gut-filling time) of 1.0 or 2.0 h, respectively. Defecation of labelled food was allowed for 3.0 h after the feeding period for the smaller size and for 6.0 and 9.0 h for the larger size. Non-labelled food was provided for gut clearance.

Due to the very short gut-filling time, the values of μg/mg in the animals after a 1.0 or 2.0 h feeding period do not indicate the ingestion rate but are actually a composite of gut contents plus tissue accumulation. The data of Table 47 for size class 3.6-4.5 mm indicate that respiratory loss of ^{14}C was slow as there was only a small decline in μg dry wt algae/mg in the animals between sampling periods 6.0 and 9.0 h after defecation. On this basis it was assumed that the body activity remaining after defecation represents the majority of assimilated material.

In experiment 5 (Table 48) Tricorythodes of 2-, 3-, and 4-mm size classes were sampled at hourly intervals after feeding on labelled food. Because animals were not sampled immediately before attaining a full gut (a difficult task with a 7-min gut-filling time), the usual methods of determining assimilation efficiency are not applicable. However, if it is again assumed that respiratory loss of ^{14}C activity during any one hour period is small, then by subtracting the value of μg dry wt algae/mg animal at $t = x$ hours from the value at $t = (x + 1)$ hours and averaging all these values for each size individually, the average rate of assimilation can be computed. These values are provided in Table 62. The mean assimilation rate on blue-green algae was 24.25 μg AFDW/mg/h which is equivalent to 0.1184 cal/mg/h (based on 4.882 cal/mg AFDW). The gut fraction can be

TABLE 62. The Average Rate of Assimilation per Size Class of *Tricorythodes* (Experiment 5) in Terms of μg DW and AFDW/mg/h for the First 6.0 h of Consumption - September 5, 1972.

Size (mm)	μg DW/mg/h	μg AFDW/mg/h
1.6 - 2.5	28.45	24.53
2.6 - 3.5	31.29	26.98
3.6 - 4.5	24.63	21.24
	28.12 (Ave)	24.25 (Ave)

derived by subtracting the amount assimilated from the combined gut-plus-tissue accumulation during the feeding period (Table 63). If the digestion time is 7 min, the amount assimilated during a 1.0 h feeding period is a product of the mean hourly assimilation rate and the time elapsed since gut-filling. This procedure was done for accumulation values on blue-green algae at 5 = 1.0 and 2.0 h. Since these figures represent the gut fraction, they also represent the ingestion rate per 7 min. The average assimilation rate during the first 6.0 h of feeding was 28.12 μg DW/mg/h or 3.28 μg DW/mg/7 min. Dividing the absolute assimilation in 7 min by the amount ingested in 7 min yields an estimation of AE (Table 64).

TABLE 63. Gut Fractions (μg DW/mg) Calculated from Data of *Tricorythodes* (Experiment 5). Estimates are Derived by Subtracting Absolute Assimilation During 1 or 2.0 h (Based on an Average Rate of 28.12 μg DW/mg/h) from the Total Accumulation During 1.0 or 2.0 h. Values are Corrected for Digestion Time of 7 min.

Size Class (mm)	Using 1.0 h Values	Using 2.0 h Values
1.6 - 2.5	54.14	60.09
2.6 - 3.5	40.48	23.01
3.6 - 4.5	24.05	23.40

TABLE 64. Assimilation Efficiency Computed from Ingestion Rates Derived from Table 63 and the Assimilation Rate from Table 62 for *Tricorythodes* (Experiment 5).

Size Class (mm)	Using 1 h Values	Using 2 h Values
1.6 - 2.5	6.06	5.46
2.6 - 3.5	8.10	14.26
3.6 - 4.5	13.64	14.02

The gut fractions from Table 63 are somewhat smaller than those measured directly by weighing guts and those calculated previously by the indirect ^{14}C method (Table 61). The former values range from 2.30 to 6.01%. The lowest value calculated gravimetrically was 4.56%. Including the thin gut wall in direct measurements of gut weight could explain the discrepancy but it is still felt that a gut fraction of 8.7% of body weight is more realistic. Nonetheless, assimilation efficiency values determined using these lower gut fractions are equivalent to AE values determined in experiment 4. If the digestion time were 1.0 h, then the gut fraction values which would be calculated range from 4.82 to 8.49% and AE values range from 3.86 to 6.86% ($\bar{x}$ = 5.57%) for the three size classes.

A second method exists for determining ingestion rate and assimilation efficiency from data of experiment 6 where blue-green algae were the food. Since the digestion time of *T. minutus* is 7 min and the animals were sampled at hourly intervals starting at t = 1.0 h, the line connecting the values for total body accumulation (μg/mg) at each hourly interval actually represents tissue loading (assimilation). For the 2.6-3.5-and 3.6-4.5-mm size classes, animals were sampled hourly between t = 1.0 h and 6.0 h and again at 22.5 h. The smallest size class (1.6-2.5 mm) was sampled at t = 1.0, 2.0, and 4.0 h. A regression of μg/mg accumulated versus time was calculated for each size class (Table 65).

T-tests indicate that a positive relationship exists ($P < 0.05$) between food accumulation and time for the 2.6-3.5 and 3.6-4.5-mm size classes and that the regression does not pass through the origin ($P < 0.05$) for the two

larger classes. This means that the tissue loading curve is not the same as the curve for gut-loading, which must pass through the origin. In addition, a z-test indicates that there is no significant difference in slopes for the two larger size classes ($P < 0.05$). The correlation coefficients (r) for the regressions are highly significant ($P < 0.01$).

TABLE 65. Regression of µg DW/mg Accumulated vs. Time During Feeding of Three Size Classes of *T. minutus* (Experiment 5) on Blue-green Algae - September 5, 1972

Size Class (mm)	No. (n)	Regression Equation	r	95% Confidence Interval of Y Intercept	95% Confidence Interval of Slope
1.6 - 2.5	3	Y = 56.15 + 26.04 X	0.994	a ±92.97	b±35.14
2.6 - 3.5	7	Y = 64.49 + 14.71 X	0.962	a ±44.44	b ±4.81
3.6 - 4.5	7	Y = 66.46 + 12.41 X	0.961	a ±29.98	b ±4.12

Substituting 0.1166 h (7 min) into the regression equations will provide the amount (µg/mg) filling the gut at the completion of gut-loading. This value also represents the gut fraction and can be multiplied by 8.57 gut-fillings/h to calculate the theoretical ingestion rate. The theoretical ingestion rate is actually the slope of the gut-filling curve and is derived from µg/mg (at t = 0.1166 h) divided by 0.1166 h. The slopes of the regression lines for tissue loading specify the µg/mg accumulated per h. Assimilation efficiency can then be derived by dividing the slope of the tissue accumulation curve by the slope of the gut-filling curve (Table 66).

