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BIOENERGETICS OF SIMULIIDAE (DIPTERA) LARVAE IN
THE BUFFALO RIVER (EASTERN CAPE PROVINCE).

Thesis
submitted in the Fulfilment
of the requirements for the Degree of
MASTER OF SCIENCE
of Rhodes University

by
PENELOPE JANE SCOTT

January 1990

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Today I salute the clever simuliid
though not as robust as your average tipuliid

By circlet of hooks they are firmly attached
a feat many lesser bugs never have matched

They feed in the current on tiny debris
known fondly as F-POM by you and by me

It's hard to believe that this filtering collector
is the juvenile precursor of a dread gnat sceptor

For once they emerge from their "antlered" cocoon
to buzz 'round about' some swampy lagoon

They're the scourge of fishermen, and aboriginal native
so, control of the fly has become quite creative

After spraying chem poisons we've come to our senses
and dealt them a blow with *B. thuringiensis*

But this wonderful paradox still persists all the same
We can't kill them in nature - nor grow them as tame!

(K. W. Cummins, 1987)

ERRATA SHEET FOR M.Sc THESIS TITLED "BIOENERGETICS OF SIMULIIDAE
(DIPTERA) LARVAE IN THE BUFFALO RIVER (EASTERN CAPE PROVINCE)"

BY P.J. SCOTT.

The following errata should be noted :

Pg 11 Mention of previous studies is cited as page 23 but is actually page 24.

Pg 38 "unfavourable hydro - conditions" should read "unfavourable hydrological conditions"

Pg 38 "lower digestion times" should read "lower digestion rates"

Pg 72 Total suspended solids should be in "g/l" not "g/h"

Pg 85 "processers" should read "processors"

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DECLARATION

This is to certify that the bioenergetic studies carried out in this thesis are my own unaided work. A survey of the Buffalo River was undertaken with the assistance of my supervisor, Dr Jay O'Keeffe. Flow rate, suspended solid concentrations and simuliid density data from the main Buffalo River Research Programme carried out by the Institute for Freshwater Studies at Rhodes University, was used and has duly been acknowledged in the text. The list of Simuliidae found in the Buffalo River (Appendix A) was compiled by R.W. Palmer. This study represents original work by the author and has not been submitted, in any form, to another University.

ACKNOWLEDGEMENTS

My sincere thanks are due to my supervisor Dr J.H. O'Keeffe for his continuing encouragement, advice and valuable criticism throughout this study.

I would also like to thank Mr Rob Palmer for his thought-provoking discussions, valuable advice and help in the field. I am grateful for his reading and critically commenting on drafts of this thesis.

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My parents for provision of my earlier education and support over the past years. Finally I would like to thank Bernd Teigler for his constant encouragement, understanding and support over the last two years which made completion of this thesis possible.

ABSTRACT

Ingestion, assimilation and egestion rates of different sized filter-feeding blackfly larvae *Simulium medusaeforme/hargreavesi* (Diptera: Simuliidae) were determined. The effects of food type, food concentration and larval size on ingestion rates were tested. These bioenergetic parameters were used to obtain an indication of the importance of blackfly larvae in processing of particulate organic matter in the Buffalo River. Mean ingestion rates for *S. medusaeforme/hargreavesi* larvae feeding in the field ranged from 900 - 1600 $\mu\text{g}/\text{mg}/\text{h}$ compared with those for larvae feeding in the laboratory on the same food type (260 - 680 $\mu\text{g}/\text{mg}/\text{h}$) and on algae, *Chlorella sp.* (590 - 1110 $\mu\text{g}/\text{mg}/\text{h}$) and *Selenastrum sp.* (340 - 1140 $\mu\text{g}/\text{mg}/\text{h}$). Ingestion rates obtained in this study were much higher than those reported by previous workers. These high ingestion rates are thought to be related to the low organic fraction of the suspended solids in transport in the Buffalo River. Larger larvae were found to ingest between three and six times as much food as smaller larvae. Ingestion rates were affected by the presence of nematodes and by imminent pupation. Assimilation rates on algae ranged from 2.3 - 49.0 $\mu\text{g}/\text{mg}/\text{h}$ and were comparable with results of previous workers. The low assimilation efficiencies obtained for larvae feeding on algae (0.4 - 15.1 %) are due to the high ingestion rates. From a survey of the Buffalo River larvae were found to ingest between 0.00011 - 0.15 % of the suspended solids per metre of stream bed, about 1 - 7 times more than what has been reported by other workers. In the upper reaches of the Buffalo River larvae were potentially able to ingest all the suspended solids in the size class 0 - 250 μm within a distance of 3.2km. Blackfly larvae play an important qualitative role in the

functioning of a river system as they remove seston from transport and facilitate the action of gut microflora. Blackfly larvae in association with micro-organisms and other invertebrates are responsible for the majority of the retention and mineralisation of organic matter in the river.

CHAPTER 1

INTRODUCTION

1.1 General biology and ecology of Simuliidae

In this section the biology, ecology and economic importance of blackflies are briefly summarised.

The Family Simuliidae are small, stout-bodied midges commonly known as 'blackflies', 'buffalo gnats' or 'river midges'. Many species of blackflies are important bloodsucking pests of man and livestock. In West and Central Africa the adult blood-sucking females of the *Simulium damnosum* complex are of economic importance as carriers of the filarial parasite *Onchocerca volvulus* Leuckart which causes the human disease river blindness (Crosskey, 1973). In southern Africa *Simulium chutteri* is found in large numbers in the Vaal, Orange (Chutter, 1968; de Moor, 1982a; Car & de Moor, 1984) and Great Fish Rivers (Coetzee, 1982). Excessive blood feeding by female adult Simuliidae can cause secondary infections in livestock (Steenkamp, 1972).

Blackflies are world-wide in distribution, and have their subimaginal stages restricted to running water. The larvae are highly adapted by their size, streamlined shape, method of attachment and feeding behaviour to life in fast flowing water (Hynes, 1970a, b) (Figure 1.0, A). Larvae maintain a favourable feeding position in the water current by attaching themselves firmly to a variety of substrates. Silk producing glands are used to spin a mat of silk onto the substratum. The posterior circlet of abdominal hooks are used to attach to the silk mat, and the body is twisted by 180° so that the ventral surface of the head

and fans face the current (Chance, 1970). This characteristic feeding position minimises energy expenditure (Wallace & Merritt, 1980).

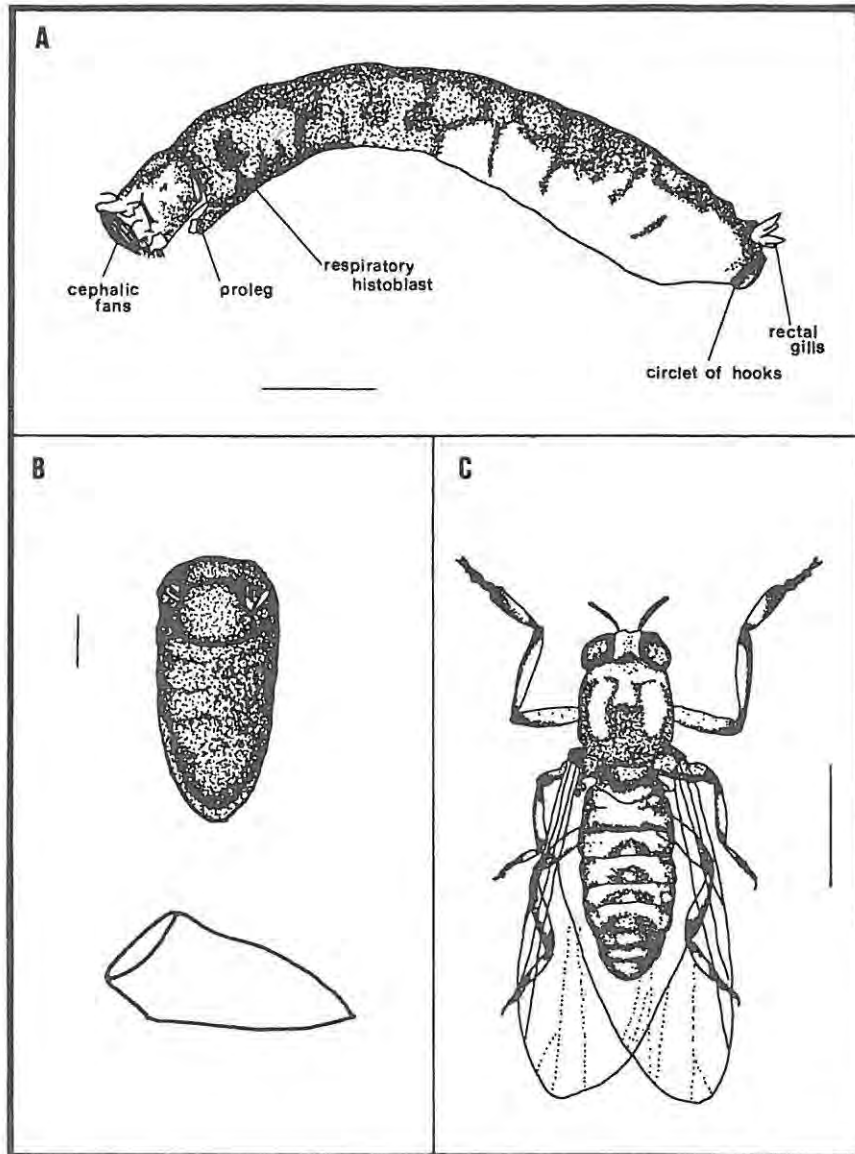


FIGURE 1.0 Representation of the life stages of a blackfly.

A: Pharate pupa of *S. hargreavesi*.

B: Top and outline view in profile of a pupa of *S. hargreavesi* in shoe-shaped cocoon.

C: Adult female of *S. hargreavesi* (adapted from Barraclough & Londt, 1985).

Scale bar = 1 mm.

The majority of simuliid larvae rely on filtering as their primary feeding mode however they are capable of engaging in other feeding strategies to fulfill their nutritional requirements.

Simuliid larvae filter particles from the water column ranging in size from 0.1 μ m to 300 μ m (Colbo & Wotton, 1981). Simuliid larvae are able to ingest material from as small as bacteria (Fredeen, 1960, 1964) to as large as filamentous algae (Burton, 1973), the majority of studies however report simuliid larvae ingesting particles of < 100 μ m (Fredeen, 1964; Chance, 1970; Wotton, 1976, 1977, 1978a). Simuliid larvae are particularly well adapted for filter-feeding as most possess a pair of cephalic fans (Figure 1.1). Each fan consists of a number of long curved primary rays. The primary rays have microtrichia on their inner curved surfaces (Figure 1.2) that point into the water current. The pattern of trichiation varies within the fan and within species (Chance, 1970). The idea that the pattern of microtrichia on rays might be of taxonomic significance was suggested by Yankovsky (1978) however Currie and Craig (1987) have stated that this is unlikely. Detailed accounts on structure and function of cephalic fans are given elsewhere (see Chance, 1970; Colbo & Wotton, 1981).

A sticky mucosubstance is spread over the microtrichia (Ross & Craig, 1980) to facilitate filtration. However in order for optimal filtration to occur the filters must remain clean. The fans are held open in the water current and periodically flicked (retraction and extension of the cephalic fan). The duration of the retraction of the fan is used for cleaning the fan. Mandibular and labral brushes transfer food to the cibarium where it is retained



FIGURE 1.1 Ventral view of head capsule of larval *S. medusaeforme/hargreavesi* showing cephalic fans. Scale bar = 100 μm .

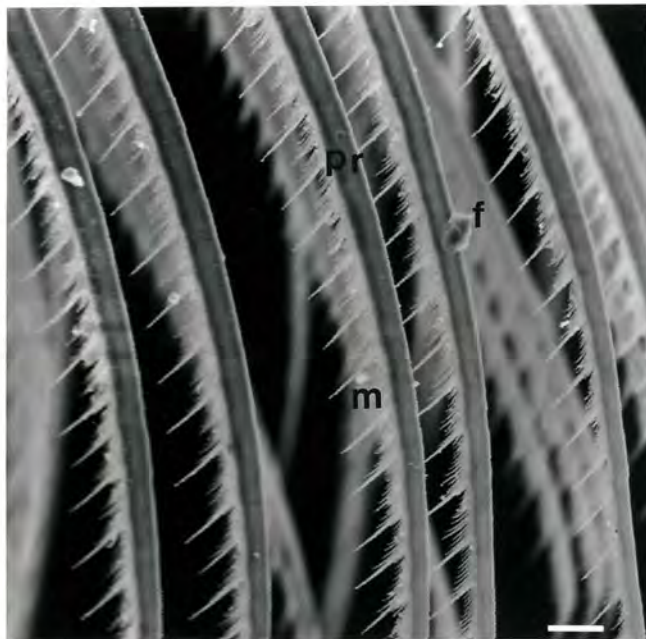


FIGURE 1.2 Scanning electron micrograph of cephalic fans of *S. medusaeforme/hargreavesi* showing primary rays (pr), microtrichia (m) and food particle (f) adhering to the fan. Scale bar = 10 μm .

until a bolus is formed and swallowed. Fans are generally flicked alternately (Chance, 1970). Flick rate is known to increase with increasing food concentration (Hart & Latta, 1986).

The second most important feeding strategy of simuliid larvae is grazing or scraping of algal mats and food deposits from the substratum. The larva attaches itself by its proleg to the substratum and uses its mandibles and labrum to scrape algae from the substratum (Chance, 1970). Grazing is an active process and once the periphyton at one feeding site is depleted the larva must relocate to continue feeding.

Predation by simuliid larvae has also been reported by a number of authors. However the predation is thought to be opportunistic as there appear to be no specific morphological adaptations specific to this feeding mode (Currie & Craig, 1987).

The pharate pupa (which looks like a final instar larva), (Hinton, 1958), spins a silken cocoon within which the larva pupates (Figure 1.0, B). Pupae respire through variously shaped thoracic respiratory filaments which are often species-specific (Freeman & de Meillon, 1953). It was originally thought that the rectal gills played a part in larval respiration (Imms, 1965), however they are now considered as having an osmoregulatory function (Crosskey, 1973). Respiration occurs cutaneously in larvae.

1.2 Rationale and aims of this project

This section places the study in context with a major research programme on the Buffalo River, eastern Cape (Figure 1.3) and outlines the aims of the study.

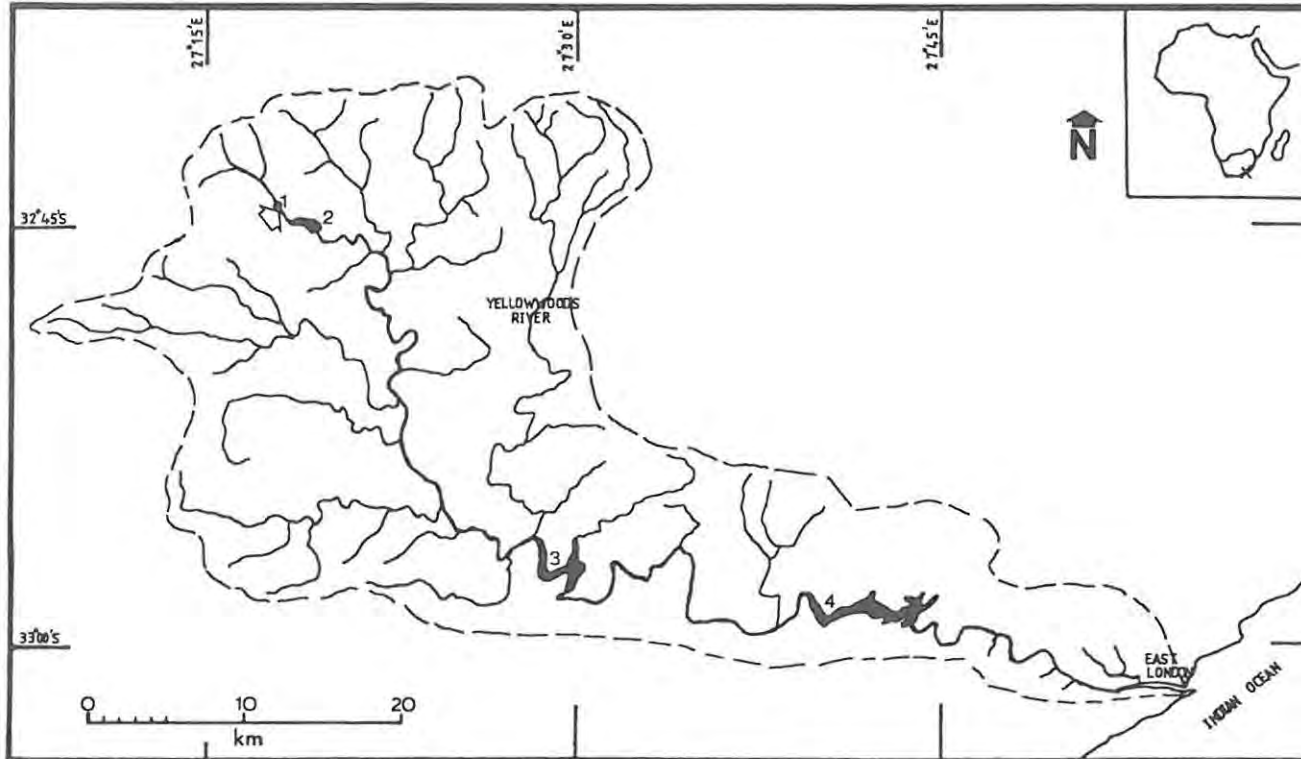


FIGURE 1.3 Map of the Buffalo River, Eastern Cape, showing the four major dams (1 = Maden, 2 = Rooikrantz, 3 = Laing, 4 = Bridle Drift Dams). The sampling site below Maden Dam is indicated by the arrow. The dashed line represents the catchment boundary. Inset: map of Africa showing the location of the Buffalo River.

In 1986 the Institute for Freshwater Studies (IFWS) initiated the Buffalo River Research Programme to investigate the effects of impoundments on the downstream reaches of the river. The study showed that blackfly larvae are a dominant part of the riffle-dwelling invertebrate fauna and occur in high densities below impoundments. The presence of dense populations of blackfly larvae in tailwaters is a well documented feature of regulated rivers world-wide (Briggs, 1948; Cushing, 1963; Ward, 1974; Armitage, 1976; Carlsson, Nilsson, Svensson, Ulfstrand & Wotton, 1977; Sheldon & Oswood, 1977; Wotton, 1978a & b, 1979, 1982; Lake & Burger, 1983).

In order to understand the physiological mechanisms regulating insect distribution, abundance and production in streams a bioenergetic study at the level of the individual or species population is essential (McCullough, Minshall, Cushing, 1979a). Ingestion, assimilation and egestion rates of blackfly larvae in the Buffalo River and the laboratory were determined. Variables such as food concentration and type, larval size and the effects of laboratory conditions were examined to see how they influence these rates.

The following specific questions have been addressed in this thesis:

- 1.1) At what rate do larvae ingest food in the laboratory and how do these feeding rates compare with field ingestion rates?
- 1.2) At what rate do larvae assimilate food in the laboratory?
- 1.3) At what rate do larvae egest faeces in the laboratory?

- 2.1) How do the above rates vary for different larval sizes?
- 2.2) How are these rates affected by different food types?
- 2.3) How do different food concentrations affect these rates?
- 3.1) Can these laboratory measurements of bioenergetic parameters be extrapolated to assess the role of blackfly larvae in processing seston in the field ?

The results of the present study have been used in combination with data on density and habitat availability of blackfly larvae from the main Buffalo River Research Programme to make estimates of the percentage of suspended organic matter processed by blackfly larvae in the Buffalo River.

1.3 The role of filter-feeding blackfly in flowing water
Filter-feeders are represented among diverse phyla (eg. Arthropoda, Bryozoa, Coelenterata, Echinodermata, Mollusca, Porifera,) all of which have developed structures and methods (setae, chaetae, nets, sticky mucus, tentacles, bristles) for removing particulate matter from suspension. Although the role of filter-feeders in marine ecosystems is well documented (see Jorgensen, 1955) the importance of filter-feeders in freshwater lotic systems is less well understood.

Blackfly larvae are considered to be non-selective feeders, the only constraint being the size of particles ($0.1\mu\text{m}$ - $300\mu\text{m}$) which they can ingest (Colbo & Wotton, 1981). In order for coarse particulate organic matter (CPOM $>1\text{mm}$) to be made available to filter-feeders it must first be converted to fine particulate organic matter (FPOM $<1\text{mm}$). FPOM is made available to blackfly larvae through the following processes:-

- 1) physical abrasion of CPOM (microbial activity increases this rate)
 - 2) direct enzymatic activity of microbes on CPOM
 - 3) feeding activity of invertebrates - egestion and fragmentation
 - 4) direct FPOM inputs from terrestrial run-off
 - 5) flocculation of DOM (dissolved organic matter)
- (Lush & Hynes, 1973; Anderson & Sedell, 1979) (see Figure 1.4).

