THE UNIVERSITY OF CALGARY

ECOLOGY AND BIOENERGETICS OF TWO <u>AMELETUS</u> . (SIPHLONURIDAE: EPHEMEROPTERA) POPULATIONS

ΒY

MICHAEL JOSEPH BENTON

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SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

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THE UNIVERSITY OF CALGARY

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "Ecology and Bioenergetics of Two <u>Ameletus</u> (Siphlonuridae: Ephemeroptera) Populations", submitted by Michael J. Benton in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Supervisor, Dr. Gordon Pritchard Department of Biological Sciences

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Dr. Bernard W. Sweeney Stroud Water Research Center

Dr. Frederick J. Wrona Department of Biological Sciences

Dr. Joseph M. Culp / Department of Biological Sciences

Dr. William A. Ross Faculty of Environmental Design

Date 9 July 1987

ABSTRACT

The life histories, bioenergetics, and ecology of <u>Ameletus celer</u> from a eurythermic stream and <u>A</u>. <u>similior</u> from a stenothermic stream were reconstructed from field and laboratory data.

Current speed and substrate particle size influenced microdistribution differentially between sites. A. similior was significantly heavier at a given head width, but fecundity, egg size, and egg mass did not differ. Growth ratio decreased with increased body size, and moult interval decreased with temperature. The use of constant temperatures and day-degrees in describing growth, and the use of thermal efficiency in growth and development as a measure of fitness are discussed. Feeding rate was positively correlated to body size, but did not differ between species at temperatures common to their respective habitats. Head width and temperature influenced weight-specific metabolism, but there was no effect of species or movement level. Ventilation behavior was more common in large Ameletus and increased respiration, but did not change with temperature. Sex ratio was female-biased in A. similior, and may be an adaptation toward balancing female-specific parasitization. Energy density (J/mg)

iii

decreased with body size. Egg energy density was higher in <u>A. similior</u>.

Using predictable moult intervals and growth ratios, a realistic instar number and growth curves which closely follow those constructed from field data were estimated. Weight-specific respiration (R) was not constant over the life cycle as reported for some other species, but was more uniform in <u>A</u>. <u>similior</u>. However, R/assimilation (A) was more constant in <u>A</u>. <u>celer</u>. Growth (G), net growth efficiency (NGE), and A were generally higher in <u>A</u>. <u>celer</u>. Consumption (C) was higher than previous estimates for aquatic insects. Reproductive effort (RE) was greater in <u>A</u>. <u>celer</u>, but its net RE was lower due to lower egg energy density. This suggests that mayfly fitness may not always be synonymous with fecundity as commonly argued.

Predaceous stonefly presence caused a reduction in <u>A</u>. <u>similior</u> mean velocity, thereby reducing predator encounter rate. Parasitized mayflies increased mean velocity, probably due to nutrient stress, and had a higher predator encounter rate. Two strategies for reducing encounters, based on locomotory and defensive abilities, are proposed, and their possible effects on energy budgets are discussed.

iv

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A number of other colleagues contributed their time, assistance, and friendship: Kelvin Conrad, Jamie Dixon, Tom Gates, Nancy Glozier, Cindy Prescott, and Dr. Sandy Walde. Dr. Ed McCauley was instrumental in developing and analyzing the experiments described in Chapter 3, and has been a tireless source of new ideas and enthusiasm. Dr. Lawrence Harder assisted with the statistical analyses. Janis Lemiski monitored growth experiments and helped "pick" most fine samples, while Ramnik Arora did much of the taxonomic work. Heather Proctor diligently proofread Chapter One.

I especially thank Patty Westlake for help in the lab, in the field, and on the home front. Her hard work made many phases of this research much easier. I also thank my

v

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Finally, I dedicate this work first to my supervisor and friend, Dr. Gordon Pritchard, whose calm and considered advice, guidance, and support have taught me the value of careful thinking, regard for self, and perserverance in the pursuit of a goal. Words cannot express my appreciation. I also dedicate this work and the accomplishment it stands for to Amy Baker who once took my hand, pulled me up, and showed me the way to fulfilling a dream. Scientific discovery consists in the interpretation for our own convenience of a system of existence which has been made with no eye to our convenience at all.

Norman Wiener

Knowledge which is required under compulsion obtains no hold on the mind.

Plato

Some minds learn most when they seem to learn least. A certain placid, unconscious, equable taking-in of knowledge suits them, and alone suits them.

Walter Bagehot

Shame on the cant and hypocrisy of those who can teach virtue, preach righteousness, and pray blessings for those only with skins colored like their own.

C. Lenox Remond

In our personal lives when we find that we are speaking in our own accent, speaking our own minds, living by our own opinions, we feel the pulse beat of our own personality coming to life again. We feel that in having discovered or arrived at ourselves, we have discovered a continent which is all our own.

Irwin Edman

. . . his reasons were emotional, and emotions, he was concluding, particularly his own, could constitute the highest form of rationality.

Joseph Heller

CONTENTS

	<u>Paqe</u>
ABSTRACT ACKNOWLEDGEMENTS TABLE OF CONTENTS LIST OF TABLES LIST OF FIGURES CHAPTER ONE - LIFE-HISTORY AND ENERGY BUDGET COMPON	iii v viii x xi VENTS
INTRODUCTION METHODS Collection Sites Water Temperature and pH Study Organisms Collection Weights and Measurements Calorimetry Respirometry Growth Feeding Rate RESULTS Water Temperature and pH Life Cycle Body. Size and Mass Fecundity, Egg Size and Egg Mass Microdistribution Growth Feeding Rate Respirometry Calorimetry DISCUSSION Sex Ratio Microdistribution Feeding Growth Respiration Calorimetry Calorimetry METHODER THO = SYNTHESIS	1 8 8 10 12 14 15 9 02 22 23 26 7 8 01 31 37 4 8 37 77
INTRODUCTION	82 83 83 87

.