TABLE 66. Accumulation of Food at the Gut-filled Time (0.116 h or 7 min) and AE Values Calculated by Dividing Slope of Tissue Accumulation Curve by Slope of Gut-filling Curve from *Tricorythodes* (Experiment 5) Blue-green Algae

Size Class (mm)	µg/mg at t = 0.1166 h	AE
1.6 - 2.5	59.19	5.13
2.6 - 3.5	66.21	2.59
3.6 - 4.5	67.91	2.13

The range of AE values calculated by the regression method (2.13 to 5.13%) is lower than values calculated in Table 64 based on a 7-min digestion time. The slopes for the regression of the tissue-loading curve for the two larger size classes are 14.71 ± 4.81 and 12.41 ± 4.12 (95% confidence limits, respectively). Assimilation rates calculated from these values (Table 67) are 0.06190 ± 0.02026 and 0.05244 ± 0.01733 cal/mg/h. The slopes of the regression equations for the two larger size classes of Tricorythodes are lowered by inclusion of the values for accumulation at t = 22.5 h. The mean assimilation rate during the first 6.0 h of feeding was 28.12 µg DW/mg/h (Table 62). A curvilinear plot of accumulation versus time (Figs. 10 and 11) provides an improved fit to the data (Table 68). When x = 7 min the amount accumulated is 19.63 and 15.19 µg/mg, respectively, for the two size classes. The ingestion rates based on these values are 168.23 and 130.18 µg DW/mg/h, respectively, and AE values based on the assimilation rate during the first 6.0 h of feeding would be 16.72 and 21.60%, respectively. If the gut-filling time were 30 min, then the hourly ingestion of food would be 63.62 and 54.72 µg/mg/h, respectively, and the AE's would be 44.20 and 51.39%.

TABLE 67. Assimilation Rates on Blue-green Algae Calculated from Slopes of Regression of Tissue-loading vs. Time in Tricorythodes (Experiment 5). 95% Confidence Limits for Assimilation Rates are Given.

Assimilation Rate	Size Class 2.6 - 3.5 mm	Size Class 3.6 - 4.5 mm
µg DW/mg/h	14.71 ±4.81	12.41 ±4.12
µg AFDW/mg/h[a]	12.68 ±4.15	10.70 ±3.55
cal/mg/h[b]	0.06190 ±0.02026	0.05224 ±0.01733

(a) Based on organic fraction 0.8623; in blue-green algae
(b) Based on 4.882 cal/mg AFDW (Cummins and Wuycheck 1971)

TABLE 68. Curvilinear Regression of µg DW/mg Accumulated vs. Time During Feeding on Blue-green Algae in Tricorythodes (Experiment 5)

Size Class (mm)	No. (n)	Quadratic Equation	r
2.6 - 3.5	7	$Y = -0.70\ x^2 + 32.20\ x + 15.89$	0.980
3.6 - 4.5	7	$Y = -0.80\ x^2 + 32.21\ x + 11.45$	0.995

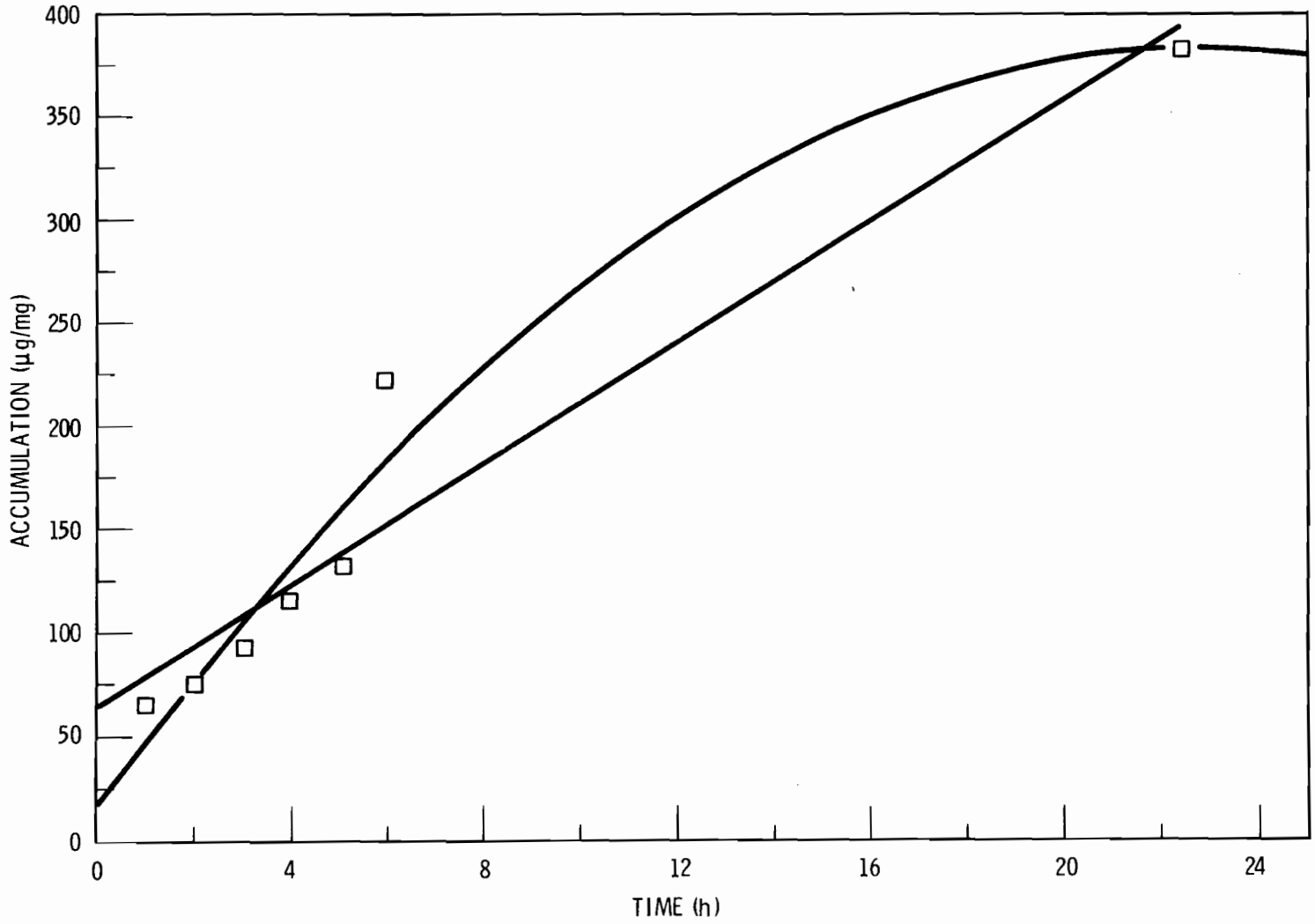


FIGURE 10. Linear and Quadratic Plot of Accumulation of Food (Blue-Green Algae) vs. Time by *Tricorythodes* (2.6-3.5 mm size) (Experiment 5).