Filter-feeders provide an important mechanism for the retention and processing of organic matter and play a role in the secondary productivity of the aquatic environment (Cummins, 1973, 1975; Wallace, Webster & Woodall, 1977). The efficiency of removing and processing seston appears to vary among localities. For example Maciolek & Tunzi (1968) found that blackfly larvae were responsible for removing 60% of seston within a 0.4 km stream section below a lake in an alpine stream. Ladle, Bass & Jenkins, (1972) calculated that at peak simuliid densities (80×10^6 simuliids per 200m stream reach), 1246kg of solids were ingested to produce 7kg of blackfly tissue. Assuming no re-ingestion of faeces and no input of material other than that carried by the inflowing water, a theoretical distance of 0.6km was calculated for complete removal of suspended solids by filtering blackfly larvae. McCullough, Minshall & Cushing (1979b) used mean biomass and numbers of invertebrates in riffles and mean ingestion rates for *Simulium* and *Hydropsyche* to calculate that filter-feeders in a 1m^2 area were able to remove about 1% of the seston flowing over them each day in Deep Creek, Washington.

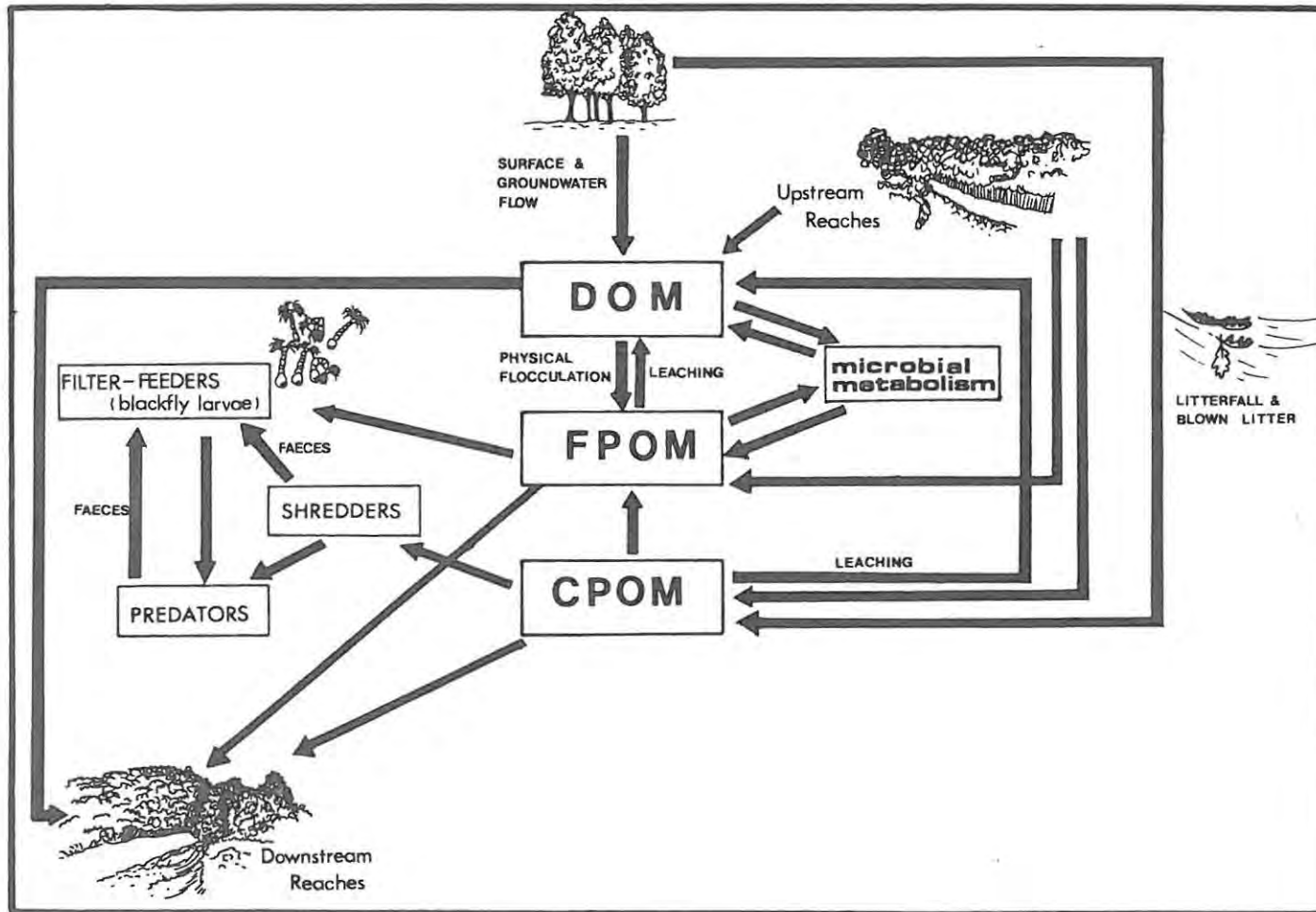


FIGURE 1.4 Model of the energy flow for a stream ecosystem. Note the input of material and energy from terrestrial and upstream sources, the importance of coarse (CPOM), fine (FPOM) and dissolved (DOM) organic matter, and the role played by microbes, shredders and filter-feeders.

Blackfly larvae influence the food available to other invertebrates in the river as they capture and alter the composition of FPOM. Faecal pellets produced by blackfly larvae can be further processed by collectors (Figure 1.4).

Blackfly larvae are also an important source of food for carnivorous insects (Jones, 1949; Koslucher & Minshall, 1973), and certain fish (Frost, 1950; Hynes, 1950; McCormak, 1962).

Although several bioenergetic studies on macro-invertebrates in river systems have been reported (McDiffett, 1970; Lawton, 1971; Stockner, 1971; Otto, 1974), few are available on simuliids. Studies that have been done on this subject are listed on page 23. Previous studies concerned with simuliid energetics are presented in Table 1.0. Where available the experimental conditions and any interesting findings have been included.

TABLE 1.0 Summary of previous bioenergetic studies on simuliids.

AUTHORITY	Spp	LOCATION	TEMPERATURE (°C)	DIET	INGESTION		ASSIMILATION		COMMENTS
					METHOD	RESULTS	METHOD	Ae %	
Ladle, Bass & Jenkins 1972	<u>Simulium</u> spp.	Field - Bere stream. Southern England	8 - 21	Suspended solids	charcoal				Peak population of simuliids- 0.64gday ⁻¹ of food assimilated produce 0.066gday ⁻¹ and ingest 12.8 gday ⁻¹ (assuming Ae 5%)
Mulla & Lacey 1976	<u>S. tescorum</u> <u>S. vittatum</u> <u>S. argus</u>	Field- Colorado River. California	12.8 - 30	Natural seston	fluorescent pigment				Larvae showed no rhythmic feeding activities.
Wotton 1978a b	<u>Metacnephia tredecimatum</u> <u>Simulium</u> spp.	Field - lake outlet. Swedish Lapland Field - River Teuronjoki. Finland	12	Natural seston	French chalk Powdered charcoal	1129-1922 µg C per day	Indirect- growth and respiration measurements	1.75-1.86	Ingestion rate affected by nematodes & imminent pupation
McCullough, Minshall & Cushing 1979b	<u>Simulium</u> spp.	Laboratory	11.6 - 19 (never >5°C above stream temp.)	Mixed diatoms	Powdered charcoal	1.36-54.74 µg/mg/h	Dual label Ash Ratio	52.7-65.8 7.6-56.1	<u>Simulium</u> comparable to bacteria in terms of metabolic removal of organic carbon from food sources
Schroder 1981	<u>S. ornatum</u>	Laboratory	5 - 15	<u>Synechococcus</u> <u>Stichococcus</u> <u>Staurastrum</u> <u>Scenedesmus</u> <u>Nitzschia</u>			Radiotracer	36 14 C 68 68 70-78	
Ladle & Hansford 1981	<u>S. wilhelmia</u> <u>S. wilhelmia</u> <u>S. austeni</u>	Laboratory	14	Natural seston Diatoms <u>Stephanodiscus</u>			Radioactive tracer	79.6-99.2 27.8-59.4	Found diatoms the most satisfactory food source for larvae in terms of growth and survival
Edwards & Meyer 1987	<u>Simulium</u> sp.	A Coastal Plain blackwater river. Georgia	11 - 20	Bacteria			Labelled bacteria with [³ H]-methyl thymidine	3.94x10 ⁵ -6.18 x10 ⁷ cells/h.	Assimilation of bacterial carbon is quantitatively important in the diet of blackfly in the Ogeechee River - accounted for 20 - 60% of daily growth
Morin, Back Chalifour, Boisvert & Peters 1988	<u>S. venustum</u> <u>S. decorum</u> <u>S. tuberosum</u>	Field-outlet of Petit Lac Fraser. Quebec	14 - 18	Natural seston	Powdered charcoal	72 µg/mg/h	Measured changes in seston flux above and below a large population	17 -25	Larvae ingested 0.8-1.4% of the seston per linear metre of the stream

CHAPTER 2

STUDY SITE, STUDY ANIMALS AND THE FOOD TYPE SELECTED

2.1 Study site

The Buffalo River rises in the Amatola Mountains at an altitude of 1300m and flows for 140km, reaching the Indian Ocean at East London (27°45'E, 32°02'S) as a fourth order stream (1:250 000 scale). Four storage dams (Maden, Rooikrantz, Laing and Bridle Drift) regulate and modify the river along its course. The Yellowwoods River, the largest tributary, joins the Buffalo River at Laing Dam (Figure 1.3). The catchment area of the Buffalo River covers 1 230km². The mean annual rainfall in the catchment area ranges from 2000mm per annum in the upper closed canopy forest to 500mm in the middle and lower reaches. Annual water temperature ranged between 6 and 30°C (6-16°C in winter, 12-30°C in summer) in the headwaters to between 8 and 35°C (21-35°C in summer, 8-22°C in winter) in the lower reaches (Palmer & O'Keefe, 1989).

The section of the Buffalo River directly below Maden Dam (Figure 2.0) was selected for regular sampling of simuliids. This site was selected because of its easy accessibility and abundance of blackfly larvae. In summer Maden Dam was often overflowing (Figure 2.1) and large numbers of simuliids developed immediately downstream.



FIGURE 2.0 Sampling site downstream of Maden Dam where blackfly larvae were collected.



FIGURE 2.1 Maden Dam in summer when the dam is overflowing.

2.2 The species of blackfly larvae studied

This section deals briefly with simuliid taxonomy and describes the larvae studied.

2.2.1 Blackfly Taxonomy

Within the Family Simuliidae Newman, separation of adults of different species on morphological criteria is often extremely difficult and the females of many species are inseparable. Freeman and de Meillon's (1953, pg 22) view was that "when two or more pupal types are all easily derivable from each other and all have identical adults, or when adult differences are inconclusive then the different pupae should all be considered forms of one species." At this time the relationship between forms was not well understood and it was thought that they might be partially induced by environmental factors. Recent non-morphological studies (e.g. chromosomal cytology and gas chromatography) (see Crosskey, 1987) have shown that a number of types previously designated as single species are in fact sibling species groups. Sibling species are reproductively isolated sympatric forms which are morphologically very similar or indistinguishable and which possess specific biological characteristics (Mayr, 1942).

In the Buffalo River there are at least 18 morphologically distinct simuliid species (Appendix A). The chromosomes of the larval silk glands are currently being used in a cytotaxonomy study which may reveal a number of new simuliid species (R. W. Palmer, pers comm).

In the Buffalo River below Maden Dam *Simulium hargreavesi* Gibbins and *Simulium medusaeforme* Pomeroy occurred sympatrically in large numbers. *S. hargreavesi* was first described as a distinct species by Gibbins (1934) but as

it often occurred sympatrically with *S. medusaeforme* and the variation of specimens between localities was great it was thought more realistic to retain the names but to consider *S. hargreavesi* a form of the one species *medusaeforme* (Freeman & de Meillon 1953). In a recent annotated world check list of blackfly Crosskey (1987) listed *S. medusaeforme* and *S. hargreavesi* as two separate species. During the main Buffalo River Research Programme *S. medusaeforme* and *S. hargreavesi* were recorded as two separate species as well (see Appendix A).

During the main Buffalo River sampling programme (1986 - 1988) *S. medusaeforme* and *S. hargreavesi* larvae were two of the most abundant species occurring along the length of the river. As the larvae of *S. medusaeforme* and *S. hargreavesi* cannot be distinguished from each other except for the final instar (the respiratory histoblasts are different - see Figure 2.2, C & D), it was decided for purposes of this study to name the larvae being studied *S. medusaeforme/hargreavesi*.

Identifications were made according to Freeman & de Meillon (1953) and Crosskey (1960, 1969). Larval identifications were verified where possible by rearing through to adults. In experiments where the larvae were killed a subsample of larvae were reared out.

2.2.2 General description of the larvae

Final instar larvae of *S. medusaeforme/hargreavesi* have an average body length (measured from the posterior circlet of hooks to the base of the head capsule) of 5.73mm (range 5.20 - 6.48mm). The larval head is strongly pigmented (Figure 2.2, A) and the postgenal cleft is very

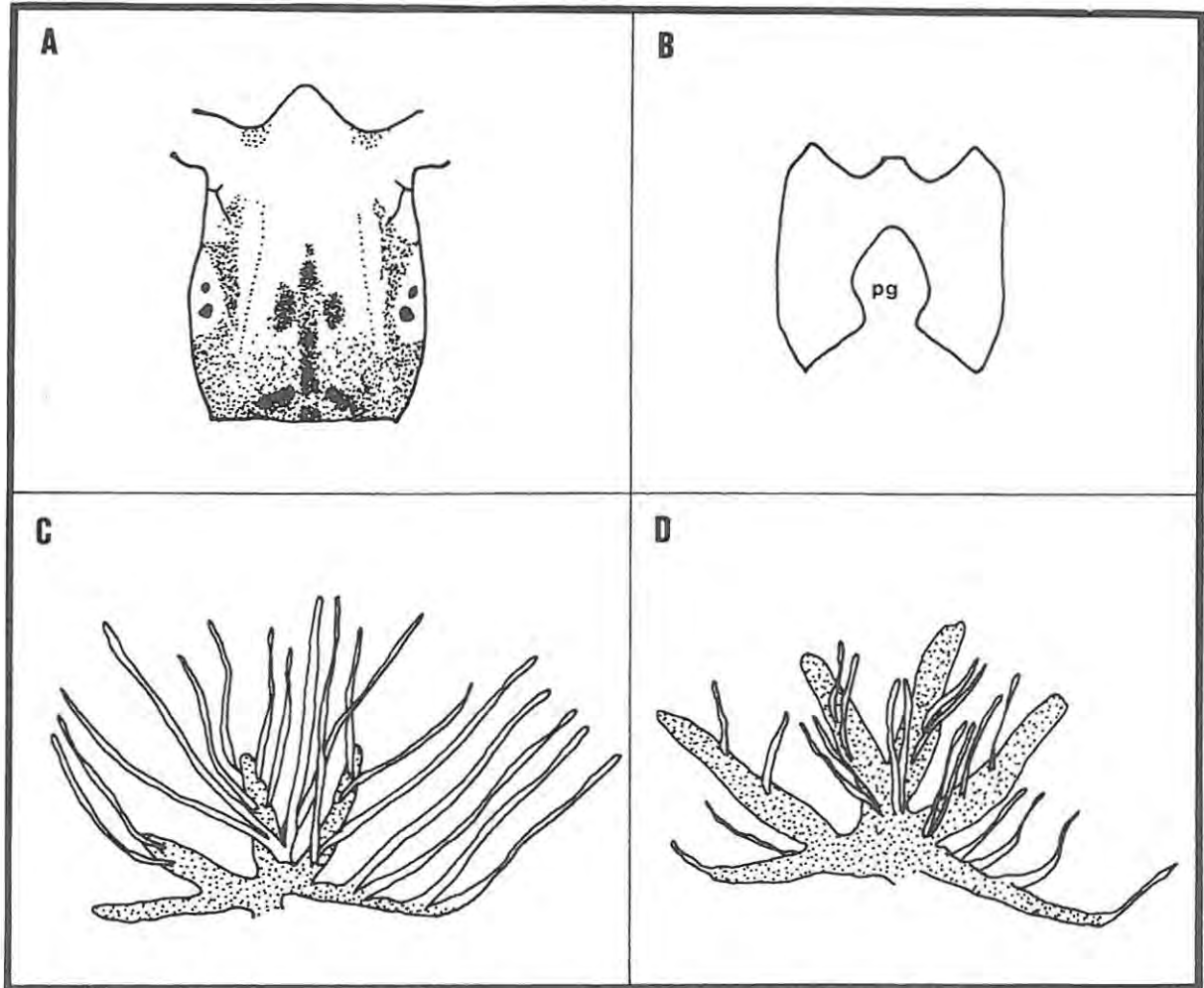


FIGURE 2.2 A: Pigmentation of the dorsal head capsule of larvae of *S. medusaeforme/hargreavesi*.
 B: Ventral view of the outline of head capsule of *S. medusaeforme/hargreavesi* - note the postgenal cleft (pg). (A & B, after Crosskey, 1969)
 C: Pupal gill form of *S. medusaeforme*.
 D: Pupal gill form of *S. hargreavesi*.
 (C & D, after Freeman & de Meillon, 1953).

conspicuous (Figure 2.2, B). The thoracic cuticle is bare but simple setae cover the posterior segments of the abdomen. Ventral papillae are absent (Figure 1.0, A).

The larvae are often found attached in high numbers to trailing grasses, dead leaves, sticks, stones and rock in cascades and turbulent waters.

2.3 Sampling techniques

This section includes sampling techniques as well as methods of determining the size of the larvae.

2.3.1 Sampling of the larvae

Larvae were collected by hand either by removing stones and trailing vegetation to which larvae had attached, or by using plastic strips which had previously been attached in fast flowing sections of the river. The larvae were transported back to the laboratory in buckets within two hours of collection. The buckets were placed in a constant temperature room (20°C) and airstones were introduced.

2.3.2 Measurement of the larvae

After initial investigations and measurements it was decided to divide the larvae into size classes rather than instars. A size class may incorporate an overlap of instars but it was assumed that physiological activities (such as feeding rate) would be related to size rather than instar. Larvae with black respiratory histoblasts were regarded as pharate pupae and were analysed separately.

Larvae were sized using a sclerotised structure. Previous workers have used a number of different structures, but the most popular are cephalic apotome (width of the head) (Chutter, 1972; Craig, 1975; de Moor, 1982a & b), head

capsule length (Fredeen, 1976) and postgenal length (Fredeen, 1976; de Moor, 1982a & b). In this study the head capsule width was measured as the entire width of the head capsule (not just the width of the fronto-clypeus in larvae) and the head capsule length was measured from the anterior edge of the mandibular phragma to the posterior edge of the post-occiput. A significant positive relationship was found between head capsule length and head capsule width for *S. medusaeforme/hargreavesi* (Figure 2.3). As either parameter could be used successfully it was decided for purposes of this study to use head capsule width to size the larvae. Larvae were divided into the following six size classes: (0.24 - 0.31); (0.32 - 0.39); (0.40 - 0.47); (0.48 - 0.55); (0.56 - 0.63) and > 0.64mm.

All measurements were made with the aid of an ocular micrometer on a Wild dissecting microscope. Head capsule widths were measured at 12x magnification (1 unit = 80µm). All measurements were made to the nearest 0.5 micrometer unit. The head capsule widths of *S.medusaeforme/hargreavesi* were used to ascertain individuals' dry body weight from a regression of dry body weight on head capsule width (Figure 2.4). Any larva with an empty gut was not included in the calculation. The degree of gut fullness and packing was not noted and this could account for the large variation found in the weight of larvae of a particular size (Figure 2.4).

2.4 Selection of experimental food types

Larvae feeding in rivers remove suspended solids from the water. However, in order to do feeding experiments the food source needs to be controlled so that its concentration and particle size can be determined. For this purpose it was decided to use a monoculture of algae.

