CANADIAN THESES ON MICROFICHE

THÈSES CANADIENNES SUR MICROFICHE

National Library of Canada
Collections Development Branch

Canadian Theses on
Microfiche Service

Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada
Direction du développement des collections

Service des thèses canadiennes
sur microfiche

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

THIS DISSERTATION HAS BEEN MICROFILMED EXACTLY AS RECEIVED

LA THÈSE A ÉTÉ MICROFILMÉE TELLE QUE NOUS L'AVONS REÇUE

Canada

NL 339 (r. 85/09)

THE UNIVERSITY OF ALBERTA

COLONIZATION AND ABUNDANCE OF EPHEMEROPTERA NYMPHS ON LOTIC SUBSTRATES, WITH SPECIAL REFERENCE TO TEXTURE, COLOUR, AND DIEL PERIODICITY

by

RICHARD J. CASEY

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA

FALL 1986

ISBN 0-315-32580-1

THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR RICHARD J. CASEY

TITLE OF THESIS COLONIZATION AND ABUNDANCE OF EPHEMEROPTERA NYMPHS ON LOTIC SUBSTRATES, WITH SPECIAL REFERENCE TO TEXTURE, COLOUR, AND DIEL PERIODICITY

DEGREE FOR WHICH THESIS WAS PRESENTED MASTER OF SCIENCE

YEAR THIS DEGREE GRANTED FALL 1986

(SIGNED) .....

PERMANENT ADDRESS:

.....

.....

.....

DATED .....19 .....

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled COLONIZATION AND ABUNDANCE OF EPHEMEROPTERA NYMPHS ON LOTIC SUBSTRATES, WITH SPECIAL REFERENCE TO TEXTURE, COLOUR, AND DIEL PERIODICITY submitted by RICHARD J. CASEY in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE.

Supervisor

Date 10 October 1986

ABSTRACT

I investigated the colonization of stream substrates by Ephemeroptera nymphs in Dyson Creek, Alberta. Stream substrates used in my study were of two textures (smooth and rough) and two colours (dark and light); the organisms studied were *Drunella coloradensis* (Ephemerellidae), Heptageniidae (*Cinygmula* and *Epeorus*), *Baetis* (Baetidae), and *Ameletus* (Siphlonuridae). I tested the hypothesis that *D. coloradensis* nymphs and other abundant benthic organisms, as they increase in size, choose a dark substrate to remain cryptic and a substrate of greater texture to reduce the risk of being swept from the substrate by the water flow.

Colonization by *D. coloradensis* nymphs was investigated by separating the immatures into four developmental stages. Substrates in baskets were retrieved from a study riffle after 14 day colonization periods. For all developmental stages the intoduced rough-dark substrate was least preferred and the Dyson Creek substrate was most preferred. However, these preferences were statistically significant for intermediate developmental stages only. Reduced numbers of nymphs on the rough-dark substrate may be attributed to reduced water velocity and a build up of fine material on the rocks inhibiting colonization. The preference for the Dyson Creek substrate is thought to be related to the small size and increased number of substrate interstices, which account for a build up of detritus in current-protected interstices. For nymphs of developmental stage three, the introduced smooth-light substrate and the Dyson Creek substrate were most preferred, but it is not known why the nymphs chose the smooth-light substrate relative to the smooth-dark substrate, since the nymphs would be conspicous to a visually-foraging predator against a light-coloured background.

Colonization was investigated for the other Ephemeroptera nymphs in Dyson Creek by making direct observations on the substrate baskets, using a glass-bottomed box. Total Fauna (i.e. all of the Ephemeroptera taxa in my study) most preferred the smooth-light substrate and least preferred the smooth-dark substrate after a short and long colonization period. In general, this trend in substrate preference was evident for each taxon in the Total Fauna group.

In the second part of my research, I documented the diel periodicity in densities of Ephemeroptera nymphs by making direct observations of the nymphs when they were on top of the undisturbed Dyson Creek substrate. Observations were made twice during a new moon and twice during a full moon; the times of darkness and full daylight were noted. Other abiotic factors were recorded including incident light, percent cloud cover, water temperature. Drift of nymphs was recorded at a later date. The factors above were recorded to determine if they were related to the observed diel periodicity.

In response to the artificial light used for observations at low illumination, Total Fauna, *Cinygmula*, and *Baetis* nymphs were not found to be negatively phototactic. In general, for Total Fauna and each taxon in Dyson Creek, the diel periodicity in density was similar between moon phases and densities were greater in the dark relative to the light period. Small and large size classes of Heptageniidae and *Baetis* nymphs showed trends in diel density similar to the trends when the sizes were considered together. Incident light, percent cloud cover, and drift were not found to be related to the diel periodicity of the nymphs. However, water temperature was positively correlated with the densities of most groups of nymphs. These factors and the potential effect of predation and polarized light are discussed as possible mechanisms in the diel periodicity in the density of immature aquatic insects.

ACKNOWLEDGEMENTS

I would like to sincerely thank my supervisor Dr. Hugh F. Clifford for the opportunity to do this research, his advice, encouragement, and thorough editing throughout my work. I thank my supervisory committee Drs. D. A. Boag and M. Hickman, and my defence chairperson Dr. S. J. Hannon for their helpful comments on my work.

Various graduate students were very helpful when I was formulating my research topics and I would especially like to thank Dr. Jan Ciborowski, Vytenis Gotceitas, and Dan Soluk. I thank Drs. Jan Ciborowski and Doug Craig and Gael Ogilvie for reviewing and providing helpful comments on parts of this work. Drs. R. K. Allen and C. P. Hawkins provided information and assistance for the identification of *Drunella coloradensis* nymphs.

Thanks to Drs. Jan Ciborowski and Lynda Corkum who braved Alberta winter conditions to introduce me to Dyson Creek, and Dr. Boag for the opportunity to work and live at the R. B. Miller Biological Station. Thanks to the 1983 and 1984 crews at the R.B.M.B.S. and fellow graduate students, especially Kerry Irons, at the U of A who have given me many fond memories. I thank Gertie Hutchinson for her cheery help in technical matters.

I wish to thank my parents who have always encouraged me in my zoological pursuits on land and in water. Maybe some day I will learn to swim!

I acknowledge the financial support from an NSERC operating grant to H. F. C. and University of Alberta teaching assistantships and summer bursaries to myself.

Table of Contents

Chapter		Page
I.	GENERAL INTRODUCTION	1
	A. REFERENCES	4
II.	COLONIZATION OF ARTIFICIAL SUBSTRATES OF DIFFERENT TEXTURES AND COLOURS BY EPHEMEROPTERA NYMPHS USING RETRIEVAL AND DIRECT OBSERVATION TECHNIQUES.	6
	A. INTRODUCTION	6
	B. STUDY SITE	8
	C. MATERIALS AND METHODS	10
	Substrate Basket Experiment	10
	Direct Observations	12
	D. RESULTS	14
	Substrate Basket Experiment	14
	Direct Observations	16
	E. DISCUSSION	18
	Substrate Basket Experiment	18
	Direct Observations	23
	F. REFERENCES	40
III.	DIEL PERIODICITY IN DENSITIES OF EPHEMEROPTERA NYMPHS ON THE SUBSTRATE AND THE RELATIONSHIP WITH ABIOTIC AND BIOTIC FACTORS.	46
	A. INTRODUCTION	46
	B. MATERIALS AND METHODS	48
	C. RESULTS	50
	Data Treatment	50
	Phototaxis	51
	Moonlight	51
	Dark/Light Densities	53

Diel Peak and Trough Densities 54
Drift 55
Size Class Differences 55
Abiotic Factors 56
D. DISCUSSION 57
Nymphal Behaviour 57
Phototaxis 57
Moonlight 58
Dark/Light Densities 59
Drift 61
Size Class Differences 63
Abiotic Factors 64
Concluding Remarks 67
E. REFERENCES 91
IV. CONCLUDING DISCUSSION 97
A. REFERENCES 103

LIST OF TABLES

Table		Page
II-1	Mean and standard deviation of the physical measurements of the substrate particles, and the water velocity and depth at the four substrate types, the rough-dark substrate, the Dyson Creek substrate, the smooth-dark substrate, and the smooth-light substrate.	26
II-2	Mean abundance and standard deviation on colonization periods one to nine, for the four developmental stages of *Drunella coloradensis* on each substrate type.	27
II-3	Multiple comparison test results of the abundance of the four developmental stages of *Drunella coloradensis* nymphs on each substrate type.	28
II-4	Mean abundance and standard deviation for each taxon group observed after the short and long colonization periods on each substrate type, with probability levels of the Friedman test to determine differences between the substarte types.	29
II-5	Mean density and standard deviation for the two nymphal size classes of taxon groups observed after the short and long colonization periods on each substrate type, with probability levels of the Friedman test to determine differences between the substrate types.	30
II-6	Multiple comparison test results of the density for each taxon group on each substrate type after the short and long colonization periods.	31
III-1	Mean and standard deviation of the abiotic factors measured during the four 24 h studies on new moon one study, on full moon one study, on new moon two study, and on full moon two study.	68
III-2	Total number of organisms observed and indices of the proportional densities of taxon groups of Ephemeroptera nymphs in the dark over light period for the four 24 h studies of observations.	69
III-3	Mean times of density peaks and troughs of the taxon groups of Ephemeroptera nymphs for the four 24 h studies of observations.	70

LIST OF FIGURES

Figure		Page
II-1	Mean water velocity and 95 % confidence intervals at the 24 sites for the substrate basket experiment, during the period from June 1983 to July 1984	32
II-2	Mean water depth and 95 % confidence intervals at the 24 sites for the substrate basket experiment, during the period from June 1983 to July 1984	34
II-3	Mean water temperature and ranges during the period from June 1983 to July 1984.	36
II-4	Diagrammatic representation of a substrate basket in place in the stream bed, the substrate basket experimental design, and the arrangment of substrate baskets for the direct observations.	38
III-1	Densities of Total Fauna per 800 cm^2 of substrate at 30 min intervals during the four 24 h studies of observations on new moon one study, on full moon one study, on new moon two study, and on full moon two study.	71
III-2	Densities of *Cinygmula* and Heptageniidae during the 24 h studies of observations.	73
III-3	Densities of *Baetis* and *Baetis-Ameletus* during the 24 h studies of observations.	75
III-4	Densities of *Drunella coloradensis* during the 24 h studies of observations.	77
III-5	Model of the mean density of Total Fauna per 800 cm^2 at the mean times of density peaks and troughs, during the four 24 h studies of observations in Dyson Creek.	79
III-6	Mean and range of two concurrent drift samples of Ephemeroptera nymphs taken at 3 h intervals over a 24 h period.	81
III-7	Densities of small *Cinygmula* and Heptageniidae nymphs during the 24 h studies of observations.	83
III-8	Densities of large *Cinygmula* and Heptageniidae nymphs during the 24 h studies of observations.	85
III-9	Densities of small *Baetis* and *Baetis-Ameletus* nymphs during the 24 h studies of observations.	87
III-10	Densities of large *Baetis* and *Baetis-Ameletus* nymphs during the 24 h studies of observations.	89

I. GENERAL INTRODUCTION

The colonization of substrates by benthic macroinvertebrates is important to our understanding of the ecology of stream invertebrates, because substrate is a major physical component of a lotic ecosystem. Our knowledge of the rates and processes of colonization and the actual choice, or preference, of substrate type by macroinvertebrates has increased greatly in recent years (see reviews by Rosenberg and Resh 1982, Minshall 1984, Sheldon 1984). Substrate preference has been studied by examining the physical characteristics of substrates, for example, size of substrate particles, surface area, substrate shape, and microspatial complexity (heterogeneity). Other potentially important characteristics of substrates are texture and colour, and these have not been studied in much detail.

Substrate texture has been studied as gross texture, for example, Hart (1978) described a type of spatial heterogeneity of a substrate as having a series of grooves on the surface. On a smaller scale, micro-texture may be important, for example, by increasing the ability of organisms to hold onto a substrate or as a collecting mechanism for saltating particles from the water column. These saltating particles may be in the form of inorganic and/or organic matter. Micro-texture has received little attention, possibly because of the absence of quantitative methods to measure texture. Previously, texture has been measured qualitatively (e.g. Nilsen and Larimore 1973) or semi-quantitatively (Erman and Erman 1984). In my study, texture was quantified using a 'profile meter', that has been developed and used elsewhere (Clifford et al., in preparation, and see Chapter II).

Colour of substrate has also been suggested to be important to a stream invertebrate's choice of substrate (e.g. Hynes 1970a). Because most aquatic invertebrates, especially immature aquatic insects, are cryptically coloured, the colour of the background substrate is probably of considerable importance, for example, in reducing predation by visually-feeding predators (see Popham 1941).

I used two methods to determine the importance of micro-texture and colour to colonization by immature aquatic insects. The first was to use methodology associated with the use of artificial substrates, i.e. using substrate basket units that could be placed

in the stream bed and sampled at regular intervals. Instead of artificial substrates, I used natural rocks from streams; these rocks had similar physical characteristics, but differed in the physical characteristics to be tested. Natural rocks were chosen because artificial substrates may be selective for particular organisms, and what colonizes them may not represent the benthic community. For example, when artificial substrate and benthic samples are compared in the same study, they are often very different in taxon composition (Rosenberg and Resh 1982). By using the methodology associated with artificial substrates, the sampling protocol and substrates (with limitations of availibility) were standardised in the experimental design. The second method employed was to make direct observations of colonization by aquatic insects on the same substrates used in the substrate basket experiment. Direct observations are more likely to reflect the actual situation on the substrate rather than the disturbance caused to the substrate, for example, when using a sampling procedure such as a quantitative sampler.

Diel periodicity of aquatic insect larvae has been studied extensively in the last 25 years. Most of the early work in the 1960's was associated with the independent discoveries of diel periodicity in drift of lotic invertebrates by Tanaka (1960), Waters (1962), and Müller (1963). Many of the later studies attempted to determine whether drift and the diel density of macroinvertebrates were related. Results usually indicated that there was no relationship between drift and density periodicity in the field or in the laboratory (see Chapter III).

Studies of diel periodicity of benthic invertebrates indicate different results. For example, some studies of aquatic insects report increased activity pattern at night, other studies found a decrease at night, and others report no changes in activity between day and night. But comparisons between studies may be hindered by differences in methodology, such as benthic samplers, direct observations, and artificial substrates. Also, large sampling intervals were used in several studies, for example, two samples per 24 h period (see Chapter III).

Recent studies have used direct observations to obtain information on the diel density of aquatic invertebrates (Statzner and Bittner 1983, and see Chapter III). I chose

this method because it would appear to have the least effect on the natural behaviour and distribution of organisms on the substrate. My study was designed to determine whether a diel periodicity in density of immature aquatic insects occurred in a stream, and if so whether this diel periodicity was related to diel periodicity of abiotic factors and drifting organisms. I attempted to determine the importance of the abiotic environment in diel periodicity by measuring several potentially important abiotic factors. For example, moonlight has been suggested to influence drift (see reviews of Hynes 1970b, Waters 1972, Müller 1974); therefore I made observations during new moon and full moon phases. Abiotic and biotic factors measured including other possible mechanisms are discussed in relation to the observed diel density patterns.

A. REFERENCES

Clifford, H. F., R. J. Casey, V. Gotceitas, and M. Dunnigan. A device to quantify texture of substrata, with special reference to stream substrata. In preparation.

Erman, D. C., and N. A. Erman. 1984. The response of invertebrates to substrate size and heterogeneity. Hydrobiology 108: 75-82.

Hart, D. D. 1978. Diversity in stream insects: regulation by rock size and microspatial complexity. Verh. Internat. Verein. Limnol. 20: 1376-1381.

Hynes, H. B. N. 1970a. The ecology of running waters. University of Toronto. 555 p.

Hynes, H. B. N. 1970b. The ecology of stream insects. Ann. Rev. Entomol. 15: 25-42.

Minshall, G. W. 1984. Aquatic insect-substratum relationships. p. 358-400. *In* V. H. Resh and D. M. Rosenberg [ed.] The ecology of aquatic insects. Preager Publishers, NY.

Müller, K. 1963. Diurnal rhythm in 'organic drift' of *Gammarus pulex*. Nature 198: 806-807.

Müller, K. 1974. Stream drift as a chronobiological phenomenon in running water ecosystems. Ann. Rev. Ecol. System. 5: 309-323.

Nilsen, H. C., and R. W. Larimore. 1973. Establishment of invertebrate communities on log substrates in the Kaskaskia River, Illinois. Ecology 54: 366-374.

Popham, E. J. 1941. The variation in the colour of certain species of *Arctocorisa* (Hemiptera, Corixidae) and its significance. Proc. Zool. Soc. Lond., Ser A 3: 135-172.

Rosenberg, D. M., and V. H. Resh. 1982. The use of artificial substrates in ths study of freshwater benthic macroinvertebrates, p. 175-235. *In* J. Cairns Jr. [ed.] Artificial substrates. Ann Arbor Science Publishers Inc., Ann Arbor, MI.

Sheldon, A. L. 1984. Colonization dynamics of aquatic insects, p. 401-429. *In* V. H. Resh and D. M. Rosenberg [ed.] The ecology of aquatic insects. Praeger Publishers, NY.

Statzner, B., and A. Bittner. 1983. Nature and causes of migrations of *Gammarus fossarum* Koch (Amphipoda) - a field study using a light intensifier for the detection of nocturnal activities. Crustaceana 44: 271-291.

Tanaka, H. 1960. On the daily change of the drifting of benthic animals in a stream, especially on the types of daily change observed in taxonomic groups of insects. [Japanese, English Summary] Bull. Freshwater Fish. Res. Lab. 9: 13-24.

Waters, T. M. 1962. Diurnal periodicity in the drift of stream invertebrates. Ecology 43: 316-320.

Waters, T. M. 1972. The drift of stream insects. Ann. Rev. Entomol. 17: 253-272.

II. COLONIZATION OF ARTIFICIAL SUBSTRATES OF DIFFERENT TEXTURES AND COLOURS BY EPHEMEROPTERA NYMPHS USING RETRIEVAL AND DIRECT OBSERVATION TECHNIQUES.

A. INTRODUCTION

There have been many studies on the colonization of freshwater benthic macroinvertebrates on substrates in lotic environments (see review by Minshall 1984). In most studies, artificial substrates have been used simply as a method of sampling the benthos. More recent work has concentrated on colonization dynamics and specific physical attributes of the substrates that might affect the preference, or choice, of a substrate by benthic macroinvertebrates (see reviews by Rosenberg and Resh 1982, Minshall 1984, Sheldon 1984). Some of the latter studies have included substrate size (e.g. Cummins and Lauff 1969, Rabeni and Minshall 1977, Shelly 1979, Khalaf and Tachet 1980, Reice 1980), particle size mixtures (e.g. Wise and Molles 1969, Williams 1980, Erman and Erman 1984), surface area (e.g. Minshall and Minshall 1977, Khalaf and Tachet 1980), and substrate shape and microspatial complexity (e.g. Hart 1978). Minshall (1984) has extensively reviewed recent work on the substrate characteristics thought to be important to organism-substrate relationships. Other abiotic factors of substrates have been suggested to be important to benthic macroinvertebrate colonization but have received little attention. Amongst these are surface roughness (or texture) (Erman and Erman 1984) and colour of inorganic substrates. There has been some work on substrate texture of organic substrates, for example, macrophytes (Nilsen and Larimore 1973). Substrate colour has received little attention except for anecdotal (e.g. Hynes 1970) or indirect references (e.g. Hughes 1966).

Surface roughness is a measure of surface irregularities of substrate particles. Roughness may include macroscale or microscale irregularities. In my study, I am considering the importance of micro-texture. Micro-texture is considered to be important to many benthic organisms, for example, because the invertebrate may attain a more secure foothold on a rougher substrate and hence reduce the chance of being

swept downstream. Differences in tarsi structure and function of dorsoventrally flattened mayfly nymphs may be related to substrate preference (Madsen 1968, Percival and Whitehead 1929). Elliott (1967) has suggested that the absence of certain insect species in the drift may be related to differences in the nymph's ability to hold onto a substrate. Also, rough substrates may indirectly affect benthic organisms by increasing the surface area available for colonization and by creating a more favourable environment for silt and detritus deposition.

Colour is defined here as a relative measure of dark- and light-contrasting substrates and how these contrasting substrates may be important to colonization by benthic organisms. Many immature aquatic insects are cryptically coloured, and this may be a response to predation pressure from visually-foraging predators (Hutchinson 1981, Peckarsky 1982, Allan 1983, Otto 1984). An organism may reduce predation by choosing a suitable background colour on which to forage in the daylight, thereby potentially reducing predation (Popham 1941, Otto 1984). Popham (1941) observed a direct relationship between the colour of corixids and the background colour in the field and in the laboratory. In the laboratory, a fish predator attacked more corixids that contrasted with the background colour. As an organism grows, it will be more obvious to visually-feeding predators. Thus, larger animals would be expected to reduce predator attacks by choosing a background colour of substrate to remain cryptic.

The stream bed materials of the eastern slopes of the Canadian Rocky Mountain foothills of Alberta provided an opportunity to test whether insect nymphs could be making a choice of a particular substrate type. These stream bed particles are diverse in their geological types; they include sandstone, shale, limestones, and conglomerates, and they are composed of rocks of various sizes, shapes, textures and colours.

I chose *Drunella coloradensis* Dodds (Ephemerellidae: Ephemeroptera) as the principal study organism because the nymphs are generally cryptically coloured with variable dark mottled markings (Dodds 1923, Walley 1930, Allen and Edmunds 1962). The dark colouration is usually more evident on older nymphs. Also, *D. coloradensis* is available in large numbers in streams of the Canidian Rocky Mountains. Gilpin and Brusven (1970) stated that their darker colour makes the *D. coloradensis* nymphs

difficult for a visually-foraging predator to see the nymphs on dark rocks. Behaviourally, *D. coloradensis* nymphs have been classified as clingers and sprawlers (Cummins et al., 1984). This suggests the nymphs are thigmotactic and texture may be important to the nymphs.

I tested the hypothesis that as *D. coloradensis* nymphs and other abundant benthic organisms increase in size they choose a dark substrate to remain cryptic and a substrate of greater texture to reduce the risk of being swept from the substrate by the water flow. I used two approaches, both involving substrate baskets. The first was to count four developmental stages of *D. coloradensis* nymphs on substrates of different texture and colour over a set colonization period. I attempted to standardise the physical characteristics of the substrates by taking measurements of size and texture. Also the sampling method and environment were standardised by placing rock substrates in wire baskets in a uniform habitat. The second approach was to make direct observations of various insect nymphs on the same substrate baskets, where the nymphs were allowed to move between adjacent baskets. From these observations, I expected taxa to choose particular substrate characteristics based on nymphal morphology and behaviour.

B. STUDY SITE

The study was conducted in Dyson Creek (50° 39' N, 114° 38' W), a second order Alberta stream (map scale = 1:50,000) in the eastern foothills of the Canadian Rocky Mountains. Dyson Creek rises in alpine vegatation on exposed sandstone bedrock at about 2,100 m elevation. The stream flows for about 12 km through two main land zones (after AENR 1979) before joining with the Sheep River at an elevation of about 1,450 m. The zone above the study site is comprised of steep slopes of sandstone overlain with shale and till deposits. These slopes are heavily forested with lodgepole pine (*Pinus contorta*), smaller stands of white spruce (*Picea glauca*) and aspen (*Populus tremuloides*). The area downstream and including the study site is mostly glacial till on less steep slopes. The main vegetation here is lodgepole pine with stands

of aspen. Willow (*Salix*) is common along much of the stream banks. The stream bed materials are predominantly sandstone with varying amounts of other rocks including limestones and shales. These are the predominant bedrock materials of the area (for further details see AENR 1979).

The study site (elevation = 1555 m), was a slow flowing riffle located about 200 m downstream from a waterfall-pool-riffle-pool series, and upstream from a log-dammed pool. At the study site the stream flowed in a northeasterly direction. The south bank of the riffle rises steeply for about 4 m to a flat area of bushes, white spruce and pine. The north bank was level for several meters away from the stream edge before rising steeply; this slope was forested with mainly poplars. There was little overhanging vegetation along the banks except for some willow on the south bank.