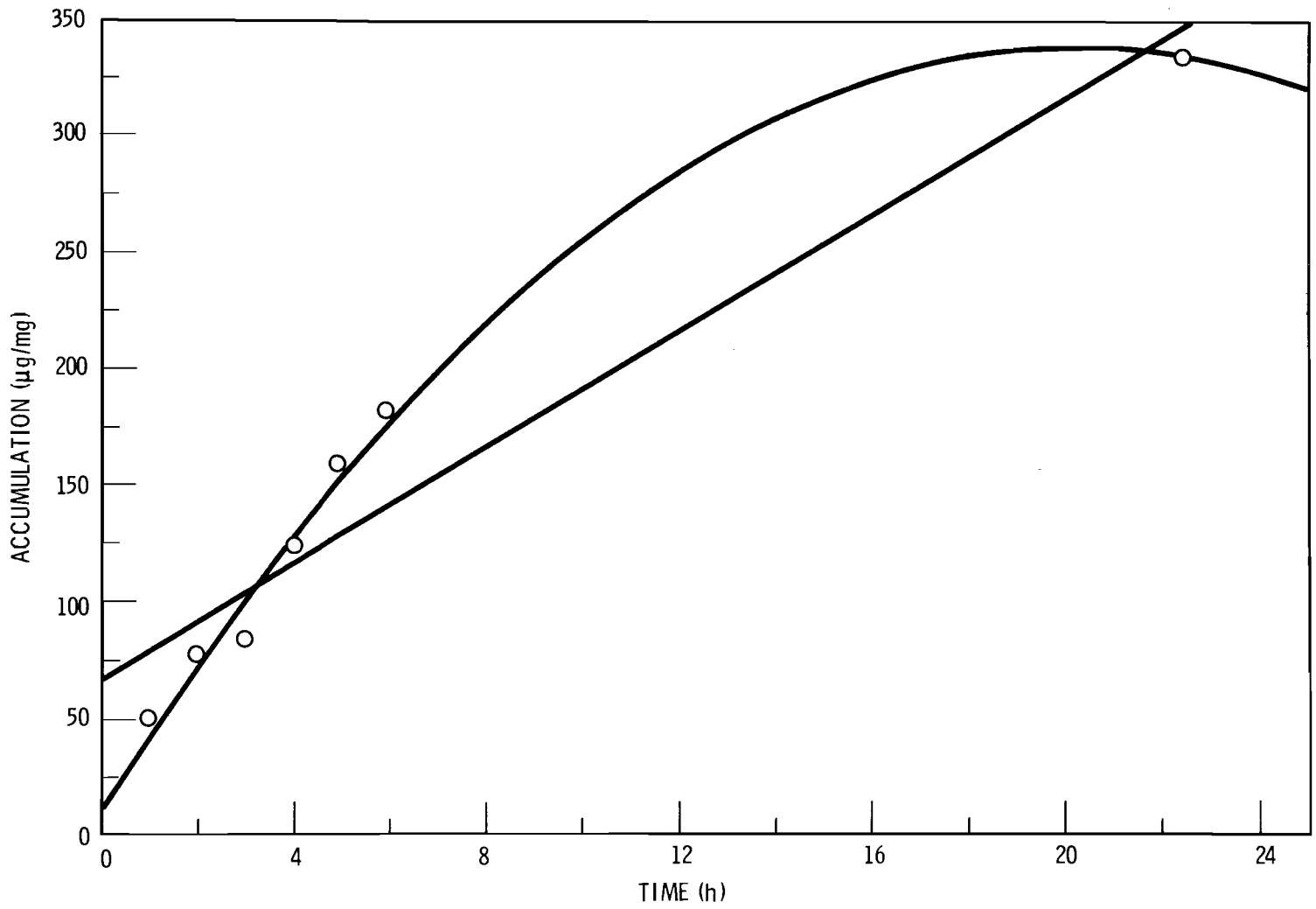


FIGURE 11. Linear and Quadratic Plot of Accumulation of Food (Blue-Green Algae) vs. Time by *Tricorythodes* (3.6-4.5 mm size) (Experiment 5).

Determination of Assimilation Rate and Assimilation Efficiency Using Dual-Labelled Foods

Data presented in Tables 49-55 indicate accumulation of material in μg DW/mg animal at various hourly intervals using dual-labelled diatoms or watercress. The average percent ash of diatoms was 81.53% which was only slightly less than the percent ash measured in natural stream seston which is composed primarily of diatoms. The average percent ash of watercress was 15.39%. Accumulation of material by Tricorythodes may be converted to μg AFDW/mg animal by multiplying the dry weight by these factors. Again, due to the 7-min gut-filling time, the ^{14}C method cannot be applied independently to estimate assimilation efficiency. The dual-label method, however, is especially useful for measuring AE of an animal with a very short digestion time. An initial sample of food is taken to determine the activity ratio of ^{51}Cr to ^{14}C and after the animal is allowed to feed for any period longer than the digestion time (instead of shorter than the digestion time as required by the ^{14}C method) a nonquantitative collection of feces is adequate to determine the fecal activity ratio.

In Tricorythodes experiments 6 and 11 a portion of the animals were sampled at intervals to determine the rate of accumulation of food and the remainder were allowed to defecate for specified times to permit feces collection and computation of AE. Experiments 7 and 9 were designed to measure only the rate of accumulation, while experiments 8 and 10 were designed to measure only AE.

Calculations of Assimilation Rates

The data from experiments 7 and 11 (Tables 50 and 54) can be manipulated in two ways to yield useful results. First, an estimate of assimilation rate can be obtained by subtracting the ^{51}Cr-derived μg/mg accumulation from that derived by the ^{14}C tag. Assuming that ^{51}Cr absorption is zero and that respiratory ^{14}C loss is negligible, then the difference of ^{14}C-minus ^{51}Cr-derived accumulation divided by the time of feeding should be the true assimilation rate. To be more precise, the difference of ^{14}C-minus ^{51}-Cr derived accumulation should be divided by the feeding time minus gut-filling time (7 min) because ^{14}C and ^{51}Cr values will be equal at the end of the gut-filling time. In experiment 11 a control for ^{51}Cr assimilation is available so that ^{51}Cr-derived accumulation rates can be corrected for absorption. At the computed ^{51}Cr assimilation rate of 1.938%, 2.02 μg/mg equivalent of ^{51}Cr are absorbed in 1.0 h and one would expect 10.10 μg/mg to be absorbed in 5.0 h. This appears to be a reasonable correction as the ^{51}Cr assimilation rate in experiment 7 was equivalent to 3.02 μg/mg/h during 4.0 h of feeding.

The calculated assimilation rates for ash-free dry weight are nearly constant (Table 69) over the 5.0 h feeding period and are similar between experiments 7 and 11 where diatoms were used as food. The range of assimilation rates for corrected and uncorrected values combined is 1.06-2.35 μg AFDW/mg/h. The mean uncorrected assimilation rate is 1.51 μg AFDW/mg/h and the mean corrected value is 1.88 μg AFDW/mg/h. The grand mean of assimilation rates is 1.70 μg AFDW/mg/h (equivalent to 0.007684 cal/mg/h). The average assimilation rate of the two larger sizes of Tricorythodes fed on Anabaena (see Table 67) was 11.69 ± 3.85 μg AFDW/mg/h which is between 4.61 and 9.14 times greater (95% confidence limits) than the mean assimilation rate with diatoms. There was 4.67 times more organic matter per mg of Anabaena than for the diatoms, and the mean ingestion rate (μg AFDW/mg) of the two larger sizes of Tricorythodes on blue-green algae was 2.3 to 6.7 times greater than similar rates on diatoms (Table 70). It appears that assimilation rate is roughly proportional to the rate of intake of AFDW of food.

TABLE 69. Assimilation Rates of Tricorythodes (Experiments 7 and 11) (Tables 50 and 54) Feeding on Diatoms in Terms of μgDW and AFDW/h. Corrected and Uncorrected Values for ^{51}Cr Assimilation are given. All Values are Uncorrected for Gut-filling Time.

	Experiment 7		Experiment 11	
Time (h)	Mean Difference ^{51}Cr-^{14}C Derived Values (μg DW/mg)	Assimilation Rate (μg DW/mg/h)	Mean Difference ^{51}Cr-^{14}C Derived Values (μg DW/mg)	Assimilation Rate (μg DW/mg/h)
0.75			5.31	7.08 (1.31)[a]
			6.83[b]	9.10 (1.68)
1.0	10.71	10.71 (1.98)	5.71	5.71 (1.06)
	12.73	12.73 (2.35)	7.73	7.73 (1.43)
5.0	40.80	8.16 (1.51)	46.11	9.22 (1.70)
	50.90	10.18 (1.88)	56.21	11.24 (2.08)

(a) Values in parentheses are μg AFDW/mg/h
(b) Second set of values at each time interval are corrected for ^{51}Cr Assimilation at rate of 2.02 μg/mg/h.