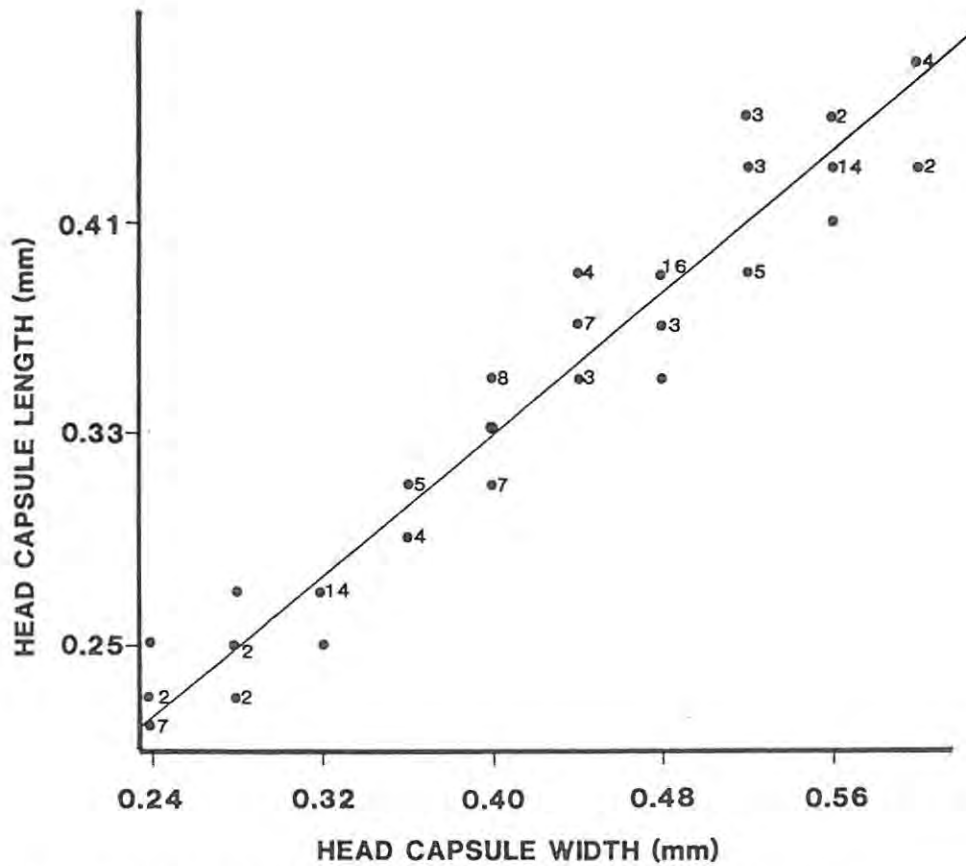


FIGURE 2.3 Relationship between head capsule length and width for different sized *S. medusaeforme/hargreavesi* larvae. When more than one larva was measured for each data point the number measured is included. $y = 0.065 + 0.661x$, $r^2 = 0.96$.

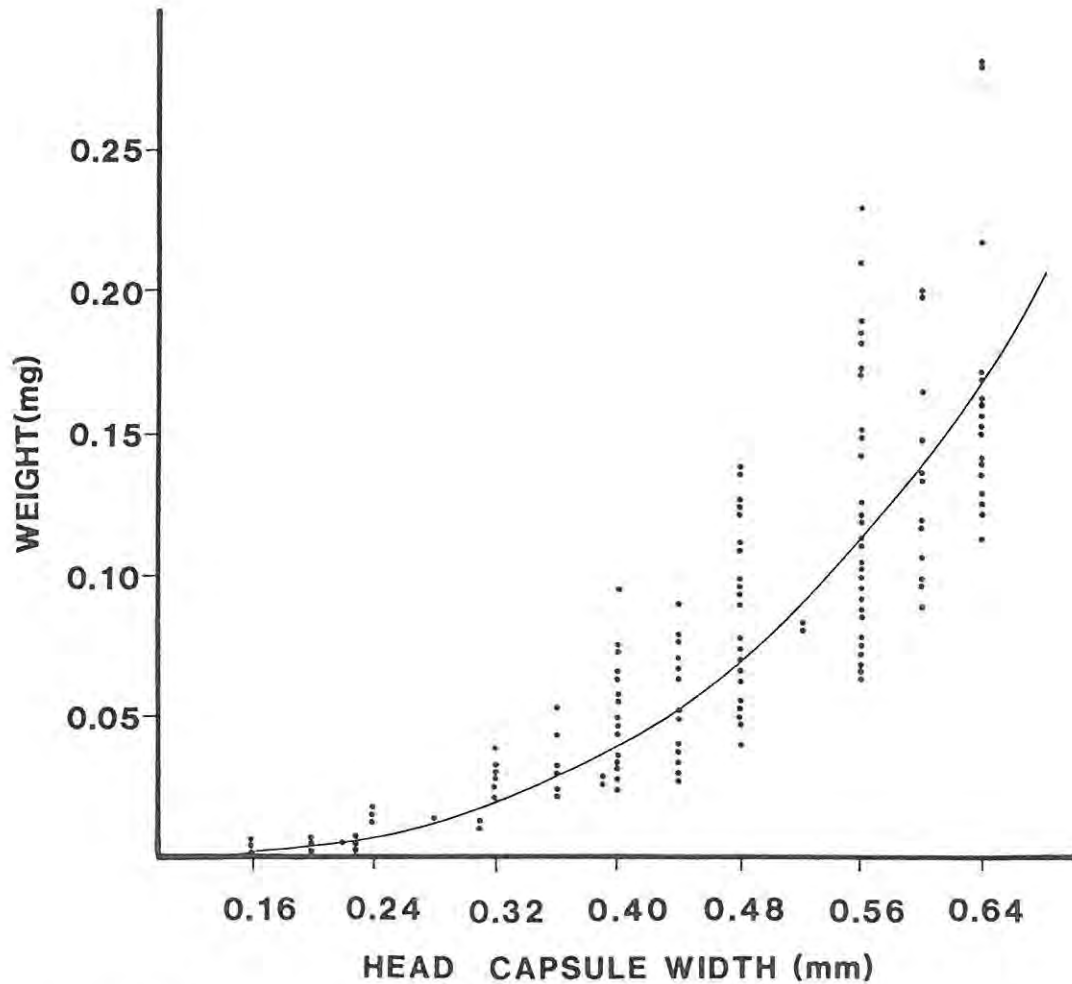


FIGURE 2.4 Relationship between dry body weight and head capsule width for different sized *S. medusaeforme/hargreavesi* larvae. Each data point represents a single larva.

$$y = (0.4257)x^{3.12844}, r = 0.96.$$

In this study assimilation experiments with radioactive ^{14}C were done so it was important that the chosen algal species was able to take up the label efficiently and be in the size range that the larvae could ingest. A single algal species culture was chosen (as opposed to a mixed culture) so as to eliminate any problems associated with the differential uptake of the label by different algal species.

Ease of culture and local availability were also important criteria in the choice of a suitable algal species. Examination of the algal species used by other workers showed that either diatoms or a green algal species was used. For purposes of this study two green algal species were chosen from the same Family but with different cell size and shape - *Selenastrum* sp. and *Chlorella* sp.

Chlorella is a non motile unicellular algae with a cell size ranging between 2 and 10 μm . *Selenastrum* is a non motile sickle shaped algae with a cell size of 10 to 15 μm . Although both species are green algae they were found to thrive on a medium for blue-green algae (BG 11) (Allen, 1968).

The algae were grown in a culture vessel (Figure 2.5) under continuous illumination from a fluorescent light. Compressed air fed into the bottom of the container was used to keep the cells in suspension.

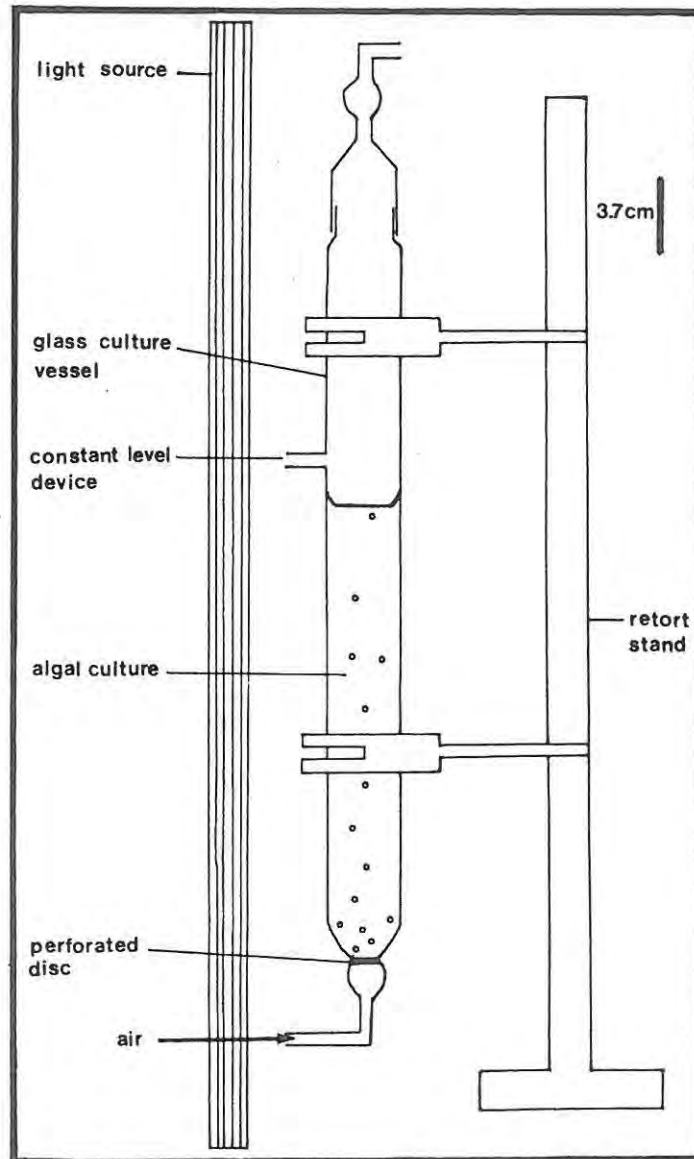


FIGURE 2.5 Apparatus used for growing an algal culture. The culture vessel containing the algae is placed in front of a fluorescent light source. Scale bar = 3.7 cm.

CHAPTER 3

INGESTION

3.1 INTRODUCTION

A number of factors influence the rate of larval food consumption. These include food quality and quantity (McCullough, 1975; Carlsson *et al.*, 1977; Kurtak, 1978; Schroder, 1980; Merritt, Ross & Larson, 1982), particle size (Kurtak, 1978; Wotton, 1978a), temperature (Mulla & Lacey, 1976; Kurtak, 1978; Lacey & Mulla, 1979; Merritt *et al.*, 1982), current velocity (Fredeen, 1964; McCullough, 1975; Kurtak, 1978; Schroder, 1979), species, developmental stage, prior feeding history and innate biological rhythms (McCullough, 1975; Mulla & Lacey, 1976).

The rate at which food is ingested by simuliids has received considerable attention, particularly in studies of bioenergetics and assimilation rates (Ladle *et al.*, 1972; McCullough, 1975; Wotton, 1978b; McCullough *et al.*, 1979b; Ladle & Hansford, 1981; Schroder, 1981; Morin, Back, Chalifour, Boisvert & Peters, 1988). Average ingestion rates reported in the literature range from 72 $\mu\text{g}/\text{mg}/\text{h}$ for larvae feeding on seston (Morin *et al.*, 1988) to between 12 and 78 $\mu\text{g}/\text{mg}/\text{h}$ for late instar larvae feeding on cultured diatoms (McCullough *et al.*, 1979b).

The aim of this study was to measure ingestion rates of different sized larvae, an area in which comparatively little work has been done. Wotton (1978a, b) studied the passage of charcoal bands along the gut of different sized larvae. He noted that the passage of the charcoal bands along the guts were more rapid in smaller larvae where the gut is narrower. Schroder (1981) measured assimilation,

ingestion and egestion rates for different sized larvae. He found that young, smaller larvae ingested small algal cells and older larvae ingested larger algal cells.

In this study the ingestion rate of larvae feeding on different food types was also investigated in order to determine if larvae ingest different food at different rates and whether larval size influences these rates. Particle concentration is known to affect ingestion rates (Kurtak, 1978; Lacey & Mulla, 1979; Schroder, 1980; Hart & Latta, 1986). The effect of food concentration on ingestion rate was examined in this study by feeding larvae two algal types at four different concentrations.

Although temperature is thought to affect feeding rates, no attempt was made to determine the effect of temperature on ingestion rates in this study. All experiments were carried out at the same temperature, 20°C. This was selected as representative of the average temperatures at the sampling site where water temperatures between 7°C and 30°C were recorded from 1986 to 1988 (Palmer & O'Keefe, 1989).

3.2 METHODS

3.2.1 Field experiments

The ingestion rate of *S. medusaeforme/hargreavesi* larvae was determined under natural conditions by observing the passage of a charcoal marker through the gut. This method was used by Ladle *et al.* (1972) and has since been used successfully by a number of workers (Wotton, 1978a & b; McCullough *et al.*, 1979b; Ladle & Hansford, 1981; Hart & Latta, 1986). No evidence of regurgitation or other disturbances of the gut contents have been observed by applying this experimental procedure.

The site at which the ingestion experiment was to be undertaken needed to fulfill a number of criteria:-

- i) a high concentration of blackfly larvae
- ii) laminar flow over the larvae
- iii) water temperature as close as possible to 20°C.

Powdered charcoal was added to a bucket of river water and stirred thoroughly before it was siphoned at a constant rate into the river above the filter-feeders. Once the last of the charcoal 'cloud' passed over the larvae a period of 10 minutes was timed during which the larvae fed on suspended material in the river. After the feeding period the process was repeated and another charcoal slurry was introduced above the larvae. On completion of the second feeding period the larvae were removed and killed immediately in 70% ethanol.

In the laboratory, larvae were placed in a 10% solution of KOH for a few minutes in order to improve the visibility of the charcoal bands. Larvae infected by nematode parasites and larvae which had just moulted (evident by a transparent head capsule) were not incorporated into the results because Wotton (1978a) noted that the presence of nematode parasites significantly affected the ingestion rate of the larvae and newly moulted larvae appeared not to feed (pers. obs.). Any larvae which did not exhibit charcoal banding were omitted.

Total body length (from the postgenal cleft to the circlet of hooks on the posterior abdomen), head capsule width and the amount of food ingested was measured for each larva.

The latter was measured as the length of the food material between the anterior edge of the initially ingested charcoal band and the posterior edge of the second charcoal band.

As the gut of simuliids is considered cylindrical (Wotton, 1978a) the volume of food ingested during the feeding period can be determined by the formula for a cylinder

$$V = \pi r^2 h \quad (1)$$

where r is the radius of the gut (which is virtually constant along the length of the animal) and h is the length of food material consumed during the feeding period.

This technique is regarded as a reliable method for estimating the amount of food ingested by larvae (Dadd, 1968; Chance, 1977; Wotton, 1978a, b; Ladle & Hansford, 1981; Hart & Latta, 1986). The effects of digestion and preservation on the gut contents and the extent of gut packing were not taken into account. However, since the experiment only lasted for 10 minutes, it was assumed that digestion was minimal.

3.2.2 Laboratory experiments

3.2.2.1 Ingestion rate on river water

Larvae were fed on river water in the laboratory and these results were compared with results obtained for larvae feeding in the field. River water contains suspended solids of organic (detritus, plankton, micro-organisms, algae) and inorganic nature (clay, sand, silt). In the laboratory, algae (as opposed to charcoal) was used to mark the guts in order to determine the ingestion rate on river water.

The larvae were placed into a glass separating funnel with 60 ml double distilled water to which an algal suspension was added and left to ingest for a period of one to two hours. Circulation was achieved by bubbling compressed air through a very fine plastic pipette tip. After this period the feeding medium was drained out by opening the tap at the bottom of the separating funnel. The tap was then closed and the medium replaced by river water poured into the top of the funnel. This method ensured minimal disturbance to larval feeding. Larvae were left to feed for 10 minutes and then killed in 70% ethanol. The guts of the larvae were dissected out and the contrast between the green algal cells ingested in the gut and the brown suspended solids ingested enabled the volume of suspended solids ingested to be calculated.

3.2.2.2 Ingestion rate on algae

Larvae were placed in double distilled water in the same feeding vessel as described above for 30 minutes to clear their feeding apparatus of food. An algal suspension of known concentration was then added to the larval feeding container and the larvae were left for a period to feed. At the end of the feeding period the larvae were killed in 70 % alcohol and the guts dissected out. When the larvae fed on the algal cells the gut became full of green cells which formed a distinct band. The natural gut contents and the algal cells were clearly separated so that the volume of algae ingested over a measured time period could be determined.

3.2.2.3 Ingestion rate at different algal concentrations

A range of cell concentrations was used for both algal species. To obtain the desired concentrations algal cells were first concentrated by centrifuging and then diluted

to the required concentrations. Concentrations used for *Chlorella* and *Selenastrum* were: 4.3, 7.5, 13.5, 17.5 x 10⁶ cells/ml and 5, 8, 14.3, 17 x 10⁶ cells/ml, respectively. As the feeding time for each experiment was relatively short (5 - 14 minutes) it was assumed that cell concentrations did not decline over each experimental period.

3.2.2.4 Conversion of the volume of food ingested to weight
The volume of food ingested was converted to weight in order to express the ingestion rate as a function of larval dry weight. This was done by feeding a number of larvae on either *Chlorella*, *Selenastrum* or river water, for a period exceeding gut clearance time. This ensured that the gut contained only the one food substance. The gut of the larvae were then dissected out, the peritrophic membrane removed, and the volume of food determined (equation 1). The gut of known volume was then transferred to a preweighed, numbered tin foil square. After oven drying at 60°C for 24 h the tin foil squares were then reweighed on a Cahn microbalance ($\pm 1\mu\text{g}$). The density of the gut contents was calculated from these weight to volume ratios. Linear regressions (with zero intercepts) were fitted for each food type (Figure 3.0, A - C).

Student t-tests were used to examine the significance between mean ingestion rates on the various food substances at different larval sizes. Significance was tested at the 5 % level ($p < 0.05$).

3.3 RESULTS

The volume of food ingested per unit of time was proportional to the size of the larvae (Figure 3.1). Larger larvae (0.44 - 0.65mm) feeding on river water, *Chlorella* and *Selenastrum* ingested between three and six times as much food per unit time as smaller larvae (0.24 - 0.43mm). Ingestion rate also increased with increasing food density. The marked decrease in ingestion rate of the largest size class (0.64mm) of larvae in the field is attributed to all larvae in this size class being pharate pupae (see Table 3.0). In contrast the largest size class of larvae feeding on river water in the laboratory were non-pharate larvae.

TABLE 3.0 The mean volume of material ingested (with standard deviation) in 10 minutes by *S. medusaeforme/hargreavesi* larvae feeding in the field. Pharate pupae (larvae with black respiratory histoblasts, indicating imminent pupation) and non-pharate larvae from the same experiment and size range were measured.

	Number of larvae measured	Mean head capsule width (mm \pm S.D.)	Mean volume of material ingested in 10 minutes (mm \pm S.D.)
Larvae with black respiratory histoblasts (pharate pupae)	12	0.56 \pm 0.00	0.232 \pm 0.02
Larvae of the same size range without black respiratory histoblasts	12	0.57 \pm 0.01	0.386 \pm 0.12

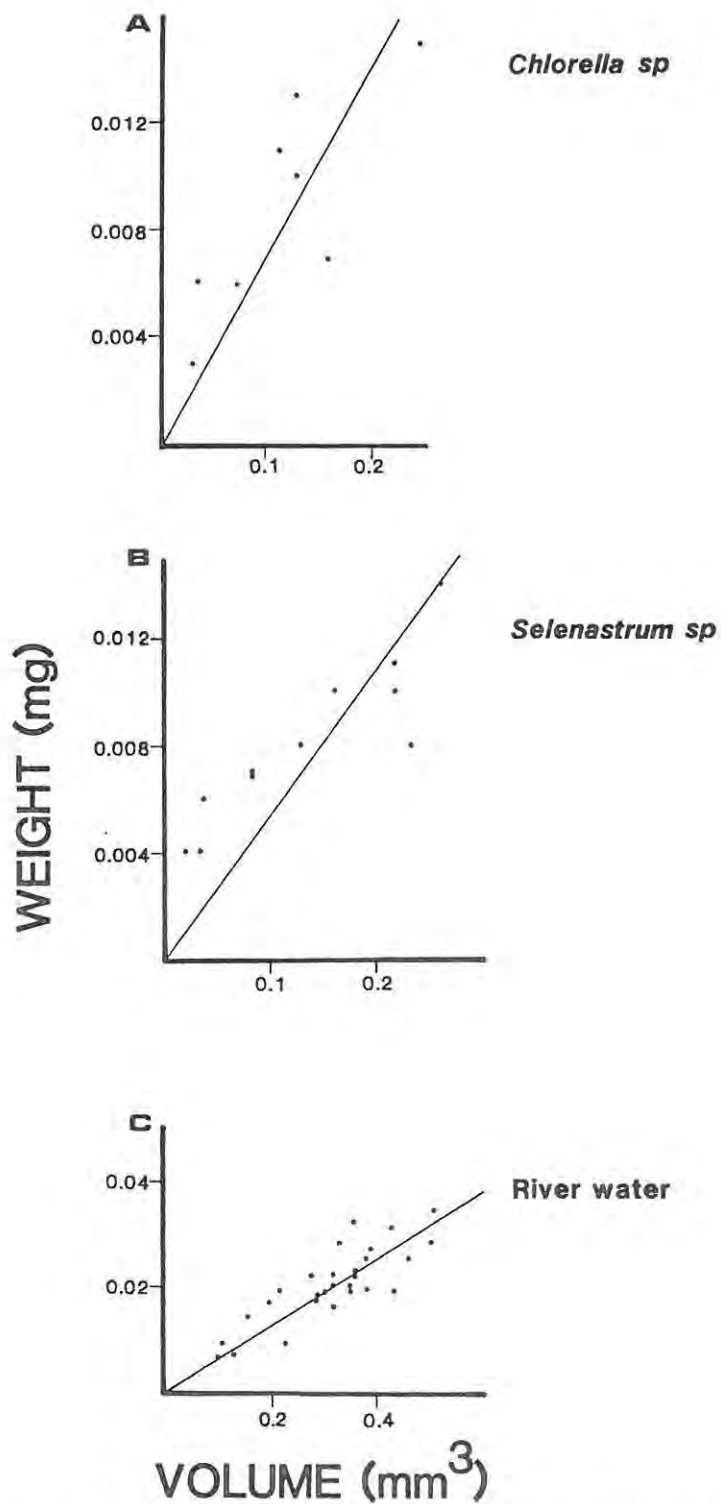


FIGURE 3.0 Relation of the weight of the gut contents to the volume of the material ingested in the gut of different sized larvae feeding on various food types.