,

DISCUSSION B. ENERGY BUDGET METHODS RESULTS AND DISCUSSION SUMMARY	89 92 92 95 112
CHAPTER THREE - MAYFLY LOCOMOTORY RESPONSES TO PREDATOR PRESENCE AND ENDOPARASITIC INFECTION: THE EFFECTS ON PREDATOR ENCOUNTER RATE	
INTRODUCTION METHODS RESULTS Predator Movement Prey Movement Mode Mode-Direction Direction Mean Velocity and Time Spent Stationary Predator-Prey Encounter Rate Encounters, Attacks and Captures DISCUSSION Effects of Predator and Parasitism on	115 119 122 122 122 123 123 124 124 124 125
Mayfly Activity Effect of Parasitism on Encounter, Attack and Capture Rates SUMMARY	125 129 131
TABLES	134
FIGURES	144
BIBLIOGRAPHY	173

.

•

.

.

•

,

LIST OF TABLES

Title Page Table Monthly and annual mean high and low 1 134 temperatures at the Elbow River study site ... Monthly and annual mean high and low 2 temperatures at the Ford Creek study site 135 Monthly and annual degree-hours at the 3 136 Ford Creek and Elbow River study sites Relative mean larval sizes associated with 4 current speeds and substrate particle sizes at the Ford Creek and Elbow River study 137 sites 5 Slopes and y-intercepts of natural log-transformed head width/weight-specific respiration regressions at experimental 138 temperatures Whole-body, egg, and exuvia calorific 6 139 values for <u>Ameletus</u> Complete energy budget for A. celer 7 at the Ford Creek study site 140 Complete energy budget for <u>A</u>. <u>similior</u> 8 142 at the Elbow River study site

LIST OF FIGURES

<u>Figure</u>

<u>Title</u>

.

l	Map of relative locations of Elbow River and Ford Creek	144
2	Map of Elbow River study site	145
3	Monthly mean high and low temperatures at the Elbow River study site	146
4	Map of Ford Creek study site	147
5	Monthly mean high and low temperatures at the Ford Creek study site	148
6	Diagram of mayfly enclosures used in growth experiments and current created by bubbling air under slanted styrofoam base	149
7	Monthly pH readings at the Ford Creek and Elbow River study sites	150
8	Monthly size-frequency distributions of <u>A. celer</u> at the Ford Creek study site	151
9	Monthly size-frequency distributions of <u>A. similior</u> at the Elbow River study site	152
10	Natural log-log relationship of head width to dry mass in <u>A. celer</u> at the Ford Creek study site	153
11	Natural log-log relationship of head width to dry mass in <u>A</u> . similior at the Elbow River study site	154
12	Comparison of natural log-log head width to dry mass relationships in <u>A</u> . <u>celer</u> and <u>A. similior</u>	155
13	Relationship of fecundity to log-transformed head width in Ameletus	156

e

14	Relationship of growth ratio to natural log-transformed head width in <u>Ameletus</u>	157
15	Natural log-log relationship of gut mass to head width in <u>Ameletus</u>	158
16	Relationship of feeding rate to natural log-transformed head width in <u>Ameletus</u>	159
17	Relationship of weight-specific respiration to natural log-transformed head width in <u>Ameletus</u> at experimental temperatures	160
18	Three-dimensional representation of the relative effects of body size and temperature on weight-specific respiration	161
19	Relationship of whole-body energy content to natural log-transformed head width in <u>Ameletus</u>	162
20	Approximate growth curves for <u>A. celer</u> using a constant temperature-moult interval regression, and 30-day moult interval for near-zero temperature	163
21	Approximate growth curves for <u>A</u> . <u>similior</u> using a constant temperature-moult interval regression, and 30-, 60-, and 90-day moult intervals for near-zero temperature	164
22	Comparison of instar-specific exuvium dry mass for <u>A</u> . <u>celer</u> and <u>A</u> . <u>similior</u>	165
23	Instar-specific ingestion rates for <u>Ameletus</u>	166
24	Instar-specific respiration rates for <u>A. celer</u> and <u>A. similior</u>	167
25	Weight-specific respiration rates by instar for <u>A. celer</u> and <u>A. similior</u>	168
26	Plexiglas artificial stream	169
27	Percent activity spent walking, swimming, and drifting with and against the current by <u>Ameletus</u>	170

28	Percent activity spent moving with and against the current by <u>Ameletus</u>	171
29	Mean velocity of nonparasitized and parasitized <u>Ameletus</u> in the absence and presence of a stonefly predator	172

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CHAPTER ONE

LIFE-HISTORY AND ENERGY BUDGET COMPONENTS

INTRODUCTION

The calculation of individual energy budgets is fundamental to the study and understanding of two important areas of ecology. Individual energy budgets may be used to estimate the flow of energy through a population, between trophic levels, or within a community (e. g. MacKay, 1985; Wolters, 1985). In this approach to ecological energetics it is the amount and efficiency of energy utilization, and the form and amount of energy lost to the system or made available to other trophic levels that is of interest (Phillipson, 1966).