TABLE 70. Assimilation Efficiencies Calculated from Data (Experiments 7 and 11) (Tables 50 and 54) on Mean Assimilation Rate and Ingestion Rate for *Tricorythodes* Feeding on Diatoms at 18°C. Values Corrected for ^{51}Cr Absorption are Given Below Uncorrected Values at Each Time Interval. Values in Parentheses are μg AFDW/mg/h and AE in Terms of AFDW. Ingestion Rate Based on 7 min Digestion Time

EXPERIMENT 7

Time (h)	Mean Assimilation Rate (μg AFDW/mg/h)	Mean Ingestion Rate (μg DW/mg/h)	AE (%)
1.0	1.98	200.37 (37.01)	0.99 (5.35)
	2.35	183.06 (33.81)	1.28 (6.95)
5.0	1.51	303.89 (56.13)	0.50 (2.69)
	1.88	217.34 (40.14)	0.87 (4.68)
			4.92 (Ave)

EXPERIMENT 11

0.75	1.31	169.51 (31.31)	0.77 (4.18)
	1.68	156.57 (28.94)	1.07 (5.81)
1.0	1.06	121.61 (22.46)	0.87 (4.70)
	1.43	104.30 (19.26)	1.37 (7.41)
5.0	1.70	218.45 (40.35)	0.78 (4.22)
	2.08	131.80 (24.36)	1.57 (8.53)
			5.81 (Ave)

Assimilation rate values in Table 69 are uncorrected for the digestion time. For example, if the digestion time is 7 min, the mean difference at t = 1.0 h of ^{14}C- minus ^{51}Cr-derived values would be due to assimilation during the 53 min period after gut-filling. Assimilation rates from experiments 7 and 11 corrected for a digestion time of 7 min are 1.65 μg AFDW/mg/h and 2.06 μg AFDW/mg/h (corrected for ^{51}Cr absorption); the respective assimilation rates corrected for a 30-min digestion time are 2.71 and 3.40 μg AFDW/mg/h. Correction for 30-min digestion time would make assimilation rates (Table 69) and AE values (Table 70) 1.8 times greater than uncorrected values.

Calculations of Assimilation Efficiency

In addition to determining assimilation rate from these data, assimilation efficiency can also be estimated from the ratio of ^{14}C- to ^{51}Cr-derived

accumulation (μg/mg). For example, if 15 μg/mg were accumulated as derived by ^{14}C and 10 μg/mg were accumulated as derived by ^{51}Cr, then this ratio is 1.5. Knowing that the gut-filling time is 7.0 min, one can calculate what the AE would be if the ratio is 1.5 when these animals were sampled at t = 14.0 min. AE in this case is 50% since 5 μg/mg were accumulated in the tissues and the gut holds 10 μg/mg as determined by ^{51}Cr. Again this assumes ^{51}Cr adsorption to be zero but the ^{51}Cr-derived accumulation can also be corrected for adsorption as before. If this accumulation ratio $^{14}C/^{51}Cr$ is 1.5 at t = 21 min, then the AE is 25% because 5 μg/mg were added in 2 gut-fillings after the initial gut loading (i.e., 2.5 μg/mg were added in one gut-filling. Since 10 μg/mg were ingested in one gut-filling also, assimilation is 2.5/10 or 25%. The formula used to calculate AE in these cases is:

$$AE = \left(\frac{^{14}C\text{-derived accumulation}}{^{51}Cr\text{-derived accumulation}} - 1\right)(100) \div (\text{no. of gut-fillings at sampling time} - 1).$$

Since mean assimilation rates in terms of μg AFDW/mg/h are already calculated in Table 69, assimilation efficiency can be calculated in experiments 7 and 11 by dividing the quantity assimilated per h by the ingestion rate per h based on a 7-min digestion time (Table 70). Ingestion rates were calculated by multiplying the mean ^{51}Cr-derived accumulation in μg/mg by 8.56 gut-fillings/h. AE was calculated by dividing μg AFDW assimilated/h by an ingestion rate/h in terms of dry weight of algae and ash-free dry weight of algae. The former calculation would give an AE comparable to one derived by the gravimetric method uncorrected for ash while the latter AE should be comparable to efficiencies calculated by the ^{14}C method (as cpm assimilated/cpm ingested) and the dual-label method. Assimilation and ingestion rate values corrected and uncorrected for ^{51}Cr absorption were used. The ^{51}Cr absorption rate correction again was 2.02 μg/mg/h.

Although the differences between means of corrected and uncorrected assimilation efficiencies (Table 70) are statistically significant ($P < 0.05$), the actual difference is very small. An AE of 5.45% AFDW is the mean of all corrected and uncorrected values and will be assumed to be a reasonable approximation of the true AE. Assimilation efficiencies computed for blue-green algae (see Tables 61, 64, and 66) are approximately the same as for diatoms. AE calculated for blue-green algae by all methods ranges from 2.13 to 16.62%, although the majority of values fall into a smaller range of 2.13 to 9.54%.

Assimilation Efficiencies Derived From the Dual-Label Method Compared to Independent Methods

The average of the six AE determinations on diatoms using the dual label method is 37.6%, which is considerably higher than the 5.45% calculated by other methods. Realizing that the dual-label method gave anomalous negative results in several *Simulium* experiments, these AE values for *Tricorythodes* should be viewed with caution even though most values are clustered around the mean. However, reduction in the activity density of food sources for *Tricorythodes* was negligible compared to that in *Simulium* experiments. An example of the errors which might occur in the calculation of AE where a 10% leaching of ^{51}Cr occurs is shown below. If AE is 5% and twice the cpm ^{51}Cr equals the cpm ^{14}C then AE would be computed by the Calow-Fletcher method as

$$1 - \frac{50/100}{50/95} = 0.05.$$

If only 10% of ^{51}Cr counts are lost then the calculated AE becomes

$$1 - \frac{50/100}{45/95} = (-)0.055.$$

If this loss can be accounted for, then a suitable correction can be made. It is possible that the percent loss could vary with type of food. Guthrie and Tindall (1968) state that the pH in the gut of the cockroach *Blattella germanica* varies from 4.8 to 6.2 as the diet varies from carbohydrate to protein. Chromium-51 could be leached from food easily in an acid gut, thus reducing the AE value. On the contrary if the AE values for diatoms are too high, a loss of ^{14}C from the feces could be the reason. If the calculated AE (uncorrected) is 40%, the corrected value would be 14.3%, provided that 30% of the ^{14}C leached out of the feces.

Assimilation of Blue-Green Algae

The relatively high assimilation rates for blue-green algae in *T. minutus* are interesting in light of the controversy over the use and nutritional quality of blue-greens by aquatic invertebrates (see Arnold 1971). Arnold found AE of *Daphnia* to be 100% on *Anabaena flos-aquae*. Production and survival were also high on *Anabaena* but were very low on *Synechocystis* and *Gloeocapsa*, which appeared to have toxic properties. Schindler (1968) found AE of *Anabaena* to be much lower than for *Chlamydomonas* and *Chlorella*. Schindler (1971) felt that the complex growth forms (large trichomes and aggregates with mucilaginous sheaths) of blue-green algae cannot be used by *Daphnia* because mechanical interference with feeding may limit ingestion. The lower amounts of blue-green algae accumulated by

T. minutus in experiments 1, 2, and 3 indicate the increased difficulty of feeding on the tough, leathery blue-green mats (this material was chopped into small fragments for subsequent experiments). Stockner (1971) also noted interference with feeding on a blue-green mat with Hedriodiscus. Hargrave (1970) found only a 15% AE for Hyalella on Anabaena, which corresponds to the derived AE value for T. minutus (Table 54 and elsewhere). Monakov (1972), in a review of Russian work on assimilability of various algae, stated that two species of Anabaena were found to be of no use to protozoans but Valvata (a snail) utilized Aphanizomenon with highest efficiency and Chlorella with low efficiency. In addition, assimilation of two species of blue-green algae by several cladoceran filtrators was high and the assimilability of Chlorella low. Utilization of some blue-green algae depended on the state of decay as the tough cellulose cell wall in live cells limited assimilation.