The study site on Dyson Creek was approximately 30 m long. It was chosen primarily because the substrate at this site was of a relatively uniform pebble size (mean size = 4.7 cm, range = 2.1-8.2 cm). The substrate at the study site was strikingly different from that found in the remainder of the stream bed, which was predominantly cobble and pebble (for further details see Gotceitas and Clifford 1984). I assumed that current velocity and depth would vary little spatially, in the riffle. Mean water velocity and mean depth in the riffle over the study period were 21.3 cm s^{-1} and 11.9 cm, respectively (Figures II-1 and -2).

Water temperature typically increased after ice breakup in late April to early May. Maximum water temperatures were in late summer; temperatures then decreased to winter levels by October (Figure II-3). During the period from late October to early May, the stream was normally ice-covered and water temperature was close to 0° C. The mean width of the riffle was 4.9 m with a range of 3.6 to 6.6 m; the maximum width was measured during the greatest mean water depth (Figure II-2). The mean discharge was 0.21 m^3 s^{-1} with a range of 0.04-0.49 m^3 s^{-1} (the widths and discharges were measured from May through July 1984). Intermittent high and low readings of water velocity and depth reflected ice breakup and snow melt in the upper reaches of Dyson Creek (Figures II-1 and -2). Turbidity was low, except during the spring runoff. Throughout 1983 to 1984 there was no major disturbance to the stream's substrate.

C. MATERIALS AND METHODS

Substrate Basket Experiment

The experimental design included four types of substrate, a smooth-dark (SD) substrate, a smooth-light (SL) substrate, a rough-dark (RD) substrate, and the substrate from Dyson Creek (DC). The first three substrates were collected from local streams where the desired substrates were more abundant than in Dyson Creek. I chose the substrates based on their mineral composition, size, shape, and colour. Mineral composition of substrates was used because this would determine textural differences between the substrates (see below). The SD substrate was a black limestone, the SL substrate was a white limestone, the RD substrate was a dark olive-green sandstone, and the DC substrate was made up of the larger particles at the study site, which was a predominantly dark coloured mixture. The size of the substrates was determined by measuring the maximum and minimum lengths through the central point of each rock, and the diagonal length (i.e. between the maximum and minimum) for each rock. Mean length (or diameter) was calculated from these measurements. The size of rock used was about the size of rocks on which *D. coloradensis* nymphs are normally found (Gilpin and Brusven 1970, Allan 1975, Hawkins, 1984). I usually chose oval shaped rocks. Colour was determined by visually matching all substrates of one colour and shade together (except the DC substrate).

Although the differences in substrate texture were based on their mineral components, limestone versus sandstone (except for the DC substrate), a quantitative measure of texture was obtained using a 'profile meter' (Clifford et al., in prep.). The profile meter measured the roughness of substrates by driving a stylus across the surface of a rock. The vertical movement of the stylus was recorded by a linear voltage displacement transducer and plotted on a chart recorder. The chart record (or profile) gave an approximate visual interpretation of a substrate surface. Profiles were digitized and statistically analysed. An approximate measure of the surface area of the substrate particles was calculated using the formula for the area of a sphere. This was

done for particles in only one basket of each substrate type; the baskets were chosen randomly. Using the measure of area, total surface area available for colonization per basket was calculated.

The four substrates were placed in 1.3 cm screen wire baskets, 25 x 25 x 10 cm, with larger rocks overlain by smaller rocks in each basket. The baskets were filled with substrate level to the top of each basket. Six replicates of each substrate type were used. The 24 substrate baskets were placed in the study riffle, with the top of the basket flush with the stream bed surface (Figure II-4, A). They were arranged in the following scheme beginning with the most upstream baskets: RD, SD, DC, SL, RD, ... etc, in rows of three trays, making up three longitudinal strata of eight baskets each (Figure II-4, B). Therefore, there were two replicates of each substrate type in the three longitudinal strata. The mean lateral distance between the substrate baskets was 1.0 m and the mean upstream-downstream distance between the baskets was 2.9 m. The mean distance between the outer baskets in the design and the stream bank was 0.9 m (measurements based on the minimum stream width).

The baskets were allowed to be colonized by benthic organisms for 14 day periods. There were two exceptions: the third colonization period was 15 days and the ninth colonization period was 13 days. There were nine trials, five during 1983 (June to September) and four in 1984 (May to July). This was necessary to assure that most of *D. coloradensis* nymphal life history stages would be included. *Drunella coloradensis* is a univoltine species in Dyson Creek. The eggs hatch during the winter, the nymphs grow rapidly after ice breakup (ca. May) and the adults emerge in August and September. The results for the nine colonization periods were ordered consecutively to correspond with four developmental stages (DS1 to DS4) of *D. coloradensis* nymphs. The developmental stages were used because the number of true instars are difficult to determine in ephemeropterans. Developmental stage one (DS1) nymphs lacked wing pads, developmental stage two (DS2) nymphs had wing pad lengths shorter than the distance between the base of the pads. Developmental stage three (DS3) nymphs had wing pads longer than the distance between the base, and developmental stage four (DS4) nymphs had darkened wing pads (after Clifford 1970).

At the end of a colonization period, the baskets were taken out beginning at the downstream baskets and moving upstream. A stainless steel three-sided shovel (28 x 28 x 10 cm) was scooped under the substrate basket, while holding a net (frame = 30 x 30 cm, length = 1 m, mesh = 0.037 mm) immediately downstream, and the basket was lifted out of the water. The substrate in each basket was processed immediately on the stream bank. Detritus (mainly leaves) did not accumulate in large quantities amongst the substrate particles; however, in spring and early summer leaves and organic material accumulated on the top-sides of some the baskets. Any detritus attached on the outside of the basket was removed and not considered as part of a sample. The rocks were taken out of the basket and placed in enamel dishes. The residue in the shovel apparatus and net were also washed into the dishes, and each rock was washed over the dish using a baster. After carefully examing the rocks for any remaining nymphs, the substrates were replaced in the basket. The organisms in the enamel dishes were sieved (mesh = 0.037 mm) and preserved in 90 % ethanol. The substrate basket was then replaced in the stream. Processing each substrate basket took about 20 min. In the laboratory, *D. coloradensis* nymphs were sorted using a dissecting microscope (magnification 60 x) and then separated (using 240 x magnification) into developmental stages.

Water velocity (at 0.6 x depth using an Ott C-1 current meter) and depth were recorded weekly, over each basket. Temperature was recorded using a thermograph (Peabody Ryan, model H). Spot temperature readings were also taken.

Direct Observations

Sixteen baskets were randomly selected from the previous substrate colonization experiment (above) after the baskets had been in the riffle for 8 days. All organisms and debris were washed off the rocks, and then the baskets were placed in the study riffle in four groups of the substrate types (SL, SD, RD,and DC). Each group of four baskets was arranged in a matrix so that they were touching. The four substrate types in the matrices occupied all possible positions in the arrangement relative to the current

direction and stream banks (Figure II-4, C). Mean distance between the upstream and downstream edge of each group of baskets was 1.1 m, and there was a mean distance of 0.8 m to the stream bank.

I made direct observations of organisms on the substrates in July and August 1984 using a Plexiglass box (50 x 25 x 30 cm) with a glass bottom and adjustable legs. Eight 10 x 10 cm quadrats were placed in the bottom of the box, and the organisms were counted within these areas. At any one time two substrate baskets were covered by the observation box (four 10 x 10 cm quadrats covering each basket). The 16 baskets were viewed in a downstream to upstream direction. Observations were made at 30 min intervals after the box was left in place for 20 min over the substrate baskets. Before making observations, I remained motionless kneeling over the observation box for 2-3 min, and I counted the organisms for 1 min in each 10 x 10 cm quadrat.

Observations were done for two colonization periods after 2-5 days (short colonization), and 13-14 days (long colonization). Observations were recorded on three occasions in 1984: July 19-22 (days 2, 3 and 5), August 14-15 (days 2 and 3), and August 25-26 (days 13 and 14). For each observation date, the organisms on the substrate were counted in each of the 16 baskets. All observations were recorded at approximately the same time of the day (0700-1300 h) to reduce any diel change in the density of organisms (see Chapter III). The organisms were identified to the lowest taxon, and the body length (excluding the cerci) of each organism was recorded with the aid of a ruler in the bottom of the observation box.

Abiotic parameters were recorded at 2 h intervals throughout the observation period. These parameters were incident light (about 10 cm above the water surface), percent cloud cover, precipitation, and water temperature. Also, water velocity and water depth were recorded at irregular intervals.

D. RESULTS

Substrate Basket Experiment

Mean diameters of the four substrates could not be successfully transformed to achieve homogeneity of variances, hence the nonparametric Kruskal-Wallis test was used to compare the four substrates. The mean diameters of the substrates were significantly different (Kruskal-Wallis test, $p < 0.01$). The mean diameter of the Dyson Creek substrate was less than the other substrates (Table II-1), because the Dyson Creek particles were collected in the study site where the mean diameter was small. The quantitative measure of substrate texture was transformed using the fourth root transformation (Downing 1979) and the data was normally distributed and the variances were homogeneous. Then the one-way ANOVA test was used to compare the substrates.

Textures were significantly different (ANOVA, $p < 0.01$, Table II-1). The textures were categorised into two groups (Newman-Keuls test, $p < 0.05$), the Dyson Creek substrate with the rough-dark substrate, and the smooth-light substrate with the smooth-dark substrate; the former group had a rougher texture than the latter group (Table II-1). The average size of the sand grains in the rough-dark (sandstone) substrate was about 0.33 mm. The Dyson Creek substrate was rougher than the smooth-light and smooth-dark (limestone) substrates because the Dyson Creek substrate contained a high proportion of sandstone and conglomerates. The mean surface area of each substrate particle was greatest for the rough-dark substrate and least for the Dyson Creek substrate, the smooth-dark substrate and the smooth-light substrate had areas of intermediate size (Table II-1).

The water velocity and depth over the 24 substrates were analysed using the one-way ANOVA test. It was not necessary to transform the data because the data approached normality and the variances were homogeneous (Table II-1). Mean water velocity over the substrate trays was high in May, June and July and decreased to lowest readings in September and October (Figure II-1). Water velocity was

significantly different between the substrate types (ANOVA, $p < 0.01$), and the rough-dark substrate was exposed to significantly slower velocities than the other substrates (Newman-Keuls test, $p < 0.05$, Table II-1). Mean depth of water over the substrate baskets was stable or decreasing over the experimental period, except for spring spates in May and June, 1984 (Figure II-3). Water depth over the baskets was significantly different between substrate types (ANOVA, $p = 0.04$, Table II-1); however, the multiple range test did not show any significant differences between the substrates (Newman-Keuls test, $p < 0.05$). The range of mean depths over the four substrate types was 2 cm which is unlikely to influence substrate colonization (Table II-1).

Mean number and standard deviation (of six replicates) of the developmental stages of *D. coloradensis* for each treatment and sample date are presented in Table II-2. Within each developmental stage, the number of nymphs on the four substrates showed similar trends between dates. Spatial dispersion of *D. coloradensis* nymphs was determined for each developmental stage and each substrate type (using the cells in Table II-2). The chi-square test was used to test the variance to mean ratio against a Poisson series, a model for a random distribution (Elliott 1977). Agreement with the Poisson series was accepted ($p < 0.05$) in 71 of the 72 cells in Table II-2. This suggests that the nymphs in all developmental stages were randomly distributed on the substrates in the baskets. Also, one day after the nine colonization periods, six quantitative samples were taken, and a Poisson series fitted the data in all but one case.

To determine the preferences for the substrates by the nymphs of the developmental stages, the nonparametric Friedman test was performed on each developmental stage. In the analysis, treatments were the substrate type and blocks were the replicates for each sample date (Conover 1980). Colonization of the substrate types was significantly different only for the DS2 and DS3 nymphs (Table II-3). A multiple-comparison test ($p < 0.05$) was performed on the results of the DS2 and DS3 nymphs (after Conover 1980). There were significantly fewer DS2 nymphs on the rough-dark substrate than on the other three substrates; and there were fewer DS3 nymphs on the rough-dark substrate and the smooth-dark substrate than the Dyson Creek substrate and smooth-light substrate (Table II-3). Although the number of DS1

and DS4 nymphs were not significantly different between the substrates, there was a tendency for the lowest mean number of nymphs to be on the rough-dark substrate and greatest mean number on the Dyson Creek substrate (Table II-2).

In general for all developmental stages, the rough-dark substrate was least preferred and the Dyson Creek substrate was most preferred.

Direct Observations

During the direct observations, water depth and velocity at each substrate basket were similar. I observed nymphs of three families of Ephemeroptera: Heptageniidae, Baetidae, and Siphlonuridae. These taxa were the dominant benthic organisms in all observations. Four *D. coloradensis* nymphs were observed but they were not considered in the analysis. The results were analysed by groups: Total Fauna (this group included all of the Ephemeroptera taxa in my study, except *D. coloradensis*), Heptageniidae (mostly *Cinygmula* and small numbers of *Epeorus*), *Baetis* (Baetidae), and a *Baetis-Ameletus* group (mostly *Baetis*). Data for *Cinygmula* and *Epeorus* nymphs and in August for *Baetis* and *Ameletus* nymphs were lumped, because of the large number of small nymphs present making identification of genera difficult. Direct observation data were analysed using the Friedman test and a multiple comparison test ($p < 0.05$, Conover 1980) where appropriate. Multiple comparisons were not made for the small and large size classes. Treatments and blocks in the design were the substrate types and dates, respectively, within a colonization period. The short and long colonization periods and two size classes were analysed separately. Mean number and standard deviation of nymphs per substrate basket for each taxon and colonization period are presented in Table II-4; data for the two size classes are shown in Table II-5. In the analysis of the small and large heptageniids and baetids, data within each date were lumped because of small sample sizes.

Densities of Total Fauna on the substrate types were significantly different for the short (2-5 days) and long (13-14 days) colonization periods (Table II-4). After the short colonization period, there were greater densities of Total Fauna on the

smooth-light substrate than on the remaining substrates; the smooth-dark substrate was the least preferred substrate (Table II-6). After the long colonization period, there were fewer organisms on the smooth-dark substrate than on the other substrates (Table II-6). For both the short and long colonization periods, the mean density of Total Fauna on the smooth-light substrate was approximately twice that of the smooth-dark substrate, which had the lowest densities for any of the four substrates (Table II-4).

The large heptageniid nymphs were statistically most abundant on the smooth-light substrate for the short colonization period. Small nymphs also showed a preference for the smooth-light substrate during the short colonization period (Table II-5). For the long colonization period, none of the size classes, including the 1-12 mm size of heptageniids, were different between the substrates (Tables II-4 and -5). Numbers of *Baetis* nymphs (1-12 mm) were different between the substrates after the short colonization, and numbers of *Baetis-Ameletus* nymphs (1-12 mm) for both colonization periods (Table II-4). *Baetis* preferred the rough-dark substrate over the other substrates, and *Baetis-Ameletus* showed preferences for the smooth-light substrate and rough-dark substrate (the smooth-dark substrate was least preferred), after the short colonization period (Tables II-4 and -6). Following the long colonization period, the smooth-dark substrate was least preferred substrate by *Baetis-Ameletus* nymphs (Table II-6). However, when the small and large size classes were considered separately, only the *Baetis-Ameletus* group for small and large were different for the long colonization period (Table II-5). The smooth-light substrate and the rough-dark substrate had the greatest number of *Baetis-Ameletus* nymphs in the small and large size classes respectively (Table II-5). Generally for both colonization periods, the *Baetis-Ameletus* nymphs least preferred the smooth-dark substrate (Table II-5).

E. DISCUSSION

Substrate Basket Experiment

The 14 day colonization period was considered sufficient to allow new colonizers to attain a population equilibrium and to reduce potential changes (e.g. deposition) to the substrate. Rosenberg and Resh (1982) reviewed recent literature on the time it took organisms to reach equilibrium densities in containers of various mineral substrates, including gravel, pebble, and cobble substrates. They found for Total Fauna per study, on average, populations in containers reached equilibrium in 14 days (range 9-20 days). However, colonization dynamics probably varies with species and populations of species. *Drunella coloradensis* nymphs of my study have many similarities with *Drunella grandis*, and results for *D. grandis* are used in the discussion for comparative purposes. Similarities between these species include distribution (Allen and Edmunds 1962, Edmunds et al., 1976) and habitat (Allen and Edmunds 1962, Gilpin and Brusven 1970). Diet is apparently variable between populations of these species (Gilpin and Brusven 1970, Hawkins 1985). Because dispersion of the developmental stages of *D. coloradensis* on the four substrates in the baskets and in the stream bed were randomly distributed, the colonization results are likely real and due to substrate manipulations rather than the distribution being contagious.

The choice of prey by a visually-foraging predator could be influenced by prey size, movement, and contrasting colours relative to the background. For example, these factors may be directly related to food selection by fish in lotic systems (see review of Healy 1984). The fauna of lotic habitats most likely evolved in the presence of predatory fish (Allan 1983). Therefore, prey would be expected to have many adaptations to these predators. In my study area on Dyson Creek fish were absent, although the stream was previously stocked on several occasions in the 1940's. Alternatively, it is possible that the substrate preferences of insect nymphs in the absence of fish may be different than when fish are present. For example, Charnov et al., (1976) demonstrated a movement of baetid nymphs into shaded or covered regions

in tanks when a fish predator was introduced. Predators in Dyson Creek include invertebrates such as Perlodidae (Plecoptera), *Rhyacophila* (Rhyacophilidae: Trichoptera), and vertebrates namely the dipper, *Cinclus mexicanus* (Passeriformes: Aves). Generally, terrestrial vertebrates such as the dipper, which is a visually-feeding predator, are not considered as having a significant effect on the abundance of benthic macroinvertebrates. However, Ealey (1977) examined the stomach contents of six dippers during their breeding season on the Sheep River into which Dyson Creek drains. He found that macroinvertebrates, excluding the only other component in the diet i.e. terrestrial invertebrates, comprised 82 % of the stomach contents. Predation pressure was expected to be least on small nymphs because of the prey's small size and an increased cost to benefit ratio to a predator. Assuming that the substrate types were similar in all characteristics (except for those characteristics being manipulated), the developmental stage one nymphs were expected to forage equally on all four substrates or to have, relative to more mature nymphs, a reduced preference for the darker and rougher substrates. The developmental stage one nymphs had no preference for the four substrates, which was what I predicted. However, the physical characteristics and the environment of the substrates were found not to be the same, and the substrate was indirectly modified (see below).

More mature *D. coloradensis* nymphs (developmental stages two, three, and four) were expected to show tendencies to colonize darker and rougher substrates in response to predation pressure and ability to hold onto the substrate. I predicted that the larger and hence more mature (developmental stages three and four) *D. coloradensis* nymphs would prefer the rough-dark, Dyson Creek, and smooth-dark substrates. I expected these large nymphs would least prefer the smooth-light substrate. However, all of the developmental stages of nymphs, especially developmental stages two and three, least preferred the rough-dark substrate. This may have been due to the low water velocity at this substrate. Two of the six baskets containing the rough-dark substrate were in areas of low flow and this accounted for the lower mean velocity. Because of the reduced velocity, the results might be biased since *D. coloradensis* nymphs may prefer high water velocities. Rabeni and Minshall (1977) found lower

numbers of *D. grandis* nymphs in substrate trays in a pool than in a riffle. Their distribution pattern also occurred when the current was experimentally decreased by 38 cm s^{-1}. In another study in the same stream, Minshall and Minshall (1977) found a similar pattern for *D. grandis*. Obviously for *D. grandis* there appears to be a habitat preference. However, in my study the difference between the lowest and highest mean velocities over the experimental period was only 8 cm s^{-1}. Reduced velocity may have modified the substrates by increasing the deposition of fine organic matter and lesser amounts of inorganic material.

The sediment on the two rough substrates (rough-dark and Dyson Creek) was very patchy and less than 1 mm thick. Deposition may have been caused by the rougher substrates trapping saltating particles in minute crevices. *Drunella coloradensis* nymphs may be attracted to such deposits as a food source or the deposit may physically repulse the nymphs. Detritus and diatoms are included in the diet of *D. coloradensis* (Gilpin and Brusven 1970, Hawkins 1985) but these food sources would have to be limiting before one would expect a direct relationship with colonization. Siltation can have various effects or it can have no effect on the zoobenthos (see Minshall 1984). Cummins and Lauff (1969) found minor effects or an increase of numbers for certain species of immature insects when siltation increased. Rabeni and Minshall (1977) found a reduction in the number of nymphs for several insect taxa, including *D. grandis*, when there was only a very thin layer (< 1 mm) on the substrate. Percival and Whitehead (1965) suggested that a light covering of fine detritus may cause the organism to loose it's hold on the substrate. Although large detrital particles, notably leaves, occasionally accumulated in the substrate of my baskets, they were not considered because leaves in streams are not a normal habitat of *D. coloradensis* nymphs.

Drunella coloradensis nymphs in developmental stage three were found in largest numbers on the smooth-light and Dyson Creek substrates. The Dyson Creek substrate particles had a smaller diameter and surface area than the other substrates, and this may have been important since amount of surface area has been shown to be important to colonization by benthic invertebrates. Minshall (1984), in his recent review

of organism-substrate relationships, compared studies using single rocks and groups of rocks. Fewer invertebrates were found on individual large rocks than on small rocks. However, when rocks were combined in trays, greater densities of invertebrates were found on large substrates. I did not measure the number of particles in all of the baskets; hence the total surface area per basket is not known, and a statistical comparison of density of nymphs between substrates is not possible. However, the Dyson Creek substrate most likely had the greatest total surface area per basket because there were about twice as many Dyson Creek particles per basket than for the other substrates. Thus, the actual densities of invertebrates in the Dyson Creek substrate baskets may be less than those on the larger substrates when surface area is accounted for. Minshall and Minshall (1977) found similar densities of *D. grandis* in trays of small and large rocks, although there was a tendency for more nymphs to be on smaller rocks. Rabeni and Minshall (1977) found *D. grandis* nymphs had a strong preference for small rocks. Other experimental results on groups of rocks in trays generally have found more nymphs on small and medium sized rocks (Williams and Mundie 1978, Wise and Molles 1979, Khalaf and Tachet 1980). My results for all developmental stages of *D. coloradensis* showed greatest numbers of nymphs were on the smallest substrate, the Dyson Creek substrate.

Minshall (1984) suggested that the 'inhabitability' of the interstices may be influencing colonization. This inhabitability could be related to the size and number of interstices. The rough-dark substrate had the largest mean diameter and therefore had the least number of interstices and largest spaces between particles. The smaller interstices in the Dyson Creek substrate may cause a reduced current and a subsequent build up of fine detritus. This is important to benthic organisms, since the amount of detritus and the abundance of insect nymphs are often positively related. The nymphs may be feeding on detritus not exposed to the current. Another function of interstices would be to provide refuges for benthic invertebrates. For example, interstices may serve as a place for prey to escape from or to avoid predators (e.g. Hildrew and Townsend 1977, Stein 1977). The increase in numbers of interstices and therefore detritus may explain why the developmental stage two and developmental stage three

nymphs preferred the Dyson Creek substrate.

Erman and Erman (1984) examined the colonization of three rock types of increasing surface texture from quartzite, to granite, to sandstone. They found that the total numbers of mayfly and stonefly nymphs increased significantly from the smoothest to roughest substrates. In another study using tile substrates, which had much greater texture than my substrates, Clifford et al. (in prep.) found more individuals and more taxa on rough tiles than on smooth tiles. I found no clear trend in my study although the greatest number of nymphs were on the roughest substrate, the Dyson Creek substrate, but the least number of nymphs were on the next roughest substrate, the rough-dark substrate.

Comparing the smooth substrates, the smooth-light and smooth-dark substrates were the most similar of the four substrates in terms of their physical characteristics and their exposure to environmental factors at the study site. *Drunella coloradensis* nymphs were most conspicuous on the smooth-light (white) substrate, and it was expected that they would be easily seen on these rocks by a visually-foraging predator. However, the smooth-light substrate was the second most preferred substrate for nymphs of all developmental stages, especially for the developmental stage three nymphs, which were more abundant on the white substrate than on the smooth-dark (black) substrate. It is difficult to determine why *D. coloradensis* nymphs preferred the smooth-light substrate relative to the other substrates. However, it is possible that there was a location effect between the substrates, other than the physical factors measured for the two substrate types.