A: *Chlorella* sp. $y = 0.0724x$, $r^2 = 0.82$

B: *Selenastrum* sp. $y = 0.0546x$, $r^2 = 0.33$.

C: Riverwater $y = 0.0628x$, $r^2 = 0.68$

The regression lines were forced through the origin.

Although ingestion rates of larvae feeding on *Chlorella* were generally higher than the ingestion rate on *Selenastrum* (Figure 3.1), a student t-test on each pair of points from each size class showed no significant difference between ingestion rates on each algal type. The ingestion rate on river water in the field was significantly different ($p < 0.05$) from the ingestion rate on *Selenastrum* and *Chlorella* over a number of size classes. The ingestion rate on river water in the laboratory is significantly lower ($p < 0.05$) than the ingestion rates on algae.

No significant increase in ingestion rate was observed with increasing *Selenastrum* cell concentration (Figure 3.2). The ingestion rate on *Chlorella* at the lowest cell concentration (i.e. 4.5×10^6 cells/ml) is significantly lower than the ingestion rates at the other concentrations (Figure 3.3), but otherwise the ingestion rate does not increase significantly over the concentration range tested (Figure 3.3).

Ingestion rates of larvae in the field are the highest (900 - 1600 $\mu\text{g}/\text{mg}/\text{h}$) (Figure 3.4). These rates are higher than in the laboratory on the same food type (260 - 680 $\mu\text{g}/\text{mg}/\text{h}$) or on algae, *Selenastrum* (340 - 1140 $\mu\text{g}/\text{mg}/\text{h}$) and *Chlorella* (590 - 1100 $\mu\text{g}/\text{mg}/\text{h}$) (Figure 3.4). Smaller larvae ingested more *Selenastrum* per mg body weight than the larger larvae (Figure 3.4). When feeding on *Chlorella* however, the greatest ingestion rates occurred in the second highest size class (Figure 3.4). The highest ingestion rates (1611.5 $\mu\text{g}/\text{mg}/\text{h}$) were achieved by the intermediate sized larvae feeding on suspended solids in the river (Figure 3.4).

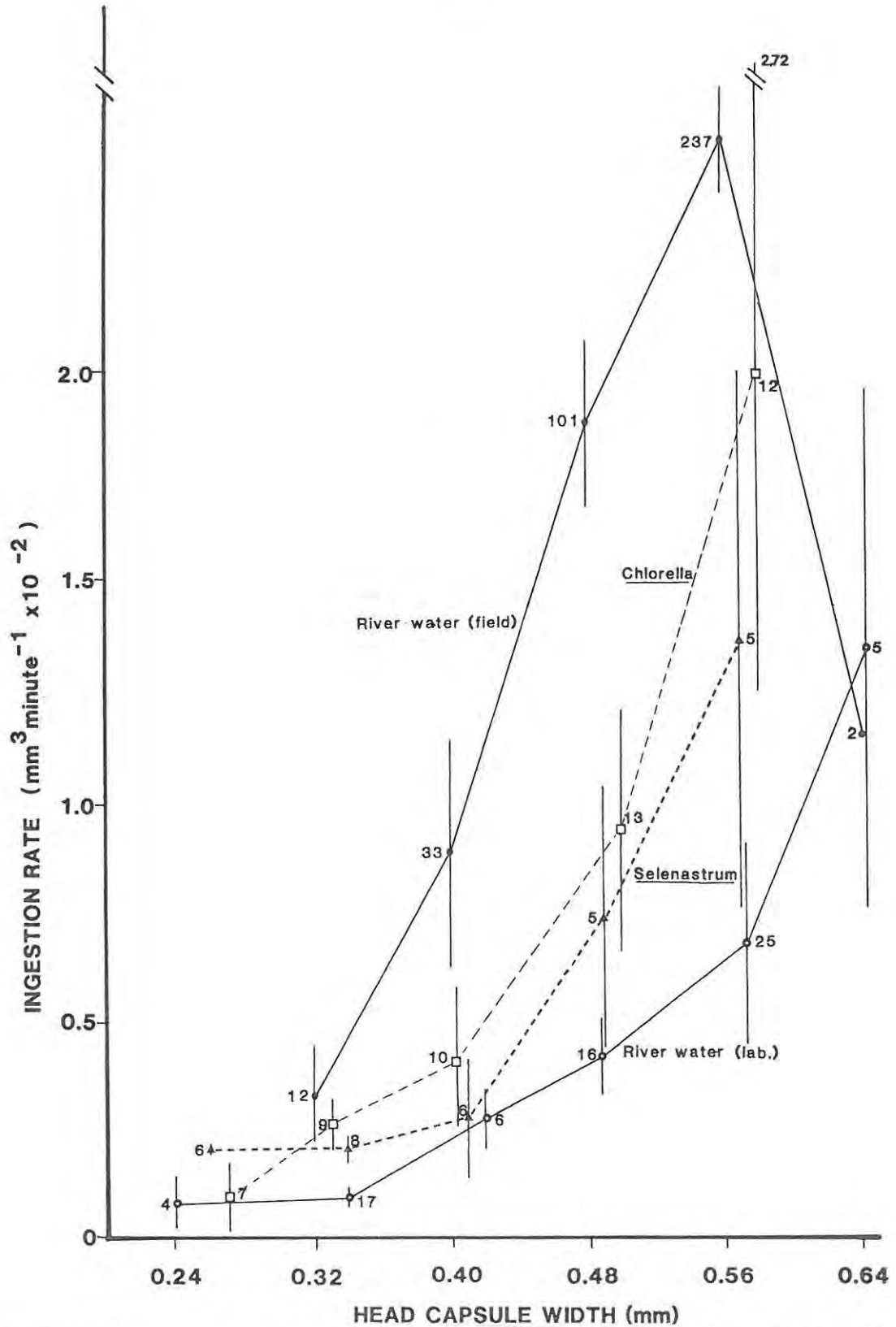


FIGURE 3.1 The mean ingestion rates of different sized *S. medusaeforme/hargreavesi* larvae feeding on river water in the field (●—●) and laboratory (○—○) 10mg l⁻¹, *Chlorocella* sp. (□—□) 12mg l⁻¹ and *Selenastrum* sp. (▲—▲) 80mg l⁻¹. The 95% confidence limits and the number of larvae measured are included.

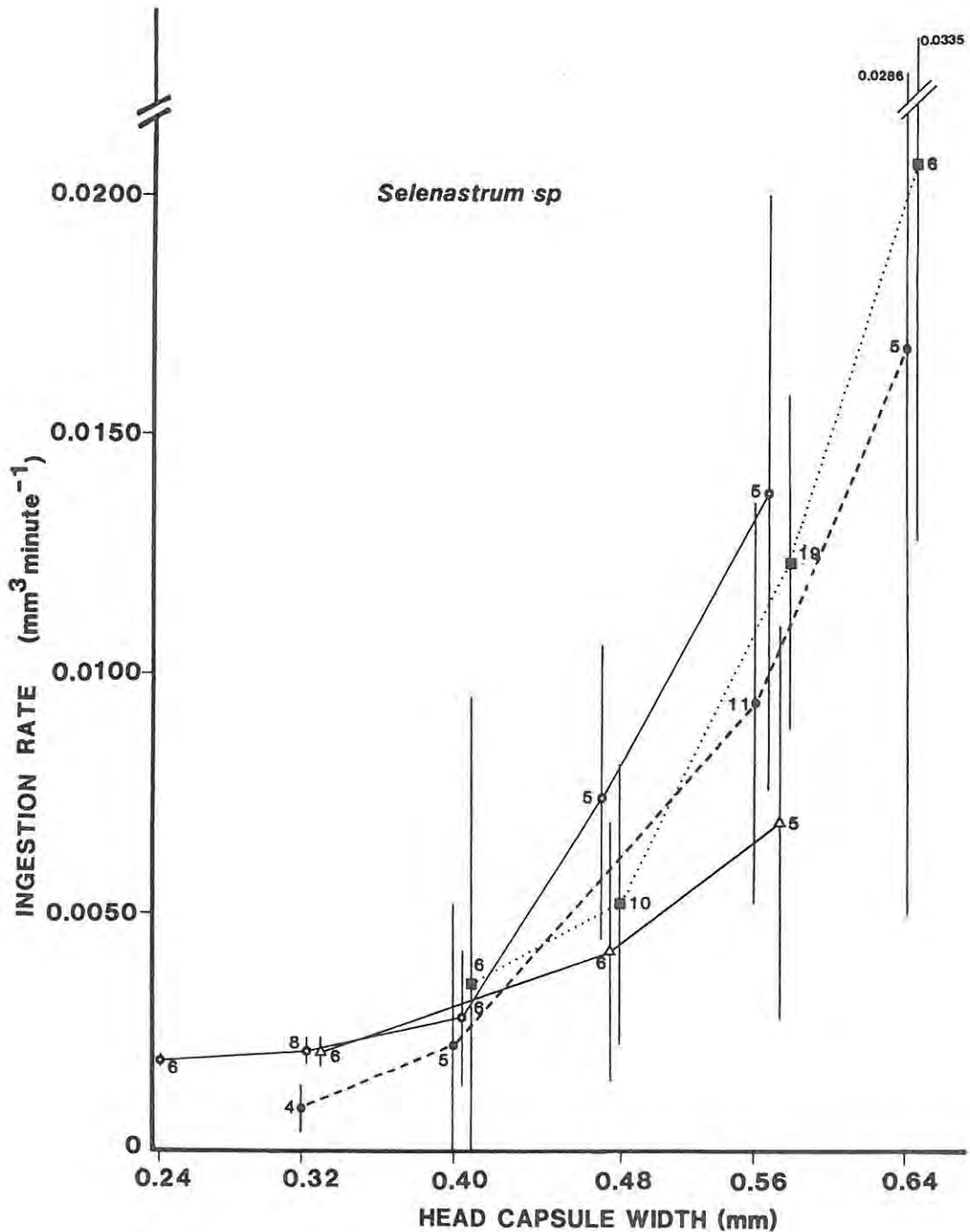


FIGURE 3.2 The mean ingestion rate of different sized *S. medusaeforme/hargreavesi* larvae feeding on *Selenastrum* algae at varying concentrations, 5×10^6 cells/ml (•---•), 8×10^6 cells/ml (○—○), 14.3×10^6 cells/ml (△—△) and 17×10^6 cells/ml (■····■). The 95% confidence limits and the number of larvae measured are included.

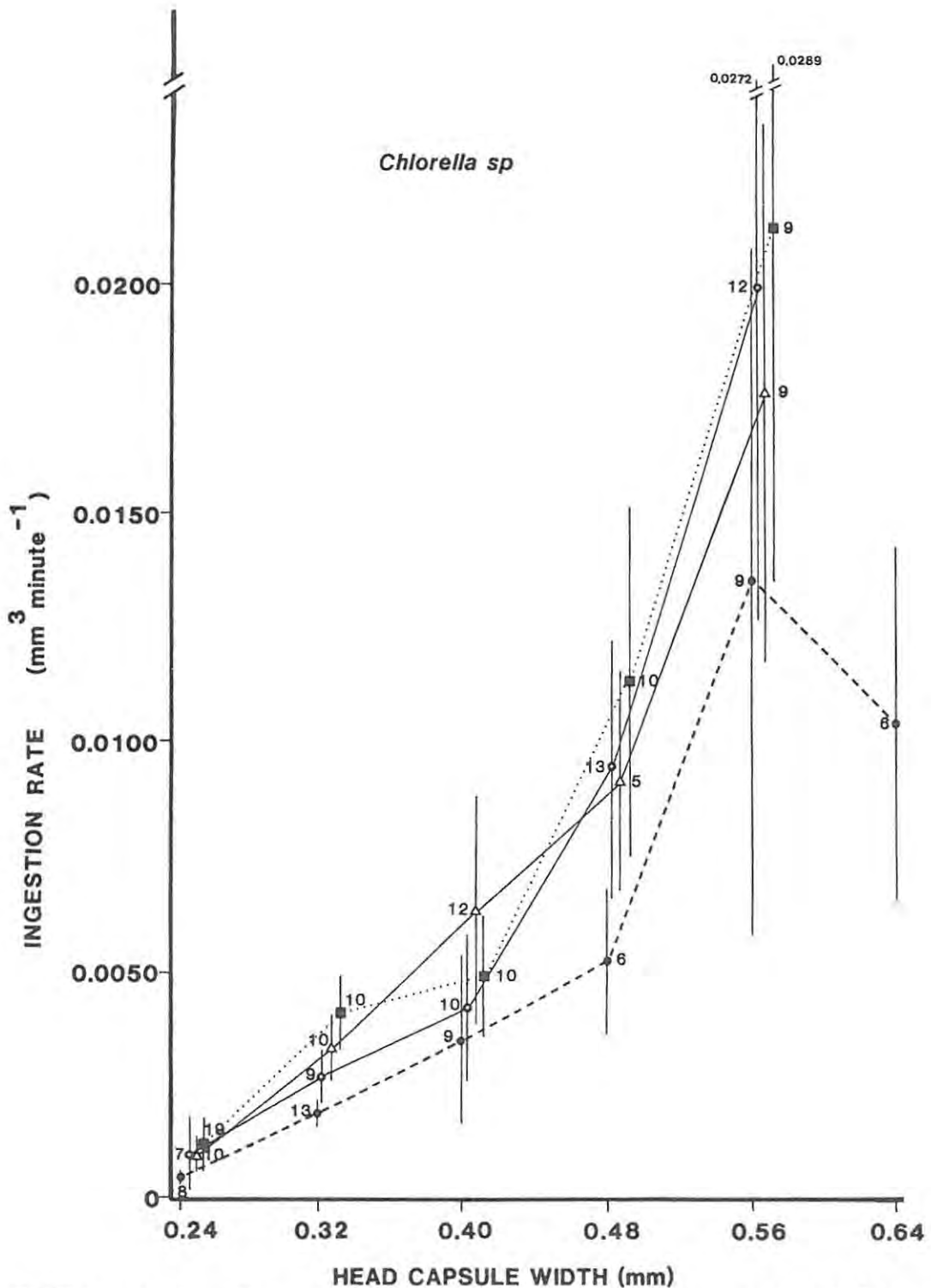


FIGURE 3.3 The mean ingestion rate of different sized *S. medusaeforme/hargreavesi* larvae feeding on *Chlorella* at varying concentrations. 4.3×10^6 cells/ml (---○---), 7.5×10^6 cells/ml (—○—), 13.5×10^6 cells/ml (—△—) and 17.5×10^6 cells/ml (—■—). The 95% confidence limits and the number of larvae measured are shown.

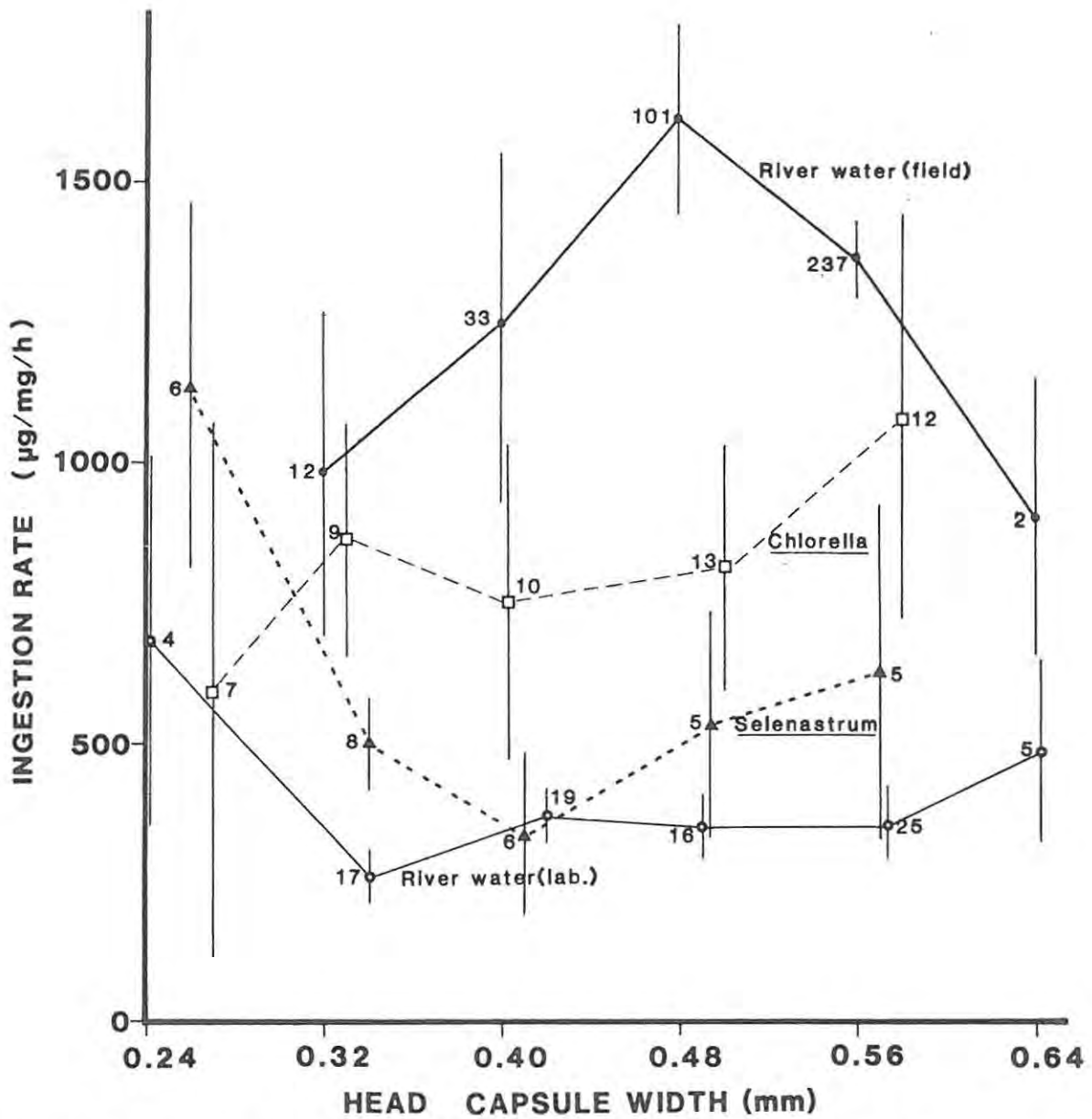


FIGURE 3.4 The ingestion rate, as a function of body weight, of different sized *S. medusaeforme/hargreavesi* larvae feeding on river water in the field (•—•) and laboratory (○—○), *Chlorella* (□—□) and *Selenastrum* (▲—▲). The 95% confidence limits and the number of larvae measured are included.

3.4 DISCUSSION

In this study it was found that larger larvae ingested a greater volume of food over the same time period than smaller larvae, due to their proportionally bigger guts (Figure 3.1). The area of the cephalic fan, although not measured in this study, could also affect the volume of food ingested per unit time. Average ingestion rates (as presented by McCullough *et al.*, 1979b; Morin *et al.*, 1988) should therefore be viewed with caution.