In contrast, an individual energy budget may be used as a measure of energy allocation among life-history traits. Because natural selection, life-history characteristics, and energy are inextricably related, this approach provides insight into how selective pressures shape life histories in certain habitats. It also reveals how phenotypic plasticity is manifested in response to environmental factors (e. g. Sweeney and Schnack, 1977; Vannote, 1978; Sweeney and Vannote, 1981). It is this

physiological ecology approach to energetics that the work at hand takes.

It is generally accepted that an organism's fitness is measured by its reproductive success and genetic representation in future generations relative to its competitors'. An individual attains a certain level of fitness by a coadapted set of characteristics, evolved through natural selection, which meets the ecological demands of the environment. Such a set of traits has been termed a life-history strategy (Stearns, 1976).

Animals may be envisioned as physiological resource allocation systems which partition a finite amount of input energy between basal metabolism, storage, repair, growth, reproduction, defense, and foraging (Rollo, 1984; Townsend, 1987). Given a finite resource input, there must be compromises in assigning energy to competing physiological demands (Rollo, 1984). However, reproduction must be considered the most important life-history component as it is the principal way in which an individual affects future generations (Stearns, 1976). Therefore, selection should drive the amount of energy available to reproduction (reproductive effort) to some optimal level which will maximize the number of offspring which survive to reproduce (Price, 1974), occasionally even at the expense of the soma or adult longevity (Calow, 1979).

There are a number of alternative ways, in the form of energetic trade-offs, to achieve an optimal reproductive effort, but animal groups are usually locked into phylogenetic patterns of energy allocation (Butler, 1984). These patterns may be considered long-standing adaptations, but they also serve as constraints. Mayflies are obligately semelparous, have a very short adult life, and do not feed in the adult stage. This general pattern may have arisen because resources for egg production were obtained more efficiently as aquatic larvae than as terrestrial adults (Butler, 1984), or because predation or competition pressures were much greater in the adult stage. As a consequence, mayflies must reproduce quickly. Although Price (1974) argued that such ephemeral insects are examples of animals exhibiting a reproductive effort which is highly constrained, even within a species a variety of energy allocation patterns may be found, each one approaching optimality in its particular habitat (Dearn, 1977). In mayflies this may include scattering eggs in small batches as opposed to laying a single egg mass, or even entering the water to oviposit at specific sites (Brittain, 1982). Fecundity and eqg size may also be sensitive to local or recent selective pressures, but this has not yet been demonstrated (Parker and Begon, 1986). Such variation in reproductive characteristics within the

constraints of the species' evolutionary history may represent adaptive responses to contemporary ecological conditions (Butler, 1984).

It is prudent to note here that some authors have warned against ascribing all observable life-history traits directly to natural selection (Gould and Lewontin, 1979; Gould, 1980, 1982). Certain life-history features can have secondary benefits which may not have been involved in the evolution of that feature. An example of such an "exaptation" among mayflies might be the synchronous emergence of many species (Butler, 1984). While this phenomenon may serve to enhance mating opportunities or swamp predators, it may be largely a function of genetically and thermally controlled enzyme and endocrine kinetics which have arisen under a certain set of environmental conditions.

The habitat, too, can act to constrain possible physiological allocations (Southwood, 1977), and fitness can be reduced by these environmental constraints (Vannote and Sweeney, 1980). Conversely, reproductive flexibility may be insurance against total reproductive failure in an unpredictable environment (Hunter, 1975; Baird <u>et al</u>., 1986). To exactly what extent life-history variation is due to phenotypic plasticity rather than to genetic

differences, however, may be extremely difficult to determine (Baird <u>et al</u>., 1986).

The connection between metabolism and heat production was known as early as the late eighteenth century, and nearly all work on energetics was limited to respiratory heat loss in human and domestic stock until well into the twentieth century (Wiegert and Petersen, 1984). Early research in insect energetics was also concerned principally with respiratory energy loss (Sayle, 1928; Fox et al., 1937; Edwards, 1946). Krogh (1916) and later Zeuthen (1953) were instrumental in establishing the relationship between respiration rate and body size. Complete energy budgets were reported as early as 1920 (Hiratsuka, 1920 cited in Wiegert and Pattersen, 1984) but budgets for aquatic insect larvae were largely absent from the literature until considerably later (stoneflies: McDiffett, 1970; Heiman and Knight, 1975; damselflies: Lawton, 1971; caddisflies: Otto, 1974; dobson flies: Brown and Fitzpatrick, 1978). Complete energy budgets for mayflies were limited through that period (Trama, 1957; Sweeney, 1978; McCullough <u>et al</u>., 1979). Subsequent research has not added a great deal to the literature in terms of new complete mayfly energy budgets (but see Sweeney and Vannote, 1981). Rather, existing information on the effects of seasonal temperature regimes on the

energetic costs of respiration, larval growth, and adult size and fecundity has been synthesized into a model which qualitatively predicts optimal thermal conditions for mayflies based on life-cycle timing (Sweeney and Vannote, 1978; Vannote and Sweeney, 1980). Only recently has the effect of food quality in conjunction with various rearing temperatures been investigated to test their effects on mayfly fitness (Sweeney and Vannote, 1984; Sweeney <u>et al</u>., 1986). Energy allocation in the form of true adaptation (i. e. genetic variation) has not been empirically tested in mayflies, presumably because of the confounding effect of phenotypic variability in response to environmental conditions.