Few studies have experimentally investigated the importance of substrate colour to colonization by aquatic insects. Hughes (1966) examined the role of light in choice of microhabitat by *Baetis* and *Tricorythus* (Tricorythidae: Ephemeroptera) nymphs; he used white and black backgrounds in an experimental stream. About 58 % and 55 % of *Baetis* and *Tricorythus* nymphs, respectively (calculated from Figure 4 in Hughes 1966), were positioned on a black surface relative to a white surface. Thus, Hughes' data shows only a slight tendency towards nymphs choosing a black substrate. In my study, the most conspicuous nymphs, when on the light coloured substrate, were the large

devolopmental stage four *D. coloradensis* nymphs. In addition to their large size, these nymphs exibited dark wing pads. But the nymphs did not prefer the dark coloured substrates. As was found for the less mature nymphs, the lowest mean numbers of developmental stage four nymphs were on the rough-dark substrate and the highest mean numbers were on the Dyson Creek substrate, which was dark coloured.

The substrates of my study were made up of different minerals and therefore the chemical constituents of the rocks may have influenced the choice of substrate. But in an extensive study of the benthic fauna of 52 streams in Scotland, Egglishaw and Morgan (1965) found a remarkable similarity in species composition and abundances between seven limestone and five sandstone streams. Although their study was not experimental, it suggests that the minerals in the limestone and sandstone may not be important to colonization by benthic organisms.

My results do not conclusively suggest that the more mature *D. coloradensis* nymphs prefer rougher texture where mature nymphs, especially intermediate sized nymphs, most preferred only one of the rough substrates (i.e. Dyson Creek substrate) and the other rough substrate was least preferred (i.e. rough-dark substrate). The colour of the substrate was important for intermediate size nymphs, where nymphs were more abundant on the light coloured substrates. The choice of substrate appears to be influenced by abiotic factors. However, biotic factors such as the availibility of food and the presence and absence of fish may also be important.

Direct Observations

I expected the other taxa studied to show similar substrate preferences to those predicted for *D. coloradensis*, assuming that all insect nymphs evolved similar mechanisms for reducing predation and ability to remain on substrate surfaces. The taxa observed were cryptically coloured, and behaviourally they have been classified as clingers (similar to *D. coloradensis*) by Cummins et al., (1984). The insect nymphs were expected to choose the dark substrates to reduce predation and to have various preferences for the rough substrates, because of possible tactile differences between

taxa. However, the Total Fauna nymphs did not show a strong preference for the rough substrates; the smooth-light substrate was the most preferred and the smooth-dark substrate was the least preferred substrate by Total Fauna. Having observed this general pattern for Total Fauna, it was possible to explain the results more clearly by examining the size classes of individual taxa.

Since the heptageniids are thigmotactic, the rough substrates, with their accumulations of sediment, may not be a preferred substrate and may in fact hinder a nymph moving over a rock surface (e.g. Percival and Whitehead 1929). This may be especially true of *Cinygmula*, which is a fast-moving nymph and is generally found in silt-free lotic habitats (Edmunds et al., 1976, Gilpin and Brusven 1970). However, my finding that large nymphs were more abundant on the light coloured substrate relative to the dark coloured substrate was unexpected, although this was only evident for the short colonization period (see below). My observations most likely did not miss nymphs on the dark substrates because I determined whether my nymphs were cryptic to my view by shining the bright white light on the dark substrate.

Although *Baetis* and *Ameletus* nymphs belong to separate families, they are, unlike the heptageniids of my study, both streamlined and good swimmers. These streamlined nymphs hold their body above the substrate and use their tarsi to hold onto the surface of a substrate. Since *Baetis* and *Ameletus* nymphs are commonly observed on the tops and sides of rocks facing into the current, I expected they would have a stronger preference than the heptageniids for the rough substrates to maintain their position on the rocks. This was supported by my results. The rough substrates had more sediment, and from a visual examination these deposits contained a lot of fine organic material. Such deposits might be the food source of *Baetis* and *Ameletus* nymphs. Also, these substrates have an increased surface area for potential colonization by algae. These nymphs belong to the collector-gatherer (grazers) functional feeding group, feeding on detritus and algae. Some *Baetis* nymphs also are grazers (Gilpin and Brusven 1970, Cummins et al., 1984). The smooth-dark substrate was the least preferred substrate of *Baetis* and *Ameletus* nymphs. I cannot explain why the heptageniids, *Baetis*, and *Ameletus* nymphs preferred the the light-coloured

substrate. Since the light (white) and dark (black) substrates were physically and chemically similar and subjected to the same environmental conditions, I postulate that algae production is greater on the white substrates, and this is due at least in part to more light being reflected from the white surface relative to that of the black rocks (see Chapter IV).

For Total Fauna and individual taxa, there was an increase in the mean number of nymphs from the short to the long colonization periods. However, it is not known whether the populations were at equilibrium after the 14-15 day period. Although colonization rate may vary between taxa, the dynamics of colonization are not well known. For example, Shaw and Minshall (1977) found that after 64 days many stream insect populations had not stabilised. But for both the substrate basket experiment and direct observation study, some interesting trends were evident. For the substrate basket experiment, the more mature *D. coloradensis* nymphs preferred the roughest substrate, i.e. the smaller rocks with lots of interstices. But these large nymphs also preferred the light coloured substrates over the dark substrates, especially the rough-dark substrate. Similar results were obtained for the other taxa in Dyson Creek using direct observations. But for the rough substrates the heptageniids preferred the Dyson Creek substrate and the *Baetis* and *Ameletus* nymphs choose the rough-dark substrate. The heptageniids, *Baetis*, and *Ameletus* nymphs also choose the smooth-light substrate, and this was similar to the preference of intermediate developmental stages of *D. coloradensis* nymphs.

Table II-1. Mean and standard deviation (in parentheses) of the physical measurements of the substrate particles, and the water velocity and depth at the four substrate types, the rough-dark (RD) substrate, the Dyson Creek (DC) substrate, the smooth-dark (SD) substrate, and the smooth-light (SL) substrate.

	Substrate Type							
Physical Factor	RD		DC		SD		SL	
Diameter (cm)	8.3	(3.9)	6.0	(1.9)	7.7	(2.1)	7.6	(1.8)
Surface Area (cm^2)	260.6	(236.2)	123.5	(78.5)	196.9	(104.6)	188.3	(88.0)
Texture (roughness units)	4.7	(4.1)	5.9	(6.8)	2.0	(3.5)	2.3	(2.2)
Water Velocity ($cm\ s^{-1}$)	15.8	(13.5)	24.1	(14.9)	24.3	(14.7)	20.7	(12.4)
Water Depth (cm)	11.2	(7.0)	11.9	(7.3)	13.1	(7.8)	11.1	(6.8)

Table II-2. Mean abundance and standard deviation (in parentheses) on colonization periods one to nine (CP1 to CP9, see Materials and Methods for details), for the four developmental stages (DS1 to DS4) of *Drunella coloradensis* on each substrate type (RD, DC, SD, and SL). See Table II-1 for details.

Developmental Stage	Colonization Period	Substrate Type: RD		DC		SD		SL	
DS1	CP1	33.7	(33.5)	36.3	(14.5)	36.0	(19.8)	39.7	(13.9)
	CP2	10.0	(5.6)	20.0	(10.5)	12.8	(8.1)	10.0	(5.1)
	CP3	2.7	(2.3)	1.8	(1.5)	2.3	(1.4)	2.2	(1.2)
	CP4	1.0	(0.9)	1.0	(1.3)	1.7	(1.6)	1.3	(2.8)
DS2	CP1	7.5	(7.3)	9.3	(7.0)	9.7	(6.6)	12.2	(7.4)
	CP2	28.5	(18.3)	60.5	(26.0)	36.3	(21.5)	35.2	(9.4)
	CP3	12.5	(8.6)	17.5	(4.6)	15.5	(11.0)	16.0	(5.0)
	CP4	12.7	(10.9)	24.3	(14.3)	28.0	(13.0)	26.8	(16.6)
	CP5	6.7	(8.5)	14.5	(6.1)	10.2	(6.3)	9.7	(4.5)
	CP6	0.3	(0.5)	0.7	(0.5)	0.2	(0.4)	0.3	(0.5)
DS3	CP5	0.0	----	0.9	(0.6)	0.2	(0.4)	0.2	(0.4)
	CP6	8.0	(8.0)	9.7	(4.8)	8.3	(3.5)	12.7	(6.5)
	CP7	7.5	(5.4)	14.2	(7.8)	8.5	(3.9)	12.8	(7.8)
	CP8	3.7	(3.0)	6.3	(3.1)	2.0	(2.5)	5.3	(2.9)
	CP9	0.0	----	0.3	(0.8)	0.0	----	0.3	(0.5)
DS4	CP7	0.5	(0.8)	1.0	(0.6)	0.8	(1.2)	0.8	(1.2)
	CP8	1.5	(0.6)	2.7	(2.1)	1.7	(2.4)	2.3	(2.0)
	CP9	0.8	(0.8)	0.8	(0.4)	1.2	(1.2)	0.8	(0.4)

Table II-3. Multiple comparison test results on the abundance of the four developmental stages (DS1 to DS4) of *Drunella coloradensis* nymphs on each substrate type (RD, DC, SD, and SL). Only significant test results ($p < 0.05$) are shown where the abundance is greater than (>) on the other substrates. See Table II-1 for details.

	Substrate Type			
Developmental Stage	RD	DC	SD	SL
DS1	----	----	----	----
DS2	----	> RD	> RD	> RD
DS3	----	> RD > SD	----	> RD > SD
DS4	----	----	----	----

Table II-4. Mean abundance and standard deviation (in parentheses) for each taxon group observed after the short and long colonization periods on each substrate type (RD, DC, SD, and SL), with probabilty levels of the Friedman test to determine differences between the substrate types (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ and NS = $p > 0.05$). See Table II-1 for details.

Taxon Group	Colonization Period	Substrate Type RD	DC	SD	SL	Friedman Test
Total Fauna	Short	7.8 (4.4)	8.1 (4.9)	5.6 (6.2)	12.4 (7.2)	***
	Long	21.5 (6.7)	20.6 (5.9)	12.1 (4.3)	25.9 (11.0)	*
Heptageniidae	Short	2.8 (2.1)	3.8 (3.2)	2.8 (3.2)	7.7 (5.1)	***
	Long	8.8 (6.1)	11.6 (5.5)	8.5 (2.1)	12.6 (8.2)	NS
Baetis	Short	2.1 (1.3)	1.3 (1.0)	0.8 (1.1)	0.9 (1.1)	**
Baetis-Ameletus	Short	9.3 (3.6)	8.8 (1.8)	5.8 (4.5)	10.9 (2.5)	*
Baetis-Ameletus	Long	12.8 (2.8)	9.0 (3.5)	3.6 (2.8)	13.3 (6.8)	**

Table II-5. Mean density and standard deviation (in parentheses) for the two nymphal size classes (small and large) of taxa observed after the short and long colonization periods on each substrate type (RD, DC, SD, and SL), with probability levels of the Friedman test to determine differences between the substrate types (* = $p < 0.05$, ** = $p < 0.01$, NS = $p > 0.05$). See Table II-1 for details.

Taxon Group	Colonization Period	Substrate Type: RD		DC		SD		SL		Friedman Test
Heptageniidae (Small)	Short	1.0	(1.2)	0.7	(1.1)	0.4	(0.5)	2.3	(2.4)	NS
	Long	1.1	(1.4)	1.6	(2.3)	0.5	(0.5)	1.5	(1.4)	NS
Heptageniidae (Large)	Short	1.8	(2.2)	3.1	(3.0)	2.4	(3.0)	5.5	(4.5)	*
	Long	7.6	(6.0)	10.0	(4.8)	8.0	(2.0)	11.1	(7.9)	NS
Baetis (Small)	Short	0.6	(0.9)	0.3	(0.7)	0.0	----	0.4	(0.5)	NS
Baetis-Ameletus (Small)	Short	6.8	(3.6)	5.5	(2.2)	4.0	(4.3)	8.5	(3.5)	NS
Baetis-Ameletus (Small)	Long	6.5	(1.9)	6.1	(4.5)	1.9	(1.9)	8.8	(5.7)	**
Baetis (Large)	Short	1.6	(1.5)	1.0	(0.9)	0.8	(1.1)	0.6	(1.2)	NS
Baetis-Ameletus (Large)	Short	2.5	(1.2)	3.3	(1.8)	1.8	(1.5)	2.4	(2.1)	NS
Baetis-Ameletus (Large)	Long	6.3	(2.1)	2.9	(1.4)	1.8	(2.0)	4.5	(3.1)	*

Table II-6. Multiple comparison test results of the density for each taxon group on each substrate type (RD, DC, SD, and SL) after the short and long colonization periods. Only significant test results ($p < 0.05$) are shown where the density of organisms is greater than (>) on the other substrates. See Table II-1 for details.

Taxon Group	Colonization Period	Substrate Type			
		RD	DC	SD	SL
Total Fauna	Short	> SD	> SD	----	> SD > RD > DC
	Long	> SD	> SD	----	> SD
Heptageniidae	Short	----	----	----	> SD > RD > DC
	Long	----	----	----	----
Baetis	Short	> SD > SL > DC	----	----	----
Baetis-Ameletus	Short	> SD	----	----	> SD > DC
Baetis-Ameletus	Long	> SD	> SD	----	> SD

Figure II-1. Mean water velocity and 95 % confidence intervals at the 24 sites for the substrate basket experiment, during the period from June 1983 to July 1984.

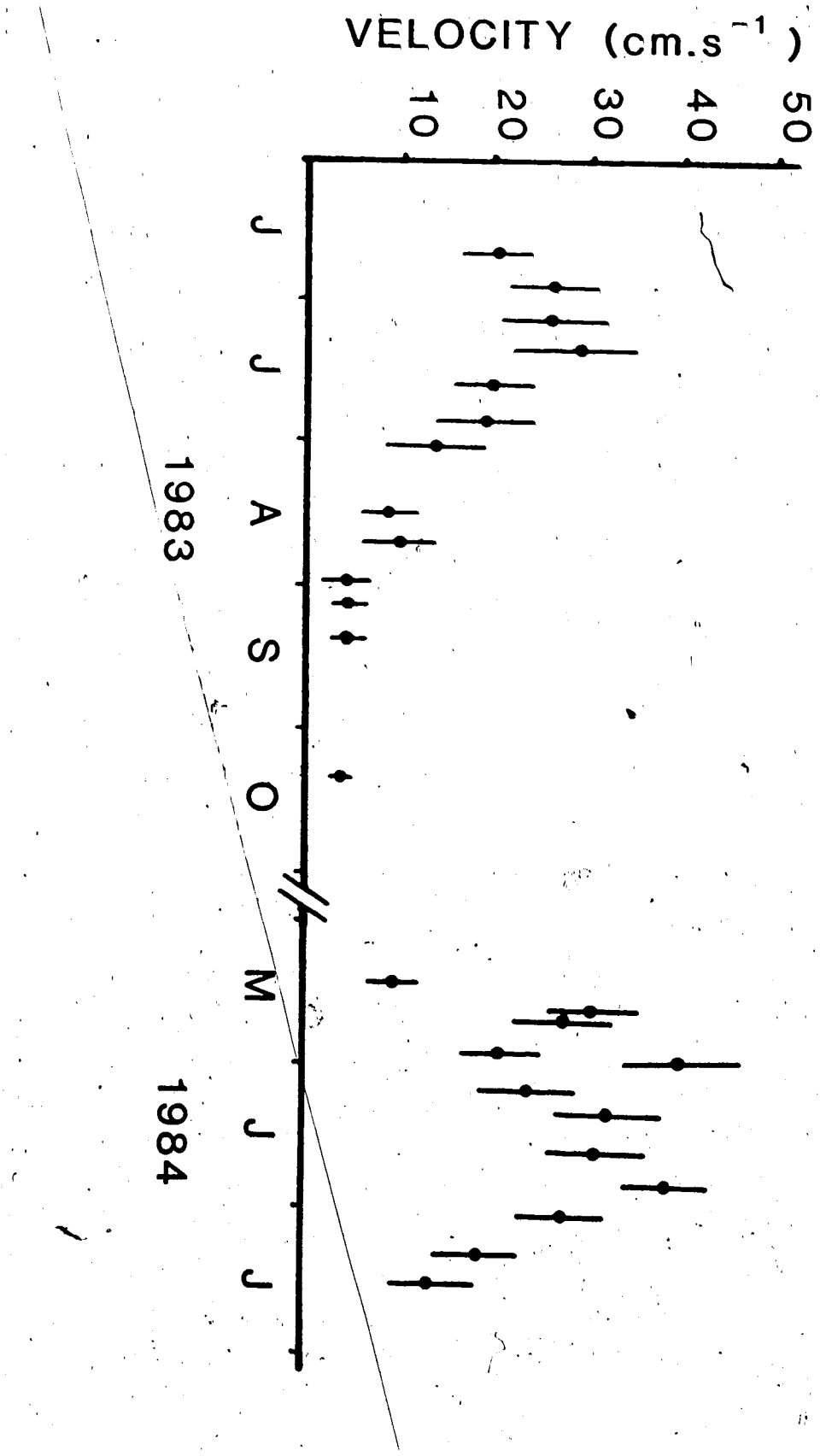

VELOCITY (cm.s⁻¹)
10
20
30
40
50
J
J
A
S
O
M
J
J
1983
1984

Figure II-2. Mean water depth and 95 % confidence intervals at the 24 sites for the substrate basket experiment, during the period from June 1983 to July 1984.

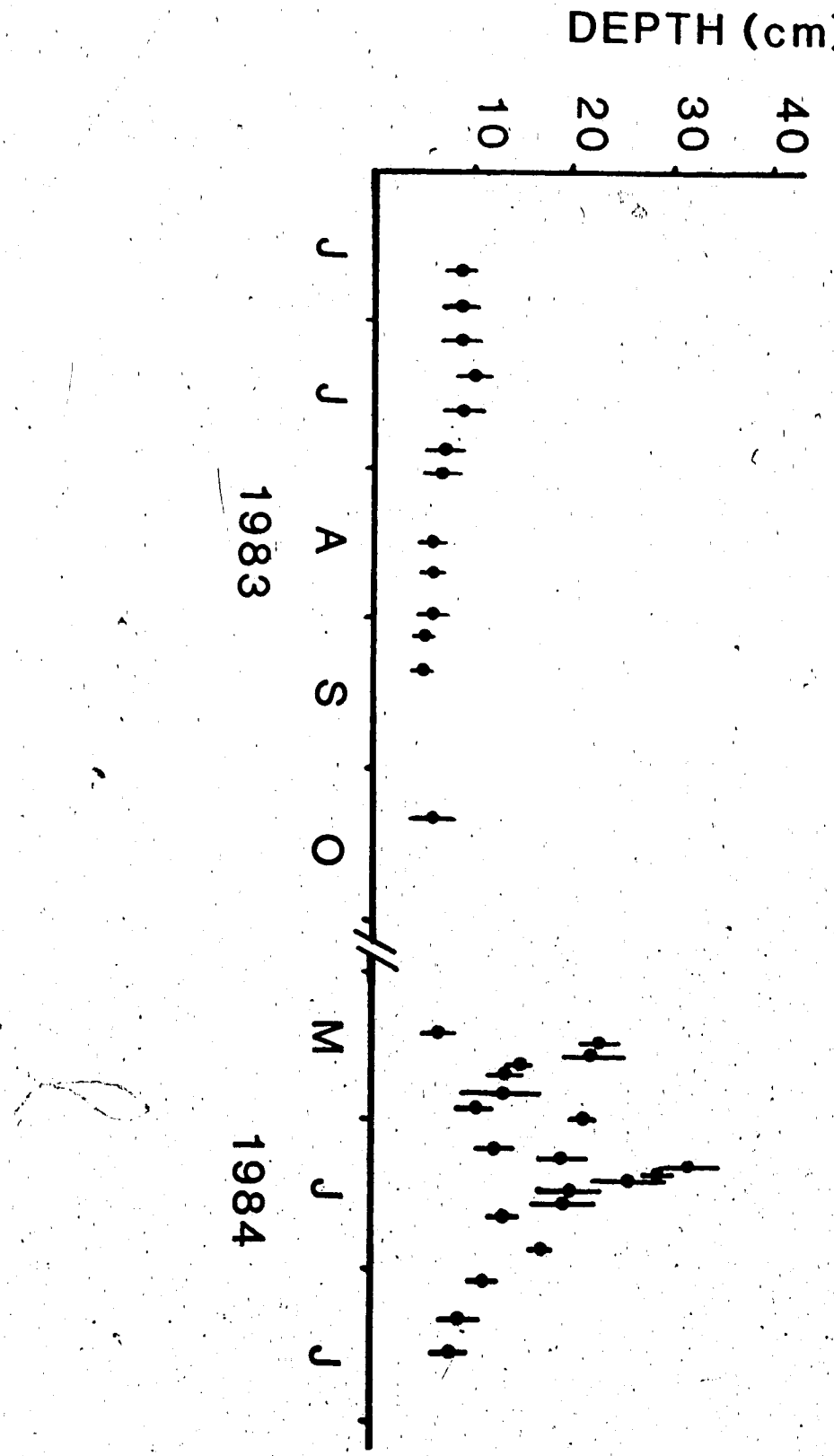

DEPTH (cm)
10
20
30
40
J
J
A
S
O
M
J
J
1983
1984

Figure II-3. Mean water temperature and ranges during the period from June 1983 to July 1984. Single closed circles are spot temperatures and the dark bar indicates ice cover.