Energy Budget

To evaluate the accuracy of AE determinations, a total energy budget was constructed for Tricorythodes minutus (Table 71) using unpublished data from R. L. Newell on rate of growth in Deep Creek at 18°C and from D. Brass on caloric content of nymphs, combined with assimilation efficiencies and ingestion and respiration rates obtained in this research.

Unpublished data from R. L. Newell indicate that for Tricorythodes nymphs (7 ♀, 5 ♂) isolated in growth chambers in the field at station 2 of Deep Creek, a mean of 7.59 days was required for nymphs to grow 1 mm. The increase in total body length was followed from 2.5-6.0 mm. Using the regression equation

$$\log_e Y = \frac{-1.4110 + \log_e X}{0.3010}$$

calculated for 200 T. minutus nymphs where X = length (mm) and Y = weight (mg) (D. McCullough and R. L. Newell, unpublished data), it was determined that the mean weight of 3.0-mm animals is 0.354 mg and 4.0-mm animals, 0.921 mg. The increase in dry weight during this interval was 0.567 mg. Subtracting the 8% gut weight from this yields 0.522 mg tissue growth during the 7.59-d interval, which is equivalent to 2.866 μg/indiv/h or 4.495 μg/mg/h. This represents the average growth rate during the 3.0- to 4.0-mm interval. Using the caloric equivalent of 4.767 cal/mg DW derived by Brass (unpublished M.S. thesis) for Tricorythodes from Deep Creek, this growth rate is equal to 0.01366 cal/indiv/h (or 0.02142 cal/mg/h).

A mean respiration rate of 2.51 μℓ O_2/mg/h was derived for Tricorythodes using a galvanic cell O_2 analyzer standardized by micro-Winkler O_2 determination. This value is roughly midway in the range of O_2 consumption

TABLE 71. A Comparison of the Bioenergetics of *Stenonema pulchellum* (Trama 1957) and *Tricorythodes minutus*

	Stenonema		Tricorythodes	
	4.0 - 5.0-mm nymphs; growth at 20°C; respiration at 20°C; calculations for mean wt 0.735 mg		3.0 - 4.0-mm nymphs; growth at 18°C; respiration at 20°C; calculations for mean wt of 0.6375 mg	
	cal/mg/h		cal/mg/h	
G	0.004054	based on 5.295 cal/mg	0.02142	based on 4.767 cal/mg DW (Brass, unpublished M.S. thesis)
R	0.008900	based on 1.78 μℓ O_2/mg/h and 5.0 cal/mℓ O_2	0.01230	based on 2.51 μℓ O_2/mg/h
Ex			0.003181	based on 3.185 cal/mg AFDW (Stockner 1971)
A	0.01295		0.03690	
	Efficiencies (%)			
G/A	31.3		58.05	
R/A	68.7		33.33	
Ex/A	-		8.62	
G/R	45.6		174.00	
R/A	-		66.67	
P/R	-		200.00	
	Assimilation Efficiencies and Ingestion Rates*			
AE	45.12 (53.1 Ave for all sizes) based on I = 8.91 μg DW/mg/h		6.45 based on 7 min digestion time, 8% gut fraction) I = 0.5724 cal/mg/h	
			27.6 based on 0.5 h digestion time and 8% gut fraction I = 0.1336 cal/mg/h	
			55.2 based on 1.0 h digestion time and 8% gut fraction I = 0.06679 cal/mg/h	

*For *Stenonema* caloric value of diatoms = 3.218 cal/mg; for *Tricorythodes* caloric value of diatoms = 4.520 cal/mg AFDW and diatoms contain 18.47% organic matter.

values for many species of mayflies calculated for 20°C by Trama (1957) from existing literature values. These respiration rates were originally calculated at 10°C and were extrapolated to 20°C using a Q_{10} factor of 2.0, a conversion which may have led to slightly high rates at 20°C. With an assumed R.Q. of 0.88, typical of carbohydrate and protein metabolism, energy is used equivalent to 4.90 cal/ml O_2 consumed. At a respiration rate of 2.51 μℓ O_2/mg/h, and with a mean dry weight of 0.6375 mg for the 3.0-4.0-mm interval, the mean respiration rate during this interval is 0.01230 cal/mg/h.

With mayflies the cast skin comprises a large portion of the energy expenditure. Total lengths and dry weights of 16 cast skins were measured. The dry weights of the nymphs which shed these skins were calculated according to the regression equation for length on dry weight. The cast skin comprised an average of 8.31% (S.D. = 1.87, n = 16) of the dry weight of the nymph. The mean percent ash of cast skins was 50.33% (S.D. = 13.05, n = 15). The average T. minutus nymph grows from 0.5 mm to 6.0 mm during its lifetime with 18-22 molts (R. L. Newell, personal communication). This length change was divided into 20 equal increments. Assuming growth to be linear, nymphs grow 0.275 mm per molt. The dry weight of nymphs at each size was calculated from the regression equation and the weight of the cast skin was determined by multiplying dry weight by 0.0831. The combined weight of cast skins from the last 15 molts equals 1.605 mg, which is 45.9% of the maximum dry weight of a nymph. The weight of skins shed during four molts in the 3.0-4.0-mm interval is 0.2335 mg, which is equivalent to 1.282 μg/indiv/h averaged over the 7.59 spent in this interval. Using the caloric equivalent of 3.185 cal/mg AFDW calculated for Hedriodiscus exuviae (Stockner 1971) and a mean percent ash of 50.33%, the energy lost per h as exuviae is 0.003181 cal/mg/h.

The total energy assimilated during the 3.0-4.0 mm-interval was derived by adding growth, respiration, and exuviae. Energy lost as excretion was not measured. Knowing the gut fraction and digestion time, a theoretical ingestion rate can be determined for diatoms. Multiplying the ingestion rate (μg DW/mg/h) by the percent organic matter in the food yields μg AFDW/mg/h; this value multiplied by the caloric equivalent of the food yields cal ingested/mg/h. An estimate of assimilation was made by multiplying the ingestion rate (cal/mg/h) by the radiotracer derived AE. Calories assimilated/mg/h was calculated independently by summing G + R + Ex = A. The AE needed to provide this amount of energy was then calculated by dividing cal A/cal I.

The energy budget derived for Tricorythodes minutus was compared with the energy budget of Stenonema pulchellum (Table 71) described by Trama (1972). Although the energetics of this mayfly were studied over 16 years ago, there has been no comparable budget constructed for a mayfly and very few budgets for insects of flowing waters in general.

Energy Budgets of Stenonema and Tricorythodes Compared

A slightly larger rate of O_2 consumption was used to compute respiration for T. minutus, accounting for the greater respiratory energy expenditure than in Stenonema. The rate of energy storage in growth is nearly 5.3 times greater in T. minutus than in S. pulchellum. Tricorythodes grows 1 mm in length in 7.59 d; 33.3 d are required for Stenonema to grow the same amount. The assimilation rate of Tricorythodes is 2.85 times that of Stenonema which is evidenced primarily by a much greater growth rate. It was found that the energy lost as cast skins during the life cycle of Tricorythodes and presumably of all mayflies comprises a large portion of the total energy budget. The combined weight of skins shed in the last 15 of 20 molts is equivalent to 45.86% of the maximum weight of a nymph and is 31.44% of the total production (G + Ex). When the exuviae are included in the total energy budget, it is found that they account for 8.62% of the total energy assimilated. Lawton (1971) found that energy lost as exuviae accounted for only 2.8% of the assimilated energy during the life cycle of a damselfly nymph. Lasker (1966), however, found that one-third of growth is lost as exoskeleton in Euphausia pacifica.