More studies of energy budgets at the level of the individual are prerequisite to an adequate understanding of the mechanisms regulating insect abundance, distribution, and production (McCullough <u>et al</u>., 1979). Such studies are also necessary for a full understanding of life-history theory, its interrelated components of genetic differentiation and phenotypic plasticity, and optimal behavior and environmental conditions. Clearly the fusion of these ideas is in its infancy.

This research attempts to add to the small body of complete mayfly bioenergetics in a number of unique ways. First, while taxonomically similar mayflies have been

compared in similar habitats (Sweeney and Vannote, 1981), this study compares two congeneric species in very different habitats. Second, energy budgets for mayflies inhabiting alpine streams have not been calculated. This work concerns populations in two high-altitude streams. Third, aquatic insect energy budgets have generally involved animals from daily and annually fluctuating temperature regimes. In this study, one population inhabits a spring-fed, cold water stream which neither freezes over during winter nor warms above approximately 6^oC in summer. Fourth, size-specific life-history and bioenergetic parameters such as respiration rate, calorific values, and feeding rates have almost always been plotted against body mass. Here I have used a linear measurement of body size, head width, because of its ease of measurement, its independence of water or gut content, and its ease of conversion to mass when that is desirable. Using head width, animals may also be measured alive and used for further work. Finally, bomb calorimetry and respirometry were conducted using the most up-to-date equipment. These new methods should eliminate many of the errors inherent in previous work.

In this study I attempted to determine how the life cycles and life-history traits of each population were related to the characteristics of its particular habitat.

I also investigated how differences in the amounts and proportions of energy allocation were related to habitat effects and, when necessary or reasonable, to possible strategy (i. e. genetic) differences. In addition, I attempted to discover whether larval growth and adult emergence were synchronized to thermal regimes which maximized fitness.

The first chapter of this thesis covers the various field and laboratory experiments used in constructing the life histories and basic energy budgets of the subject species, and compares and explains the results. The second chapter deals with the synthesis of the energy budgets. Using calculable growth ratios and moult intervals, I estimate the instar number and growth curves for each population. I also compare the overall life-histories and energy budgets of the two populations. The final chapter investigates the locomotory responses of one species to predator presence and nematode endoparasitic infection, and how these responses affect predator encounter rates.

METHODS

Collection Sites

Animals for this study were collected from two locations in the Bow/Crow Forest in southwestern Alberta, Canada. The first site was approximately 50 m downstream

from the source of the Elbow River (50⁰38'N 115⁰00'W, elevation 2103 m) (Figures 1 and 2). This alpine headwater is chiefly spring-fed and stenothermic. Diurnal water temperature fluctuations are negligible (<1^OC) (Figure 3); monthly average temperatures range from 2°C during winter to 6^oC in summer (Table 1). Despite mid-winter air temperatures as low as -40°C, coupled with the chill factor from high winds, the water does not freeze (pers. obs.). This is due to the proximity of the site to the underground water source. Substrate composition varies considerably over short distances. Generally, riffles consist of cobble and boulders while slower runs and pools contain sand and silt with isolated aggregations of large cobble and boulders. Water depth is also quite variable, ranging from a few centimetres in some riffles to over a metre in slow, deep-channel runs. Surface current velocity is quite fast in shallow riffles (up to 25.0 cm/sec), but is nearly zero in some backwater pools. Mats of algae cover most rock substrate, and thick beds of moss occur along both banks at the waterline.

The second site was located on Ford Creek, 2.7 km upstream from its confluence with the Elbow River (50⁰48'N 114⁰51'W, elevation 1707 m) (Figures 1 and 4). Ford Creek is a second order, eurythermic sub-alpine stream. Water temperature fluctuates daily and seasonally (Figure 5 and

Table 2). The stream does freeze over during winter (pers. obs.), but it is not known if anchor ice forms. Substrate composition at this site is more uniform than at the Elbow River site, consisting mainly of cobble with some large boulders. Water depth and surface current velocity are also more uniform in Ford Creek. Depth ranges from 5 to 25 cm over most of the collection area, and current velocity is mostly in the 5 to 15 cm/sec range as riffles constitute a major proportion of site. Stream banks are rock and soil with overhanging shrubs and trees.

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Water Temperature and pH

Daily and seasonal water temperatures were recorded at both collection sites with Peabody Ryan Model "J" thermographs which were submerged and secured at the stream bottoms. pH was recorded monthly using a Markson Model 80 Mini-pH-Meter.

Study Organisms

Two species of siphlonurid mayflies were the main subjects of this study. Although it is not abundant there, <u>Ameletus similior</u> McDunnough is the principal mayfly species at the Elbow River site. Larvae were usually found on the undersides of large cobbles and small boulders in slow riffles and pools. Examination of gut contents revealed that <u>A</u>. <u>similior</u> feeds mainly on diatoms, although some unidentified detritus was sometimes also present. The

life cycle of <u>A</u>. <u>similior</u> lasts three years at the Elbow River site. Emergence is somewhat asynchronous and occurs chiefly in late summer. Also in late summer and in early autumn many large, late-instar larvae were observed to be infected with endoparasitic mermithid nematodes. A single nematode eventually fills the abdominal and thoracic cavities of each affected larva. These parasitic juveniles almost always finally kill their hosts by burrowing out of the body cavity, usually between the thorax and the first abdominal segment (pers. obs.), prior to becoming free-living adults.