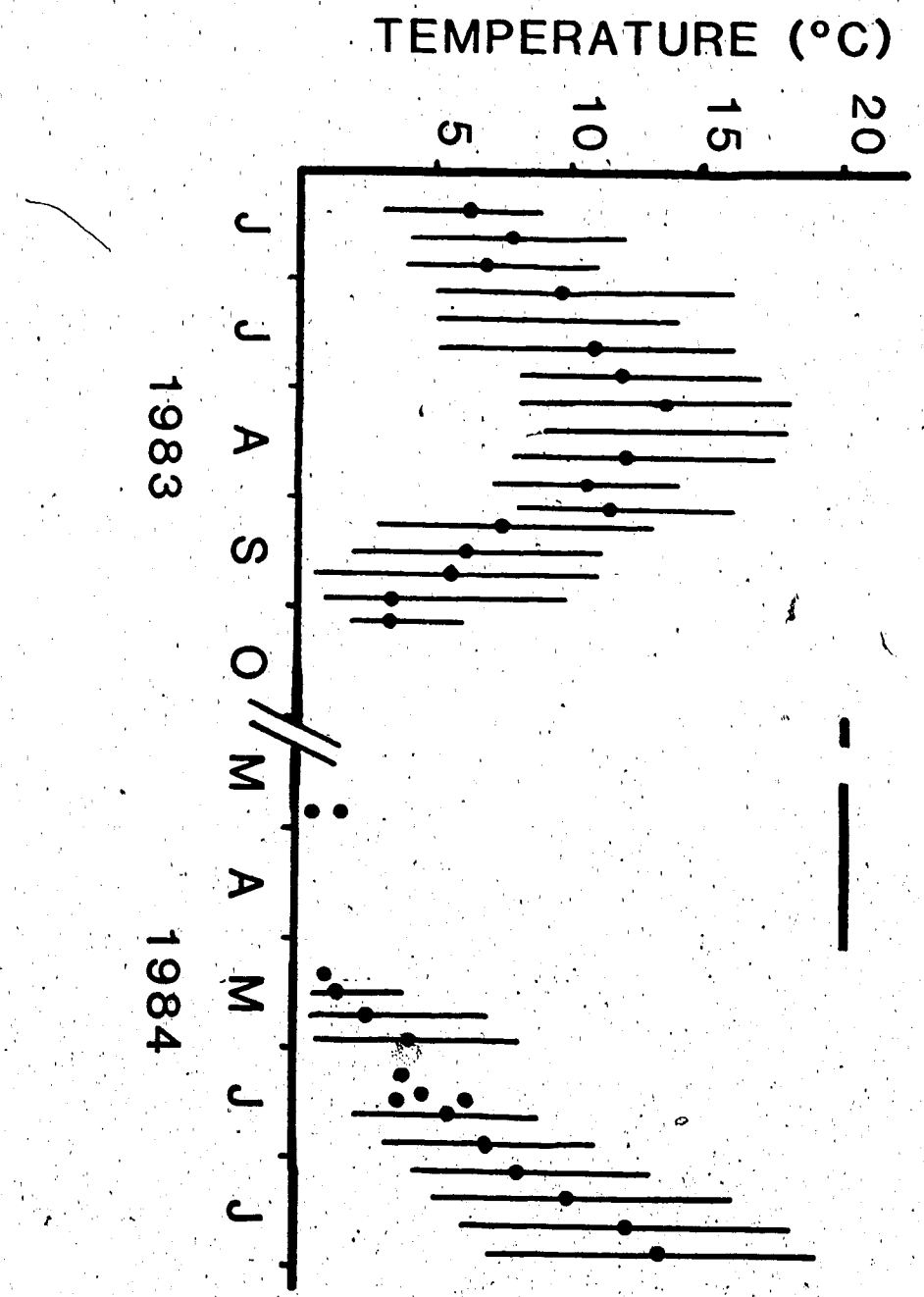
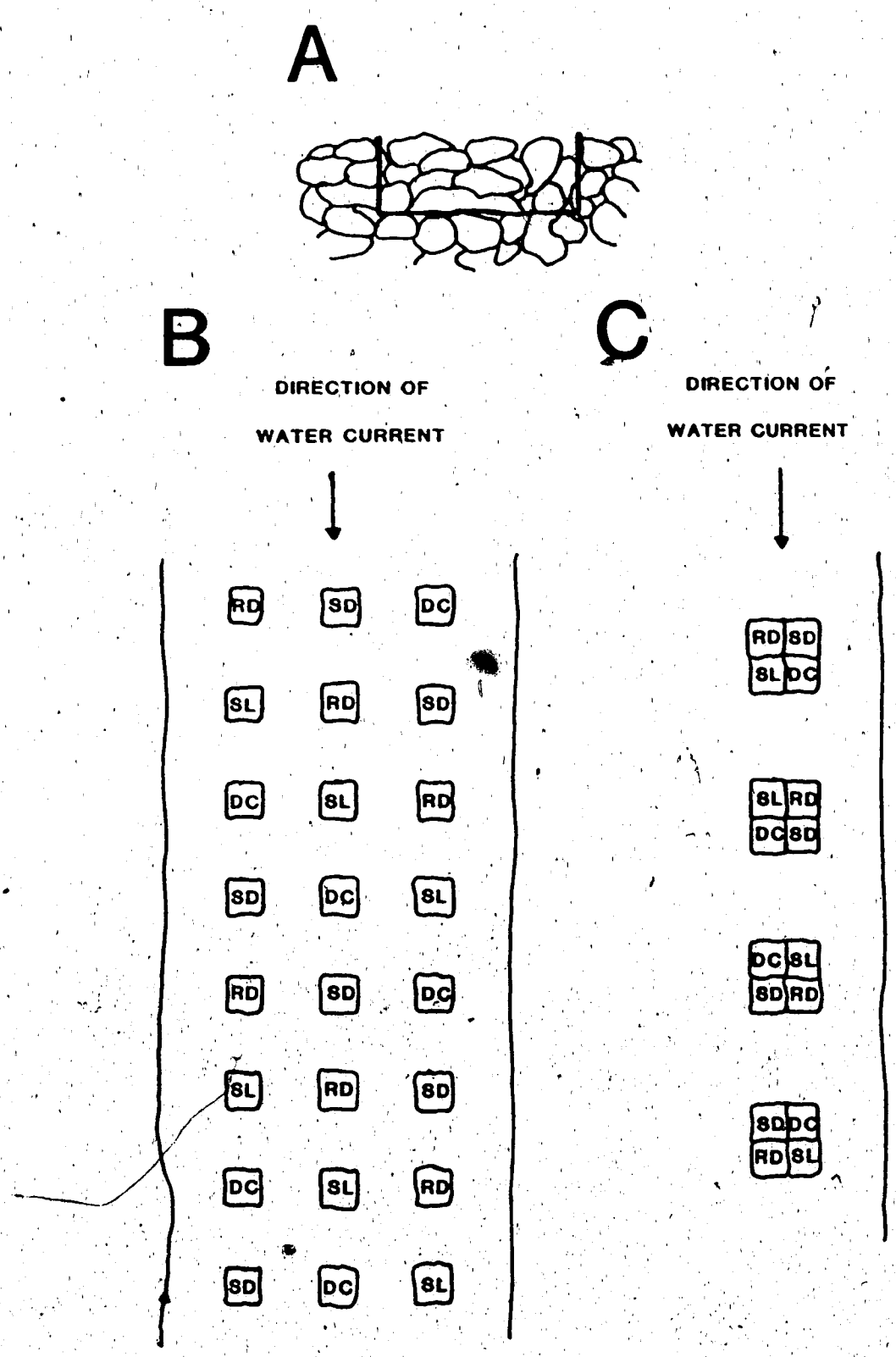

TEMPERATURE (°C)
5
10
15
20
J
J
A
S
O
M
A
M
J
J
1983
1984

Figure II-4. Diagrammatic representation (not drawn to scale) of (A) a substrate basket in place in the stream bed, (B) the substrate basket experimental design, and (C) the arrangement of substrate baskets for the direct observations. The four substrate basket types were the rough-dark (RD) substrate, the Dyson Creek (DC) substrate, the smooth-dark (SD) substrate, and the smooth-light (SL) substrate.


A
B
C
DIRECTION OF
WATER CURRENT
DIRECTION OF
WATER CURRENT
RD SD DC
SL RD SD
DC SL RD
SD DC SL
RD SD DC
SL RD SD
DC SL RD
SD DC SL
RD SD
SL DC
SL RD
DC SD
DC SL
SD RD
SD DC
RD SL

F. REFERENCES

Alberta Energy and Natural Resources. 1979. Ecological land classification and evaluation Highwood-Sheep. E.N.R. Rep. No. 93. 63 p.

Allan, J. D. 1975. The distributional ecology and diversity of benthic insects in Cement Creek, Colorado. Ecology 56: 1040-1053.

Allan, J. D. 1983. Predator-prey relationships in streams, p. 191-229. *In* J. R. Barnes and G. W. Minshall [ed.] Stream ecology: application and testing of general ecological theory. Plenum Press, New York, NY.

Allen, R. K., and G. F. Edmunds Jr. 1962. A revision of the genus *Ephemerella* (Ephemeroptera, Ephemerellidae). V. The subgenus *Drunella* in North America. Misc. Publ. Entomol. Soc. Amer. 3: 145-179.

Charnov, E. L., G. H. Orians, and K. Hyatt. 1976. Ecological implications of resource depression. Am. Nat. 110: 247-259.

Clifford, H. F. 1970. Analysis of a northern mayfly (Ephemeroptera) population, with special reference to allometry of size. Can. J. Zool. 48: 305-316.

Clifford, H. F., V. Gotceitas, and R. J. Casey. Roughness and color of substratum particles as a possible factor in the colonization of stream invertebrates, in preparation.

Clifford, H. F., R. J. Casey, V. Gotceitas, and M. Dunnigan. A device to quantify texture of substrata, with special reference to stream substrata, in preparation.

Conover, W. J. 1980. Practical nonparametric statistics. 2nd ed. John Wiley and Sons, NY. 493 p.

Cummins, K. W., and G. H. Lauff. 1969. The influence of substrate particle size on the microdistribution of stream macrobenthos. Hydrobiology 34: 145-181.

Cummins, K. W., G. F. Edmunds Jr., and R. W. Merritt. 1984. Table pp 122-125. *In* R. W. Merrittt and K. W. Cummins. [ed.] An introduction to the aquatic insects of North America. Kendall/Hunt Publishing Co., Dubuque, Iowa.

Dodds, G. S. 1923. Mayflies from Colorado, description of certain species and notes on others. Trans. Amer. Ent. Soc. 49: 93-114.

Downing, J. A. 1979. Aggregation, transformation, and the design of benthos sampling programs. J. Fish. Res. Board Can. 36: 1454-1463.

Ealey, D. M. 1977. Aspects of the ecology of a breeding population of dippers (*Cinclus mexicanus*; Passeriformes) in southern Alberta. M.Sc. Thesis, Department of Zoology, University of Alberta, 198 p.

Edmunds Jr., G. F., S. L. Jensen, and L. Berner. 1976. The mayflies of North and Central America. University of Minnesota Press, Minneapolis. 330 p.

Egglishaw, H. J., and N. C. Morgan. 1965. A survey of the bottom fauna of streams in the Scottish Highlands. Part II. The relaationship of the fauna to the chemical and geological conditions. Hydrobiology 26: 173-183.

Elliott, J. M. 1967. The life histories and drifting of the plecoptera and ephemeroptera in a Dartmoor stream. J. Anim. Ecol. 36: 343-362.

Elliott, J. M. 1977. Some methods for the statistical analysis of samples of benthic invertebrates. 2nd ed. Freshwater Biol. Assoc. Scientific Publ. 25. 160 p.

Erman, D. C., and N. A. Erman. 1984. The response of stream invertebrates to substrate size and heterogeneity. Hydrobiology 108: 75-82.

Gilpin, B. R., and M. A. Brusven. 1970. Food habits and ecology of mayflies of the St. Maries River in Idaho. Melandria 4: 19-40.

Gotceitas, V., and H. F. Clifford. 1983. The life history of *Dicosmoecus atripes* (Hagen) (Limnephilidae: Trichoptera) in a Rocky Mountain stream of Alberta, Canada. Can. J. Zool. 61: 586-596.

Hamilton, H. R., and H. F. Clifford. 1983. The seasonal food habits of mayfly (Ephemeroptera) nymphs from three Alberta, Canada, streams, with special reference to absolute volume and size of particles ingested. Arch. Hydrobiol./Suppl. 65: 197-234.

Hart, D. D. 1978. Diversity in stream insects: regulation by rock size and microspatial complexity. Verh. Internat. Verein. Limnol. 20: 1376-1381.

Hawkins, C. P. 1984. Substrate associations and longitudinal distributions in species of Ephemerellidae (Ephemeroptera: Insecta) from western Oregon. Freshwat. Invertebr. Biol. 5: 181-188.

Hawkins, C. P. 1985. Food habits of species of ephemerellid mayflies (Ephemeroptera: Insecta) in streams of Oregon. Am. Midl. Nat. 113: 343-352.

Healy, M. 1984. Fish predation on aquatic insects, p. 255-288. *In* V. H. Resh and D. M. Rosenberg [ed.] The ecology of aquatic insects. Praeger Publishers, NY.

Hildrew, A. G., and C. R. Townsend. 1977. The influence of substrate on the functional response of *Plectrocnemia conspera* (Curtis) nymphs (Trichoptera: Polycentropodidae). Oecologia 31: 21-26.

Hughes, D. A. 1966. The role of responses to light in the selection and maintainence of microhabitat by the nymphs of two species of mayfly. Anim. Behav. 14: 17-33.

Hutchinson, G. E. 1981. Thoughts on aquatic insects. BioScience 31: 495-500.

Hynes, H. B. N. 1970. The ecology of running waters. University of Toronto Press. 555 p.

Khalaf, G., and H. Tachet. 1980. Colonization of artifial substrata by macro-invertebrates in a stream and variations according to stone size. Freshwat. Biol. 10: 475-482.

Madsen, B. L. 1968. A comparitive ecological investigation of two related mayfly nymphs. Hydrobiology 31: 337-349.

Minshall, G. W. 1984. Aquatic insect-substratum relationships, p. 358-400. *In*.V. H. Resh and D. M. Rosenberg [ed.] The ecology of aquatic insects. Praeger Publishers, NY.

Minshall, G. W., and J. N. Minshall. 1977. Microdistribution of benthic invertebrates in a rocky mountain (U. S. A.) stream. Hydrobiology 55: 231-249.

Nilsen, H. C., and R. W. Larimore. 1973. Establishment of invertebrate communities on log substrates in the Kaskaskia River, Illinois. Ecology 54: 366-374.

Otto, C. 1984. Adaptive head coloration in case-making caddis nymphs. Freshwat. Biol. 14: 317-321.

Peckarsky, B. L. 1982. Aquatic insect predator-prey relations. BioScience 32: 261-266.

Percival, E., and H. Whitehead. 1929. A quantitive study of the fauna of some types of stream-bed. J. Anim. Ecol. 17: 282-314.

Popham, E. J. 1941. The variation in the colour of certain species of *Arctocorisa* (Hemiptera, Corixidae) and its significance. Proc. Zool. Soc. Lond., Ser A 3: 135-172.

Rabeni, C. F., and G. W. Minshall. 1977. Factors affecting microdistribution of stream benthic insects. Oikos. 29: 33-43.

Reice, S. R. 1980. The role of substratum in benthic macroinvertebrate microdistribution and litter decomposition in a woodland stream. Ecology 61: 580-590.

Rosenberg, D. M., and V. H. Resh. 1982: The use of artificial substrates in the study of freshwater benthic macroinvertebrates, p. 175-235. *In* J. Cairns Jr. [ed.] Artificial substrates. Ann Arbor Science Publishers Inc., Ann Arbor, MI.

Shaw, D. W., and G. W. Minshall. 1980. Colonization of an introduced substrate by stream macroinvertebrates. Oikos 34: 259-271.

Sheldon, A. L. 1977. Colonization curves: application to stream insects on semi-natural substrates. Oikos 28: 256-261.

Sheldon, A. L. 1984. Colonization dynamics of aquatic insects, p. 401-429. *In* V. H. Resh and D. M. Rosenberg [ed.] The ecology of aquatic insects. Praeger Publishers, NY.

Shelly, T. E. 1979. The effect of rock size upon the distribution of species of Orthocladiinae (Chironomidae; Diptera) and *Baetis intercalaris* McDunnough (Baetidae; Ephemeroptera). Ecol. Entomol. 4: 95-100.

Stein, R. A. 1977. Selective predation, optimal foraging, and the predator-prey interaction between fish and crayfish. Ecology 58: 1237-1253.

Walley, G. S. 1930. Review of *Ephemerella* nymphs of western North America (Ephemeroptera). Can. Ent. 62: 12-20.

Williams, D. D. 1980. Some relationships between stream benthos and substrate heterogeneity. Limnol. Oceanogr. 25: 166-172.

Willliams, D. D., and J. H. Mundie. 1978. Substrate size selection by stream invertebrates and the influence of sand. Limnol. Oceanogr. 23: 1030-1033.

Wise, D. H., and M. C. Molles Jr. 1979. Colonization of artificial substrates by stream insects: influence of substrate size and diversity. Hydrobiology 65: 69-74.

III. DIEL PERIODICITY IN DENSITIES OF EPHEMEROPTERA NYMPHS ON THE SUBSTRATE AND THE RELATIONSHIP WITH ABIOTIC AND BIOTIC FACTORS.

A. INTRODUCTION

Prior to the 1960's, there were few studies on diel periodicities of aquatic insects. Notable exceptions were studies by Moon (1940) and Harker (1953). In the 1960's interest was probably stimulated by the discovery of diel periodicity in the drift (i.e. in the water column, as opposed to being on the substrate) of stream invertebrates. Since that time, diel periodicities of drift have been demonstrated for many taxa and in many parts of the world (see reviews Waters 1972, Müller 1974, Statzner et al., 1984). However, the mechanisms involved in this diel periodicity are still not well understood. Two patterns of periodicity on the substrate have been identified: diel positioning changes and diel activity levels (Wiley and Kohler 1984). Diel density changes of the benthic invertebrates on the top surfaces of the substrates (i.e. diel positioning changes) are considered here.

There is much debate as to whether diel drift periodicity is accounted for by an assumed accidental displacement of the benthos or by an active release of the organisms from the substrate (e.g. Wiley and Kohler 1984, Allan et al., 1985). Whether drift is due to accidental or active departure from the substrate, it would be expected to be directly related to diel densities of the benthos on the substrate. If drift and density are unrelated, accidental displacement can be rejected as a mechanism to explain the diel periodicity in drift. Several hypotheses have been proposed to explain diel density changes of the benthos on the substrate. A premise in these hypotheses is that the benthic animals are present on the top surfaces of the substrate mainly at night. Phototaxis, responses to predation, foraging periodicity, and respiratory needs of organisms may account for this phenomenon (Hynes 1970, Waters 1972, Wiley and Kohler 1984). Many immature aquatic insects have been reported to be negatively phototactic. Therefore the organisms would be present beneath the substrate particles during the daylight and migrate to the top surfaces of the substrate in

the dark. Visually-foraging predators, such as fish, are thought to depress zoobenthos densities on top of the substrate during daylight hours. High densities of organisms on the top of the substrate may also be related to feeding periodicity. Because many aquatic insects are grazers, feeding on the attached algae and fine organic material on the upper surfaces of the substrate, a nocturnal foraging strategy by prey might reduce risk of predation. Nocturnal activity of prey may be more evident in larger prey since these will be most likely chosen by a vertebrate predator. A nocturnal decrease in dissolved oxygen in the substrate may cause a migration from the bottom surfaces of the substrate, or from deep in the substrate, to more exposed surfaces at night (Wiley and Kohler 1980). These mechanisms may be controlled by endogenous factors or exogenous or both. For example, Elliott (1968) stated that density changes on the top of the substrate were exogenously controlled (i.e. by light intensity) but that activity was endogenously controlled.

Many approaches have been taken in studying diel density changes of benthic organisms. They include, the use of samplers and artificial substrates (Moon 1940, Clifford 1972, Kovalak 1978, 1979, Campbell 1980, Kohler 1983, Graesser and Lake 1984), and direct observations in the laboratory (Harker 1953, Elliott 1968, 1969, Bailey 1981) and in the field (Wiley and Kohler 1981, Statzner et al., 1984, Statzner and Mogel 1984, 1985, Mogel et al., 1985, Allan et al., 1985). In my study, I chose direct observations, since this method would potentially cause the least disturbance to the substrate and ultimately to the organisms. Data of such observations most likely represent the most natural situation (see Discussion). My study was designed to determine whether the stream benthos in Dyson Creek, Alberta, exhibited a diel periodicity in density changes on the top of the substrate. Abiotic factors were also measured, and observations were made during two new moon phases and two full moon phases. Also, drift was measured to determine if these abiotic and biotic factors were related to the diel periodicity of benthic organisms. Mechanisms that may contribute to the observed diel densities are discussed.

B. MATERIALS AND METHODS

The area of the stream chosen was about 4 m downstream of a previous study (see Chapter II for a detailed description). A 30 m length of the stream bed close to the south bank was chosen because in this area the substrate size (mean = 4.7 cm, range = 2.1-8.2 cm, water velocity and depth were uniform (Table III-1). The substrate particles were generally well-covered with epilithon, and the particles were rarely disturbed by the current except when the stream was in spate (see below).

Direct observations were made with a rectangular observation box (50 x 25 x 30 cm) with Plexiglass walls (thickness = 1.2 cm) and a glass bottom (thickness = 0.5 cm). Around the top of the box, four pipes (length = 4 cm) were attached to a wooden frame (6.8 x 1.8 cm). Four stainless steel legs (length = 65.5 cm, diameter = 1.3 cm) were each secured into each pipe by wingnuts. The leg-length could be adjusted, using the wingnuts and this allowed the level of the glass bottom to be positioned relative to the ambient water depth. This was necessary to obtain a clear view of the bottom substrate with a maximum depth of water between the glass and the substrate. The mean distance between the glass bottom of the box and the top of the substrate was about 6 cm. The box was carefully positioned on the substrate to minimize disturbance to the substrate under the box. To determine whether the box was creating unnatural water currents a suspension of fluorescent particles was released around and underneath the box; I observed no currents due to the box. Occasionally between observations an anti-fog wax was applied to the inside of the glass bottom to prevent condensation. At night (ca. 2100-0600 h M.D.T.), and including dusk and dawn, observations were made with a red light. The light source was an automobile headlight (face = 12 x 12.5 cm, with a H 3 halogen bulb, 12 V, 55 W), with a handle attached to the back of the light; the power source was a heavy duty 12 V battery. A sheet of neoprene (area = 17 x 17.5 cm, thickness = 0.5 cm) with a porthole (diameter = 4 cm) cut in the centre was attached to the front of the light. Between the neopryene and the light, a photographic glass-mounted filter (Tiffen AA # 29, diameter = 52 mm) could be secured. The filter transmitted deep red to infra red wavelengths (transmission range = 600-900 nm). The headlight was painted mat black except for an area at the front

(diameter = 8 cm) to allow the light to pass through the filter. The sides and back of the light were enclosed with foam and electrical tape and also painted mat black to prevent light escaping from the apparatus. In 1983, preliminary results at night were obtained by using a flashlight with hard red plastic (diameter = 6 cm, thickness = 1 mm) taped over the lens (diameter = 4.5 cm). The results demonstrated the flashlight beam was weaker relative to that of the headlight; this reduced the area illuminated by the light and caused increased shadows and consequently more required observer movement. The flashlight was not as effective as the headlight apparatus described above. I determined whether the density of organisms observed on the substrate was different when observations were made using red light (with filter), white light (no filter), and no light. These observations were made between 0750 h and 1300 h on two days (June 19 and 20, 1984) at 30 min intervals at different and adjacent sites. Seven sequences of the three observation methods, which were randomly chosen, were used.

To determine diel densities of the benthic organisms, direct observations were made in 1984 on four series of dates: a new moon one (NM1) study on June 27-29 and a new moon two (NM2) study on July 25-28, and a full moon one (FM1) study on July 11-13 and a full moon two (FM2) study on August 8-11. Each series of observation dates made up a 24 h day. Observations for each 24 h study were taken in four 6 h blocks to make up a 24 h day. Twelve readings were taken at a 30 min interval in each of the 6 h blocks and there were 12 h between any of the four 6 h blocks in a 24 h day. The first 6 h block on the successive four series of observation dates was alternated to begin early (0630 h) or late (1830 h) in the day for the two new moons and the two full moons. Within any 6 h block, observations were taken at 0.5-1.0 m parallel from the stream edge at 12 sites, begining at the most downstream site. Twelve sites were chosen to reduce the chances of recording the same organism twice. The longitudinal axis of the box was placed parallel with the stream edge and there was about 10 cm between each of the 12 sites. The bottom of the box contained two 20 x 20 cm quadrats (which were each subdivided into four 10 x 10 cm quadrats each, hence a total of eight quadrats), a stopwatch, and a ruler (scale = 1 mm). The box was placed at a site and left for about 20 min prior to starting observations. Before taking

observations I spent 2-3 min motionless, kneeling in position over the box. Only rarely did some of the nymphs react to my approach by moving to the sides or bottom surfaces of the rocks, but usually the nymphs returned to the upper surfaces of the rocks. All observations were recorded, in a coded format, using a portable tape recorder to increase the efficiency of collecting data and to minimize movements that might disturb the benthic organisms. Individual organisms were counted during eight 1 min periods in each 10 x 10 cm quadrat; the most downstream quadrats were observed first. The organisms were identified and their body length (less the cerci) recorded using a ruler in the bottom of the observation box. Other factors relating to the organisms that were recorded were the activity of the insect nymphs, i.e. if they were stationary or moving or feeding, and the orientation of nymphs on individual rocks relative to the substrate surfaces and current direction (R. J. Casey upublished data).

During the test of the effect of the red light and the four 24 h studies of observations, the following abiotic factors were recorded: incident light (Gossen Lumasix meter, about 10 cm above the water surface), an estimate of the percent cloud cover, water temperature and water depth. Water velocity was measured at the end of the observation periods. Diel drift densities were recorded in 1985. Two drift samples were taken at 3 h intervals over a 24 h period on August 5-6 in an area 300 m upstream of the study area. The drift nets had a large opening (frame = 1.0 x 0.5 m, mesh = 0.211 mm). They were 2 m long and had a detachable cup at the end of the net. They were placed about 10 cm above the stream bed.