A large P/R ratio (2.00) was found for Tricorythodes. The respiration rate used in computing the energy budget is slightly larger than that of Stenonema and is probably in the middle of the range for mayflies. A P/R ratio of 0.78 was computed for the herbivore-detritivore population of Silver Springs from literature values compiled by Coffman et al. (1971) and a ratio of 1.34 was found for carnivores in Root Spring (Table 1). A P/R ratio greater than one can be calculated from many of the energy budgets computed for single species tabulated by Welch (1968). For example a P/R ratio of 1.6 was calculated for Tubifex (from Ivlev 1939); 1.27-2.7 for Daphnia (from Richman 1958); 1.75 for Lestes sponsa (from Fisher 1967). Lawton (1971) found a P/R ratio of 1.03 for Pyrrhosoma in Pond F. The lower mean P/R ratio (P not including Ex) for Stenonema (0.363) may be typical of slow-growing animals. The large difference between ingestion rates of Stenonema and Tricorythodes is a major distinction between these animals. Trama (1957) does not indicate that he measured the digestion time for Stenonema although the use of the ^{32}P tracer to measure ingestion rates requires this. Measuring the ^{32}P activity of nymphs after 12 h of feeding indicates the ingestion rate only if the digestion time is 12 h or greater.

The AE of 53.1% calculated by Trama for Stenonema also appears to be high for herbivores. If the digestion time is actually 1.0 h, then the AE would be 4.4%. Cummins et al. (1973b) (see Cummins 1973) studied the ingestion rate of Stenonema spp. on conditioned hickory leaves and found a maximum ingestion rate of 23.2% of dry body weight/day. Assuming a maximum gut fraction of 11.1% of dry body weight as in Simulium, a digestion time of approximately 12 h is calculated for Stenonema. A 12 h digestion

time was also found for Glossosoma nigrior by Cummins et al. (1973a). The digestion time found for Tricorythodes is very short (7 min) but is roughly comparable to digestion times found for Simulium by Fredeen (1964) and Ladle et al. (1972) and for certain deposit feeders (Hargrave 1972) which feed on low quality food. Uncertainty in the digestion time makes the estimate of ingestion rate and AE very difficult (Table 71). As the digestion time varies from 7 min to 1.0 h, the AE on diatoms from Rattlesnake Springs (18.47% organic) varies from 6.45% to 55.2%. These AE values are based on calories assimilated and ingested. The 7 min digestion time estimate was based on the rate of gut-filling on a marked food source after 24 h of preconditioning on that food source (unmarked). It is possible that initially high rates of ingestion after transference to the marked food could have resulted in the low digestion time estimate. Other data in the previous experiments suggest that a 0.5 to 1.0 h digestion time is more appropriate. In this case, the mean AE of 37.2% derived by the dual-label method may be correct as it would indicate a digestion time between 0.5 and 1.0 h. An experiment measuring the gut-filling and tissue accumulation rates of Tricorythodes was run recently (August 26, 1974) at 20°C using a single species of diatom (Nitzchia). Nymphs were maintained on labelled food prior to the experimental feeding. The uptake curve (Fig. 12) indicates that within 24 min the gut was full (61.86 μg/mg) and no additional food was accumulated by assimilation for the duration of the experiment. A similar result was obtained in experiments 2 and 3 (Table 26 b and c) in which accumulation reached a plateau near 60 μg/mg and later during prolonged feeding continued to increase to levels which were similar between experiments. The food supply was, however, of a higher quality for animals consuming Nitzchia ($\bar{x}$ = 56.12% organic, n = 3) than diatoms from Rattlesnake Springs. This may have caused the cessation of feeding after gut-filling. A concurrent experiment using the ^{14}C method indicated that the AE on this food was 57.38%.

Using the assimilation rate calculated in Table 71, the AE values for various ingestion rates were calculated. The shorter the digestion time, the smaller is the AE. Tricorythodes is a collector which feeds on material of low quality in the stream. The mean percentage ash of the gut contents of Tricorythodes (n = 3) was 78.56%. R. L. Newell (personal communication) found the gut of Tricorythodes to contain about 50% sand, which could be of no value nutritionally. Food of such low quality could account for either short digestion times and low AE's or longer digestion times and high AE's. The quality of food available in the surface sediment at station 2 of Deep Creek (September 29, 1974) was of much lower quality than the experimental diatom food source. Random samples of surface sediment (particles < 351 μm) from pools and riffles contained a mean of only 3.24 (n = 4) and 2.27% (n = 4) organic matter, respectively. The scarcity of Tricorythodes at this sampling time may be correlated with the low food supply. If digestion time remains constant as the food quality decreases