At Ford Creek, <u>Ameletus celer</u> McDunnough was chosen for this study because of its availability and taxonomic similarity to <u>A</u>. <u>similior</u>. The microdistribution of <u>A</u>. <u>celer</u> is also similar to <u>A</u>. <u>similior</u>. Its life cycle is two years at Ford Creek. Emergence is more synchronous than at Elbow River and occurs principally in August. While an occasional larva was found to be parasitized by a nematode, this was not a common occurrence and did not contribute significantly to mortality in the Ford Creek population.

[NOTE: Close examination of larvae from both Ford Creek and Elbow River shows some variation in coloration on the ninth and tenth tergites and sternites (B. Sweeney, pers. comm.). This opens the possibility of the presence

of more than one species at each study site. However, monthly samples over a two-year period produced size frequency distributions that strongly indicate distinct cohorts. In addition, all specimens reared to the adult stage were identified as <u>A</u>. <u>celer</u> (from Ford Creek) or as <u>A</u>. <u>similior</u> (from Elbow River). These identifications were verified by Dr. Francoise Harper, University of Montreal.] Collection

Collection of <u>Ameletus</u> larvae was done with a double-mesh kick net. This was a long-handled net with a triangular frame. The frame held an inner 1-mm mesh net and a concentric outer 200-um mesh net. This arrangement allowed the collection of all size classes down to a head width of 0.2 mm. The net was held vertically against the stream bottom with one hand while substrate on the upstream side of the net was agitated and wiped clean of all organisms with the other.

The collection of adult <u>Ameletus</u> was attempted using several methods. Pyramidal emergence traps were placed at various locations within each stream. The large open base was situated 1 to 2 cm above the stream surface by adjusting the four metal legs. The smaller top end was fitted with a cardboard collecting box. The top surface of the collecting box was covered with 200 um mesh to admit light, while the lower portion contained a clear plastic

baffle to funnel the mayflies up into the box and prevent easy escape.

A hand-held UV light was occasionally employed during the late afternoon and evening in an attempt to attract individual and swarming mayflies. These were then to be captured with a cotton mesh aerial net. A large (2 m x 3 m) white sheet was also used, sometimes in conjuction with the UV light, as an artificial swarm marker. The sheet was spread over streamside rocks and vegetation in order to attract swarming <u>Ameletus</u>, which were also to be collected with an aerial net.

Finally, physical searches of nearby vegetation were undertaken to collect subimagoes. These individuals often do not fly but remain grounded until after the final moult.

Samples were taken monthly from June to November, 1984 to 1986, at both collection sites. The Ford Creek and upper Elbow River areas are closed to traffic from December to May due to heavy snowfall and large-mammal migration. However, special access was obtained to sample at Elbow River in December, April, and May of 1985.

For each collected benthic sample, current velocity and substrate size were rated and recorded. Current velocity was scored 1, 2, or 3 for pool, slow run, or riffle, respectively. Substrate size was graded 1, 2 or 3 for silt/sand, cobble, or boulder, respectively. All

samples were placed into water-filled plastic bags and kept on ice while being transported to the laboratory.

Weights and Measurements

Head widths of <u>Ameletus</u> were measured at the widest point to within 0.04 mm using a Wild dissecting microscope fitted with an ocular micrometer. Animals to be weighed were first dried at 60^oC in a Fisher Isotemp Model 126G oven for at least 24 hr, then weighed to within 0.005 mg on a Cahn electrobalance.

Clutch sizes were determined by manually counting eggs from dissected final-instar larvae (identified by black wing pads) and adults. Individual eggs were measured under a Wild dissecting microscope with an ocular micrometer. Length and diameter at the widest point were recorded. To calculate egg volume, eggs were considered to be cylindrical and the equation:

 $V = (3.14r^2)(h)$

was used where V is egg volume, r is one half egg diameter, and h is egg length. Eggs were dried at 60° C for a minimum of 24 hr and weighed in batches of ten to forty on a Cahn electrobalance.

<u>Calorimetry</u>

Specimens of <u>Ameletus celer</u> and <u>Ameletus similior</u> were first dried in a Fisher Isotemp Model 126G oven at 60^OC for a minimum of 48 hr. Individuals of each species were then grouped into eight head width classes (Table 6). Whole mayflies of each head width class were formed into disc-shaped pellets approximately 2 mm in diameter and 1 mm thick using a pellet press.

Pellets were combusted in a Newham Electronics Limited Model AH12/CC micro-bomb calorimeter. This calorimeter operates on the Phillipson (1964) principle, but has several advantages over conventional models. First, the sample pellet is secured under a screw-down electrode so no platinum wires are required. Second, the calorimeter interfaces with a BBC microcomputer which generates an energy curve on the screen, eliminating the need for a pen recorder. Finally, the software automatically computes the calorific content of the sample in cal/g.

The calorimeter was first calibrated by combusting weighed pellets of benzoic acid, which has a known calorific content (6317 cal/g), and entering into the computer a correction factor determined from this process. The weighed mayfly pellets of the various head width groups were then also fired and their calorie content (cal/g) recorded. Samples of eggs and exuviae from each population were similarly tested. Caloric values were converted to joules (J) by the conversion factor 4.186 J/cal.