C. RESULTS

Data Treatment

For the analyses, the total number of organisms observed at any 30 min interval (i.e. eight 10 x 10 cm quadrats) was treated as one sample. The eight quadrats were treated as one sample, because if the quadrats were treated individually, the quadrats would not be true replicates and they would be pseudoreplicated (Hurlbert 1984). The

new moon one (NM1) study was done when the stream was in spate. At this time the mean water velocity (33.3 cm s^{-1}) and depth (8 cm) values were about twice those of the other 24 h studies (Table III-1). Also, the substrate particles were occasionally disturbed by the current during the NM1 study. During the full moon one (FM1) study, the new moon two (NM2) study, and the full moon two (FM2) study, I had difficulty identifying some of the taxa, because of large influxes of small nymphs. For all these studies, *Cinygmula* and *Epeorus* nymphs were considered in the Heptageniidae taxon. For the FM2 study the morphologically similar *Baetis* and *Ameletus* nymphs were treated as a single group. Most of these nymphs were *Baetis*. Other taxonomic groupings of nymphs were: Total Fauna (NM1, FM1, NM2, and FM2 studies), *Baetis* (probably *Baetis bicaudatus* and *Baetis tricaudatus*) (NM1, FM1, and NM2 studies), *Drunella coloradensis* Dodds (NM1, FM1, NM2, and FM2 studies), *Cinygmula* (NM1 study), and *Ameletus* (FM1 and NM2 studies).

Phototaxis

The densities of Total Fauna, *Cinygmula*, and *Baetis* nymphs observed in daylight with the red light, the white light, and without a light source were not significantly different among observation treatments (Friedman test, $p = 0.73$, 0.11, and 0.51, respectively). *Cinygmula* and *Baetis* were the dominant taxa of the 457 organisms observed in this test (35.7 % and 62.8 % of the total fauna, respectively). The mean incident light readings (daylight) and red light were similar (24,903 and 26,886 lux, respectively). The white light was about twice that of the red and ambient light (59,086 lux). When this test of the effect of light on the benthos was made, all abiotic factors were similar between dates except for percent cloud cover.

Moonlight

To determine if temporal trends in the substrate diel densities of the new moon and full moon studies were similar, I used Kendall's coefficient of concordance rank test (Siegel 1954, Conover 1980). The chi-square test was used to determine whether

Kendall's coefficient values (W) were significantly different from zero (Siegel 1954); the W values vary from zero (no concordance) to one (complete concordance). If the chi-square results were significant, it would suggest that the ranks (of 48 densities) between the individual 24 h studies were related. Results are presented in Figures III-1 to -4. The diel densities of Total Fauna for the four 24 h studies were similar (W = 0.48) and the rankings were significantly related between the two new moon and the two full moon studies (Figure III-1).

Densities of Total Fauna were generally low between 1200 h and about 1500 h; densities then increased after 1500 h and decreased after dusk, and then there were again density increases prior to dawn. After dawn, the densities were generally lowest and below the mean density for the four studies. The Total Fauna group included Heptageniidae (44.3 % of the total number), *Baetis* (17.4 %), the *Baetis-Ameletus* group (26.2 %), *D. coloradensis* (5.8 %), *Cinygmula* (4.0 %), *Ameletus* (4.0 %), and minor groups (0.8 %) (Table III-2). A total of 6,503 organisms were observed in the four 24 h studies.

Numbers of *Cinygmula* nymphs were highest from 1200 h to dusk and lowest in the dark period (Figure III-2). The heptageniid diel densities of nymphs were similar (W = 0.61) and the ranks were significantly related (Figure III-2). Although *Baetis* densities over all of the 24 h studies were similar between studies (W = 0.32), the chi-square value was not significant (Figure III-3). For the NM1 and FM1 studies, demonstrated *Baetis* nymphs were generally greater than the mean density after 1600 h and in the dark; but in the NM2 study, *Baetis* densities were greater than the mean density usually only after 1600 h (Figure III-3).

Because the stream was in flood during the NM1 study, the results of my study may have been biased. Therefore Kendall's coefficient of concordance and the chi-square test (on the difference between W and zero) were calculated for the Total Fauna and the *Baetis* nymphs for the 24 h studies, but the NM1 study was omitted. Similar W coefficients and the same chi-square test results were obtained as when the NM1 study was included in the analyses. The *Baetis-Ameletus* nymphs had a similar trend in densities to the *Baetis* nymphs in the NM1 and the FM1 studies (Figure III-3).

Kendall's coefficient was neither calculated for *Ameletus* nymphs, because they were present in low densities (Table III-2); nor for *D. coloradensis* nymphs, because there was a large number of ties in the data (Figure III-4), and these analyses would be inappropriate. *Drunella coloradensis* nymphs were at greater densities at night in the FM1, NM2, and FM2 studies. (Figure III-4, Table III-2). The increase in diel densities of *D. coloradensis* nymphs were synchronised with darkness; in contrast, the other taxon groups, especially the heptageniids, exhibited their largest densities well before the onset of darkness (Figures III-2 to -4). The results of Kendall's concordance test demonstrated that both the Total Fauna and individual taxon groups exhibited similar substrate diel densities between the new moon and full moon phases.

Dark/Light Densities

To determine if the substrate densities of Total Fauna and the individual taxon groups were proportionally greater in the dark period (night) than in the light period (day), the total densities in the dark and light periods were divided by the number of 30 min readings for each period in the four 24 h studies. Indices of the number of organisms present in the dark period divided by the light period for each 24 h study and the mean of these indices for each taxon were calculated (Table III-2). The indices of densities indicated that for Total Fauna there were more organisms at night than in the day. When the individual taxa were considered alone, other trends were evident (Table III-2). *Drunella coloradensis* nymphs showed the greatest increase in numbers at night. The *Baetis-Ameletus* group and *Baetis* nymphs had the next highest densities at night. For *Ameletus* nymphs in the dark, there was an increase in density for the FM1 study and a decrease in density for the NM2 study (Table III-2). The Heptageniidae had similar densities in the dark and the light periods (Table III-2). *Cinygmula* showed a distinct tendency for greater densities in the day, but only one 24 h study was examined (Table III-2). In short, for Total Fauna the high mean density in the dark is due mainly to high densities of *D. coloradensis* and, to a lesser extent, the *Baetis-Ameletus* and *Baetis* nymphal densities (Table III-2).

Diel Peak and Trough Densities

By using a graphical method, I determined if the taxon groups showed common high (peak) and low (trough) diel densities at 30 min intervals. This was done for Total Fauna and individual taxa, excluding *Ameletus*, by selecting the times of the three highest peaks and three lowest troughs (ties in the densities = one peak or trough) in two 12 h periods (1230 h to 2400 h and 0030 h to 1200 h M.D.T.) for each 24 h study. Three peaks and three troughs were chosen because they would represent an estimate of the average peak and trough densities in a 12 h period. I chose the 12 h periods because peaks in the drift of most organisms in streams occur either side of midnight (Waters 1972). Thus, mean times of a peak and trough were calculated for each 12 h period. Characteristic mean times of peaks and troughs were evident; and for the individual taxa, mean times varied little from the mean peaks and troughs of Total Fauna (Table III-3). Note that these mean times did not correspond to actual peaks and troughs of densities because of the variation associated in calculating the means (Table III-3, Figures III-1 to -4). *Clinygmula* nymphs had similar mean times to the other taxa, but the times of peaks and troughs were reversed relative to the other taxa (Table III-3). The percentage number of 30 min readings required in any of the 12 h periods to calculate a peak or trough ranged between 14 % and 28 %, depending on the number of ties in densities. The trough densities for *D. coloradensis* probably do not represent a real pattern because the troughs make up 40 % and 54 % of the 30 min readings for the two 12 h periods (Table III-3, Figure III-4). Because of the consistency of the mean times of the peaks and troughs of diel densities for the individual taxa, I constructed a graph of the diel periodicity in density of Total Fauna in the stream. Mean densities associated with the mean times of peaks and troughs for the four 24 h studies were plotted (Figure III-5). The mean benthos density for all studies (i.e. 34 organisms per 800 cm^2) was used as a base density between the peaks and troughs (Figure III-5). The graph of the diel periodicity in density of the Total Fauna was compared with published results of diel periodicity in densities and the drift of aquatic insect nymphs (see Discussion).

Drift

All taxa observed on the substrate by the direct observations, except *Ameletus* nymphs, were found in the drift samples. But only *Cinygmula*, *Epeorus*, and *Baetis* were common in the drift. Mean number and range of nymphs per sample interval and the proportional abundances of nymphs in the dark period divided by those in the light period are presented in Figure III-6. Drift of *Cinygmula* and *Epeorus* nymphs increased in the dark with a peak occurring at 0100 h M.D.T., although the numbers of *Epeorus* were low. For *Cinygmula* and *Epeorus*, there were proportionally more nymphs in the drift at night than in the day. *Baetis* nymphs exhibited two peaks of drift, the highest mean number being at 1600 h, the second peak was at 0100 h. However, there were still proportionally more *Baetis* nymphs present in the drift at night than during the day.

Size Class Differences

The organisms were separated into two size classes based on body length to determine if small and large nymphs showed the same trends in diel density on the substrate as when the nymphs of each taxon were treated as a single size class. Maximum body length of nymphs making up the Total Fauna group was 12 mm. The size classes chosen were 1-4 mm (small) and 5-12 mm (large); these sizes made up 57 % and 43 %, respectively, of Total Fauna. Diel densities, Kendall's coefficient, and the chi-square statistics of the small and large size classes of the heptageniid and *Baetis* nymphs are presented in Figures III-7 to -10. *Drunella coloradensis* nymphs were mostly in the large size class. Both small and large *Cinygmula* nymphs had a trend in diel densities (Figures III-7 and -8) similar to when the nymphs were treated as a single size class (Figure III-2). Most of the *Cinygmula* nymphs were large. The diel densities of small nymphs of the heptageniid group (Figures III-7 and -8) were similar to the pattern for the Heptageniidae group when they were treated as a single size class (Figure III-2); but this was not the case for the large size class of Heptageniidae nymphs. However, for both the small and large size classes of Heptageniidae nymphs, the diel densities were similar between the 24 h studies and the ranks of the densities were

significantly related (Figures III-7 and -8).

For *Baetis* nymphs, there were more small nymphs in the dark in two of three studies; these were similar to when the nymphs were treated as a single size class (Figures III-3 and -9). Large *Baetis* nymphs had similar densities over two of the three 24 h studies, but had lower densities in the dark during new moon two study (Figure III-10). For the small and large *Baetis* nymphs, the densities were not significantly related between the new moon and full moon studies (Figures III-9 and -10). The small *Baetis-Ameletus* nymphs showed a trend similar to when all the nymphs in this group were considered as one size class (Figures III-3 and -9). But nymphs in the large size class did not have any apparent pattern (Figure III-10). Kendall's coeffecient and chi-square values for the small and large size classes of the heptageniid and *Baetis* nymphs were similar to those when the sizes were considered together.

Abiotic Factors

Each of the abiotic factors recorded during the four 24 h studies was significantly different (Friedman test, $p < 0.05$) between the four studies (Table III-1). However, when the mean of each abiotic factor was calculated for the two new moon and two full moon studies, they were similar except for water velocity and depth (Table III-1). To determine if incident light, percent cloud cover, and water temperature were related to the diel densities of the nymphs on the substrate, I used the Spearman rank correlation test. Incident light and cloud cover were not correlated with the diel densities of Total Fauna, and the correlation coeffecients (r_s) for these abiotic factors (-0.08 and 0.04, respectively) were not significantly different from zero ($p > 0.05$). Water temperature was positively correlated with the diel densities of Total Fauna (r_s = 0.60), and this association was significant ($p < 0.001$). Water temperature and diel densities of Heptageniidae ($r_s = 0.48$, $p < 0.001$), *Cinygmula* ($r_s = 0.59$, $p < 0.001$), and *D. coloradensis* ($r_s = 0.16$, $p > 0.10$) were positively correlated. However, *Baetis* and *Baetis-Ameletus* densities were negatively correlated with water temperature (r_s = -0.22, $p < 0.01$ and $r_s = -0.01$, $p > 0.50$, respectively).

D. DISCUSSION

Nymphal Behaviour

When I made the observations there were characteristic reactions by the nymphs to movements by me, presumably due to nymphs perceiving my silhouette against the sky. For example, *Cinygmula* nymphs were easily disturbed when I suddenly moved. The nymphs would move quickly from the top to underneath the substrate. But if I moved slowly or only slightly, the nymphs usually did not react to my presence. *Epeorus* nymphs were less easily disturbed than *Cinygmula* nymphs by movements, and *Baetis*, *Ameletus*, and *D. coloradensis* nymphs generally did not seem to react to my movements. Similar reactions of heptageniid and *Baetis* nymphs have been reported by other workers (Madsen 1968, Allan et al., 1985).

Phototaxis

Red light is a common method used to observe benthic organisms in the dark. Where the potential effect of red light and dim white light has been tested no effect has been found (Elliott 1968, 1970, Bailey 1981). In my study, when I used the red light (600-900 nm) at night, the nymphs did not seem to react as much to my movement as they did during the daylight. There is little evidence that wavelengths greater than about 650 nm are visible to insects, one exception is the firefly *Photinus* (Burkhardt 1964).

I found equal densities of *Baetis* and *Cinygmula* nymphs when observed with red light, white light, and no light in the daylight. This suggests that the use of the red and white light did not affect the organisms and that the nymphs are not negatively phototactic. Bohle (1978) found similar numbers of *Baetis rhodani* nymphs in shaded and illuminated areas in a laboratory stream. Nevertheless my light observations are generally contrary to other workers' results for *Baetis* and heptageniid nymphs (Wodsedalek 1911, Lyman 1945, Scherer 1962, Elliott 1968). The red and white light that I used could be seen clearly (the red light less so) on the substrate when the observations were made in the early morning; shining the light beam directly on the

nymphs apparently did not cause a negative phototactic response. It would be informative to study more immature aquatic insect reactions to light, especially in the field instead of in the laboratory, because the artificial conditions of the laboratory might alter an organism's behaviour. For example, Elliott (1968) found that the nymphs of four of five ephemeropteran genera did not show a negative phototaxis when water flow in an artificial stream was stopped. When water was flowing the nymphs were negatively phototactic.

Moonlight

To my knowledge, my study is the first where the diel densities of the benthos have been compared between new moon and full moon phases. Although Allan et al., (1985) did not discuss the effect of moonlight on benthos diel density, they made direct observations on *Clnygmula* and *Baetis* nymphs at approximate new moon and full moon phases in a Rocky Mountain, Colorado stream. They made observations on two occasions in 1983, one during a new moon and the other about seven days after a full moon; in 1984 they made observations twice when there was a full moon. Their results would indicate that diel benthic densities were similar between the new moons and full moons. Hence their results are similar to mine, although their study was done in a different geographical location.

Concerning insect drift, it is not clear whether moonlight has a depressant effect on the drift of insects. Such an effect has been suggested by several workers (e.g. Waters 1962, Anderson 1966, Bishop and Hynes 1969). However, other studies indicate that moonlight has no effect on drift (Elliott 1967, Elliott and Minshall 1968, Chaston 1969, Waters 1969). I could not detect light intensity differences between a new moon and full moon when using a small light meter. Chaston (1972), when discussing possible causes of drift, suggested that the difference in incident light between a full moon and no moon may not be important to the benthos. This is because the maximum value of incident light for a full moon (0.25 lux) was below the threshold level of light (about 1.0 to 1.6 lux), as reported by Holt and Waters (1969) and

Chaston (1969), apparently needed to initiate behavioural drift. However, a lower drift threshold of 0.01-0.001 lux was found by Bishop (1969). In my study area of Dyson Creek, direct illumination from a full moon did not fall on the stream bed (where the observations were made) during darkness, because the study site was in a "moon shadow" accounted for by surrounding gorge walls. Obstructions to moonlight may be important to aquatic insects that can only perceive direct illumination (rather than dispersed light) from the moon. Thus moonlight may only have an affect in streams where direct illumination reaches the substrate. Other factors which could have affected the results include cloud cover, water velocity and depth. For example, during the full moon studies 50 % cloud cover occured during the dark period although this was rare.

Dark/Light Densities

My results indicate that nymphs of certain taxa were found in greater densities on the top of the substrate at night. Studies on the diel densities of immature lotic insects in the field and in the laboratory have also shown increases in number at night (Moon 1940, Elliott 1968, Campbell 1980, Bailey 1981, Mogel et al., 1985). But some workers have found greatest densities during the day (Graesser and Lake 1984, Statzner and Mogel 1984, 1985, Allan et al., 1985); and other studies indicate no differences between day and night (Clifford 1972, Kovalak 1978, 1979, Wiley and Kohler 1981, Kohler 1983). Some of the disparity in these published results, other than differences due to different taxa, are possibly accounted for by the method of sampling, the sample interval, and whether the study was conducted in the laboratory or field.

Sampling the benthos with a sampler as opposed to making direct observations causes disturbance to the substrate, and the organisms collected from the upper layer of the substrate are not necessarily the organisms that were on the top of the substrate prior to taking the sample (also see Discussion in Allan et al., 1985). In several of the studies cited above, the day and night samples of the benthos were taken at only one time in the day and once at night. Since my results indicate a large amount of variation

over a 24 h study, even between consecutive 30 min observation intervals, it would seem advisable to take frequent samples during a 24 h period. When there are long intervals between daily samples, the sampling times chosen are important. For example, in my study with 30 min sampling intervals, equal benthos densities occurred in the dark and light periods in Dyson Creek (also see Bailey 1981, Wiley and Kohler 1981, Allan et al., 1985). Determining the most appropriate daily sampling time would appear to be difficult given the high variance in my study. In the laboratory, it is easier to control factors such as immigration and emigration from the substrate. In the field, this is difficult, if not impossible, to control satisfactorily for a small area of stream bed (Kohler 1983). But laboratory studies cannot replicate biotic or abiotic factors as they are in the field, and some of these factors most likely influence the diel behaviour of the benthos. Thus a field study using direct observations will cause the least disturbance to a natural system and the results will most likely best describe the actual situation.

Most field studies that employed direct observations on lotic insects have demonstrated density increases in the daylight (Statzner and Mogel 1984, 1985, Allan et al., 1985). Statzner and Mogel's (1984, 1985) studies were done in Germany, and Allan et al., (1985) study was done in North America. In both the North American and German studies, the immatures of *Baetis*, *Cinygmula*, and *Micrasema* (Brachycentridae: Trichoptera) exhibited high daylight densities on the substrate and low nocturnal densities; the sharp change in diel densities in these studies appeared to be a direct response to incident light. The daylight density peaks in Dyson Creek, relative to these studies were skewed more towards the dark period, and there was proportionally more nymphs at night than during the day. In a Michigan, USA stream, Wiley and Kohler (1981) used cinematography over two consecutive 24 h periods and found no apparent diel periodicity of *Baetis vagans* nymphs. The authors attributed this to volitional movements by the nymphs.

To my knowledge, the Dyson Creek study is the first using direct observations to show a nocturnal increase in densities of Ephemeroptera nymphs; there is one study showing a nocturnal increase in a trichopteran, *Hydropsyche* (Hydropsychidae) larvae in a German stream (Mogel et al., 1985).

The taxa observed in my study are eaten by fish although there are no fish in the study area of Dyson Creek (see Chapter II). The absence of fish is not believed to have influenced the results, since aquatic insects most likely evolved in the presence of fish predators (Allan 1983). Since fish are visually-foraging predators, the insect nymphs would be expected to experience reduced predation pressure by avoiding the top surfaces of the substrate during daylight.

There are other predators in Dyson Creek (see Chapter II) which include tactile foragers such as perlodid (Plecoptera) stoneflies, some of which exhibit a diel periodicity (Walde and Davies 1985). These stonefly predators, which are fast moving foragers, most likely affect the distribution of their prey. Invertebrate predators such as perlodid stoneflies and *Rhyacophila* larvae, both of which are present in Dyson Creek, have been shown to have significant influences on the distribution and abundance of their prey which include mayflies (Wiley and Kohler 1981; Allan 1983).

In Dyson Creek, most of the organisms observed were cryptically coloured. Functionally, cryptic colouration of aquatic insects may have evolved as an avoidance mechanism to predation, especially to a visually-foraging predator that feeds in the day. There is evidence that cryptic colouration is more effective avoidance mechanism at low illumination (e.g. dawn and dusk) relative to full daylight (Otto 1984). Nymphs therefore may restrict their activity to low light levels and darkness, rather than being active during full daylight. Thus, I suggest that benthic insect nymphs may restrict most of their activities to the top of the substrate in the dark, at dusk, and at dawn to reduce predation. This would explain the diel densities observed for most taxa in Dyson Creek. In the few observational studies where the sampling interval was less than 1 h and the diel densities were greatest during the day, there was a tendency for peak densities to occur close to dusk (e.g. see figures in Statzner and Mogel 1984, 1985).

Drift

The taxa in Dyson Creek exhibited similar diel patterns in benthic densities (with the exception of *Clinygmula*). To determine if drift was related to diel density on the

substrate, the graph of the diel periodicity of the mean times of density peaks and troughs of Total Fauna on the substrate can be compared with the typical diel periodicity of drifting insects. Drift of benthic insects characteristically shows a peak in density after darkness with a second peak, often of smaller magnitude, before dawn. The nocturnal increase in drift is generally of several orders of magnitude (see reviews Hynes 1970, Waters 1972, Müller 1974). The graph of diel densities of Total Fauna on the substrate appeared to be shaped similarly to the diel periodicity documented for drift studies (i.e., two density peaks). However, the first peak in the diel substrate densities occurred about 1 h before darkness and the magnitude of density increase was small relative to that found in most drift studies (e.g, Holt and Waters 1967, Elliott 1969). The second peak in the graph of Total Fauna was at a time similar to the second peak in the drift, i.e. just before dawn. However, the overall nocturnal substrate diel density increase was of smaller magnitude than that of the drift. My drift results for Dyson Creek nymphs showed no second peak possibly due to the large sampling intervals. Also, the drift peak occurred well after the observed density peak.

The drift peaks for the Dyson Creek fauna were perhaps smaller than would be expected from other drift studies. The substrate diel density peaks of the heptageniid nymphs were most obvious and consistent between 24 h studies, and there was a suggestion that they may precede peaks in drift. But the heptageniid drift peak occurred about 5 h after the substrate density peak, and it was difficult to determine if there was a second drift peak. *Baetis* nymphs are often reported to be one of the most abundant of drifting insects, and *Baetis* nymphs usually exhibit very large behavioural drift peaks (Waters 1972). If the diel densities of *Baetis* on the substrate were related to their abundance in drift, the nymphs would be expected to show the most pronounced substrate diel density peaks. This was clearly not the case in my study.

Given the large sampling interval used in my study of drift, I suspect that more frequent samples would give a better picture of the relationship between the benthic and drift densities. There is little evidence for a relationship between drift and diel densities of immature aquatic insects on the substrate in Dyson Creek. However, I did not

measure the substrate and the drift densities of the nymphs at the same time or at the same location in Dyson Creek. But in studies where the substrate and drift densities have been measured at about the same time no clear relationship was found, in either laboratory studies (Elliott 1968, Bohle 1978) or field studies (Bailey 1981, Kohler 1983, Graesser and Lake 1984, Statzner and Mogel 1984, 1985). A possible exception is a study by Mogel et al., (1985) who found that the drfit of *Hydropsyche* (Hydropsychidae: Trichoptera) larvae appeared to be related to when activity, including migration and aggressive encounters on the substrate, were most prevalent. Such activities may indicate either an active or accidental departure from the substrate. Considering the size of my drift nets, the potential area of substrate and the volume of water sampled, drift numbers are remarkably small when compared to the direct observations of benthos densities in Dyson Creek. Since the diel drift and substrate densities do not appear to be related in my study, the accidental displacement from the substrate by the benthos was not an important factor in causing a drift periodicity of the nymphs.

Kohler (1983) stated that migrations to and from the exposed surfaces appeared not to dislodge organisms into the drift. When I made observations of the nymphal substrate densities in Dyson Creek, I noted incidents of drift occurring. Drift was recorded as nymphs landing on the substrate from the water column or leaving the substrate into the water column, or both. Of the 6503 organisms, I observed that only 51 (i.e. 0.008 %) animals drifted. This is a very low proportion of the benthos that would be expected in the drift, given my values of observed drift for a 24 h period. If drift is a result of an active release and not an accidental displacement, it is difficult to determine how this may be measured, since the observed incidence of drift is so low and the substrate diel density does not appear to be related to drift. Also, I was unable to determine if the drift I observed was delibrate or accidental.