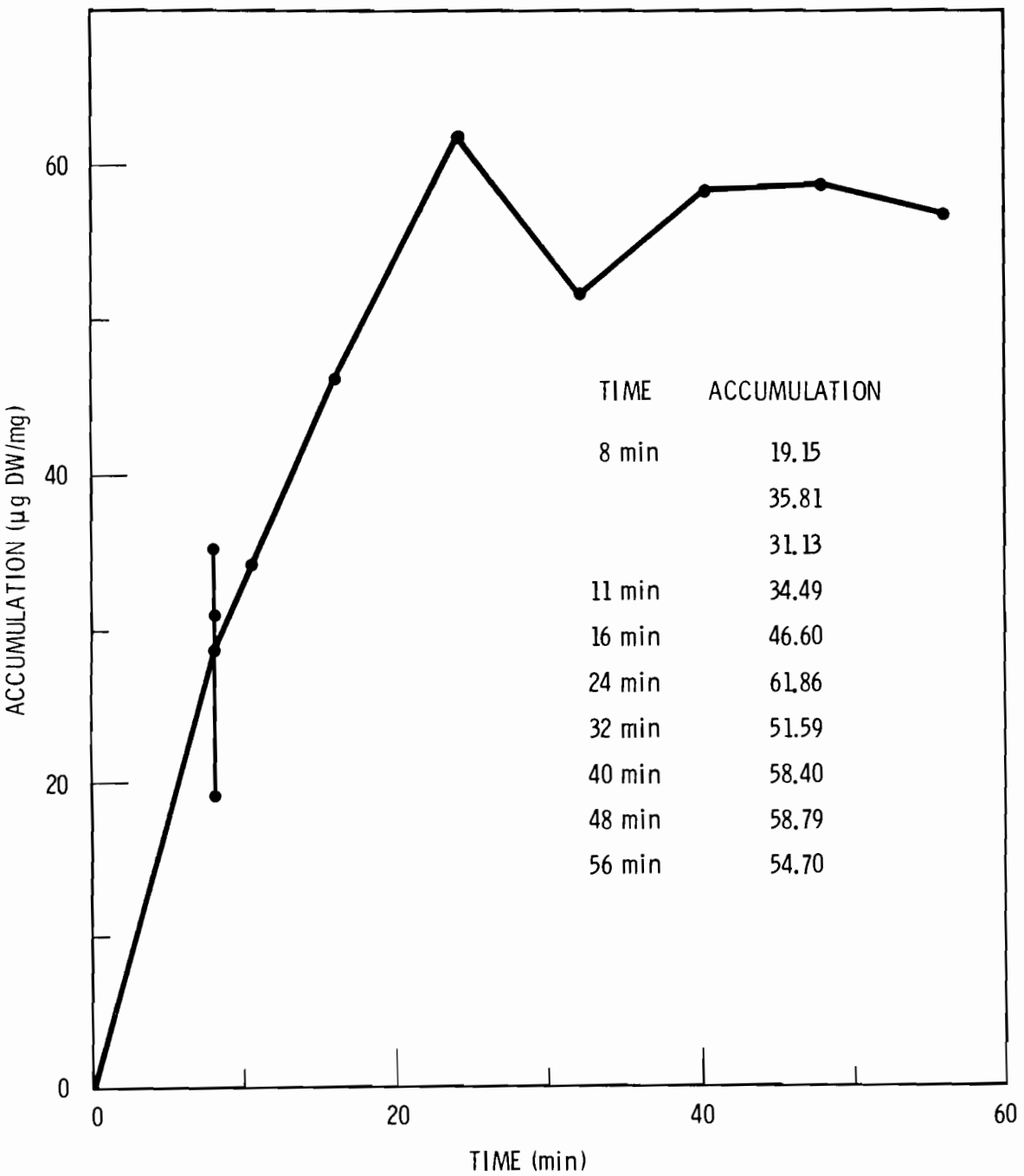


FIGURE 12. Accumulation of Diatoms (µg DW/mg) vs. time at 20°C by *Tricorythodes*.

to 2 to 3% organic matter as in Deep Creek, the assimilation rate must decrease. It is probable that Tricorythodes adjusts its ingestion rate relative to the quality of food.

Net Growth Efficiencies and Assimilation Rates from this Research and the Literature

Net growth efficiencies (G/R) may vary with season, temperature, and species, as do ingestion rates and assimilation efficiences. As a general rule the greater the ingestion rate the lower the assimilation efficiency. An increase in rates of ingestion, growth, and respiration usually is a result of measured temperature. Pandian and Delvi (1973) found a positive correlation between temperature, energy extraction efficiency, and AE. Although the relationship of AE/ingestion may vary inversely with temperature, it is felt that the rate of assimilation should remain relatively constant, for an animal requires a constant supply of energy, regardless of the quality of the food supply. Edwards et al. (1970) felt that assimilation and not ingestion rate should correlate with some power of body weight. Lower quality food necessitates higher ingestion rates at a constant AE, constant ingestion rates coupled with elevated AE, or reduced growth. Assuming that food of reasonable quality and quantity is available throughout the year, assimilation rates should be fairly similar between species and vary to a small extent seasonally with temperature and reproductive state.

Data supplied in Table 72 indicate a considerable degree of similarity in assimilation rates of various species of aquatic invertebrates (carnivores and herbivores). Values on the high end of the range (0.013-0.060 cal/mg/h) were found in Simulium, Tricorythodes, Glossosoma, Stenonema, Lepidostoma and Gammarus and a lower range of values (0.001-0.009) was found in Hedriodiscus, Pteronarcys, Ecclisomyia, Pyrrhosoma, and Potamophylax, It appears that there is a division here between fast and slow growing species. Species of Simulium typically have two or three generations per year; Glossosoma has two generations per year in Linesville Creek. Tricorythodes emerges continuously throughout the year at station 2 of Deep Creek and can complete its life cycle in 2 months. Pteronarcys and Pyrrhosoma each have a 2-year life cycle and consequently assimilate energy less rapidly. Stockner (1971) felt Potamophylax is a slow-growing caddisfly requiring nearly a full year to reach the adult stage and consequently exhibits a low range of assimilation rates during most of its life cycle. However, assimilation rates in the first two size classes are in the high range. A definite decline in assimilation rate with age (instar) is also noted in Pteronarcys, Hedriodiscus, and Pyrrhosoma. There is a positive relationship between assimilation rate and temperature in Gammarus, regardless of the size class. This relationship with temperature is not found with Potamophylax as it appears that larval size is the overriding factor. A

TABLE 72. A Comparison of Net Growth Efficiencies, Assimilation Rates, and Assimilation Efficiencies from this Research and Literature. Assimilation Rates (cal/mg/h) were Calculated from Literature Values of A or (G + R) Converted to Calories and the Mean Weight (mg) of Individuals in the Growth Interval Studied. Where Only Ingestion Rates were Provided, Assimilation Rates were Calculated as AE x cal I/mg/h.

Species	Author	Growth Efficiency (G/A or K_2)	Assimilation Rate (cal/mg/h)		AE	Remarks
Simulium spp.	present research	49.52	0.04386		57.12	Calculated by Dual-Label Method
Tricorythodes minutus	present research	58.05 (P/A = 66.67)	0.03690		6.45-55.2	(See Table 70)
Stenonema pulchellum	Trama (1957)	26.63	0.01295		53.1	K_2 - mean for 4 to 6 mm sizes assimilation rate calculated for 4 to 5 mm-size AE - mean for all sizes studied
			Winter	Instar		
Glossosoma nigrior	Cummins et al. (1973a)	22.8	0.03394	1	17.2	AE - mean for winter
			0.005708	2		
			0.03256	3		
			0.01360	4		
			0.1159	5		

TABLE 72. (Contd)

Species	Author	Growth Efficiency (G/A or K_2)	Assimilation Rate (cal/mg/h)		AE	Remarks
			Summer	Instar		
			0.05919	1	30.0	AE - mean for summer
			0.2630	2		
			0.01702	3		
			0.02765	4		
			0.04200	5		
			Spring 6			
Hedriodiscus truquii	Stockner (1971)	22.8	0.005559	2	59.03	AE - mean for both springs
			0.004224	3		
			0.002322	4		
			Spring 4			
		37.3	0.008319	2		
			0.004881	3		
			0.002790	4		
			Age(mo.)			
Pteronarcys scotti	McDiffett (1970)	34.2	0.009127 3-5	(July-Sept)	10.6	AE - mean for all sizes
			0.005375 6-7	(Oct-Nov)		
			0.003599 13-17	(May-Sept)		
				Instar		
Lepidostoma sp.	Grafius (1972)	--	0.008039	4	12.0	AE - mean
			0.01456	4 and 5		
Ecclisomyia sp.	"		0.006393		8.93	AE - mean

TABLE 72. (Contd)

Species	Author	Growth Efficiency (G/A or K_2)	Assimilation Rate (cal/mg/h)		AE	Remarks
			Mean Length (mm)	Date		
Pyrrhosoma nymphula	Lawton (1971)	48.3	0.01225 2.15	Aug	87.8	From 1966 year class, pond B
			0.001597 3.4	Nov		
			0.009347 6.85	July		
			0.007162 9.1	Aug		
			0.005979 10.9	Sept		
			0.000919 13.0	Dec		
			Mean wt(mg)	Expt. temp. (°C)		
Potamophylax cingulatus	Otto (1974)	35.6 (P/A = 42.8)	0.0408 0.15	5	20.2	G/A - mean for larvae stages AE - mean
			0.0180 1.1	5		
			0.00563 2.9	1.8		
			0.00416 4.2	1.8		
			0.00324 5.0	1.8		
			0.00293 5.5	1.8		

TABLE 72. (Contd)

Species	Author	Growth Efficiency (G/A or K_2)	Assimilation Rate (cal/mg/h) Mean wt(mg)	Expt. temp. (°C)	AE	Remarks
Potamophylax cingulatus (cont'd)			0.00610	5		
			8.2			
			0.00816	10		
			16.3			
			0.00598	15		
			25.1			
			0.00427	15		
			29.0			
			Expt. temp. (°C)			
Gammarus pulex	Nilsson (1974)	57.4	0.00542		30-40 (alder leaves)	
			2			
		70.1	0.00981		0-35 (beech leaves)	
			5			Assimilation rates are means for all sizes at each temp.
		58.6	0.0157			
			10			
		69.7	0.0300			
			15			
Hydropsyche fulvipes	(Edington and Hildrew 1971)	52.5	0.00840			Summer warm temp. regime (max. >15°C)
		40.6	0.00618			Summer cool temp. regime (max. <15°C)
Diplectrona felix		44.1	0.00676			Summer warm
		51.3	0.00552			Summer cool

normal range of assimilation rates which seems to be applicable for any insect is about 0.004 to 0.04 cal/mg/h. An appropriate assimilation rate could be selected for an insect on the basis of instar and temperature.