Respirometry

Respiration rates of Ameletus celer and Ameletus

<u>similior</u> were measured using a flow-through respirometer of the Wrona and Davies (1984) design. This apparatus allows the measurement of the oxygen content of aerated, filtered water flowing through a chamber housing the experimental animal, thus reducing or eliminating complications such as decreasing oxygen tension, waste product accumulation, and chamber agitation which are commonly encountered in closed-bottle respirometry systems.

Respiration chambers consisted of six 2-ml glass syringes through which water, cooled to the desired temperature, was pumped. Incoming dechlorinated tap water was bubbled through a glass bead column to ensure 100% oxygen saturation. Individual mayflies were confined in each of five chambers while the sixth served as a control. After a 12- to 14-hr acclimation period, five 1-ml water samples were drawn from each chamber and separately injected into a Radiometer oxygen electrode. The electrode was housed in a thermostatic jacket to keep the water samples at the same temperature as that in the respiration chamber. The partial pressure of oxygen in each water sample was then measured in torr by a Radiometer PHM 73 gas monitor to which the electrode was attached. Samples from the control chamber were similarly tested after each experimental sample. The water flow rate from the experimental chamber was also measured after each sample,

and adjusted to maintain a 5 torr minimum difference (equal to the maximum sensitivity of the apparatus) between each experimental and control water sample pair. The flow rate was usually between 0.05 and 0.50 ml/min. Water flowing from the chambers and that pumped through the electrode's thermostatic jacket was filtered, returned to a reservoir, and recycled by an Instant Ocean WM 500 refrigeration-recirculation pump.

As much as possible, experimental temperatures reflected those occurring at each study site. It was difficult, however, to obtain detectable (>5 torr) differences between control and experimental chambers at temperatures less than 6°C. Preliminary experimentation suggested that oxygen consumption of A. celer and A. similior was very similar at 6, 7, and 9°C. Extensive testing at 6^oC resulted in nearly identical y-intercepts and slopes for weight-specific oxygen consumption of both species over the entire range of head widths. Therefore, <u>A. celer</u> respiration was measured over a range of $6^{\circ}C$ to 14^oC, while A. similior was tested at 6^oC only. Respiration rates at temperatures not tested were estimated by a multiple regression equation. For <u>A</u>. <u>similior</u> this necessitated the inclusion of data for <u>A</u>. <u>celer</u>, but this is justifiable for several reasons. First, the aforementioned preliminary data strongly indicate that

there is no difference between the respiration rate of the two species at a given temperature. Cold adaptation of <u>A</u>. <u>similior</u> beyond what may occur in <u>A</u>. <u>celer</u> seems unlikely in that <u>A</u>. <u>similior</u> also occurs farther downstream in the Elbow River where temperature regimes are eurythermic, and should therefore be adapted to a much broader temperature range than that which occurs near the spring-fed source. If some additional cold adaptation were present, testing <u>A</u>. <u>similior</u> at temperatures higher than 6° C in order to obtain a separate regression line may well produce a spurious equation by subjecting the animal to temperatures which it never encounters in nature. Given the evidence, however, estimates of weight-specific respiration at temperatures lower than 6° C using data from both species should be a reasonable approximation.

Weight-specific oxygen consumption of each animal was calculated by the equation:

MO2 = (piO2-peO2)(aO2)(F)/wt

where MO2 is rate of oxygen consumption (umol/mg/hr), piO2 is partial pressure of oxygen of incoming water (torr), peO2 is partial pressure of oxygen of exiting water (torr), aO2 is solubility coefficient of oxygen in water at a defined temperature (from Dejours, 1975), F is flow rate of water through respirometry chamber (L/hr), and wt is dry weight of mayfly (mg). Oxygen consumption of each mayfly

was converted to a volumetric rate using: VO2 = (MO2)(22.414 L/mol)

where VO2 is rate of oxygen consumption (uL/mg/hr).

The activity of each experimental animal prior to the withdrawal of each water sample was monitored by an oscilloscope. An impedance converter created a low oscillating current between electrodes located at either end of each respiration chamber. Movement by the mayfly changed the impedance of the circuit and a signal was displayed on the oscilloscope screen. Movements were graded from zero to six depending upon the frequency and altitude of the display over the three minutes prior to taking each sample. Ventilating behavior, a rhythmic dorsoventral waving of the abdomen, was recorded separately from other movement.

Growth

In order to determine growth rate and frequency of moulting, larvae of <u>Ameletus celer</u> and <u>Ameletus similior</u> were reared at three temperatures: 5, 10, and 15°C. Experimental animals were collected from July to September, 1986. Starting head widths ranged from 0.8 to 1.6 mm. Each mayfly was kept in a cylindrical enclosure of 1-mm nylon mesh 3.5 cm in diameter and 9.0 cm high. Thirty enclosures were affixed to a rectangular styrofoam sheet, and this sheet was submerged in an 18.5 L aquarium to a

depth such that the water level was slightly below the tops of the enclosures. By angling the styrofoam sheet slightly and operating a large airstone on the aquarium bottom, a slow water current across the enclosures was maintained (Gee and Bartnik, 1969) (Figure 6). Fifteen mayflies of each species were kept at each of the three experimental temperatures. Temperatures were controlled by housing the aquaria in Puffer-Hubbard Uni-Therm 800 refrigeration units where 12 hr/12 hr light/dark cycles were maintained. Pure <u>Synedra</u> diatom cultures (Carolina Biological Supply No. 15-3095) were kept as food for experimental <u>Ameletus</u>. Diatoms were grown on 2-cm circular filter papers, and were replaced in the enclosures every other day.