Larvae ingested suspended solids in the river faster than algae in the laboratory (Figure 3.1), and this is likely to be related to the difference in particle size and food quality. In the Buffalo River the organic fraction of seston ranged from 44% in the upper reaches to 12% in the lower reaches (Palmer & O'Keeffe, *in press*, a). Seston is therefore less nutritional than a pure algal culture where carbon is assumed to account for 50% of algal dry weight (Reynolds, 1984). A number of workers have found ingestion rates to be enhanced when animals feed on food with a low proportion of organic matter (Baker & Farr, 1977; Ladle & Hansford, 1981; Muthukrishnan & Pandian, 1987).

The ingestion rate of larvae feeding in the field shows a sharp decrease in the largest size class, which were pharate pupae. There is a cessation of feeding by pharate pupae as they develop from a feeding (larvae) to a non-feeding (pupae) stage of their life cycle. This decrease in ingestion rate is in accordance with Wotton's (1978a) findings. He noted that the largest larvae were pharate pupae and showed a lower ingestion rate compared with similar sized larvae not in the same condition.

The higher ingestion rate obtained on river water in the field compared to the laboratory can probably be attributed to the conditions in the laboratory. Larvae in the laboratory were kept in water aerated by airstones, this together with the fact that the water was continually recycled throughout a feeding experiment could contribute to unfavourable feeding conditions for the larvae. Larvae feeding in the field are found in unidirectional flowing water. During laboratory experiments the larvae are unavoidably handled before the onset of a feeding experiment and this together with unfavourable hydro-conditions could result in the larvae being stressed and therefore not feeding as they would under natural (i.e. field) conditions.

McCullough *et al.* (1979b) were the only other workers to compare laboratory and field results. They found that larvae had lower digestion times in the laboratory compared with the field and this was thought to be caused by the inability of cephalic fans to open due to unsuitable currents in the laboratory. In this study ingestion rates (using the same food type) have been tested and compared in the laboratory and the field. The difference in ingestion rates between the laboratory and field on the same food type is an important factor that needs to be taken into account when extrapolating laboratory based results to the field.

Since algal ingestion rates in the laboratory were higher than seston ingestion rates in the laboratory it is possible that ingestion rates on algae in the field could be much higher than in the laboratory, possibly even higher than ingestion rates on seston in the field.

Ingestion rates on algae in the field were not tested due to the difficulty of having just algae and no suspended solids in the feeding current passing over the simuliids. It appears that both laboratory conditions and food type affect ingestion rates.

There is a limited amount of literature available on the effect of food concentration on the ingestion rate of blackfly larvae. Lacey & Mulla (1979) and Schroder (1980) obtained similar functional responses for blackfly larvae, concluding that ingestion rate reached a plateau at high particle concentrations. This was in agreement with Hart and Latta (1986) who noted an increase in ingestion rate with rising food density below 100mg l^{-1} but little change in ingestion rates were observed above this density.

At the lowest cell concentration for *Chlorella*, (4.3×10^6 cells/ml) the ingestion rate is significantly lower ($p < 0.05$) than the ingestion rates at other concentrations (Figure 3.3). The ingestion rates at the other three concentrations are not significantly different from each other (Figure 3.3). To compare these results with Hart and Latta (1986) the density of algae was determined by using a conversion factor (of 1 cell = 15pg) for *Chlorella* (Hart, 1988). At the lowest cell concentration (4.3×10^6 cells/ml) the food density is 64.5mg l^{-1} . The density of food at the other three concentrations are all greater than 100mg l^{-1} . It is possible that the plateau referred to by Hart and Latta (1986) at 100mg l^{-1} is reached at the concentration of 7.5×10^6 cells/ml and as a result any increase in cell concentration above this threshold would not affect the ingestion rate. No similar trend for *Selenastrum* is apparent (Figure 3.2).

For comparative purposes the ingestion rate (expressed in μg of food per unit weight of larvae per hour) is more

suitable than absolute values (i.e. μg eaten per larva) (Waldbauer, 1968). It has already been noted that the volume of food ingested is proportional to the size of larvae (ie. larger larvae ingest more than smaller larvae). However, when the ingestion rate to body weight ratio of the larvae is examined an increase in the volume of food ingested with an increase in larval size is not apparent (Figure 3.4).

The ingestion rates obtained on suspended solids in the field in this study are higher than rates obtained in the field by McCullough *et al.* (1979b) and Morin *et al.* (1988). It is possible that these high ingestion rates are due to the high turbidity of the Buffalo River. Southern African rivers are generally turbid, due to the erosive effects of poor catchment management and the nature of the underlying geology (Noble & Hemens, 1978; Kirk & Akhurst, 1984). The concentration of total particulate material in the Buffalo River ranged from a median of 3 mg l^{-1} to 45 mg l^{-1} , and at the sampling site it varied from $1 - 12 \text{ mg l}^{-1}$ (Palmer & O'Keeffe, in press, a). In Rattlesnake Creek, Washington where McCullough *et al.*, (1979b) worked, the seston varied from $0.97 - 2.5 \text{ mg l}^{-1}$ and in the outlet stream in Quebec where Morin *et al.*, (1988) worked, the seston concentration ranged from 0.6 to 1.6 mg l^{-1} .

It is possible that the different curves (Figure 3.4) for the ingestion rate as a function of body weight and time could be due to particle size selection by different sized larvae. Schroder, (1981) found that small larvae ingested a greater number of algal cells (1 to $3\mu\text{m}$ in diameter) than large larvae ingested. Large larvae preferred algae of 25 to $50\mu\text{m}$ in diameter, a transition being formed by cells of $14\mu\text{m}$ in diameter. Wenk and Dinkel (1981) found that small larvae preferred particles ranging from 5 to $12\mu\text{m}$ while large larvae ingested all particles between 1

and 40 μ m in diameter. The algal culture fed to the larvae consisted of a range of cell diameters due to active cell growth. However, the range of cell size always fell between 1 and 15 μ m. In seston there is a much greater range of particle size and this could explain the greater ingestion rate obtained by the intermediate sized larvae.

CHAPTER 4

ASSIMILATION

4.1 Introduction

Assimilation is the incorporation of digested food and its metabolic products into tissues. The assimilation rate (A) (μg food assimilated per mg of larvae per hour) gives an indication of the metabolic level of the larvae. Like ingestion rate, a number of factors may modify assimilation rate: larval feeding rate, water temperature, food type, larval size, and developmental state.

Literature values of assimilation efficiency (Ae) for simuliids are extremely variable. Assimilation efficiencies range from 1.8% for natural detritus (Wotton, 1978 b) to 99.2% for diatoms (Ladle & Hansford, 1981). In this study assimilation efficiencies on two algal types were determined for *S. medusaeforme/hargreavesi* larvae.

4.2 A review of techniques for determining assimilation

Several methods are available for measuring assimilation rates. These are divided into chemical, gravimetric and radiotracer analyses - their limitations and advantages are discussed below.

4.2.1 Chemical analyses

This method involves measuring the change in concentration of some component (e.g. organic matter, calories or nitrogen) of the food and faeces (Conover, 1966; Hargrave, 1970; Pandian & Delvi, 1973; Otto, 1974). Included in chemical analyses is the indicator technique, which measures the increase in concentration of an inert non-absorbed substance (e.g. chromic oxide) as it

passes through the gut. The difference in concentration of the non-absorbed substance between the food and faeces indicates the degree of assimilation (Corbet, Greenhalgh, McDonald & Florence, 1960; McGinnis & Kasting, 1964). The indicator technique has an advantage over the other techniques because it allows the determination of assimilation efficiency using a small sample of the faeces.

The chemical techniques are based on the assumption that all the measured components within the faeces are derived entirely from the food. This assumption is not entirely justified as animals lose some metabolic secretions in the faeces. These methods therefore may underestimate assimilation efficiency.

4.2.2 Gravimetric technique

The gravimetric technique is frequently used (Smith, 1959; Fewkes, 1960; Lawton, 1970; Mason, 1970; McDuffett, 1970; Stockner, 1971; Mackay & Kalff, 1975), and involves estimating assimilation from the difference between the weight of food consumed and the weight of resultant faeces.

The gravimetric technique uses the balance equation $A = I - F$ (A = assimilation, I = ingestion, F = faeces) and is based on two assumptions (Johannes & Satomi, 1967):

- i) that faeces contain organic matter entirely derived from food
- ii) that all unassimilated food is released in faecal pellets.

The problems associated with this method are blotting and reweighing food accurately, leaching (the removal, by percolating water, of some component from food

or faeces) water loss and growth of the food source. To obtain a sufficient quantity of faeces the experiment needs to extend over a greater time period than the other methods. This results in additional problems as the quantity of food may change, microbial activity on the food may affect protein and caloric content and leaching then needs to be accounted for. The gravimetric method is not as sensitive as the radiotracer techniques.

4.2.3 Radiotracer techniques

The conventional carbon-14 (^{14}C) method (see Sorokin, 1968) has been used by a number of workers (Rodina & Troshin, 1954; Sorokin, 1966; Sorokin & Panov, 1966; Schindler, 1968; Hargrave, 1970; Arnold, 1971; Schindler, 1971; Ladle and Hansford, 1981). Animals feed on ^{14}C labelled food and then clear their guts by feeding on non-labelled food for a period exceeding digestion time. Radioactive carbon remaining in the animal's tissue indicates food assimilated during the feeding time.

Assumptions made are:

- i) that the consumption rate is constant
- ii) that the respiratory losses of ^{14}C are minimal

Losses of labelled organic material from the animal can be ignored if the body burden of ^{14}C is kept low and if the experiment is short enough (McCullough, 1975). Radiotracer techniques are considered time consuming as they require the complete removal of the isotope from the gut.

The problem of respiratory loss of ^{14}C in the conventional method is overcome by using the twin tracer technique. The twin radiotracer indicator technique

measures the ratio of radioactive chromium (^{51}Cr) (an inert non-absorbable tracer) to ^{14}C (the assimilated tracer) in samples of the food and faeces of the experimental organism (Calow & Fletcher, 1972). This method was considered superior to the other methods as neither quantitative recovery of faeces nor complete gut clearance is required.

Schindler (1968) working on *Daphnia* found that assimilated ^{14}C was only respired 16 to 24 hours after the first radiolabelled food was ingested. In this study it was assumed that the same was true for simuliids and so the loss of ^{14}C due to respiration was not a problem as experiments only lasted for a maximum of eight hours.

The conventional ^{14}C method was chosen for use in this study for two reasons. Radioactive chromium (^{51}Cr) is both a dangerous and expensive isotope to work with, and the Beckman Liquid Scintillation Counter, to which access was available, is not programmable to detect gamma (Auger electron) rays emitted by ^{51}Cr . In addition, when Calow & Fletcher (1972, pg 166) first verified the use of the twin tracer technique they stated that "Assimilation efficiencies estimated from the ratio $^{14}\text{C} : ^{51}\text{Cr}$ in food and faeces are directly comparable with assimilation efficiencies estimated from the differences between ^{14}C lost from the food discs and ^{14}C appearing in the faeces." If results from the twin tracer technique are directly comparable to results obtained from the conventional ^{14}C method, then using the twin tracer technique with its associated problems (safety, cost, lack of equipment) is not justified for purposes of this study.

In addition, Calow & Fletcher (1972) were not certain to what degree ^{51}Cr is taken up in freshwater microflora. Therefore, before an algal food source can be used in radio-labelling experiments, tests for potential uptake of ^{51}Cr would have to be undertaken.

For all these reasons stated above, ^{14}C alone was used to measure assimilation in this project.

4.3 METHODS

4.3.1 Radioactive labelling of the algal suspension

Two single-species algal cultures of *Chlorella* and *Selenastrum* (see section 2.4) were used to determine assimilation efficiency of simuliid larvae. The algal suspension was labelled at log-growth phase to ensure maximum and uniform ^{14}C labelling of cells. Fifty millilitres of algae were labelled by growing it in the presence of one millicurie of ^{14}C as $\text{NaH}^{14}\text{CO}_3$ (AMERSHAM (Pty) Ltd.) for 24 hours under constant illumination with frequent agitation.

Labelled algae were centrifuged at 2000rpm for five minutes and resuspended twice in fresh medium before being used as experimental food. This was necessary in order to remove any unincorporated label. At this stage the labelled culture was stoppered and placed in a cool dark place for later use.

Before use in an experiment, the weight and number of cells and radioactivity of the food were determined using subsamples of the labelled culture. These values were necessary for the computation of assimilation rates.

- i) Three 1-ml aliquots of the algal culture were pipetted onto preweighed and ashed Whatman GF/A (2.5 cm diameter) filters. These were then oven dried at 60°C for 24 hours and reweighed to determine the weight of algal cells ($\mu\text{g/ml}$).
- ii) The number of cells in 1ml of algal culture was counted using a haemocytometer (cells/ml). The average weight of cells was estimated from dry weights and cell counts.
- iii) Three 1ml aliquots were filtered onto membrane filters (0.2 μm pore diameter) and placed in separate scintillation vials with 12 ml insta-gel, a universal liquid scintillation cocktail for aqueous samples. These vials were read by the scintillation counter (see section 4.3.3) and the radioactivity of the food determined.

4.3.2 Experimental procedure

Simuliid larvae were acclimated to experimental conditions by placing them in a glass beaker with 100 ml water containing non-labelled algal suspension at the same concentration as the experimental algal suspension for at least 24 hours. Larvae were transferred to a separating funnel with 60 ml double distilled water for a period of 30 to 60 minutes before the onset of the experiment to ensure that their upper guts and food gathering apparatus were free of food. This time was long enough for larvae to seek out suitable microhabitats for feeding.

At the end of the acclimation period, 10ml of labelled food suspension was introduced to the 60ml double distilled water in the feeding container. From this three 5-ml aliquots were filtered onto 25mm diameter membrane filters (0.2 μm pore size) for determination of the pre-feeding radioactivity of the feeding suspension (see

section 4.3.3). After the experimental feeding period three 5 ml aliquots of the feeding suspension were filtered onto membrane filters so that the amount of algae consumed by the larvae could be determined. As the simuliids were fed for a period which exceeded gut-filling time, values of animal radioactivity (the amount of radioactivity incorporated into the larvae) were given in $\mu\text{g}/\text{mg}/\text{h}$. The actual readings of radioactivity in the larvae were used to determine assimilation efficiency.

The larvae were rinsed in double distilled water and transferred to a separating funnel. This allowed water to be changed and faeces to be removed with minimal disturbance to feeding. Larvae were allowed to feed on non-labelled culture for a period which exceeded gut passage time to ensure that all radioactive food was removed from the gut and that the remaining radioactivity was due to assimilated ^{14}C . It is possible that a negligible amount of ^{14}C could be reingested due to the resuspension and filtration of faecal material. Faeces were collected during this time and pipetted onto membrane filters. The filters were placed in scintillation vials with cocktail as described above and the specific activity counted (see section 4.3.3).

At the end of the egestion period larvae were immobilised using carbonated water, to prevent any defaecation or removal of food from the gut (Rigler, 1971). Any radioactive food that may have been adhering to the exterior of the larvae was removed by rinsing them in a 1% solution of hydrochloric acid and finally in double distilled water.

Larvae were sized and placed in scintillation vials in groups of 10 similar sized individuals. Five drops of Soluene-350 (tissue solubiliser) were added. This dissolves all soft tissue and renders the hard head capsule transparent. The vials were then placed in the oven for 24 hours at 60°C.

Twelve millilitres of insta-gel (scintillation cocktail) was added to the vials and the samples were left to equilibrate for 24 hours before reading (see section 4.3.3).

4.3.3 Sample counting

All samples were counted on a Beckman LS 3801 Liquid Scintillation Counter programmed to read ^{14}C . A quench coefficient curve for ^{14}C was determined and entered into the programme. The need to convert counts per minute (CPM) for quenching to disintegrations per minute (DPM) before calculation of assimilation efficiency was noted by Cammen (1977). Disintegrations per minute (DPM) is a more valuable and reliable form for calculating assimilation rates as it incorporates corrections for the effect of various factors that can distort the results. Radioactive carbon sample activity was counted for 5 minute periods. Counts were corrected for background and counting efficiency. A consistently high counting efficiency ($\bar{x} = 94\%$) was obtained.

4.3.4 Indirect determination of assimilation efficiency

Assimilation efficiency of larvae feeding on suspended solids in the laboratory was calculated indirectly by subtracting the average egestion rates obtained for different sized larvae (see chapter 5) from the average ingestion rates (see chapter 3).

4.4 RESULTS

Preliminary experiments to monitor leaching of ^{14}C from algal cells showed that leaching was negligible over the feeding period. Due to the short duration of the experiments (60 - 90 minutes) respiratory losses of ^{14}C were considered minimal and were not corrected for.

Assimilation rates by different sized larvae on two labelled algal food sources, *Selenastrum* and *Chlorella*, are presented in Table 4.0. Mean values and standard deviations were presented as there was not enough data to use median values. The assimilation rates for *Selenastrum* are lower than on *Chlorella* for all size classes.

The mean assimilation rate over different larval sizes is fairly constant for both *Selenastrum* (2.3 - 6.1 $\mu\text{g}/\text{mg}/\text{h}$) and *Chlorella* (21.9 - 49.0 $\mu\text{g}/\text{mg}/\text{h}$), although these rates are slightly higher over the smaller size classes (Table 4.0).

Calculations of assimilation efficiency of larvae feeding on suspended solids in the laboratory are presented in Table 4.1. These values calculated indirectly from ingestion and egestion results are extremely variable and range between 7 and 68%.

4.5 DISCUSSION

Although the use of monocultures in assimilation experiments is not indicative of assimilation on natural mixtures of algae and detritus, it may provide insight into the ecological importance of plants in animal nutrition. The efficiency of utilisation of certain algal species varies widely among invertebrates (see Monakov, 1972).

TABLE 4.0 Summary of the experimental conditions for determining assimilation efficiency. The acclimation time (AC), feeding time (FT), temperature and the number of larvae measured are indicated. In each experiment, where a number of replicates in a larval size class were done, only the mean assimilation rate and standard deviation is shown. An overall mean assimilation rate for each larval size class on each algal type is determined as well as the assimilation efficiency. Ingestion rates for larvae feeding on different algae were obtained from the previous chapter.

FOOD <i>Selenastrum</i> sp.					ASSIMILATION RATES ($\mu\text{g}/\text{mg}/\text{h}$)					
LARVAL SIZE (HCW) mm					0.24	0.32	0.40	0.48	0.56	0.64
EXPT	AC minutes	FT minutes	TEMP ($^{\circ}\text{C}$)	No. LARVAE						
9/5/89	30	90	20.1	155		5.06 (3.08)	5.96 (0.56)	3.14 (0.24)	2.00 (0.95)	1.52 (0.95)
10/5/89	30	95	20	105		2.71 (0.83)	1.93 (0.60)	2.26 (0.72)	3.14 (0.72)	2.79 (0.89)
30/5/89	45	90	19	121	6.12 (0.66)	4.43 (3.27)	6.42 (0.10)	3.31 (1.76)	4.62 (1.76)	2.47 (1.76)
OVERALL MEAN ASSIMILATION RATE ($\mu\text{g}/\text{mg}/\text{h}$) (S.D)					4.16 (1.08)	4.76 (3.01)	2.94 (0.63)	3.25 (1.49)	2.32 (0.87)	
MEAN INGESTION RATE ($\mu\text{g}/\text{mg}/\text{h}$)					185.6	296.5	294.3	409.0	521.8	
ASSIMILATION EFFICIENCY (%)					2.24	1.61	1.00	0.80	0.44	
FOOD <i>Chlorella</i> sp.										
EXPT	AC minutes	FT minutes	TEMP. ($^{\circ}\text{C}$)	No. LARVAE						
7/6/89	60	60	20.2	90	29.8 (8.54)	44.5	45.4	15.9	13.6	16.3
8/6/89	20	90	20.0	86	132.0	40.2	7.8	31.0 (4.5)	31.0 (4.24)	28.4 (8.6)
9/6/89	45	90	20.8	87	23.5	24.6	12.5	16.7 (4.65)	17.1 (6.2)	14.6
OVERALL MEAN ASSIMILATION RATE ($\mu\text{g}/\text{mg}/\text{h}$) (S.D)					49.0 (46.9)	36.4 (10.5)	21.9 (20.5)	22.2 (8.6)	21.9 (9.2)	21.9 (8.9)
MEAN INGESTION RATE ($\mu\text{g}/\text{mg}/\text{h}$)					325.2	637.5	570.6	484.0	676.9	429.3
ASSIMILATION EFFICIENCY (%)					15.1	5.71	3.80	4.60	3.20	5.10

TABLE 4.1 Indirect calculation of assimilation (A) and assimilation efficiency (Ae) from mean ingestion (see Figure 3.4) and egestion (see Table 5.0) values for different larval size classes were obtained on river water in the laboratory. The ingestion rate (I), assimilation rate (A) and egestion (F) are all expressed in ($\mu\text{g}/\text{mg}/\text{h}$). Assimilation efficiency is expressed as a percentage.