Ivlev (1939) (see Levanidov 1949) found that net growth efficiency for various animals was very stable, varying between 43-44% ($\bar{x}$ = 43.5%). The net growth efficiencies for Simulium and Tricorythodes are similar (49.5% and 58.1%) and are somewhat greater than Ivlev's values. Slobodkin (1959) (see Kuenzler 1961) felt that net growth efficiencies between 20-28% were universal. Welch (1968) claimed that net growth efficiencies are negatively correlated with assimilation efficiencies and may range from 20-90%. He found carnivores to have high assimilation efficiencies with low net growth efficiencies and the reverse for herbivore-detritivores. High ingestion rates as in Tricorythodes could explain a low AE but this does not seem to be the case for Simulium. Simulium, Hedriodiscus, and Stenomena are herbivores with high AE values (53.1 to 59.0%). The detritivores Pteronarcys, Lepidostoma, Ecclisomyia, and Potamophylax feeding on leaf material had AE values ranging from 8.9 to 20.2%. None of the animals in Table 71 show the relationship of AE to net growth efficiency (G/R) described by Welch. If a high AE were appropriate for Tricorythodes, however, it would conform to his regression. The net growth efficiencies of Simulium, Tricorythodes, Hydropsyche, Diplectrona, Gammarus, and Pyrrhosoma are high (> 48%). A higher G/A ratio was found for Hedriodiscus in Spring 4 than in Spring 6 because lower temperatures allowed more energy to be diverted to growth. Certain fast-growing species (Simulium, Tricorythodes, and Gammarus) have high assimilation rates and G/A values, although the relationship between assimilation rate and AE is not consistent for fast- and slow-growing species. However, Fisher (1967, see Lawton 1971) found G/A values of approximately 70% in Lestes sponsa, a damselfly with exceptionally rapid larval growth. Edington and Hildrew (1973) presented an ecologically significant explanation for the temperature-related distribution pattern in streams for two hydropsychid caddisflies, Hydropsyche fulvipes and Diplectrona felix, related to their bioenergetics. H. fulvipes and D. felix are found in streams with summer warm (> 15°C) and summer cool temperatures regimes, respectively. The net growth efficiency of each species is decreased by rearing it under the alternate temperature regime, to which it is not adapted. In H. fulvipes this lowering of G/A is a result of decreased growth rate with a constant respiration rate at lower temperatures, and in D. felix this involves a substantial elevation of respiration rate with constant growth rate at higher temperatures. Apparently temperature, food quality, or species differences may influence this ratio more than whether the animal is a carnivore or herbivore as thought by Welch (1968).

Assimilation efficiencies of herbivores are generally considered to be low (5-50%) while efficiencies for carnivores generally are over 60% (Lawton 1970). Nonetheless, numerous examples to the contrary can be found (see Welch 1968). In certain cases where high AE values are calculated for

herbivores, the animals were fed on food of high quality not normally available to the animal. Schindler (1968) found that AE increased with calorific content of the food in Daphnia magna. A high ash content of food has been reported to cause a reduced AE in Calanus (see Conover 1966). Pandian and Delvi (1973) show that the energy extraction efficiency for foods with extraordinarily high ash content may be greater than the normal 7% efficiency found for most animals feeding on normal food. In addition to variation in food quality, much of the variation in AE values reported for the same species is due to the wide variety of methods used and the non-standard terminology. Weights are reported as wet or dry weight; calories--as per dry wt or AFDW; ingestion--as percent body weight/day, μg/mg/d, μg C ingested/μgC animal/d, μg/indiv/d; assimilation--as tissue loading, retention, incorporation, accumulation, energy extraction; assimilation efficiency--expressed as μg DW or AFDW, carbon, or calories assimilated per individual, mg DW or AFDW, or calorie. For these reasons AE values calculated by the gravimetric technique are not necessarily comparable to values from the radiotracer method. AE values reported from ^{14}C analysis can also be misleading. Assimilation can be calculated as cpm A/cpm I or as μg A/μg I by the ^{14}C method. In the latter case μg A represents only organic matter but μg I may represent total dry weight or μg AFDW if a correction for ash has been made. When reporting results for energy budgets, conversion factors should be supplied so that accurate comparisons can be made between species.

Whereas most carnivores have a high AE, herbivores are found to have a wide range of AE values (Table 72). The lower AE values are typically found in animals feeding on low quality food such as leaf litter (see McDiffett 1970) or sediment (see Hargrave 1970). Otto (1974) and Nilsson (1974) also noted the effect of food quality on the AE.

APPENDIX A

M-9 salts medium for culturing *Esherichia coli* (Roberts et al. 1957)

NaCl	5.0 g
KH_2PO_4	3.0 g
NH_4Cl	1.0 g
$MgSO_4 \cdot 7H_2O$	0.1 g

Carbon source 2 ml glycerol or 2 g glucose to make 1.0 ℓ

Adjust the pH to 7.0

APPENDIX B

Current speed measurements using the hot-wire anemometer in a reach and the riffle downstream from the reach where *Simulium* were collected for feeding studies.

Position of Probe	Current Speed (cm/sec)
April 20, 1973 -- Stream temperature 17-18.6°C	
Side of stream in watercress	0.6- 0.9
Next to heads of larvae on a rock with a V-shaped surface, in reach	13.1-19.5
Repeat measurement at another spot on the rock	19.5-21.4
Next to bottom in the reach	7.8-15.1
Halfway from top to bottom in reach above riffle	11.7-24.8
Water upstream from a rock with larvae, in fastest section of reach	19.5-28.6
Next to larvae on this rock in riffle	34.4-41.1
Another spot	24.8-34.4
Another spot	19.5-24.8
July 18, 1973 -- 1:00 PM Stream temperature 19.5°C	
Measurements in three distinct stations in a 4-m section of stream (Rattlesnake Springs)	
Station 1	
In watercress at side of stream	1.9- 3.1
In reach, 2.0-cm below surface and in front of rock with larvae	24.8-38.6
Next to larvae on this rock	19.5-24.8
Station 2	
Downstream in riffle, over rock with 1.0-cm water flowing over it	88.4->95
Surface of a rock with larvae, thin layer of water cover, measured next to larvae	53.1-68.3
Station 3	
Further downstream in fastest part of riffle; on top of rock	62.8-88.5
Between rocks in deep water	88.5->95
Toward bottom of a rock, next to larvae	80.5->95

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The enclosed Battelle Report BNWL-1928 is the complete M.Sc. thesis submitted by Mr. Dale A. McCullough to the Graduate School at Idaho State University. Mr. McCullough's research was performed under a NORCUS* appointment at the Pacific Northwest Laboratories of Battelle. His major professor was Dr. G. Wayne Minshall. On-site liason and supervision were provided by Dr. C. E. Cushing.

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We hope that you will find material of interest in this report.

Sincerely,

D. A. McCullough, Oregon State University
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