Prior to placing it in an enclosure, the head width of each mayfly was measured. Animals were then checked daily for exuviae, and the date of moulting was recorded. To reduce stress on the mayflies and allow for hardening of the new exoskeleton, the measurement of post-moult head width was made 24 to 48 hr after ecdysis. Individuals moulted up to five times before dying or emerging. Moults which resulted in no growth were assumed to be due to the stress of experimental conditions or handling and were not counted.

Feeding Rate

Ameletus of various head widths were kept without food

for 48 hr to allow gut-clearing. All mayflies were then transferred to an aquarium containing algae-covered stones collected from the study sites. Both gut-clearing and feeding containers were maintained at 5°C for A. similior and 10⁰C for <u>A. similior</u>. At half-hour intervals several animals were removed from the aquarium and immediately killed by immersion in 4% formalin. This method resulted in rapid death, and no regurgitation of gut contents was observed. After measuring the head width of each mayfly, its entire intact alimentary tract was removed by first cutting off the head and terminal abdominal segment, then opening the thoracic and abdominal cavities with a midline ventral cut with microscissors. The length and diameter of the fore-, mid-, and hind-guts were measured under a Wild dissecting microscope fitted with an ocular micrometer. From these measurements foregut volume and total gut volume and length were calculated. The proportion of the gut containing food was also determined. Whole guts were dried for 24 hr at 60⁰C and weighed on a Cahn electrobalance.

To estimate feeding rate, the partial correlations between feeding time and foregut and whole gut volumes, while controlling for body size, were examined. The asymptote of a gut volume versus feeding time plot, which is usually used to estimate gut-filling time, could not be used here because there was no distention of the alimentary

tract as it filled to capacity. Therefore, gut-filling time was estimated by direct observation.

RESULTS

Water Temperature and pH

Water temperature at the Elbow River site exhibited almost no daily fluctuation, and the difference between recorded monthly mean high and low temperatures was greater than 1°C only in July (Figure 3 and Table 1). Where complete temperature data were not collected (January through March), single temperature measurements and the known lack of thermal variation enabled estimates to be calculated.

At Ford Creek the daily temperature fluctuation was considerable (up to 8°C), especially during the summer months (Figure 5 and Table 2). Differences between monthly mean highs and lows were as great as 6°C. For months in which complete temperature data were not recorded (January through April), estimates were calculated based on single measurements, temperature records of nearby similar-sized streams, and known periods of freezing.

Total annual degree-hours were similar between the two sites (Table 3). The estimate for Ford Creek is only 6 percent higher than that for Elbow River. This is largely due to the relatively high winter water temperatures at the
Elbow River site. Because the water remains at or above 2°C there, monthly estimates of degree days are higher from September to February than at Ford Creek where the water freezes over. Because evidence suggests that anchor ice does not form at Ford Creek (see Life Cycle), the minimum temperature there is estimated at 1°C during the winter.

For months in which measurements were taken, pH ranges were similar for Elbow River (7.4 - 8.5) and Ford Creek (7.7 - 8.7). Monthly pH comparisons were also quite similar between sites (Figure 7).

Life Cycle

Life cycles were reconstructed from size-frequency distributions during 1984 and 1985 (Figures 8 and 9). Although size-frequency distributions were nearly identical between years at each site, it is understood that fluctuations in temperature regimes, food availability, water level and turbulence, and predator or competitor population size may act to alter the life cycle in other years.

Egg-hatching was assumed to occur during the period immediately preceding the appearance of the smallest size-class larvae (Svensson, 1977). The emergence period of adults was estimated to be the time span during which the larger larval cohort, many of which exhibited the black wing pads characteristic of the final instar, disappeared

from monthly samples. This estimate was necessary due to failed attempts to collect adult <u>Ameletus</u> in the field. Emergence traps were colonized by araneid spiders which constructed orb webs over the trap openings. Mayflies were largely prevented from entering the traps, and those that did were preyed upon. UV light and artificial swarm markers attracted swarms of mayflies, but neither of the desired species. This was especially curious at the Elbow River site where adults were captured of genera which were never collected as larvae there. Physical searches for subimagoes were also fruitless. Fortunately, final-instar larvae of each species were successfully reared to adulthood in the laboratory.

At Ford Creek, <u>Ameletus celer</u> eggs probably incubate for one year and hatch in July and August. Most of the cohort overwinters as small larvae, suggesting that either anchor ice does not form, or larvae are able to burrow and survive within the substrate. The former seems the more likely explanation given the respiratory requirements of the species. Some adults emerge the following July, but most emerge in August. In the laboratory the subimaginal stage lasts approximately 24 hr prior to moulting to the adult. A small number of larvae do not emerge after one year as evidenced by their presence in late autumn and early spring collections. The sex ratio among larvae large

enough to identify (head width at least 1 mm) was not significantly different from 1:1 (n = 353, x^2 , p>0.50).

At Elbow River the life cycle of Ameletus similior takes three years. Eggs hatch from September to November, approximately one year after oviposition. The larvae grow quite slowly in the cold water and overwinter the third year a little more than half their ultimate size. Emergence takes place during the summer and fall of the second year, mostly from August to October. As in A. celer, a single subimaginal stage of about 24 hr occurs prior to adulthood. It is in late summer that the large second-year larvae are particularly susceptible to nematode endoparasitism. As much as 75% of the cohort may be affected; infected smaller larvae were almost never observed. The sex ratio among large larvae was female-biased 1.83:1, significantly different from 1:1 (n = 316, x², p<0.001).