	LARVAL SIZE (HCW) mm			
	0.32	0.40	0.48	0.56
Ingestion rate (I)	263.7	370.9	349.5	357
Egestion (F)	244.2	160.7	143.1	115.8
Assimilation rate (A) A = I - F	19.5	230.2	206.4	241.2
Assimilation efficiency (%) Ae = (A/I x 100)	7	62	59	68

TABLE 4.2 Summary of assimilation efficiencies obtained by other workers and in this study for simuliid larvae feeding on various food types.

SPECIES	METHOD	DIET	ASSIMILATION EFFICIENCY (%)	AUTHORITY
<u>Simulium medusaeforme/hargreavesi</u>	Radioactive ¹⁴ C	<u>Selenastrum</u>	0.4 - 2.2	Present study
		<u>Chlorella</u>	3.0 - 15.1	
	Indirect- Ingestion & Egestion Measurements	Seston	7 - 68	
<u>Simulium</u> spp.	Measured seston flux changes above & below a large population	Seston	17 - 25	Morin, Back Chalifour, Boisvert, Peters 1988
<u>S. austeni</u>	Radioactive ¹⁴ C	Diatoms	27.8 - 59.4	Ladle & Hansford 1981
<u>Simulium</u>		<u>Stephanodiscus</u>	79.6 - 99.2	
<u>Simulium ornatum</u>	Radiotracer ¹⁴ C	Diatoms (<u>Nitzschia</u>)	70 - 78	Schroder 1981
		Blue-green (<u>Synechococcus</u>)	36	
		Green algae (<u>Stichococcus</u>)	79	
		(<u>Scenedesmus</u>)	68	
		Desmid (<u>Staurostrum</u>)	68	
<u>Simulium</u> spp.	Indirect- Growth & Respiration measurements	Natural detritus	1.75 - 1.87	Wotton 1978b
<u>Simulium</u> spp.	Dual Label	Mixed	57	McCullough <u>et al.</u> , 1979a
	Ash Ratio	diatoms	56	

In this study, assimilation efficiencies (Ae) obtained on green algae are much lower (0.4 - 15%) than assimilation efficiencies obtained in other studies on algae (Table 4.2). For example Schroder (1979) obtained an assimilation efficiency of 68 - 79% for larvae feeding on a green algae. Ladle and Hansford (1981) obtained an assimilation efficiency of up to 99% for larvae feeding on diatoms. This value however should be viewed with caution as diatoms have silicon frustules which are not digestible.

It appears that simuliids have considerable difficulty in digesting many types of algae. Boyd and Goodyear (1971) noted that even on food with a high calorific content (such as algae) the assimilation was low if the food was indigestible. Moore (1977) showed that Chlorophyta and Cyanophyta are not broken down to any extent in the gut and therefore do not play an important role in the nutrition of sub-Arctic blackflies. It is therefore difficult to account for the high assimilation efficiencies obtained by other workers. The lower assimilation efficiencies obtained in this study appear to be more realistic.

There are a number of factors which, to a limited degree, can affect the estimation of assimilation efficiency. The ingestion of resuspended faeces during the gut clearance period can result in an overestimation of assimilation efficiency. The omission of excreted and secreted ^{14}C from calculations, the adhesion of labelled food to body surfaces and the possibility of the larvae feeding discontinuously can all result in the underestimation of assimilation efficiency.

The assimilation rates on algae obtained in this study decrease slightly with increasing larval size (Table 4.0). In insects, earlier life stages are metabolically more active and grow faster than later stages. Small larvae therefore need to assimilate more food per unit weight than later stages, which maintain a steady state for metabolism (Muthukrishnan & Pandian, 1987). A two-fold increase in the weight or volume of an animal is usually accompanied by a 1.8 fold increase in the gut area (Gordon, 1959), resulting in a decrease in assimilation rates with increasing larval size.

The assimilation rates (A) obtained in this study are comparable with those of other workers. Morin *et al.* (1988) obtained specific assimilation rates of 3.6 - 31.8 $\mu\text{g}/\text{mg}/\text{h}$ for simuliids feeding on seston. Average assimilation rates obtained in this study ranged from 21.9 - 49.0 $\mu\text{g}/\text{mg}/\text{h}$ for *Chlorella*, and 2.3 - 4.7 $\mu\text{g}/\text{mg}/\text{h}$ for *Selenastrum*. Morin *et al.* (1988) used an average ingestion rate of 72 $\mu\text{g}/\text{mg}/\text{h}$ and obtained an assimilation efficiency of 25%. Even though my assimilation rates are comparable with other workers, the high ingestion rates obtained (see chapter 3) result in very low assimilation efficiencies. High ingestion rates result in fast gut passage and reduced assimilation (Lehman, 1976). When feeding on *Selenastrum* 11% of the larvae had filled their guts with algal cells within seven minutes of introducing the algal suspension. After 12 minutes, 22% had full guts. The ingestion rate on *Chlorella* was even greater with 53% of the larvae examined having full guts 13 minutes after introducing the algal suspension. It is possible that no significant digestion of the algal cells could occur in this short time period.

The assimilation efficiencies for seston obtained indirectly from ingestion and egestion rates (Table 4.1) are much higher than those measured by previous workers. Wotton (1978b) obtained an assimilation efficiency of between 1.75 and 1.87% for simuliids feeding on seston. This assimilation efficiency was calculated indirectly from growth and respiration measurements. These values are much lower than Morin *et al.*'s (1988) who obtained an assimilation efficiency of 17 to 25%. It therefore appears that indirect methods for estimating assimilation efficiency are not reliable and that at least two independent methods to derive assimilation rates or assimilation efficiencies should be used.

Bacteria often contribute significantly to the nutritional requirements of blackfly larvae (Ivlev, 1945; Fredeen, 1960, 1964). However, Ladle and Hansford (1981) obtained poor survival rates for simuliid larvae fed on bacteria and found diatoms to be the most satisfactory food source. It therefore appears that the species of *Simulium* and the type of food are important in determining assimilation efficiencies for simuliid larvae.

CHAPTER 5

EGESTION

5.1 INTRODUCTION

Egestion is the expulsion of that portion of the ingested food not assimilated (Cummins, 1973). The egested material consists of undigested components of the ingested food, negligible fractions of dead cells lining the gut as well as nitrogenous compounds produced from assimilated material. In most cases egestion takes place after a definite interval following ingestion.

The quantification of faecal loss is an important aspect of bioenergetic studies as it is useful for calculating the net metabolisable energy (ie. assimilation) that is available to the individuals for growth and metabolism. However, it is a difficult parameter to measure unless the faecal pellets can be quantitatively retrieved.

Simuliid larvae produce spherical, fine-particulate, compact, cohesive and smooth pellets (Ladle & Griffiths, 1980), which have a brownish orange colour similar to that of the experimental food used in this study. Ladle, Welton & Bell (1987) measured *Simulium* spp. faecal pellets and found them to have a mean diameter of $0.21 \pm 0.02\text{mm}$ (range 0.21-0.31mm) with a mean volume of $0.006 \pm 0.002\text{mm}^3$ (range 0.001 - 0.016mm³). It should be noted that faecal pellet size varies with different instars within a species.

Very little work has been carried out on faecal material in lotic environments. Hargrave (1972) noted that ingestion and egestion rates were directly related, and that ingestion rates could be predicted from egestion

rates and assimilation efficiency. Hargrave (1976) studied the role of invertebrate faeces in decomposition of river sediments. Ladle and Griffiths (1980) described the faeces of a variety of chalk-stream invertebrates. Ladle *et al.* (1987) determined the sinking rates of faecal pellets from *Simulium* spp. and a deposit feeder *Gammarus pulex*.

Faecal pellets are sites for active microbial growth (Newell, 1965; Johannes & Satomi, 1966; Hargrave, 1970; Turner, 1977) and a food source for invertebrates (Newell, 1965; Johannes & Satomi, 1966; Frankenberg, Coles & Johannes, 1967; Frankenberg & Smith, 1967) and in this way feature predominantly in the recycling of organic nutrients (Hargrave, 1976).

5.2 METHODS

No one has attempted to determine egestion rates of simuliids by recovering all the faeces egested over a definite period. This method was employed in the present study to determine whether accurate reproducible results could be obtained. This method can only be used successfully if the faecal pellets can be quantitatively removed. Incomplete retrieval of all the faecal pellets would result in an underestimation of the egestion rate.

Larvae collected from the sampling site were left overnight to feed and acclimatise in aerated river water at 20°C. A number of similar sized larvae were placed in a separating funnel with river water and left to attach to the sides of the vessel. Once all the larvae were attached the water was drained and replaced by fresh river water. The larvae were left to void their guts for a period ranging between 30 and 60 minutes. Faecal pellets sank to the bottom of the separating funnel and this facilitated

removal of faeces without disruption of their feeding activities. The water was then drained from the separating funnel into a white plastic container. The larvae were killed in 70% ethanol.

The white background of the dish allowed the faecal pellets to be viewed with ease. The faecal pellets were collected with a fine tipped pipette and filtered onto a preweighed and ashed GFA 2.5 cm diameter filter paper. The filter paper was then oven dried at 60°C for 24 hours and reweighed on a Cahn microbalance ($\pm 1\mu\text{g}$). The weight of faeces egested over a specific time period by a certain number of larvae was calculated from the difference between the initial and final weight of the filter paper. The egestion rate of simuliid larvae was expressed as the weight of faecal pellets egested (μg) per larval body weight (mg) per hour.

5.3 RESULTS

Egestion of simuliid larvae (expressed as a function of body weight) feeding on suspended solids in river water decreased with increasing larval size (Table 5.0). Smaller larvae (0.32 - 0.36 mm) egested 244.2 $\mu\text{g}/\text{mg}/\text{h}$ which is double the amount of 115.8 $\mu\text{g}/\text{mg}/\text{h}$ egested by the largest size class of larvae (0.56 - 0.63 mm) measured.

Smaller larvae (up to 0.02 mg) egested between 20 and 25% of their dry body weight per hour (Figure 5.0) in comparison to larger larvae (0.07 mg) which only egest between 10 and 15% of their dry body weight per hour.

TABLE 5.0 Summary of the essential experimental conditions in determining the mean egestion (expressed as a function of body weight and time ($\mu\text{g}/\text{mg}/\text{h}$), of different sized *S. medusaeforme/hargreavesi* larvae. In each experiment the following are indicated: the mean head capsule width (HCW) and standard deviation (SE), the number of larvae measured (NO.), the time spent egesting (TE), the water temperature (TEMP), the total weight of faeces egested (μg) and the total larval body weight (mg). Egestion ($\mu\text{g}/\text{mg}/\text{h}$) for each experiment is indicated and a mean (\bar{x}) and standard error (SE) is calculated for each of the four larval size classes.

LARVAE		No.	EXPERIMENT				EGESTION RATE	
HEAD CAPSULE WIDTH (mm)			TE (h)	TEMP (°C)	FAECES (μg)	Body wt (mg)	($\mu\text{g}/\text{mg}/\text{h}$)	
\bar{x}	S.E.						\bar{x}	S.E.
0.34	0.05	25	1	21	129	0.4203	306.9	
0.35	0.04	20	1	20	54	0.3405	158.6	244.2
0.36	0.05	15	1	19.5	68	0.2546	267.1	76.7
0.40	0.04	19	1	20	93	0.4739	196.2	
0.42	0.03	17	1	20	145	0.4990	290.6	
0.42	0.02	19	1	20	77	0.5286	145.7	
0.43	0.03	14	1	19	67	0.4366	153.4	160.7
0.44	0.05	16	1	19.5	93	1.0508	88.5	67.7
0.45	0.05	14	1	21	75	0.4938	151.9	
0.47	0.05	16	1	21	67	0.6780	98.8	
0.49	0.04	15	1	21	122	0.7034	173.4	
0.52	0.05	15	1	20	90	0.8490	106.0	
0.54	0.02	18	0.5	20	71	1.1340	125.2	
0.54	0.03	12	0.5	20	44	0.7652	115.0	143.1
0.54	0.02	18	0.5	20	139	1.1506	241.6	55.1
0.54	0.03	13	1	19.5	164	0.8324	197.0	
0.54	0.03	21	1	20	137	1.2891	106.3	
0.55	0.01	19	0.5	20	50	1.2466	80.2	
0.56	0.04	18	1	20	87	1.2658	68.7	
0.56	0	10	1	19.5	88	0.6940	126.8	
0.56	0.02	15	1	20	138	1.0957	125.9	
0.56	0.03	16	1	21	165	1.1199	147.3	115.8
0.57	0.02	14	1	20	58	1.0574	54.9	34.5
0.57	0.02	16	1	19.5	146	1.1772	124.0	
0.57	0.02	15	1	21	155	1.0767	144.0	
0.60	0.03	16	0.75	19.5	138	1.3687	134.4	

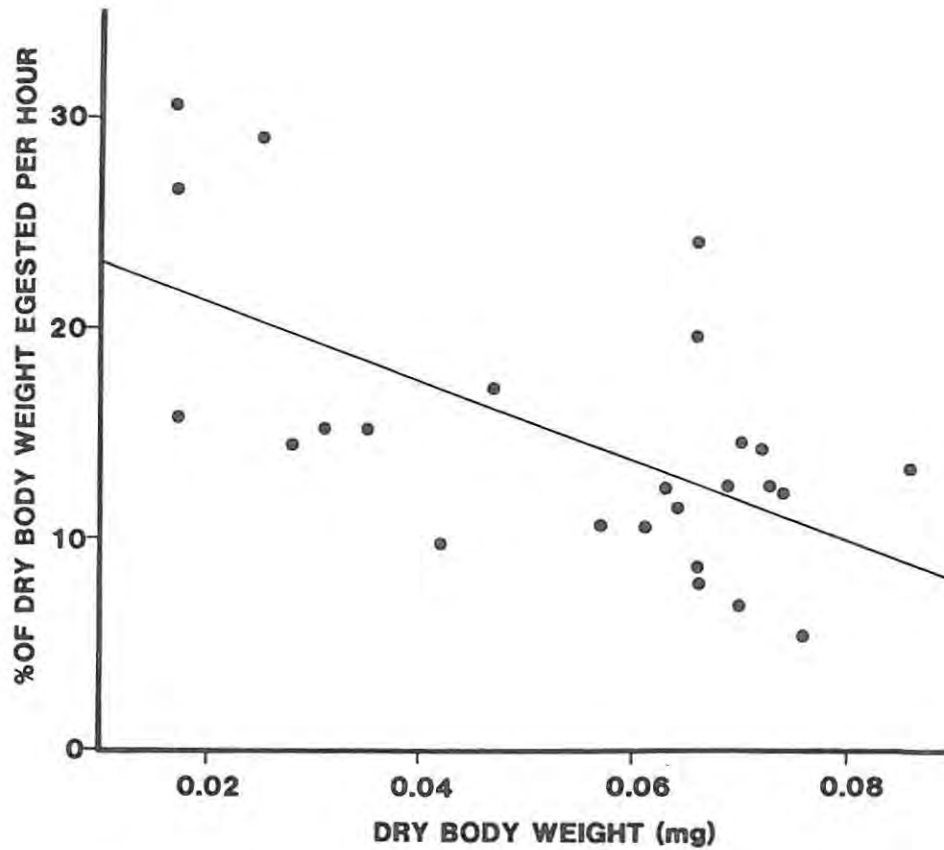


FIGURE 5.0 The percentage of dry body weight egested per hour as a function of simuliid dry body weight. Each data point represents one *S. medusaeforme/hargreavesi* larva.

$$y = 25.1 - 188x, r^2 = 0.38.$$

5.4 DISCUSSION

Smaller larvae generally feed at a higher rate (per unit body weight) and egest at a greater rate (per unit body weight) than larger larvae (Table 5.0), which are not growing as rapidly as small larvae and do not assimilate as much food as the younger larvae.

The trend for the percentage of dry body weight egested per hour to decrease as body size increases (Figure 5.0), is found in a number of aquatic deposit feeding invertebrates (North, 1954; Hughes, 1969; McDiffett, 1970; Hargrave, 1972). The proportional decrease in egestion as body size increases in a particular species probably reflects lowered growth rates with maturity. Energy intake by pharate pupae of *Simulium* is probably used entirely for respiration and silk production. These observations indicate the necessity for including all developmental stages in any estimate of population feeding rate.

An underestimation of egestion rate could result from larvae reingesting their own faecal pellets. Wotton (1980) stated that coprophagy may play an important part in the nutrition of larvae at high population densities. This is contradictory to Ladle *et al.* (1987) findings that simuliid larvae produce almost spherical faecal pellets due to their looped hind gut and that this faecal pellet form is an adaptation to prevent reingestion since the pellets fall out of suspension.

Simuliids play a role in altering the particle size available to other stream organisms as they feed on a wide range of particle sizes (0.1 - 300 μ m) (Colbo & Wotton, 1981) and produce larger compact faecal pellets (0.21 \pm 0.02mm) (Ladle *et al.*, 1987), which become available for other invertebrates to utilise.

Compaction of suspended fine particulate organic matter into faecal pellets is believed to accelerate their sinking rates (Moore, 1931; Smayda, 1969; Schrader, 1971; Fowler & Small, 1972; Ladle, 1973; Ferrante & Parker, 1977).

CHAPTER 6

AN ESTIMATION OF THE CONTRIBUTION OF SIMULIIDS TO THE PROCESSING OF ORGANIC MATTER IN THE BUFFALO RIVER.

6.1 INTRODUCTION

The ultimate purpose of a bioenergetic study is to define the importance of an organism in the processing of organic matter (through ingestion and assimilation) (McCullough *et al.*, 1979 a). For simuliids, conflicting conclusions as to their importance as processors of organic matter have been made (see chapter 1, 1.3).

It should be noted that this part of the study was not a major aim of the project but an attempt to use some of the results obtained in the previous chapters to estimate the contribution of simuliids to the processing of suspended solids down the length of the Buffalo River and to assess the role of this dominant invertebrate family in the functioning of the Buffalo River ecosystem.

Density estimates of simuliids have been combined with estimates of available habitat, total suspended solids and flow data, from the main Buffalo River Research Programme (BRRP) from March to April 1987, to arrive at figures for the total number of larvae in the river and seston availability. Ingestion and assimilation data from the present study were used to calculate the amount of organic material processed by simuliids in the river.

Due to the nature of this part of the study subjective estimates as well as extrapolation of results have been necessary (see below). These results, which should be

viewed with caution, give an indication of the amount of suspended solids being ingested by simuliids in the Buffalo River.

6.2 METHODS

6.2.1 How the Buffalo River was sectioned

For purposes of this study the Buffalo River was divided into five parts, using the impoundments as dividers. The Buffalo River was divided as follows:

- 1) upstream of Maden Dam
- 2) from Maden Dam to Rooikrantz Dam
- 3) Rooikrantz to Laing Dam
- 4) Laing to Bridle Drift Dam
- 5) Bridle Drift Dam to just upstream of the estuary.

In order to make correlation with data from the main BRRP possible each of the five parts of the Buffalo River were divided into sections with each section incorporating a sampling site. A section of the river included a sampling site and was measured from half way between the previous two upstream sampling sites to half way between the next two downstream sampling sites (see Figure 6.0). A 1:50 000 map of the Buffalo River was used to measure the length of each section of the river. This was done with a measuring wheel. In each section a zone covering a distance of between 2 and 8 km (depending on accessibility) was surveyed (Figure 6.0). In each of the zones the following measurements were made:

- i) the length of pools (see Figure 6.1)
- ii) the length and breadth of runs, cascades, riffles and waterfalls (see Figure 6.2).