Size Class Differences

During the observations in Dyson Creek, I often observed that small nymphs did not react to my movement as readily as large nymphs. This was especially evident for

the heptageniid nymphs. Larger nymphs are probably seen and preferred by fish because of their greater size, movement, and nutritional value. For the two size classes of insect nymphs in my study, I predicted that larger nymphs would show increased densities at night to reduce predation from visually-foraging predators and smaller nymphs would show an aperiodic density or a density increase in daylight. This hypothesis is similar to one proposed by Allan (1978) as a mechanism in drift, whereby larger nymphs were expected to drift at night to avoid predation and small nymphs would be aperiodic or increase in abundance in the day. In my results the diel densities of small heptageniid nymphs increased in diurnal densities and this supported my hypothesis. However, the large heptageniids did not appear to increase in the dark. For *Cinygmula*, small nymphs were aperiodic and large nymphs decreased in abundance at night. Small *Baetis* and *Baetis-Ameletus* nymphs were more common at night and the large nymphs of these groups had aperiodic densities in the 24 h studies. At the level of size classification in my study there is little evidence to suggest that the densities of small and large Ephemeroptera nymphs supported my hypothesis.

Abiotic Factors

Incident light has been shown to be important in the control of drift (e.g, Bishop 1969, Chaston 1969, Holt and Waters 1969), and light was expected to be important in the control of diel benthic densities of the nymphs in Dyson Creek. For example, in the laboratory, photoperiod has been shown to affect benthos diel densities when temperature has been relatively constant (e.g, Elliott 1968, Bailey, 1981). But in my study, incident light and cloud cover were not correlated with the diel benthic density of Total Fauna. Perhaps this was due to the high variance of light and cloud cover between the 2 h sampling interval and within any 2 h period. Also the scale at which these factors were measured may not be biologically important.

Water temperature, which exhibits a daily cycle, was correlated with the substrate diel densities of Total Fauna and most taxon groups. This would be expected since an increase in temperature may lead to an increase in activity (see below). When

water temperature increases the nymphs that would have been on the bottom of the substrate migrate to the top surfaces to feed. However, not all of the taxon groups in Dyson Creek were positively correlated with water temperature. For aquatic insects, an increase in water temperature would result in an increased metabolism, and this would be expected to be directly related to observed activity and density on the top of the substrate. But if this was the case in Dyson Creek, the second peak in substrate densities, for example, could not be explained by water temperature. Allan et al., (1985) found the substrate diel densities of *Cinygmula* and *Baetis* nymphs to be positively correlated with water temperature. In other studies, where direct observations were made, substrate diel densities and water temperature appeared to be positively correlated also (Statzner and Mogel 1984, 1985). Although incident light (and water temperature in some cases) was not correlated with the diel densities of any taxa in Dyson Creek, the photoperiod and thermoperiod may affect larval behaviour, either separately or synergistically. For example, Beck (1983) in a recent review stated that thermoperiod may be acting in concert with photoperiod, for example, to entrain a circadian rhythm. The existence of a circadian rhythm in the diel periodicity of aquatic insects has been suggested previously (e.g, Harker 1953, Elliott 1968). In streams where there is little fluctuation in temperature or light over several months, aquatic insects had activity patterns that appeared to be based on a circadian rhythm (Müller 1974).

To account for the observed diel density changes of nymphs on the substrate, I propose an extrinsic factor that, to my knowledge, has not previously been considered in regard to diel changes in density of aquatic insects. It is the angle of the plane of polarized sunlight, which has been demonstrated as a mechanism of orientation for certain arthropods (see reviews, Mazokhin-Porshnyakov 1969, Waterman 1984). The compound eye and the simple ocelli of arthropods are capable of analysing and responding to polarized light. This has been demonstrated in terrestrial and aquatic organisms such as cladocerans (e.g, Mazokhin-Porshnyakov 1969, Waterman 1984). Baylor and Smith (1953) found that trichopteran larvae were capable of orientating to a polarized beam of light. Sky-polarized light is most evident in the morning and in the

evening, and such light has been suggested as a mechanism controlling the diurnal migration of plankton (Baylor and Smith 1953). Therefore, since aquatic arthropods possess eyes suitable for analysing polarized light, they may have adapted to the diel periodicity of polarized light.

Sky-polarized light observed at a given point will vary with factors such as position of the sun, cloud cover, and the screening effects of vegetation and other landmarks (Waterman 1984). In my study, the sky was completely overcast on several occasions when the direct observations were made, and during these conditions polarization can be excluded (Waterman 1984). However, insect nymphs may be capable of "remembering" the position of the sun, relative to landmarks, based on previous experience when the sky was clear. This has been found for the honey bee under overcast skies (Waterman 1984). At the specific location of the observations in Dyson Creek there was no overhanging vegetation, and the stream substrate was subjected to direct solar radiation from about 0830-1830 h M.D.T. each day. Between 1830-0830 h M.D.T., the sun was obstructed from this area by the surrounding gorge and vegetation. In the graph of the diel density of Total Fauna, the 1830 h and 0830 h times corresponded approximately with the troughs in diel density; between 0830 h and 1830 h the benthos exhibited its greatest densities. Therefore, the observed diel density pattern may be explained by a threshold plane of polarized light or the degree of polarization initiating an increase in density before darkness and a decrease in density after dawn. I would expect symmetrical changes before dusk and after dawn, when the sun was at equivalent angles relative to the water. This expectation is supported to some extent by my results, however, there is an unexplained decrease in density of Total Fauna prior to dawn. Also, polarization alone would not appear to explain my results, for example, the decrease in density between the two nocturnal peaks. But as suggested, several factors may interact to affect the organisms.

Polarized light may be modified by several factors, for example, an increase in turbidity of the water. This occurred in Dyson Creek during the new moon one study when the stream was in spate. The increased number of suspended particles probably altered the polarized light, and this would potentially affect the nymphs. Although I did

not observe a definite diel change in density, the increased water velocity and erosion from suspended particles most likely affected the organisms also.

In view of recent studies on diel changes in density using direct observations, it would be informative to record the occurrence of direct illumination on the substrate and also to consider obstructions to this light (e.g. the water surface, vegetation, the observer, and the observation box) to determine if they play a role in diel density. While the incidence of direct illumination is easy to obtain, polarization of skylight may be very difficult to study, especially in the field.

Concluding Remarks

Although the daily and seasonal cycles of the abiotic factors may be important in influencing diel densities, biotic factors are probably of considerable importance also. Aquatic insects may have trade offs between their rate of metabolism (related to ambient water temperature) and predation pressure at different times of the day. This could be reflected in the diel periodicity of density of organisms. Some examples of biotic factors affecting diel densities include predation (see above) and crowding. For example, Bailey (1981) noted an increase in activity, including feeding and swimming, when the mayfly nymphs were crowded on the substrate. there were no fish at the study site. Although the substrate diel density patterns of my study appear to be similar between dates at a given site, further studies using direct observations are needed to determine if the diel density patterns I observed are consistent within and between geographic regions. The existence of a single abiotic or biotic factor determining diel density of the benthos is still to be demonstrated.

Table III-1. Mean and standard deviation (in parentheses) of the abiotic factors measured during the four 24 h studies of observations on new moon one (NM1) study, on full moon one (FM1) study, on new moon two (NM2) study, and full moon two (FM2) study. The probability levels of the Friedman test (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$) to determine differences between the 24 h studies, and the mean of the two new moons and two full moons are indicated.

Abiotic Factor	24 h Study								Friedman Test	Mean	
	NM1		NM2		FM1		FM2			NM	FM
Incident Light (lux)	7171	(9766)	10792	(14375)	11138	(11892)	4926	(6690)	*	8982	8132
Cloud Cover (%)	81	(16)	53	(37)	56	(29)	81	(13)	*	67	68
Water Temperature (°C)	7.7	(2.5)	13.0	(2.9)	9.5	(2.5)	12.8	(3.3)	***	10.3	11.1
Water Velocity (cm s^{-1})	33.3	(2.9)	14.7	(4.5)	17.0	(5.7)	8.6	(4.1)	**	24.0	12.8
Water Depth (cm)	7.8	(0.8)	3.3	(0.5)	4.2	(0.4)	3.5	(0.6)	**	5.6	3.8

Table III-2. Total number of organisms observed and indices of the proportional densities of taxon groups of Ephemeroptera nymphs in the dark period over light period (Dark / Light) for the four 24 h studies of observations (NM1, FM1, NM2, and FM2). The mean of the Dark / Light indices for each taxon group is indicated. See Table III-1 for details.

Taxon Group	24 h Study	Number Organisms Observed	Dark / Light	Mean Dark / Light
Total Fauna	NM1	750	1.05	
	FM1	1271	1.10	1.12
	NM2	1697	1.07	
	FM2	2769	1.26	
Cinygmula	NM1	257	0.60	----
Heptageniidae	FM1	732	0.94	
	NM2	1225	1.00	1.03
	FM2	919	1.16	
Baetis	NM1	452	1.41	
	FM1	463	1.34	1.21
	NM2	217	0.83	
Ameletus	FM1	34	1.23	0.97
	NM2	65	0.70	
Baetis-Ameletus	FM2	1700	1.25	----
Drunella coloradensis	NM1	7	3.00	
	FM1	34	1.59	
	NM2	190	2.30	2.64 [1]
	FM2	212	4.02	

[1] NM1 was not included in this calculation.

Table III-3. Mean times of density peaks and troughs of the taxon groups of Ephemeroptera nymphs for the four 24 h studies of observations (NM1, FM1, NM2, and FM2). See Table III-1 for details.

		1230 - 2400 h		0030 - 1200 h	
Taxon Group	24 h Study	Peak	Trough	Peak	Trough
Total Fauna	NM1 FM1 NM2 FM2	2030	1730	0330	0830
Clinygmula	NM1	1600	2000	0700	0500
Heptageniidae	FM1 NM2 FM2	2000	1500	0400	0900
Baetis	NM1 FM1 NM2	2100	1700	0500	0630
Baetis-Ameletus	FM2	1930	2200	0330	0830
Drunella coloradensis	NM2 FM2	2000	1330	0230	0830

Figure III-1. Densities of Total Fauna per 800 cm^2 of substrate at 30 min intervals during the four 24 h studies of observations on new moon one (NM1) study, on full moon one (FM1) study, on new moon two (NM2) study, and on full moon two (FM2) study. Kendall's coeffecient of concordance (W) and the probability level of the chi-square test (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, NS = not significant i.e. $p > 0.05$) are indicated. Horizontal bars represent darkness. The mean density for each 24 h study is shown by the horizontal line.

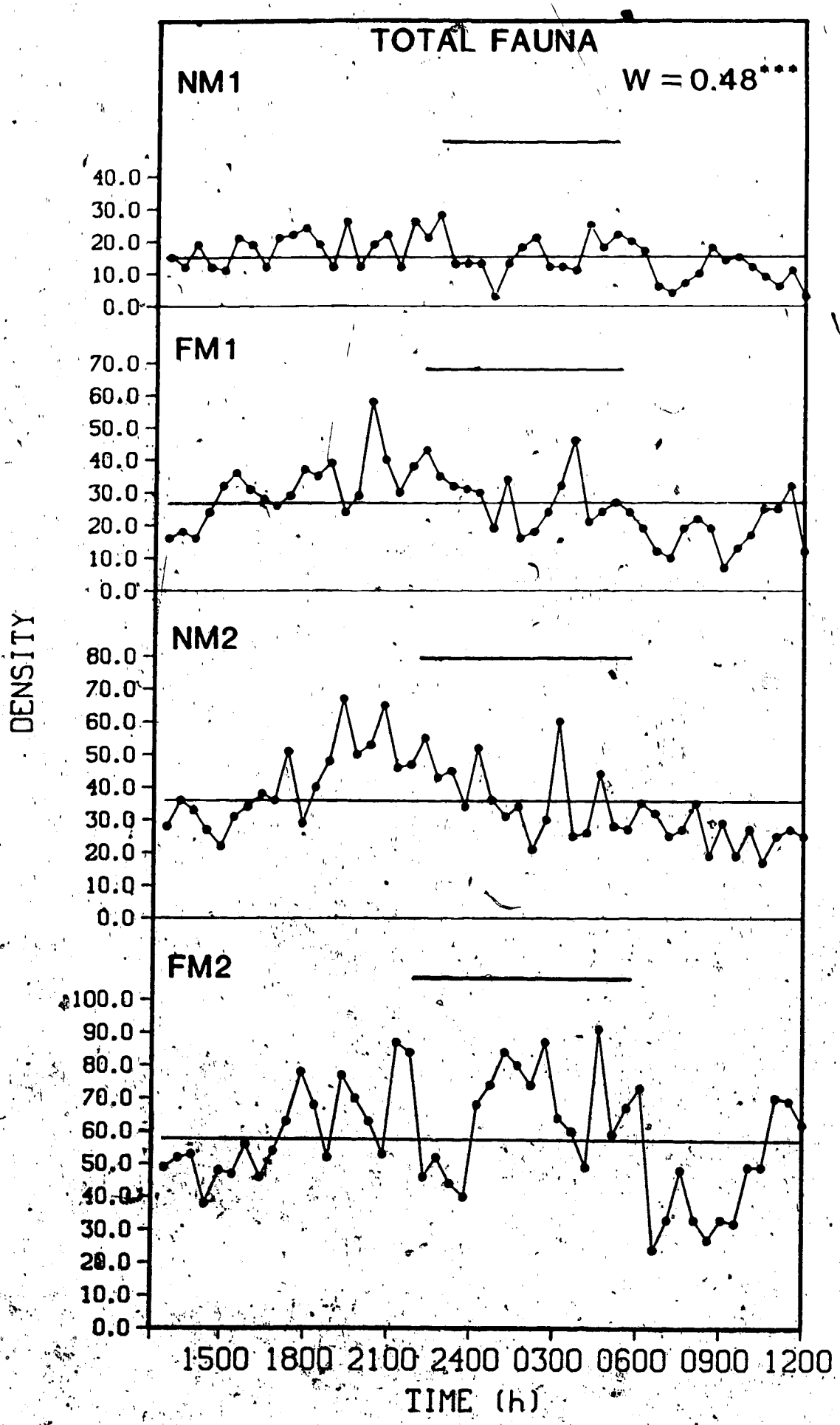

TOTAL FAUNA
NM1
W = 0.48***
FM1
NM2
FM2
DENSITY
40.0
30.0
20.0
10.0
0.0
70.0
60.0
50.0
80.0
90.0
100.0
1500 1800 2100 2400 0300 0600 0900 1200
TIME (h)

Figure III-2. Densities of *Cinygmula* and Heptageniidae nymphs during the 24 h studies of observations (NM1, and FM1, NM2, and FM2 studies). The mean density for each 24 h study is shown by the horizontal line. See Figure III-1 for details.

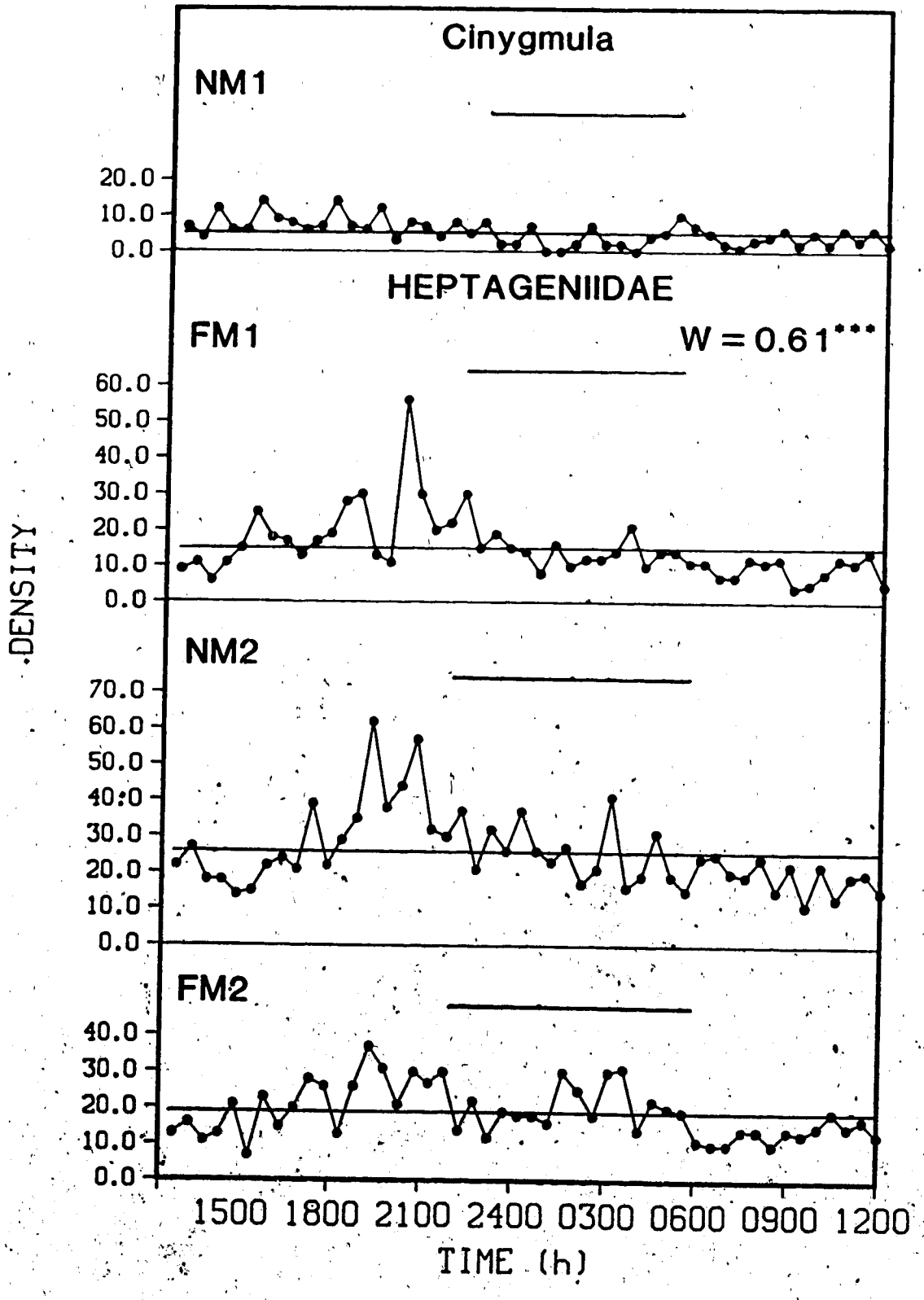

Cinygmula
NM1
20.0
10.0
0.0
HEPTAGENIIDAE
FM1
W = 0.61***
60.0
50.0
40.0
30.0
20.0
10.0
0.0
DENSITY
NM2
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
FM2
40.0
30.0
20.0
10.0
0.0
1500 1800 2100 2400 0300 0600 0900 1200
TIME (h)

Figure III-3. Densities of *Baetis* and *Baetis-Ameletus* nymphs during the 24 h studies of observations (NM1, FM1, and NM2, and FM2 studies). The mean density for each 24 h study is shown by the horizontal line. See Figure III-1 for details.

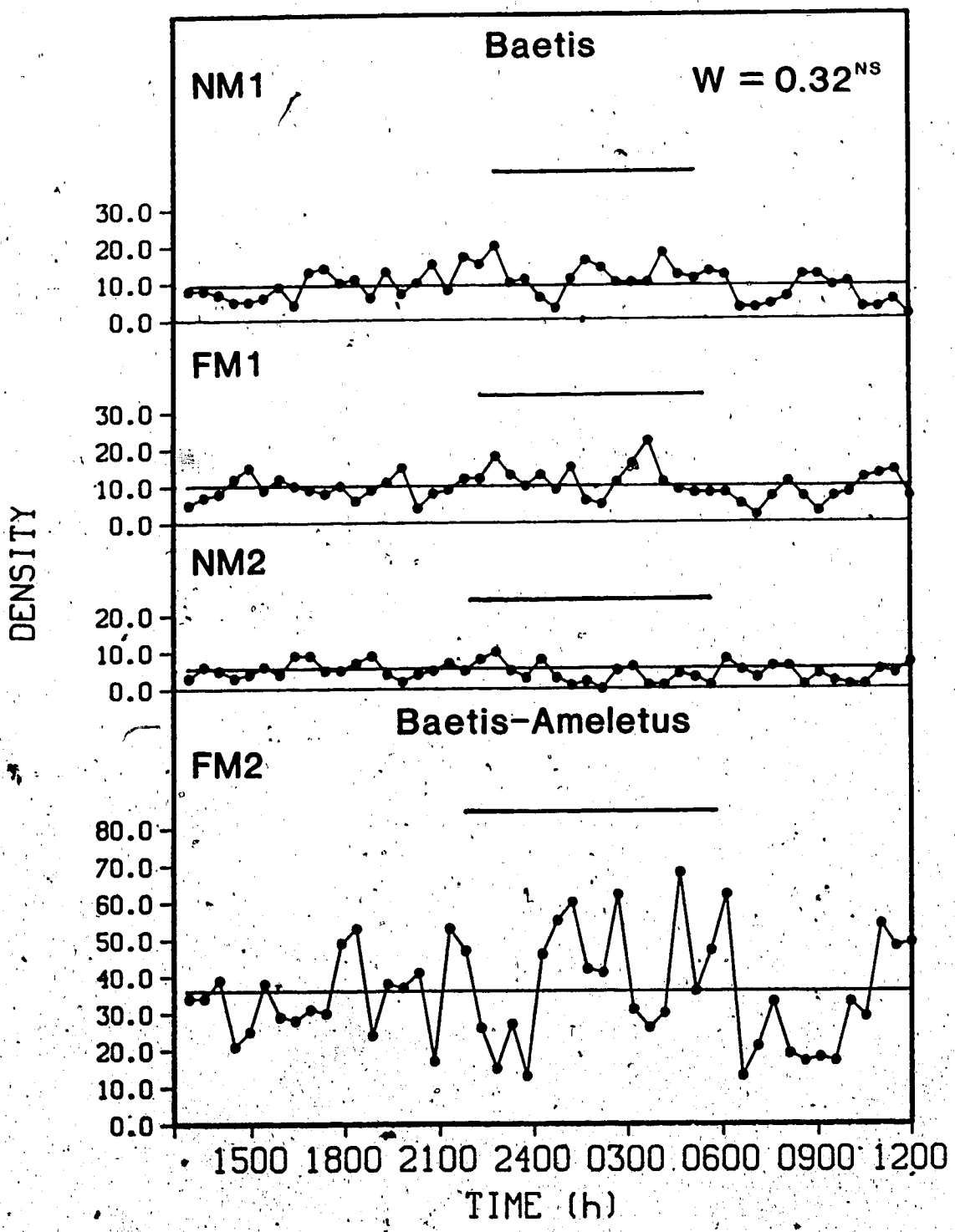

Baetis
NM1
W = 0.32NS
30.0
20.0
10.0
0.0
FM1
30.0
20.0
10.0
0.0
NM2
20.0
10.0
0.0
DENSITY
Baetis-Ameletus
FM2
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
1500 1800 2100 2400 0300 0600 0900 1200
TIME (h)

Figure III-4. Densities of *Drunella coloradensis* nymphs during the 24 h studies of observations (FM1, NM2, and FM2 studies). The mean density for the NM2 and FM2 studies were four and three organisms respectively. See Figure III-1 for details.