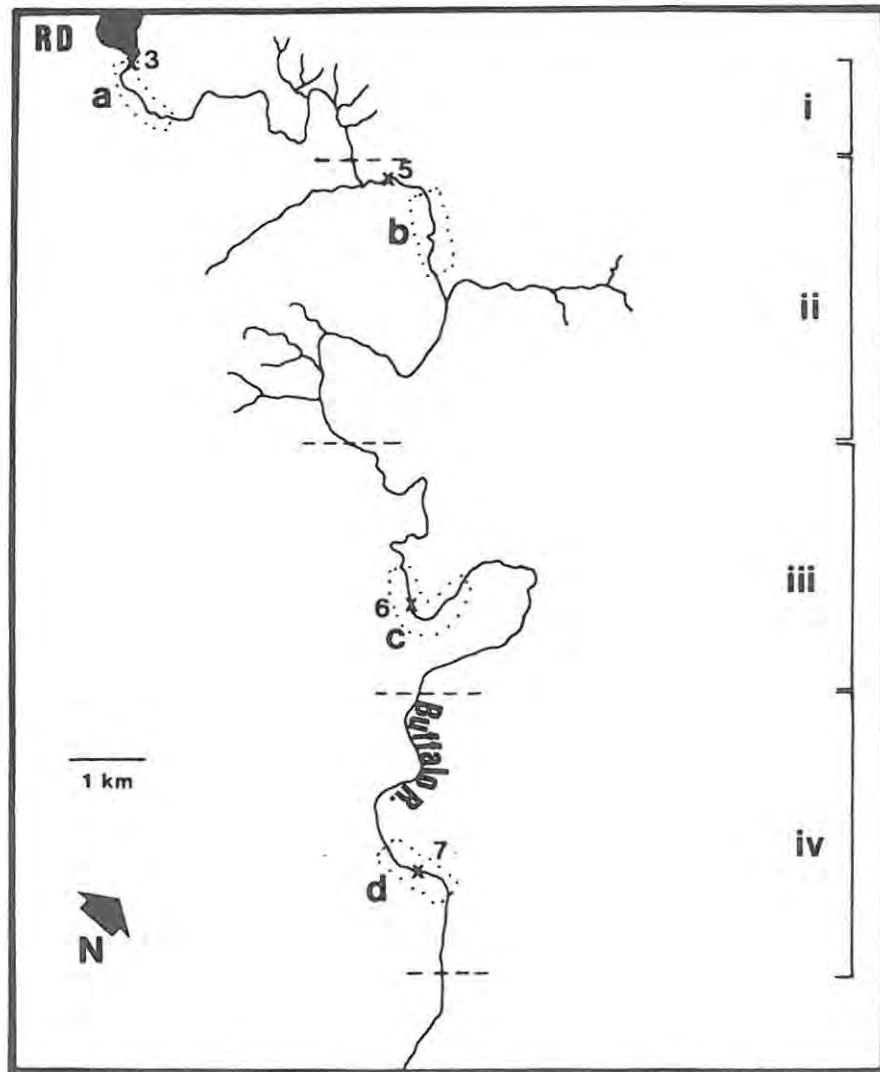


FIGURE 6.0 Map of part of the Buffalo River below Rooikrantz dam (RD). The river was divided into sections (i - iv) and representative zones (a - d) were surveyed in each section. Each section incorporated a main BRRP sampling site, the original numbering of sampling sites from the main Buffalo River Research Programme was maintained (see accompanying Table 6.0).

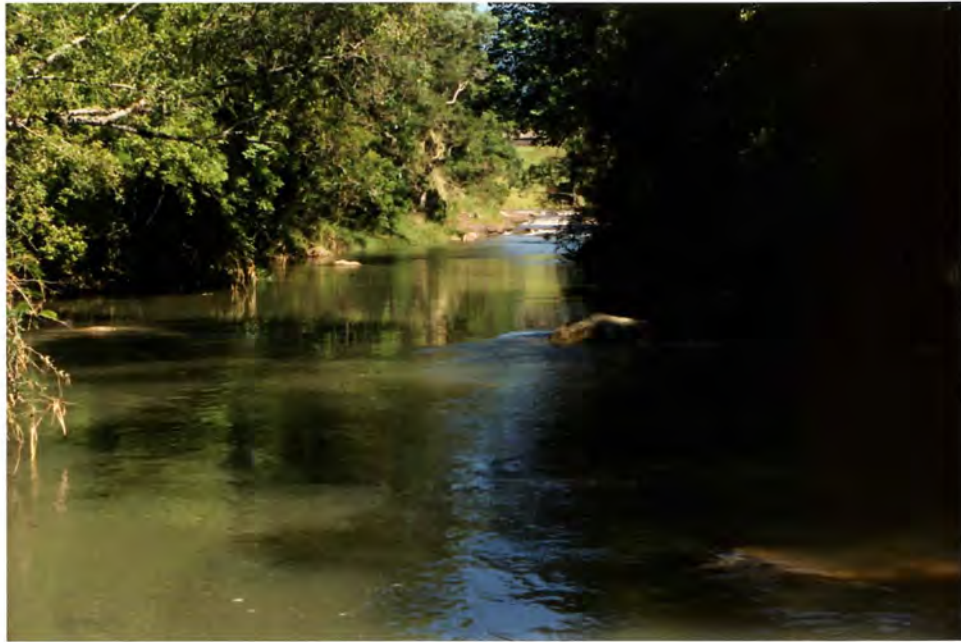


FIGURE 6.1 Photograph of the Buffalo River showing a section of the river which is not suitable for simuliid colonisation, i.e. classified as 'pool' during the survey.



FIGURE 6.2 Photograph of the Buffalo River showing a section of the river which is suitable simuliid habitat, i.e. classified as 'riffle' during the survey.

iii) For each of the simuliid habitats (i.e. runs, cascades, riffles and waterfalls) a subjective estimate of the percentage habitat available for simuliid colonisation was made. This was done by scanning the riffle and determining what percentage of it was box sample habitat (i.e. where box samples could be taken). This was necessary as all simuliid densities were based on comprehensive box sampling during the main BRRP. Only runs, cascades etc. were sampled and this could possibly result in an underestimation of the total numbers of simuliids.

As the Buffalo River is very seasonal (with low water flow occurring in winter and high flow in summer) it was decided to choose a period when flow was median and at a time when the river had not been subjected to a flood or drought in the previous six months. Median flow occurred throughout the river in March and April 1987. It should be noted that all the data used from the main BRRP and the calculations of the processing of organic material by simuliids are based on this time period of median flow and do not consider floods or periods of no flow.

6.2.2 Estimation of the total number of simuliids in each of the sections

Simuliid densities from comprehensive box sampling during the main BRRP were used to obtain average simuliid densities per m^2 at each site. The BRRP took three box samples at each site every month for three years. From the box samples larvae and pupae were separated, sorted and counted. Simuliid densities at each site were used together with the estimated area of simuliid habitat (from the zone surveyed) to calculate the mean number of simuliids in each section of river during median flow (see Table 6.0, number 2).

6.2.3 Calculation of the average ingestion rate of simuliid larvae on suspended solids in the field

As the main BRRP did not record the size classes of larvae it was not possible to determine the size structure of simuliid densities. It was assumed that an even distribution of larval numbers and size classes existed along the Buffalo River during median flow (i.e. March to April 1987) and therefore an average ingestion rate was used.

From Figure 3.1 the mean volume of seston ingested by different sized larvae in the field per minute was obtained. These volumes were first converted to weight (Figure 3.0, C) and extrapolated to an hourly rate for each size class. These rates for each size class were summed and an average ingestion rate per simuliid of 5.5×10^{-5} g/h was obtained (see Table 6.1). It should be noted that these field ingestion rates were obtained for one species at one site on the Buffalo River and were used to extrapolate for the whole river.

TABLE 6.1 Calculation of the average ingestion rate of simuliid larvae on suspended solids in the field. The volume ingested per minute was obtained from Figure 3.1. The conversion of volume to weight was obtained from Figure 3.0, C.

HEAD CAPSULE WIDTH (mm)	mm ³ /min	mm ³ /h	mg/h	g/h \bar{x}
0.32	0.0084	0.504	0.0317	5.5 x 10 ⁻⁵
0.33 - 0.40	0.0089	0.534	0.0335	
0.41 - 0.48	0.0188	1.128	0.0708	
0.49 - 0.56	0.0253	1.518	0.0953	
0.64	0.0116	0.696	0.0437	

6.2.4 Determination of food available to simuliids

The average flow rate at each site was calculated from the flow rate data from the main BRRP from March to April 1987 (see Table 6.0, number 4). It was assumed that all the water would be available for filtration by simuliids in a section of river (a distance of between 2 and 8 km).

The suspended food in the size range of 0 - 250 μ m was calculated at each site from the main BRRP data. This particle size range was selected as this is the range which blackfly larvae can ingest (Colbo & Wotton, 1981). More than 95% of suspended solids in transport in the Buffalo River are less than 80 μ m (Palmer & O'Keeffe, in press,a).

From all the above data, the total suspended solids flowing over the larvae in a particular section of the Buffalo River during median flow and the percentage ingested can be calculated (see Table 6.0, number 6).

6.2.5 Calculation of the assimilation efficiency of larvae on seston

The assimilation efficiency of simuliid larvae feeding on seston in the Buffalo River needs to be known in order to calculate what percentage of suspended solids ingested is assimilated. Wotton (1978b) calculated the assimilation efficiency indirectly for larvae feeding on material in the field and obtained very low efficiencies between 1.75 and 1.87%. Morin *et al.* (1988) obtained assimilation efficiencies for simuliids feeding on seston at a lake outlet of between 17 and 25%. Assimilation efficiencies in this study (Table 4.1) are extremely variable and it was decided not to use these values. As Wotton's (1978b) values were very low I decided to base estimates of

assimilation efficiency on values obtained by Morin *et al.* (1988). Their values for assimilation efficiency had to be adapted before use in this study owing to different conditions encountered in the Buffalo River when compared with a lake outlet. The ingestion rates obtained for simuliid larvae in this study were much higher than ingestion rates obtained by Morin *et al.* (1988). The high ingestion rate and fast gut passage time of simuliid larvae combined with the high suspended solid loads in the Buffalo River made me decide to adapt Morin *et al.* (1988) assimilation efficiency of between 17 - 25% to 10% for use in this study.

The results for assimilation in the following calculations would vary considerably if different assimilation efficiencies were assumed. The difficulty of measuring assimilation efficiency in a natural environment makes this sort of assumption inevitable. Using the assumed assimilation efficiency of 10%, the percentage of available organic suspended solids assimilated during median flow in each section in the Buffalo River could be determined (see Table 6.0, number 7).

In order to make these data comparable with those of other workers and to compensate for the different lengths and simuliid densities in each of the river sections, estimates of the percentage suspended solids ingested and assimilated per metre of stream bed were made (see Table 6.0, number 8). A theoretical distance was calculated in which complete removal of all suspended solids less than 250µm was possible in a section of river by the blackfly larvae present (see Table 6.0, number 9).

TABLE 6.0 An example of the calculations used to estimate the contribution of simuliids to the processing of organic matter in the Buffalo River (see accompanying Figure 6.0).

In this example section (iii) of the river incorporating site 6 (see Figure 6.0, 6.3, & 6.4) was used to demonstrate the calculation for estimating the contribution of simuliids to the processing of organic matter in the Buffalo River.

Information obtained from the survey:

Total length of the zone surveyed	1000 m
Total length of the riffle	226 m
Average width of the riffle	6.3 m
Percentage simuliid habitat (in the riffles section)	35 %

Information obtained from the main BRRP (at site 6 from March to April 1987)

Mean simuliid density	1082 m ⁻²
Flow rate	626400 l/h
Total suspended solids	0.007991 g/h

1) The area available for simuliid colonisation

The actual length of river section (ie. iii, see Figure 6.0) on 1:50000 map was 6400m in length. In this section the length of zone surveyed (ie. c, see Figure 6.0) was 1000m and the average width was 6.3m.

Therefore, the total area of riffles in the 6400m section of the river was

$$6400 \times 226/1000 \times 6.3 = 9112.3 \text{ m}^2$$

In the river section (iii) the estimated percentage of available habitat, in the zone surveyed, for colonisation by simuliid larvae was 35%.

Therefore, of the total amount of riffle in the river section only 35% was available for simuliid colonisation

$$9112 \times 0.35 = 3189 \text{ m}^2$$

3189 m² was available for simuliid colonisation.

2) The mean number of simuliids at site 6 for the period of median waterflow i.e. March to April 1987 is 1082 simuliids per m² (see above).

Therefore, the total number of simuliids in the section of the river was

$$1082 \times 3189 = 3450498.$$

3) The mean ingestion rate of larvae on suspended solids in the field is 5.5×10^{-5} g/h (see Table 6.1).

Therefore, in the river section the ingestion rate of the larvae was

$$3450498 \times 0.000055 \text{ g/h} = 189.78 \text{ g/h.}$$

4) The mean flow rate at site 6 during the period of March to April was 626400 l/h.

5) The average suspended solids (0 - 250 μ m) at site 6 from March to April was 0.0079915 g/l.

Therefore, the total available suspended solids in the river section (iii) of the Buffalo River during median flow was

$$626400 \text{ l/h} \times 0.0079915 \text{ g/l} = 5005.9 \text{ g/h.}$$

6) The estimated ingestion rate of all the simuliids in section (iii) of the river was 189.78 g/h.

Therefore, the percentage of available suspended solids that was ingested was

$$189.78 / 5005.9 \times 100 = 3.8 \%$$

7) The assimilation efficiency of simuliids on suspended solids in the Buffalo River is assumed to be 10% (see 6.2.5). Therefore, of the total suspended solids available to simuliids, the average percentage assimilated in this river section is 0.38 %

8) In section (iii) (see Figure 6.0) of the river an average of 3.8% and 0.38% of the suspended solids were ingested and assimilated respectively. As each section of the river was a different length the mean ingestion and assimilation rates per metre were calculated for comparative purposes.

If an average of 3.8% of suspended solids was ingested over a distance of 6400m then in one metre 0.00059% of suspended solids flowing over the simuliids are ingested and similarly if 0.38% of the suspended solids was assimilated over a distance of 6400m then in one metre 0.000059% was assimilated.

9) If 0.00059% of the suspended solids was ingested in one metre of the stream bed then assuming a constant rate of removal, the distance required for complete removal of suspended solids (i.e. 100%) is 169.5km. It should be noted that processing of suspended matter in the river by simuliids only occurs where simuliids are present (i.e. riffles, runs etc.), however a distance was calculated to incorporate a whole river section not just the riffles.

6.3 RESULTS

The calculated numbers of simuliids along sections in the Buffalo River are represented in Figure 6.3. Numbers of simuliids were high at Site 1 (130×10^6 simuliids per section of river), in the upper reaches of the river. There was a noted increase in simuliid numbers below all of the dams, except Maden Dam. At site 11 where the flow rate was the highest (Table 6.2) the number of simuliids was correspondingly high (85.81×10^6 simuliids per section of river).

The part of the Buffalo River between Maden and Rooikrantz dams is characterised by low simuliid numbers and low flow rate. Rooikrantz Dam receives overspill from Maden Dam. During periods when Maden Dam is not overflowing (i.e. late April 1987) the river below Maden Dam ceases to flow strongly. There is some seepage from the impoundment which is probably only able to sustain low simuliid numbers. The high flow rate at Site 8 (Table 6.2) is due to urban and industrial effluent inflow from the Kingwilliamstown and Zwelitsha area.

The total suspended solids and the organic fraction in the 0 - 250 μ m size range along the river during the period of median flow is represented in Figure 6.4. Total suspended solids are low in the headwaters and increase downstream. There is a sharp increase at Site 8 due to urban and industrial waste inflow from Kingwilliamstown and Zwelitsha. At site 11, below Bridle Drift Dam, the concentration of suspended solids is also high (100.5 mg l^{-1}).

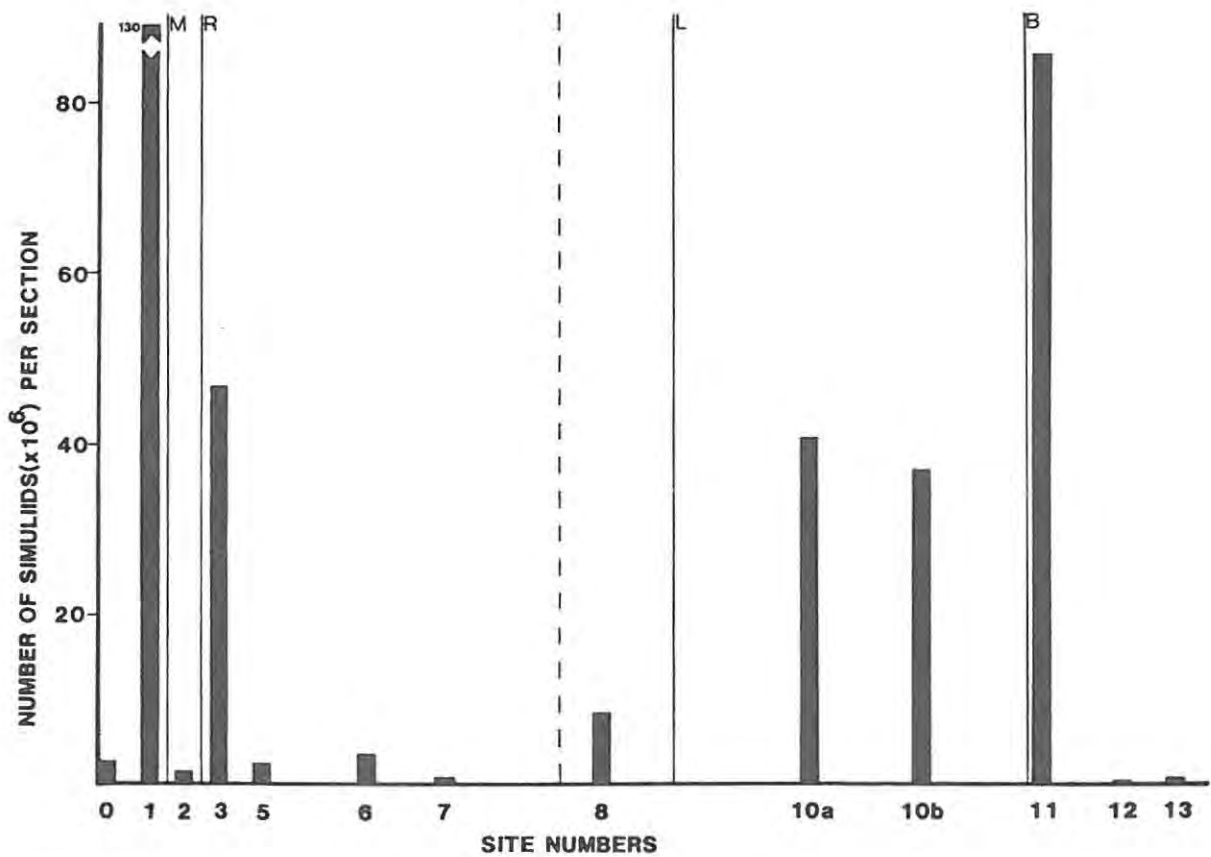


FIGURE 6.3 Representation of the mean number of simuliids in each section of the river during median flow along the river. The sampling sites are numbered from the main (BRRP) and are spaced proportionally according to actual distances apart. The position of the dams (vertical black lines) are indicated. M = Maden, R = Rooikrantz, L = Laing and B = Bridle Drift Dam. The dashed line between sites 7 and 8 indicates the position of Kingwilliamstown and Zwelitsha. Note the areas of the sections differ so actual densities are not indicated.

TABLE 6.2 Summary of the major parameters calculated in the river sections to estimate the percentage of suspended solids processed by simuliids during periods of median flow. The percentage of suspended solids processed per metre of stream bed and the theoretical distance required for complete removal of suspended solids is also shown. The numbered sampling sites (original numbering from the main BRRP) are indicated. (* As no flow rate data were available for Site 10b the flow rate from 10a was used).