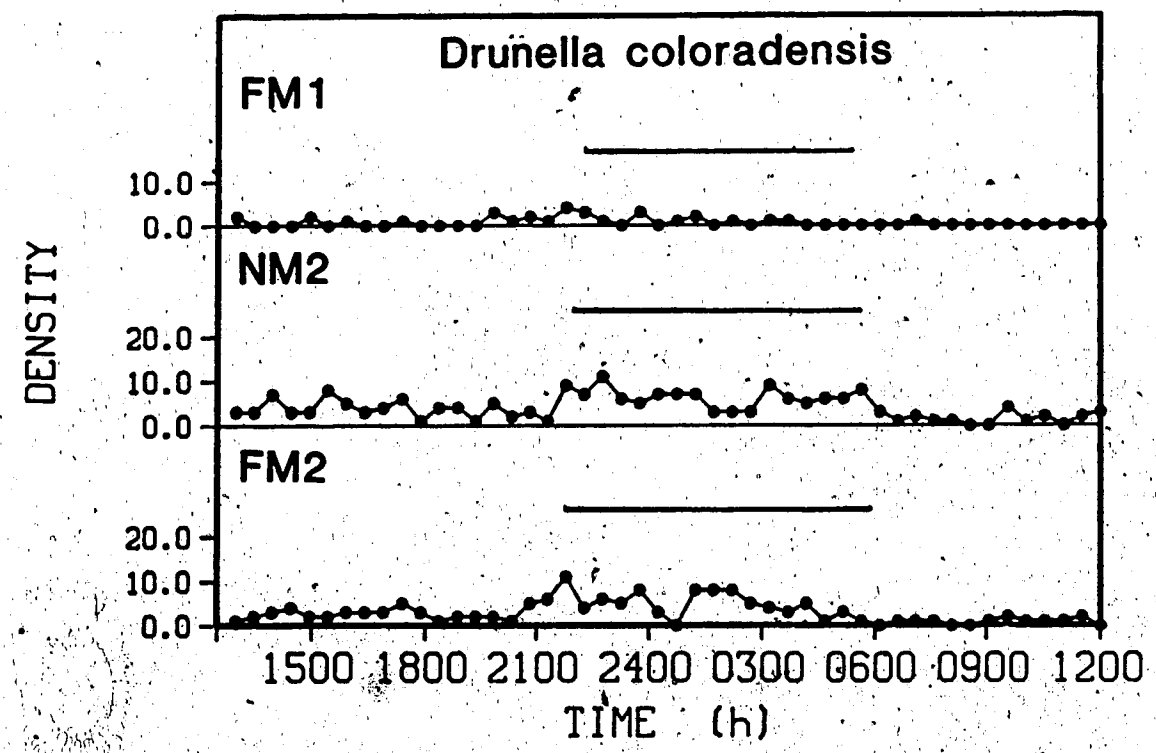

Drunella coloradensis
FM1
10.0
0.0
NM2
20.0
10.0
0.0
FM2
20.0
10.0
0.0
DENSITY
1500 1800 2100 2400 0300 0600 0900 1200
TIME (h)

Figure III-5. Model of the mean densities of Total Fauna per 800 cm^2 at the mean times of density peaks and troughs, during the four 24 h studies of observations in Dyson Creek. Mean density of the four studies was 34 orgnisms. The horizontal bar represents darkness. See text for details.

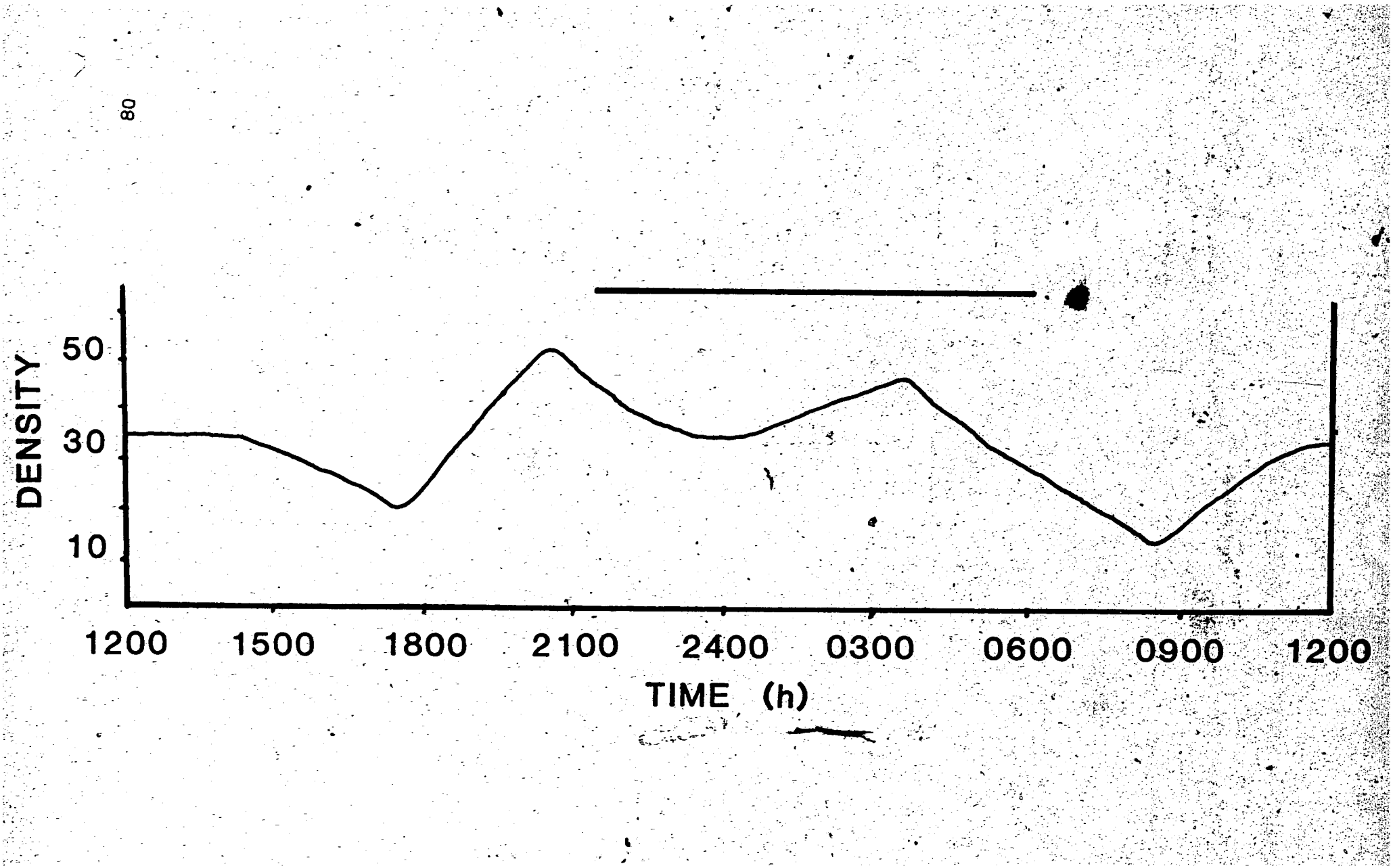

DENSITY
50
30
10
1200
1500
1800
2100
2400
0300
0600
0900
1200
TIME (h)

Figure III-6. Mean and range of two concurrent drift samples of Ephemeroptera nymphs taken at a 3 h interval over a 24 h period. The proportion of nymphs in the dark period over light period is indicated by D/L. The horizontal bar represents darkness.

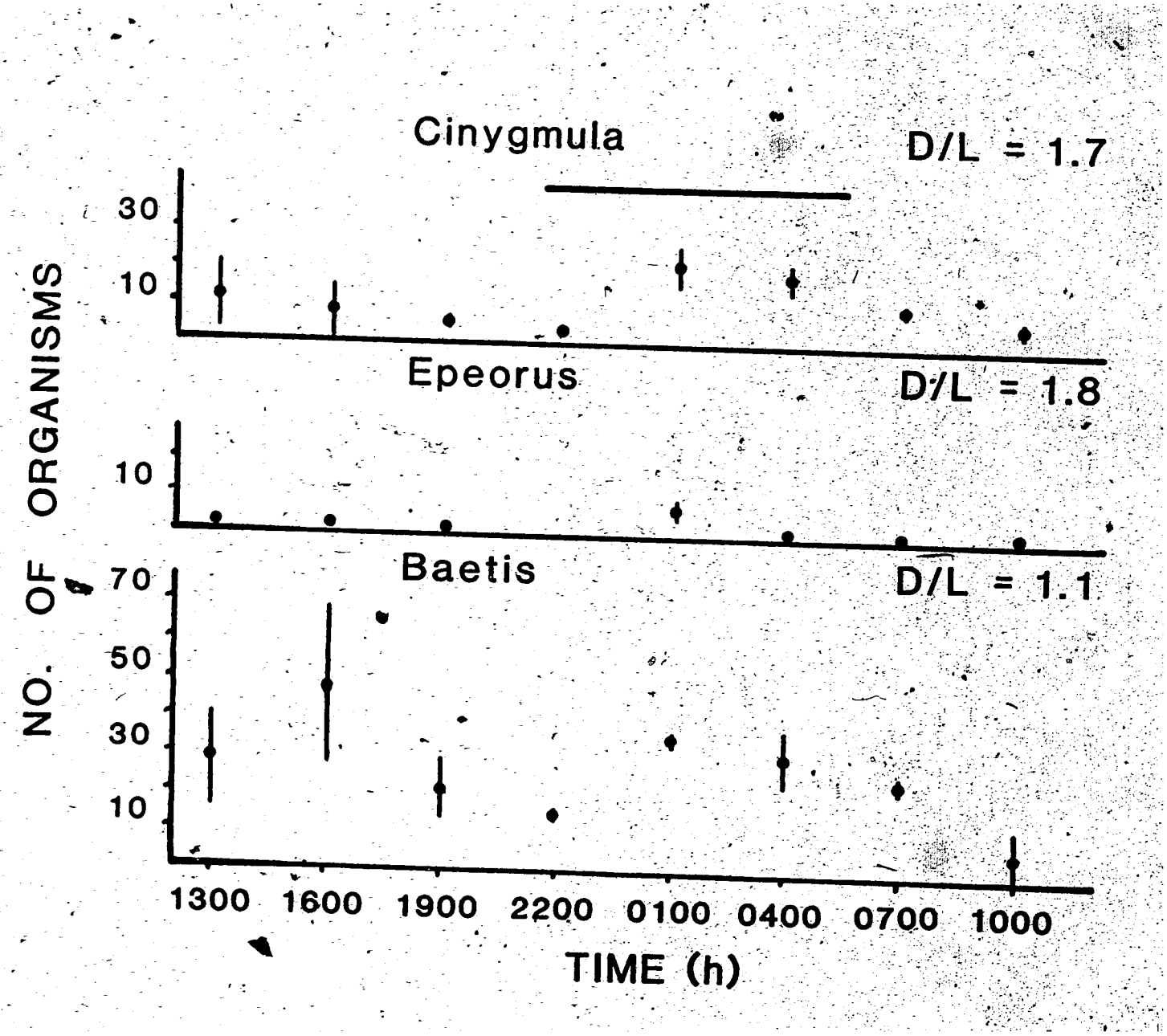

Cinygmula
D/L = 1.7
30
10
Epeorus
D/L = 1.8
10
Baetis
D/L = 1.1
70
50
30
10
NO. OF ORGANISMS
1300
1600
1900
2200
0100
0400
0700
1000
TIME (h)

Figure III-7. Densities of small (1-4 mm) *Cinygmula* (NM1) and Heptageniidae (FM1, NM2, and FM2) nymphs during the 24 h studies of observations. See Figure III-1 for details.

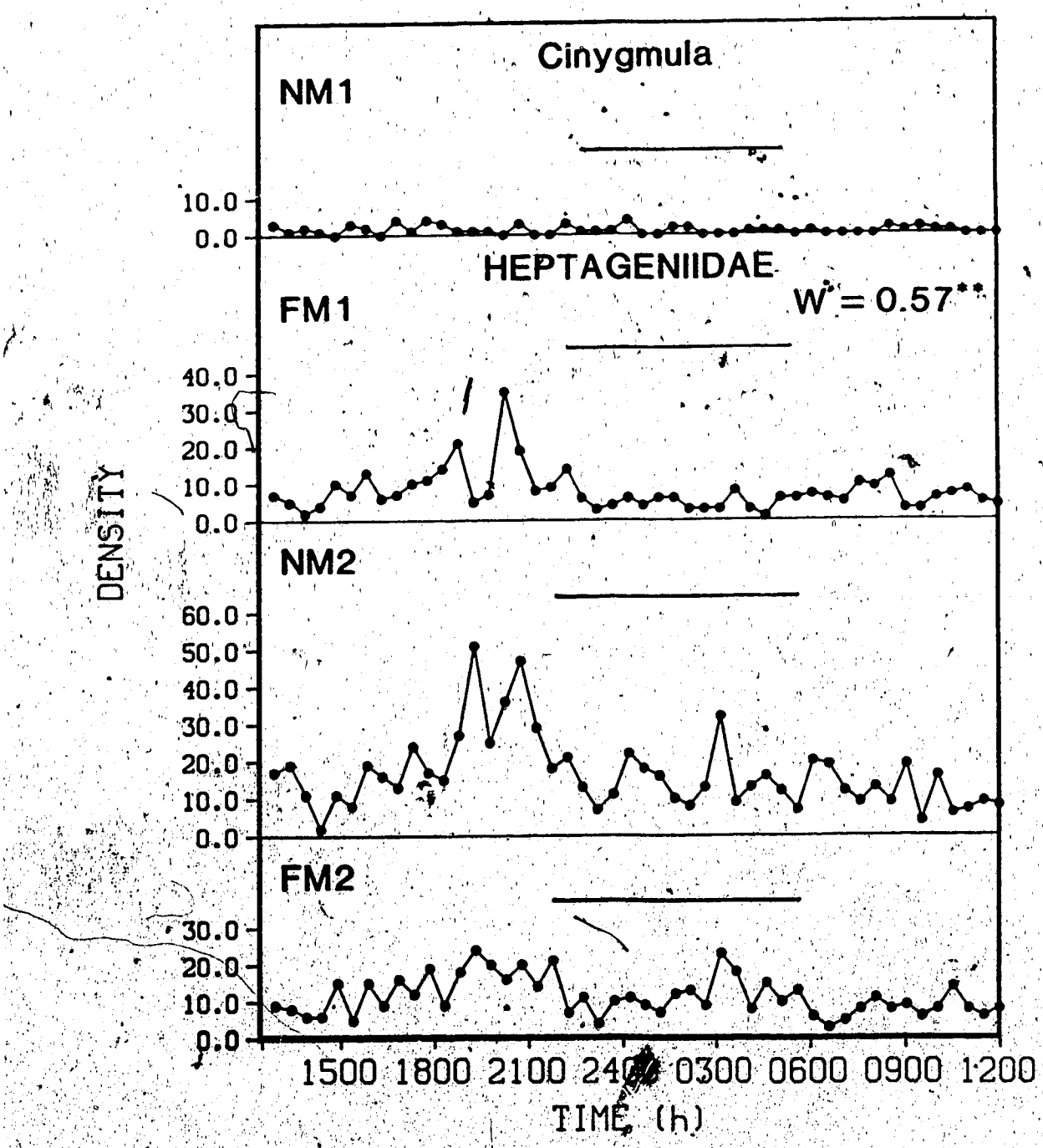

Cinygmula
NM1
10.0
0.0
HEPTAGENIIDAE
FM1
W' = 0.57**
40.0
30.0
20.0
10.0
0.0
DENSITY
NM2
60.0
50.0
40.0
30.0
20.0
10.0
0.0
FM2
30.0
20.0
10.0
0.0
1500 1800 2100 2400 0300 0600 0900 1200
TIME (h)

Figure III-8. Densities of large (5-12 mm) *Cinygmula* (NM1) and Heptageniidae (FM1, NM2, and FM2) nymphs during the 24 h studies of observations. See Figure III-1 for details.

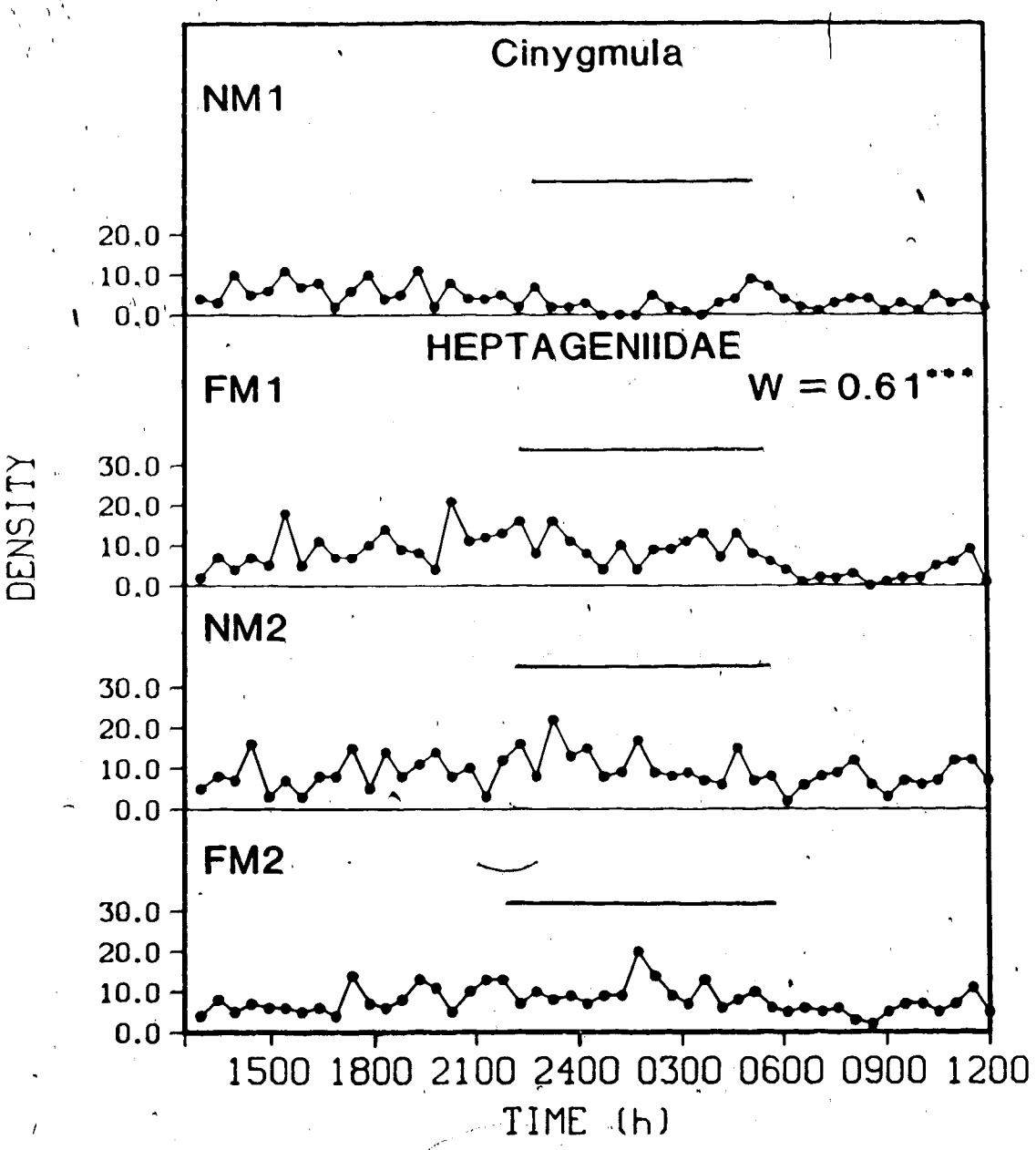

Cinygmula
NM1
20.0
10.0
0.0
HEPTAGENIIDAE
FM1
W = 0.61***
30.0
20.0
10.0
0.0
DENSITY
NM2
30.0
20.0
10.0
0.0
FM2
30.0
20.0
10.0
0.0
1500 1800 2100 2400 0300 0600 0900 1200
TIME (h)

Figure III-9. Densities of small (1-4 mm) *Baetis* (NM1, FM1, and NM2) and *Baetis-Ameletus* (FM2) nymphs during the 24 h studies of observations. See Figure III-1 for details.

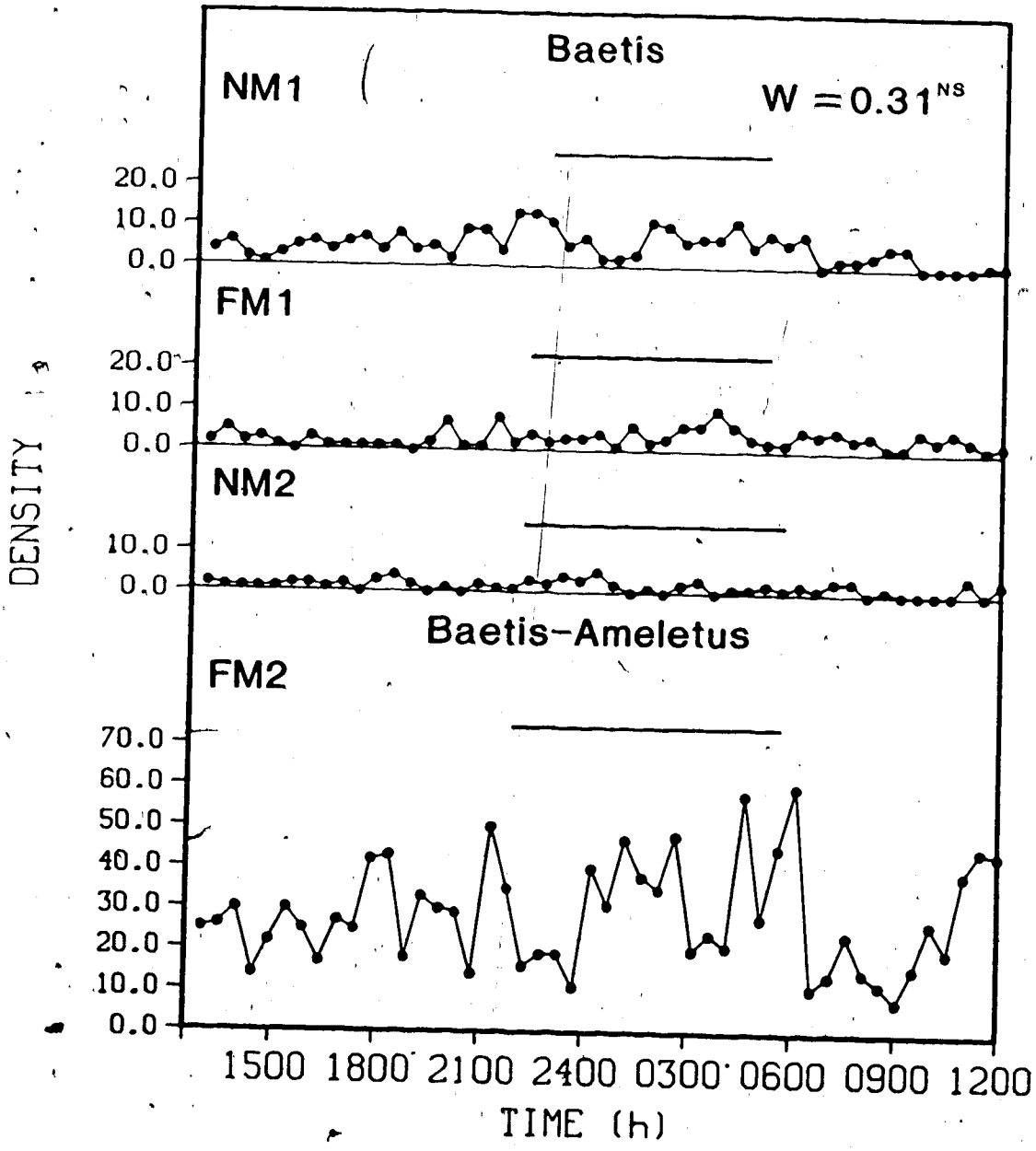
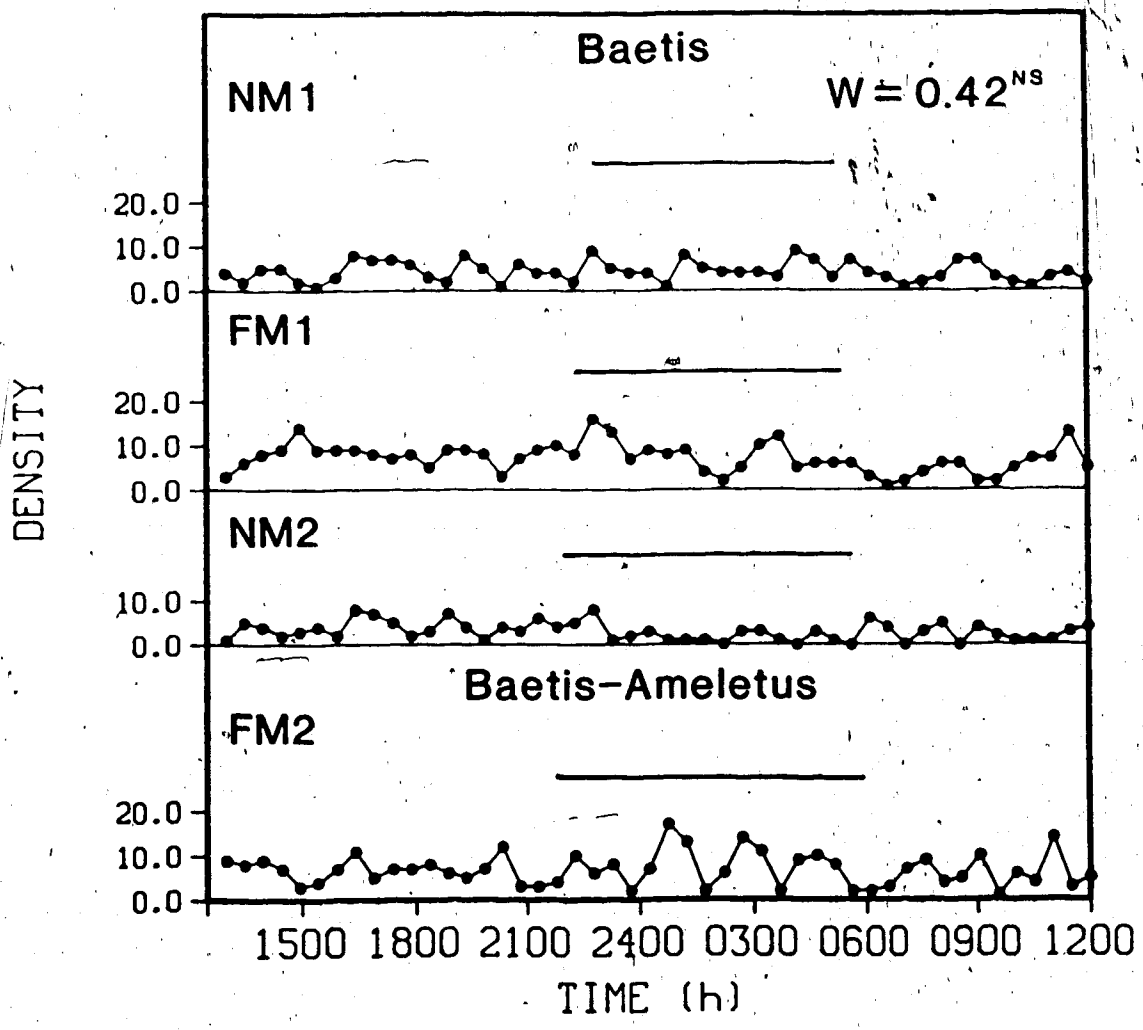

Baetis
NM1
W = 0.31 NS
20.0
10.0
0.0
FM1
20.0
10.0
0.0
NM2
10.0
0.0
DENSITY
Baetis-Ameletus
FM2
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
1500 1800 2100 2400 0300 0600 0900 1200
TIME (h)

Figure III-10. Densities of large (5-12 mm) *Baetis* (NM1, FM1, and NM2) and *Baetis-Ameletus* (FM2) nymphs during the 24 h studies of observations. See Figure III-1 for details.