Site No.	ABOVE MADEN DAM		MADEN TO ROOIKRANTZ	ROOIKRANTZ TO LAING					LAING TO BRIDLE DRIFT		BRIDLE DRIFT TO THE BEGINNING OF THE ESTUARY		
	0	1	2	3	5	6	7	8	10a	10b	11	12	13
Area available for simuliid colonisation in each section (m ²)	1074	6872	1944	5276	6314	3189	2079	2835	18240	15648	18893	97	617
Tot. No. of simuliids (x10 ⁶) in the section of river	2.96	130	1.41	46.67	2.17	3.45	0.75	8.18	40.83	37.10	85.81	0.35	0.75
Ingestion rate (g/h)	162.8	7150	77.55	2566.85	119.35	189.78	41.25	449.9	2245.65	2040.5	4719.55	19.25	41.25
Flow rate (l/h)	46800	568800	266400	522000	698400	626400	662400	1288800	1119600*	1119600*	2916000	669600	190800
Total suspended solids (g/l)	0.004616	0.001968	0.007417	0.001434	0.003052	0.007991	0.003987	0.029586	0.024667	0.022607	0.100539	0.087239	0.018428
Total suspended solids available to simuliids (g/h)	216.0	1119.7	1976.1	748.7	2131.5	5005.9	2674.3	38131.1	27617.2	25310.8	293171.7	58415.2	3516.1
% of suspended solids ingested and assimilated in each section of the river	75.4	638.4	3.9	342.9	5.6	3.8	1.5	1.2	8.1	8.1	1.6	0.033	1.2
	7.536	63.8412	0.3924	34.2911	0.5599	0.3791	0.1542	0.118	0.8131	0.8062	0.161	0.0033	0.1173
% of suspended solids ingested and assimilated per m of stream bed	0.0314	0.14847	0.00218	0.04131	0.00068	0.00059	0.00037	0.00025	0.0004	0.00078	0.00021	0.00032	0.00011
	0.00314	0.014847	0.000218	0.004131	0.000068	0.000059	0.000037	0.000025	0.00004	0.000078	0.000021	0.000032	0.000011
Theoretical distance in km for complete removal of suspended solids < 250 µm	3.2	0.67	45.9	2.4	147.1	169.5	270.3	400	250	128.2	476.2	312.5	909.1

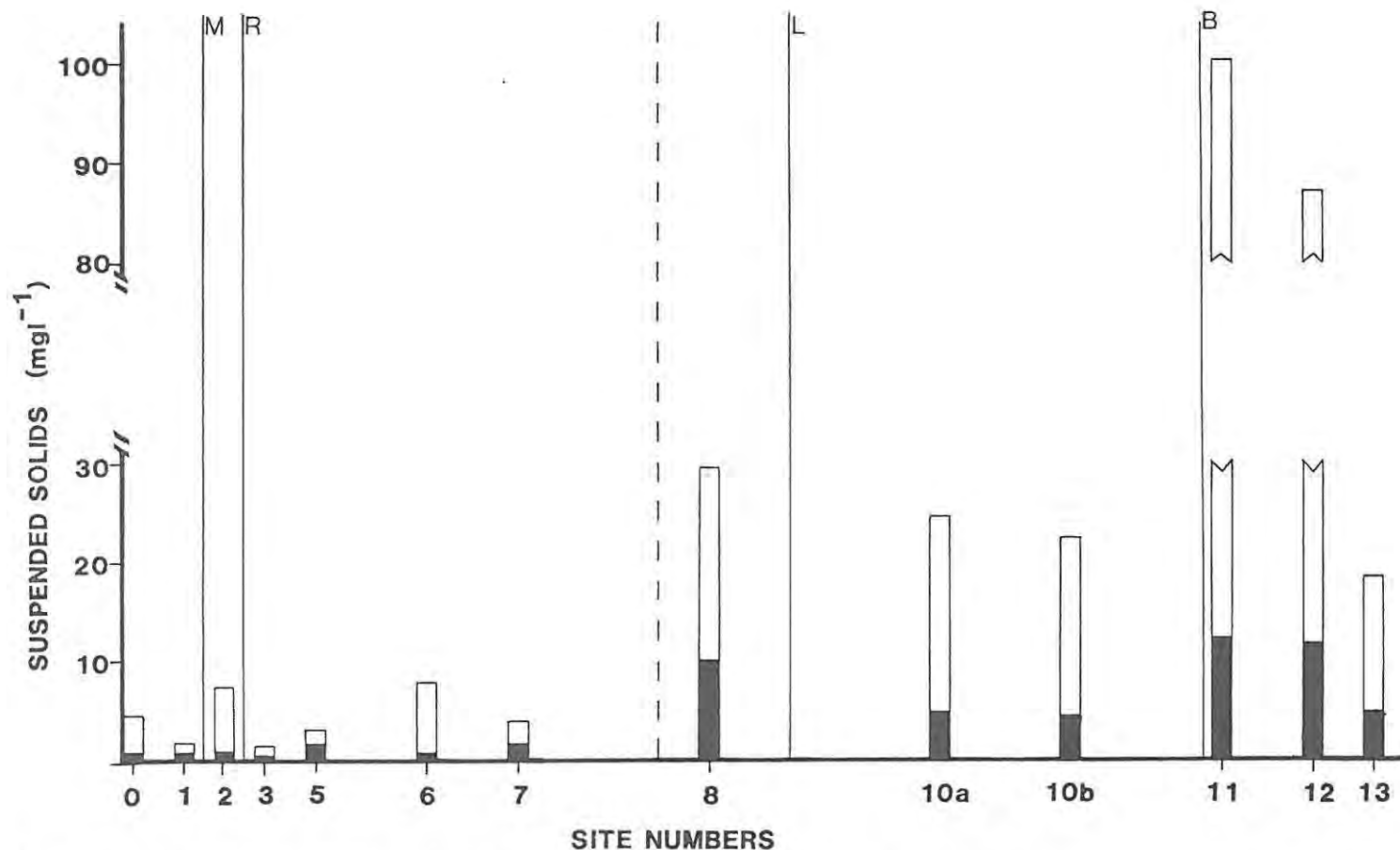


FIGURE 6.4 Mean suspended solids (< 250µm) in each section of the river during median flow. The total suspended solids (unshaded histogram) and the organic fraction (shaded histogram) of the suspended solids in each section is shown. The sampling sites are numbered from the main (BRRP) and they are placed proportionally apart. The position of the four dams are indicated by solid black lines. M = Maden, R = Rooikrantz, L = Laing and B = Bridle Drift Dam. The dashed line between site 7 and 8 represents the position of Kingwilliamstown and Zwelitsha.

The percentage of the total suspended solids ingested and assimilated by simuliids during median flow along the river in each section and per metre of stream bed is presented in Table 6.2. In the parts of the river above Maden Dam (Site 1) and immediately below Rooikrantz Dam (Site 3) the simuliids present are able to ingest between 3 and 6 times the amount of food available to them in each section of the river, the percentage of total suspended solids ingested per metre of stream bed is 0.15 and 0.041% respectively.

In the upper reaches of the river where the river was flowing a theoretical distance of between 0.67 and 3.2 km is required for simuliids to ingest all of the suspended solids available to them as food. In the middle and lower reaches a distance of between 147 and 910 km is necessary for complete removal of suspended solids < 250 μ m from the water.

6.4 DISCUSSION

The three factors likely to have the greatest influence on the processing of seston by simuliids in the Buffalo River are the densities of simuliids, the suspended solids available to them, and their rates of ingestion and assimilation.

Simuliid densities along the river are very variable (Figure 6.3). The high densities below impoundments (Sites 3, 10a & 11) are a well known feature of river ecology (Briggs, 1948; Cushing, 1963; Ward, 1974; Armitage, 1976; Wotton, 1978a, b, 1979, 1982; Sheldon and Oswood, 1977; Carlsson *et al.*, 1977; Lake and Burger, 1983). In addition to this, Bridle Drift and Rooikrantz Dams both release

water even when they are not overflowing, and this continuous waterflow results in large numbers of simuliids colonising the tailwater regions.

The high simuliid densities above Maden Dam and the low numbers near the mouth of the Buffalo River can be attributed to the percentage availability of habitat for colonisation by simuliids. Above Maden Dam, the stream is favourable simuliid habitat being shallow, swift flowing with a high percentage (70%) of riffles. In contrast, downstream of Site 11, the river broadens and deepens with very few riffles (< 20%). This section of the river is characterised by low numbers of simuliids.

Suspended solids increase along the course of the river. Maden Dam receives cool, clear, high quality water from a near-pristine, afro-montane closed-canopy forest catchment (Palmer & O'Keefe, in press, b). Rooikrantz Dam receives water from Maden Dam overspill. This water is low in suspended solids (Figure 6.4). The high value for suspended solids at Site 11 is due to Bridle Drift Dam releasing turbid bottom water.

Estimates of the ingestion rate of suspended solids by simuliids vary between 0.00011 and 0.15% per metre of stream bed. These values are higher than the values of 0.00008 - 0.019% of seston ingested per metre of stream bed obtained for filter-feeders by other workers (Maciolek & Tunzi, 1968; McCullough *et al.*, 1979b; Benke & Wallace, 1980; Haefner & Wallace, 1981; Parker & Voshell, 1983; Ross & Wallace, 1983). The higher percentage of ingestion of suspended solids per metre of stream bed obtained in this study is thought to be due to the high inorganic

fraction of the suspended solids in transport (see chapter 3). Larvae feeding on poor quality suspended solids (i.e. low organic fraction) have to process a large amount in order to meet their nutritional requirements.

In the upper sections of the Buffalo River the high simuliid densities and the low suspended solid loads enable simuliid larvae to ingest all the suspended solids ($< 250\mu\text{m}$) flowing past them during a period of median flow rate in a theoretical distance of less than 3.2km. As more than 95% of the suspended solids in transport in the Buffalo River are less than 250 μm in diameter simuliids in the upper reaches are in effect ingesting virtually all the suspended solids flowing past them within 3.2 km. This capture of FPOM in transport will not only influence the food available to collectors but also provides important energy sources for predators of simuliids. The exponential decrease in simuliid densities progressively further downstream of a dam could be due to high simuliid densities immediately below the dam rapidly removing a large proportion of suspended solids from the water column. This large amount of removal of suspended solids could limit the food available to the simuliids downstream resulting in decreasing simuliid numbers.

In order to make rough estimates of the contribution of simuliids to processing organic matter in the Buffalo River it was necessary to extrapolate data and make some subjective estimates. Temperature, a factor which is known to affect ingestion rates (Mulla & Lacey, 1976; Kurtak, 1978; Lacey & Mulla, 1979; Merritt *et al.*, 1982), was not incorporated in this study. The temperature during the experimental period ranged from a minimum of 11°C at Site 1 to a maximum of 32°C at Site 10a (Palmer & O'Keefe, 1989).

In summary, the relative importance of simuliid larvae in processing suspended solids in the Buffalo River is greatest in the headwaters, where densities are high and suspended solid loads are low, and decreases downstream. Simuliids in the upper river are very important in retarding transport (i.e. they take all material out of transport and deposit it as faeces for further microbial and invertebrate processing) (see Chapter 7). Simuliids in the upper reaches are theoretically able to remove all the available seston flowing over them within a distance of 3.2km during periods of median flow.

Although results obtained from this study have limitations, it should be noted that this is the first detailed study of this nature to be undertaken. Previous workers (Maciolek and Tunzi, 1968; McCullough *et al.*, 1979a) worked on a short section of a river, whereas in this study the whole river was considered. Ladle *et al.* (1972) determined the importance of larvae in the river only when simuliid populations were large and suspended solids were low, but in this study a period of median flow rate was used. This is also the first study of this nature to be undertaken on a southern hemisphere turbid river.

CHAPTER 7

GENERAL DISCUSSION: A CRITICAL ASSESSMENT OF BOTH THE ROLE OF SIMULIIDS IN THE BUFFALO RIVER AND THE PROBLEMS ENCOUNTERED IN THIS STUDY

South Africa will soon be facing a water shortage due to a combination of low rainfall, high evaporation rates and a growing population (O'Keeffe, 1986). This increasing demand for river resources will put severe strains on south African river systems.

As yet there is no policy for river conservation in southern Africa, but before any management policies can be initiated river ecosystems in South Africa need to be studied and understood. Cummins (1973) stated that any rehabilitation or management strategy for rivers that is to be successful must rely on fundamental knowledge of freshwater ecosystem structure and function.

The model of the community concept and flow of energy in ecological systems was first developed by Raymond Lindeman in 1942. In his model no attempt was made to separate out the important detritus and decomposer components. Wiegert and Owen (1971) modified Lindeman's original model to provide for the grazing and detritus pathways not accounted for. Lindeman's original model however, stimulated the study of energy flow and formed the cornerstone of ecology. Since the early 1950's energetics has served as a unifying concept in ecology in general as well as in stream ecology. The flow of energy and the continuous cycling of

material through the system is a vital characteristic of the structure and functioning of stream ecosystems (Cummins, 1973).

The importance of terrestrial sources of organic matter in headwaters of streams have been recognised for several decades (e.g. Maciolek (1966) found that 70 - 90% of seston in a Californian mountain stream was of allochthonous origin; Fisher & Likens (1973) found that in Bear Brook, New Hampshire over 99% of input of organic energy to consumers was allochthonous). The importance of allochthonous material in rivers highlights the necessity for land management in the catchment areas to prevent the removal of natural vegetation due to growing urban and agricultural demands.

A unique feature of rivers is the unidirectional transport of material from headwaters to the sea. Without a means of retardation and storage within the river system this organic matter (mostly allochthonous) would pass along the river into the sea with little or no utilisation. Macroinvertebrates are thought to play a significant role in this processing of organic matter. The River Continuum Concept (RCC) of Vannote, Minshall, Cummins, Sedell & Cushing (1980, pg 130) stated that "... biological communities developed in natural streams assume processing strategies involving minimum energy loss. Downstream communities are fashioned to capitalise on upstream processing inefficiencies." In other words communities are able to make maximal use of resources (particularly food) in a river. This suggests that invertebrate communities are important processors of the organic matter flowing down a river.

In rivers, the large numbers of filter-feeders feeding on a wide range of particle sizes using various filtering mechanisms are able to utilise seston flowing down a river efficiently (Wallace *et al.*, 1977). Filter-feeding simuliids in the Buffalo River were found to have an important direct impact on the suspended solids in transport in the upper reaches (see chapter 6). Simuliids were potentially able to ingest all the suspended solids ($< 250\mu\text{m}$) in a distance of 3.5km in the upper reaches. Simuliids in the middle and lower reaches were found to have lesser impact on suspended solids in transport requiring 40 - 280 times the distance to ingest all the suspended solids (see chapter 6).

The distance of 3.2km for the removal of suspended solids by simuliids in the upper reaches of the Buffalo River falls well within the 0.6 - 9.21km range reported by previous workers (Ladle *et al.*, 1972; McCullough *et al.*, 1979b)(see chapter 6). This significant impact of simuliids on the suspended solids load in the upper reaches of the Buffalo River is attributed to a combination of high ingestion rates (see chapter 3), high densities (see chapter 6), a low concentration of suspended solids and low flow rates in the upper reaches (see Table 6.2). Due to the high inorganic fraction of suspended solids in the Buffalo River, (44% in the upper reaches, Palmer & O'Keeffe, in press, a) simuliids only assimilated a small fraction (0.000011 - 0.015% per metre of stream bed) (see Table 6.2) of the ingested material, the rest being re-released as faeces. (Indirect calculations of the assimilation efficiency of different sized larvae feeding on seston in the laboratory however were higher - see chapter 4). The contribution of simuliids to direct energy processing of organic matter in the Buffalo River

is therefore less important than their role in intercepting and retarding the downstream flow of organic matter.

Probably the most important qualitative role of blackfly larvae as a component of a filter-feeding group in the functioning of a stream is through their feeding activities. Blackfly larvae are the only important members of the fauna which can make direct use of suspended bacteria (Hynes, 1970a). Bacteria are filtered from the water and converted into larger food particles available to more invertebrates, incorporating fewer trophic transfers and therefore greater energetic efficiency. Bacteria are also important in the nutrition of blackfly larvae (Baker & Bradman, 1976; Berrie, 1976). Feeding activities of blackfly larvae change FPOM to compact faecal pellets which either settle out from suspension in pools, which are major sites of decomposition in rivers (Smith, 1980), or are transported downstream. Faecal pellets are probably a major source of nutrition to benthic communities (Pomeroy, 1980). Fragmented particles and faeces formed by feeding activities represent a considerable increase in substrate surface area for microbial colonisation.

Microbial production is known to play an important part in community energetics of streams but there are as yet no methods available for its direct measurement (Benke, Hall, Hawkins, Lowe-McConnell, Stanford, Suberkropp & Ward, 1988; Meyer, McDowell, Bott, Elwood, Ishizaki, Melack, Peckarsky, Peterson & Rublee, 1988). Microbes enhance the palatability and nutritional content of food for invertebrates. Microbes also colonise detritus, a major source of food for

invertebrates in freshwater ecosystems. Detritus is a low quality food for invertebrates and passes rapidly through their guts (Berrie, 1976). Invertebrates are only able to assimilate a small portion of plant material ingested but they are able to strip the detritus of micro-organisms (high quality food) rather than utilise the detritus itself (Newell 1965).

Fisher and Likens (1973) working on Bear Brook in the Hubbard Forest of northern New Hampshire found that of the organic input into the river 66% was transported downstream in the form of CPOM, FPOM, and DOM. The remaining 34% was used locally, of which the invertebrates account for only 0.2% the rest being utilised by micro-organisms. The importance of the microbial food loop in the nutrition of invertebrates and regeneration of nutrients and energy in stream ecosystems needs to be fully examined.

The gut micro-flora of simuliid larvae are responsible for the qualitative change of food material. Food passing through their digestive tract is rapidly colonised by micro-organisms (Hargrave, 1976) and so becomes more palatable to other species. Simuliids are also an important energy subsidy for predators particularly because their frequent occurrence in drift makes them available to many predators (Cummins, 1987).

In summary, simuliids (and other invertebrates) have a limited role as direct processers of organic matter in river ecosystems. Results from Fisher and Likens's study (1973), show that micro-organisms are more important than invertebrates as processers of organic matter. The synergistic effects of invertebrates and micro-organisms appear to be central in energy processing in rivers.

Invertebrates are responsible for retarding the flow of detritus downstream, increasing surface area of particles and inoculating particles with bacteria. The indirect effects of invertebrates on energy processing are therefore more important than their direct effects. The presence of both invertebrates and microorganisms are essential for effective processing of organic matter. For these reasons, the kind of bioenergetic techniques used in this study, while providing some useful insights on simuliid larvae are unlikely to reflect the role and importance of any species fully.

A number of conceptual and practical problems with bioenergetic studies have been highlighted by this project. Firstly, all experiments which were conducted in this project were at one water temperature. Temperature is known to affect ingestion and assimilation rates and so the results obtained in this study should be viewed with this in mind.

The difference in ingestion rate obtained on river water in the laboratory and the field (see Chapter 3) indicate that conditions in the laboratory were not optimal for larval feeding. Water currents created by airstones, the lack of unidirectional water flow, the recycling of water during an experiment and the unavoidable handling of the larvae may all have resulted in stressing the larvae and therefore reducing ingestion rates. It appears that unfavourable laboratory conditions rather than the food type may have caused decreased ingestion rates. This difference in ingestion rates on the same food type under laboratory and field conditions needs to be noted for those workers who extrapolate laboratory data for use in the field.

Algae in this study were assimilated at very low rates (see chapter 4). Detritus is not only plentiful in the natural environment but forms the basis of simuliid diets. (Smears of guts from simuliids collected along the length of the Buffalo River during the year revealed that the major constituent of the gut was detritus, algal cells when present, constituted a very small fraction.) An extremely useful study would be to determine the assimilation rate of simuliids on detritus directly. A number of workers have determined assimilation efficiencies of detritus indirectly and the results range from 1.75% (Wotton, 1978b) to 25% (Morin *et al.*, 1988) (see Table 1.0). The direct computation of the assimilation efficiency of simuliids on detritus would give an indication of detritus utilisation. Detailed studies would need to be conducted on each river system for more accurate accounts of detritus utilisation in specific river systems.

The lack of studies on invertebrate and micro-organism bioenergetics and interactions in the literature is apparent and this field needs to be studied further in order to obtain a more holistic view of energy processing in river systems.

Another potential source of error in this study was the nature of the survey of the Buffalo River. Estimations and extrapolation of data were necessary but this reduces the accuracy of the study. A more extensive survey of the Buffalo River with quantitative sampling of simuliid habitat along greater lengths of the river although time consuming, would result in a more accurate picture

of the importance of blackfly larvae in energy processing. However, since this was not the major aim of the project the lack of time and manpower resources restricted us in many cases to making coarse estimates.

Invertebrates in rivers are responsible for the partial cleansing of polluted water. They are able to extract seston, DOM, FPOM and CPOM from the water column for use in their own life processes, therefore cycling nutrients and energy. This removal of seston and organic matter by simuliids helps to cleanse the water, making it available for further human use. The importance of rivers needs to be realised not only for their ecological, recreational and aesthetic attributes but also because they are the major sources of fresh water in South Africa, and therefore constitute one of the most important limiting factors in South Africa's economic prosperity.

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Appendix A: Simuliidae found in the Buffalo River.

Subgenus ANASOLEN Enderlein, 1930

1. *dentulosum* Roubaud, 1915

Subgenus EDWARDSELLUM Enderlein, 1921

2. *damnosum* Theobald, 1903 (complex)

Subgenus FREEMANELLUM Crosskey, 1969

3. *debegene* de Meillon, 1934
4. *hirsutilateris* de Meillon, 1937

Subgenus MEILLONIELLUM Rubtsov, 1962

5. *adersi* Pomeroy, 1922

Subgenus METOMPHALUS Enderlein, 1935

6. *hargreavesi* Gibbins, 1934
7. *letabum* de Meillon, 1935
8. *medusaeforme* Pomeroy, 1920
9. *natalense* de Meillon, 1950
10. *vorax* Pomeroy, 1922

Subgenus NEVERMANNIA Enderlein, 1921

Group 2: LOUTETENSE species-group

11. *rutherfordi* de Meillon, 1937

Group 3: RUFICORNE species-group

12. *nigritarse* Coquillett, 1902
13. *ruficorne* Macquart, 1838

Subgenus POMEROYELLUM Rubtsov, 1962

14. *alcocki* Pomeroy, 1922
15. *bequaerti* Gibbins, 1936
16. *impukane* de Meillon, 1936
17. *rotundum* Gibbins, 1936
18. *unicornutum* Pomeroy, 1920