Baetis
NM1
W = 0.42 NS
20.0
10.0
0.0
FM1
20.0
10.0
0.0
NM2
10.0
0.0
Baetis-Ameletus
FM2
20.0
10.0
0.0
DENSITY
1500 1800 2100 2400 0300 0600 0900 1200
TIME (h)

E. REFERENCES

Allan, J. D. 1978. Trout predation and the size composition of stream drift. Limnol. Oceanogr. 23: 1231-1237.

Allan, J. D. 1983. Food consumption by trout and stonflies in a Rocky Mountain stream, with comparison to prey standing crop, p 371-390. *In* T. D. Fontaine and S. M. Bartell. [ed.] Dynamics of lotic ecosystems. Ann Arbor Science Publishers Inc., Ann Arbor, MI.

Allan, J. D. 1983. Predator-prey relationships in streams, p 191-229. *In* J. R. Barnes and G. W. Minshall [ed.] Stream ecology: application and testing of general ecological theory. Plenum Press, New York, NY.

Allan, J. D., A. S. Flecker and N. L. McClintock. 1985. Diel epibenthic activity of mayfly nymphs, and its non-concordance with behavioral drift. Manuscript. [Submitted to Limnol. Oceanogr., Spring 1985]

Anderson, N. H. 1966. Depressent effect of moonlight on activity of aquatic insects. Nature 209: 319-320.

Bailey, P. C. E. 1981. Diel activity patterns in nymphs of an Australian mayfly *Atalophlebioides* sp. (Ephemeroptera: Leptophlebiidae). Aust. J. Mar. Freshwater Res. 32: 121-131.

Bailey, P. C. E. 1982. Diel activity pattern in nymphs of the Australian caddisfly *Helicopsyche murrumba* Mosely (Trichoptera: Helicopsychidae). J. Aust. ent. Soc. 21: 247-250.

Baylor, E. R., and F. E. Smith. 1953. The orientation of *Cladocera* to polarized light. Amer. Nat. 87: 97-101.

Beck, S. D. 1983. Insect thermoperiodism. Ann. Rev. Entomol. 28: 91-108.

Bishop, J. E. 1969. Light control of aquatic insect activity and drift. Ecology 50: 371-380.

Bishop, J. E., and H. B. N. Hynes. 1969. Downstream drift of the invertebrate fauna in a stream ecosystem. Arch. Hydrobiol. 66: 56-90.

Bohle, H. W. 1978. Beziehungen zwischen dem Nahrungsangebot, der Drift und der raumlichen Verteilung bei Larven von *Baetis rhodani* (Pictet) (Ephemeroptera: Baetidae). [English Summary] Arch. Hydrobiol. 84: 500-525.

Burkhardt, D. 1964. Colour discrimination in insects. Adv. Insect Physiol. 2: 131-173.

Campbell, I. C. 1980. Diurnal variations in the activity of *Mirawara purpurea* Riek (Ephemeroptera, Siphlonuridae) in the Aberfeldy River, Victoria, Australia, p 297-308. *In* J. F. Flannagan and K. E. Marshall [ed.] Advances in Ephemeroptera biology. Plenum Press, New York, NY.

Chaston, I. 1969. The light threshold controling the periodicity of invertebrate drift. J. Anim. Ecol. 38: 171-180.

Chaston, I. 1972. Non-catastrophic invertebrate drift in lotic systems, p 33-51. *In* R. B. Clark and R. J. Wooton [ed.] Essays in hydrobiology. University of Exeter.

Clifford, H. F. 1972. Comparison of samples of stream bottom fauna collected during the day and at night. Limnol. Oceanogr. 17: 479-481.

Conover, W. J. 1980. Practical nonparametric statistics. 2nd ed. John Wiley and Sons, NY. 493 p.

Elliott, J. M. 1967. Invertebrate drift in a Dartmoor stream. Arch. Hydrobiol. 63: 202-237.

Elliott, J. M. 1968. The daily activity patterns of mayfly nymphs (Ephemeroptera) J. Zool., Lond. 155: 201-221.

Elliott, J. M. 1969. Diel periodicity in invertebrate drift and the effect of different sampling periods. Oikos 20: 524-528.

Elliott, J. M. 1970. The diel activity patterns of caddis nymphs (Trichoptera). J. Zool., Lond. 160: 279-290.

Elliott, J. M., and G. W. Minshall. 1968. The invertebrate drift in the River Duddon, English Lake District. Oikos 19: 39-52.

Graesser, A., and P. S. Lake. 1984. Diel changes in the benthos of stones and of drift in a southern Australian upland stream. Hydrobiology 111: 153-160.

Harker, J. E. 1953. The diurnal rhythm of activity of mayfly nymphs. J. exp. Biol. 30: 525-533.

Holt C. S., and T. M. Waters 1967. Effect of light intensity on the drfit of stream invertebrates. Ecology 48: 225-234.

Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological experiments. Ecol. Monogr. 54: 187-211.

Hynes, H. B. N. 1970. The ecology of running waters. University of Toronto Press. 555 p.

Kohler, S. L. 1983. Positioning on substrates, positioning changes, and diel drift periodicities in mayflies. Can. J. Zool. 61: 1362-1368.

Kovalak, W. P. 1978. Diel changes in stream benthos density on stones and artificial substrates. Hydrobiol. 58: 7-16.

Kovalak, W. P. 1979. Day-night changes in stream benthos density in relation to current velocity. Arch. Hydrobiol. 87: 1-18.

Lyman, F. E. 1945. Reactions of certain nymphs of *Stenonema* (Ephemeroptera) to light as related to habitat preference. Ann. Ent. Soc. Amer. 38: 234-236.

Madsen, B. L. 1968. A comparative ecological investigation ot two related mayfly nymphs. Hydrobiol. 31: 337-349.

Mazokhin-Porshnyakov, G. A. 1969. Insect Vision. Plenum Press, New York. 306 p.

Mogel, R., N. Rieder and B. Statzner. 1985. Ein Gerat zur Freilandbeobachtung des nachtlichen Verhaltens von benthischen Bachtieren, mit Befunden aus der Gattung *Hydropsyche* (Trichoptera, Insecta). [English Summary] Carolinea 42: 121-128.

Moon, H. P. 1940. An investigation of the movements of fresh-water invertebrate faunas. J. Anim. Ecol. 9: 76-83.

Müller, K. 1974. Stream drift as a chronobiological phenomenon in running water ecosystems. Ann. Rev. Ecol. Systemat. 5: 309-323.

Otto, C. 1984. Adaptive head coloration in case-making caddis larvae. Freshwat. Biol. 14: 317-321.

Scherer, E. 1965. Zur Methodik experimenteller Fliebwasser-Okologie. Arch. Hydrobiol. 61: 242-248.

Siegel, S. 1956. Nonparametric statistics: for the behavioral sciences. McGraw-Hill Book Company Inc, New York. 312 p.

Statzner, B., C. Dejoux and J.-M. Elouard. 1984. Field experiments on the relationship between drift and benthic densities of aquatic insects in tropical streams (Ivory Coast). Rev. Hydrobiol. trop. 17: 319-334.

Statzner, B., and R. Mogel. 1984. No relationship between the substrate surface densities and drift of the stream caddisfly *Micrasema longulum* (Brachycentridae, Trichoptera), p 383-389. *In* J. C. Morse [ed.] Fourth international symposium on Trichoptera. Series Entomologica, Vol. 30. Dr. W. Junk Publishers, The Hague.

Statzner, B. and R. Mogel. 1985. An example showing that drift net catches of stream mayflies (*Baetis* spp., Ephemeroptera, Insecta) do not increase during periods of higher substrate surface densities of the larvae. Verh. Internat. Verein. Limnol. 22: 3238-3243.

Walde, S. J. and R. W. Davies. 1985. Diel periodicity of two predatory stoneflies (Plecoptera). Can. J. Zool. 63: 883-887.

Waterman, T. H. 1984. Natural polarized light and vision, p. 63-114. *In* M. A. Ali [ed.] Photoreception and vision in invertebrates. Plenum Press, New York.

Waters, T. F. 1962. Diurnal periodicity in the drift of stream invertebrates. Ecology 43: 316-320.

Waters, T. F. 1969 Diel patterns of aquatic invertebrate drift in streams of northern Utah. Proc. Utah Acad. Sci. Arts Lett. 46: 109-130.

Waters, T. F. 1972. The drift of stream insects. Ann. Rev. Entomol. 17: 253-272.

Wiley, M. J., and S. L. Kohler 1980. Positioning changes of mayfly nymphs due to behavioral regulation of oxygen consumption. Can. J. Zool. 58: 618-622.

Wiley, M. J., and S. L. Kohler. 1981. An assessment of biological interactions in an epilithic stream community using time-lapse cinematography. Hydrobiol. 78: 183-188.

Wiley, M. J., and S. L. Kohler. 1984. Behavioral adaptations of aquatic insects, p. 101-133. *In* V. H. Resh and D. M. Rosenberg [ed.] The ecology of aquatic insects. Praeger Publishers, NY.

Wodsedalek, J. E. 1911. Phototactic reactions and their reversal in the may-fly nymphs *Heptagenia interpunctata* (Say). Biol. Bull. 21: 265-271.

IV. CONCLUDING DISCUSSION

My studies pertained to important areas of the ecology of immature aquatic insects, namely the distribution and abundance of stream invertebrates on substrate particles in relation to substrate characteristics and the abiotic environment of a stream ecosystem. The stream invertebrates at my study site were mostly Ephemeroptera taxa; however, other aquatic insect groups were also present e.g. the Plecoptera, Trichoptera, and *Polycelis coronata* (Turbellaria). Much of my work dealt with direct observations as a means of obtaining data. I believe this method should be used more in future studies. This method causes a minimum of disturbance to the habitat while obtaining information on organisms in a relatively undisturbed system. Direct observations may be criticized as not being a preferred method in obtaining ecological data, because such observations are descriptive rather than experimental. However, direct observations may be combined with an experimental approach, for example, where I manipulated substrates in baskets and made observations on these substrates (and see Peckarsky 1983). Also, as a describtive method alone, direct observations have been little used in stream ecology. Observations of an organism's behaviour and interactions with other organisms are fundamental to our understanding of aquatic insect ecology. For example, the premise that benthic macroinvertebrates migrate to the tops of substrate particles mainly at night has only recently been examined in the field (see Chapter III).

Undoubtedly direct observation caused disturbance to the benthic organisms, especially when I approached the observation box after it had been in place before a sample observation. On numerous occasions I tested the reactions of organisms (when not doing my sample observations) to movements, and I feel that major sudden movements of my silhouette against the sky caused most disturbance to the nymphs. However, these disturbances are minimal when compared to other techniques. After an organism's initial reaction, the organism appeared to be unaware of my presence. Direct observations appear to be one of the best methods of determining a diel periodicity in density or activity of invertebrates on the stream substrate.

Colonization of substrates has been most often studied by using artificial substrates because quantitative results can be obtained. However, the efficacy of these artificial substrates has been questioned because the substrates might be selective towards particular taxa. Therefore, I chose a second method (i.e. direct observations) to determine the preference by Ephemeroptera nymphs on rocks in substrate baskets. These results were expected to compliment those from the substrate baskets; this was generally the case.

Drunella coloradensis was the only mayfly nymph studied in the substrate basket experiment, and because only four *D. coloradensis* nymphs were recorded during the direct observations, a species to species comparison between my two methodologies was not possible. But the results for mayfly nymphs in both methods indicated a preference for the smooth-light and Dyson Creek substrates; the smooth-dark substrate was usually least preferred. The main difference between results for the substrate basket experiment and direct observations was that the rough-dark substrate was least preferred by all developmental stages of *D. coloradensis*; whereas *Baetis* and *Ameletus* nymphs showed a strong preference for this substrate. Preference for the smooth-light substrate by mayfly nymphs suggests that substrate colour may be important to colonization by aquatic insects, but the reason for this is not clear. In my study, the choice of substrate based on colour by mayfly nymphs does not appear to be related to predation as a means of remaining cryptic against a dark background.

The choice of substrate by a nymph to match the background colour may be by an endogeneous or an exogeneous mechanism. In my study, an endogeneous preference for a particular substrate background seems unlikely, because the nymphs were expected to choose a darker substrate to remain cryptic and hence reduce predation. An exogeneous mechanism explaining background colour choice by nymphs may be due to algae colonization on my substrates (see Chapter II). For example, it would be informative to determine whether there were differences other than the factors measured between my smooth-light (white) and smooth-dark (black) coloured substrates. These substrates were physically and chemically the same, and they were originally obtained from the same stream location. Algae colonizing these substrates

may be influenced by reflected light from the substrates. Thus the white substrate with its higher reflected light levels may be colonized by greater amounts and/or higher quality algae than would colonize the black substrate. This could be an interesting area of research. However, if the difference between these substrates is due to algae colonization, the algae would have to be limited in quantity in the habitat to affect the distribution of the invertebrates. Edmunds (1974) noted that one of the main disadvantages of cryptic colouration is that it conflicts with essential needs such as feeding. Food acquisition by immature aquatic insects is important for growth and may override predation pressure. Thus the choice of food could be more important to these animals than background colour.

An aspect of cryptic colouration not considered in my study is colour polymorphism, which is not uncommon in immature aquatic insects. This polymorphism will reduce predation by visually-foraging predators, if the prey are feeding on backgrounds of numerous colours. Edmunds (1974) stated that colour polymorphism is the best defence against predators, for example, predators searching for prey of particular colours or patterns. Therefore, predation pressure may not be as important as I originally believed in my study. Nevertheless, future work on the importance of substrate colour to colonization should be conducted in both the presence and absence of fish predators, because substrate preference may not be an endogenous fixed response, but an endogenous plastic response depending on predator presence. For example, Charnov et al. (1976) demonstrated changes in behaviour of baetid mayfly nymphs due to the presence or absence of a fish predator.

The choice of background colour by aquatic insects requires more work to explain the mechanisms involved. In general, substrate colour appears to have been ignored in the treatment of aquatic insect substrate choice (see Minshall 1984) except for anecdotal reference to its potential importance (Hynes 1970). But in studies using introduced dark and light coloured artificial substrates, for some invertebrate taxa the dark bricks were colonized by more animals than the light coloured bricks, but these results were not conclusive (Clifford et al., in prep.).

The importance of micro-texture is not clear from my results. The Dyson Creek substrate that had the greatest texture was preferred by intermediate developmental stages of *D. coloradensis* but in general the rough-dark substrate was least preferred by these mayflies. In general for the other Ephemeroptera groups, the rough-dark substrate was preferred. Unfortunately the two rough substrates (the Dyson Creek and rough-dark substrates) were found to be of different areas, and they were exposed to different water velocities. Therefore, the importance of micro-texture is difficult to determine since other factors may have influenced the results (see Chapter II). Other studies that have examined texture include natural rock particles (Erman and Erman 1984) and artificial substrates (Clifford et al., in prep.). Erman and Erman (1984) found significantly greater total numbers of mayfly and stonefly nymphs on sandstone than on smoother particles. Clifford et al. (in prep.), although their tiles were of much greater roughness than my substrate, found that more individuals and more taxa were present on the rough tiles than on smooth tiles.

Probably one of the most important factors to consider in relation to substrate texture preference is the build up of organic and/or inorganic material, that might be trapped between the substrate irregularities. Deposition occurred on both of my rough substrates. Because this deposit may represent part of the nymphs' food, or because it may inhibit colonization, it would obviously be important even when dealing with micro-texture (illustrated by my results) as a factor in insect colonization. At this time it would seem difficult to eliminate the effect of deposition from texture studies in natural systems. Substrate size might have also influenced my results (see Chapter II). In future studies, substrates could be made to exact specifications and as similar to natural substrates as possible. For example, Hart (1978) constructed substrates from ceramic clay, thus the chemical and physical make up should be the same. Surface roughness or texture as a factor in the colonization by stream invertebrates requires more work and in particular the modification of substrate particle condition due to roughness may prove to be important. For example, the increase in surface area resulting in potentially greater algae and/or detritus colonization.

Results of my white and red light studies have suggested the mayfly nymphs in Dyson Creek were not negatively phototactic. A negative phototactic response by immature aquatic insects has often been suggested (see Chapter III). This phenomenon commonly forms the basis of the premise that invertebrates are migrating to the upper surfaces of substrates to feed in the dark. Further work on the phototactic response by aquatic insects is needed, because most of the previous studies in this area appear to have been done in the laboratory and not in the field under natural conditions. Such studies would contribute to our knowledge of the diel periodicity in density of aquatic insects by determining the role of light, which has been shown to be important in the laboratory.

Periodicity of diel density of various immature aquatic insects in field studies, where direct observations were used, has been demonstrated in different geographical areas (see Chapter III). But my results conflict with other studies on Ephemeroptera nymphs, which report nymphs being present on the top of the substrate in the daylight (Statzner and Mogel 1985, Allan et al., 1985). These contradictory results are difficult to explain especially in the case of the study of Allan et al. (1985), which was done in a Rocky Mountain stream with similar fauna to Dyson Creek. Also, in both Allan et al. (1985) and my study, water temperature and diel density of most taxa were positively correlated.

In general, there does not appear to be a single abiotic factor to explain the observed periodicities in density in my study or in similar studies when direct observations were used. It seems likely that several abiotic factors may be important, but there are many problems in examining the potential effect of these factors. Such as for moonlight, direct illumination, and not dispersed light from the moon, may be perceived by aquatic arthropods. Therefore, obstructions to moonlight could be important factors to be considered when studying the effect of moonlight. Also, abiotic factors may be acting in concert to affect diel periodicity, thus confounding interpretations.

Polarized light is one such abiotic factor. If polarized light does affect diel periodicity in density of stream invertebrates, it would appear not to influence diel

densities alone. However, polarized light could be important in diel periodicities, since the arthropod eye can analyse this light and then the aquatic animals could react to this light (see Chapter III).

As discussed in Chapter III, biotic factors could have a considerable influence on the diel periodicity in density of stream invertebrates. A common view in stream ecology is that abiotic factors are more important than biotic factors in determining stream community structure; however, more recent work suggests that biotic factors may also be important (Barnes and Minshall 1983). Although the influence of fish predators could not be examined directly, in Dyson Creek, invertebrate predators such as perlodid stoneflies could potentially have a significant influence on the diel periodicity of the benthos. These stoneflies were common in quantitative samples from my study site, but they were rarely observed in the direct observations. This suggests that the stoneflies were mostly under the substrates. But if the perlodids have a diel periodicity in activity, they would be expected to influence the distribution and abundance of their prey, the Ephemeroptera nymphs, on the top of the substrate.

In my studies I chose both experimental and describtive approachs to examine [redacted]portant area of the ecology of immature aquatic insects. I think these studies have contributed to our knowledge of the potential influence of roughness and colour to colonization by stream invertebrates, and to the diel periodicity in density of these animals.

A. REFERENCES

Allan, J. D., A. S. Flecker and N. L. McClintock. 1985. Diel epibenthic activity of mayfly nymphs, and its non-concordance with behavioral drift. Manuscript. [Submitted to Limnol. Oceanogr., Spring 1985]

Barnes, J. R., and G. W. Minshall. 1983. Stream Ecology; an historical and current perspective, p 1-5. *In* J. R. Barnes [ed.] Stream ecology: application and testing of general ecological theory. Plenum Press, New York, NY.

Charnov, E. L., G. H. Orians and K. Hyatt. 1976. Ecological implications of resource depression. Am. Nat. 110: 247-259.

Clifford, H. F., V. Gotceitas, and R. J. Casey. Roughness and color of substratum particles as a possible factor in the colonization of stream invertebrates, in preparation.

Edmunds, M. 1974. Defence in animals. A survey of anti-predator defences. Longman Inc, NY. 357 p.

Erman, D. C., and N. A. Erman. 1984. The response of stream invertebrates to substrate size and heterogeneity. Hydrobiology 108: 75-82.

Hart, D. D. 1978. Diversity in stream insects: regulation by rock size and microspatial complexity. Verh. Internat. Verein. Limnol. 20:1376-1381.

Hynes, H. B. N. 1970. The ecology of running waters. University of Toronto Press. 555 p.

Minshall, G. W. 1984. Aquatic insect-substratum relationships, p. 358-400. *In* V. H. Resh and D. M. Rosenberg [ed.] The ecology of aquatic insects. Praeger Publishers, NY.

Peckarsky, B. L. 1983. The use of behavioral experiments to test ecological theory in streams, p 79-97. *In* J. R. Barnes and G. W. Minshall [ed.] Stream ecology: application and testing of general ecological theory. Plenum Press, New York, NY.

Statzner, B., and R. Mogel. 1985. An example showing that drift net catches of stream mayflies (*Baetis* spp., Ephemeroptera, Insecta) do not increase during periods of higher substrate densities of the larvae. Verh. Internat. Verein. Limnol. 22: 3238-3243.