Body Size and Mass

Head width and dry weight were highly correlated for both species. Plots of log-transformed data gave r^2 values of 0.92 and 0.89 for <u>A</u>. <u>celer</u> (Figure 10) and <u>A</u>. <u>similior</u> (Figure 11), respectively (Linear Regressions, n's = 194 and 204, respectively, p<0.001 for both). An analysis of covariance showed the two regression lines to be significantly different (F_{2.400} = 14.86, p<0.001). While

the slopes are nearly identical, the y-intercept of the Elbow River regression is slightly higher indicating that <u>A. similior</u> is somewhat heavier over the entire range of head widths (Figure 12). It should be noted that the use of the regression equations to calculate first instar larvae dry weights results in a discrepancy of approximately 10 ug when compared to egg dry weights (see below). However, possible deviations from the calculated regression line at this small size, plus the sensitivity of the electrobalance (5.0 ug) and the tolerance of reference weights (5.4 ug) would account for this.

Fecundity, Eqq Size and Eqq Mass

The relationship between head width and fecundity was not significantly different between species (ANCOVA, $F_{2,43}$ = 1.33, p>0.50). A linear regression showed fecundity to be positively correlated to natural log-transformed head width (n = 46, r^2 = 0.39, p<0.001). The relationship between head width and fecundity is described by the equation:

y = 275.8 + 2133.4x

where y is fecundity and x is the natural logarithm of head width (Figure 13).

Mean egg volumes were 0.248 mm³ (\pm 0.006 s. e.) (n = 156) and 0.246 mm³ (\pm 0.009 s. e.) (n = 102) for <u>A</u>. <u>celer</u> and <u>A</u>. <u>similior</u>, respectively. There was no difference in

mean egg volume between species (t-test, $t_{256} = 0.16$, p>0.80). Neither was there a significant difference in egg mass between <u>A</u>. <u>celer</u> (1.33 ug <u>+</u>0.1) (n = 19) and <u>A</u>. <u>similior</u> (1.45 ug <u>+</u>0.1) (n = 19) (ANOVA, $F_{1,37} = 0.80$, p>0.30).

Microdistribution

At Ford Creek the physical distribution of different sized larvae was influenced by both substrate size and current velocity. The mean head width of larvae collected from the slowest currents (mean = 0.727 mm + 0.02) (n = 235) was significantly smaller than that of larvae taken from moderate speed or fast currents (means = $0.937 \text{ mm} \pm 0.02$ and 0.865 mm +0.03, respectively) (n's = 382 and 44, respectively) (ANOVA, F_{2.658} = 18.87, p<0.001; Duncan Multiple Range test [DMR], p<0.05) (Table 4). While there was no difference between the sizes of larvae found in the smallest and the largest substrates (means = 0.670 mm +0.15 and 0.827 mm \pm 0.07, respectively) (n's = 21 and 8, respectively), significantly larger larvae (mean = 1.014 mm +0.03) (n = 106) were collected from intermediate-sized substrate (ANOVA, $F_{2.132} = 6.94$, p<0.001); DMR, p<0.05) (Table 4). These results may be biased, however, due to the scarcity of larvae inhabiting the largest substrate class (n = 8).

At the Elbow River site, current velocity was a factor in the distribution of <u>A</u>. <u>similior</u>. Animals collected from the slowest currents were intermediate in size (mean = 0.968 mm ± 0.02) (n = 377), those from moderate speed waters largest (mean = 1.070 mm ± 0.03) (n = 317), and those from the fastest runs were smallest (mean = 0.776 mm ± 0.06) (n = 60) F_{2,751} = 12.06, p<0.001; DMR, p<0.05) (Table 4). <u>A</u>. <u>similior</u> was not found in any but the intermediate sized substrate (Table 4).

Growth

The number of days between moults was significantly affected by temperature (n = 122, 2-way ANOVA, $F_{2,121}$ = 11.76, p<0.001), but did not differ between species (2-way ANOVA, $F_{1,121}$ = 0.40, p>0.50). There was no interaction between temperature and species (2-way ANOVA, $F_{2,121}$ = 0.02, p>0.90). There was also no correlation between moult interval and body size (Correlation Coefficient = -0.03, p>0.30). At 5°C, time between moults was significantly greater (mean = 12.9 d ±2.5, n = 16) than at 10°C (mean = 6.1 d ±0.7, n = 29) or 15°C (mean = 6.5 d ±0.9, n = 27) (DMR, p<0.05).

Growth increments were converted to growth ratios by dividing post-moult size by pre-moult size. While mayfly size had a significant effect on growth ratio (Partial Correlation Coefficient = -0.31, p<0.001), there was no

difference between sites (Partial Correlation Coefficient = 0.04, p>0.20) or temperatures (Partial Correlation Coefficient = -0.10, p>0.20). The relationship between size and growth ratio was negative and linear (Linear Regression, n = 53, r^2 = 0.10, p<0.05), and is described by the equation:

y = 1.09 - .062x

where x is the natural log-transformed pre-moult head width (mm) and y is the growth ratio (Figure 14). Although a decreasing growth ratio with increasing body size is expected, in this case the fit is admittedly not very good. This may have due in part to the narrow diet of a single species of diatom, differences from study site water chemistry, or other stresses in experimental conditions or from handling. Stress moults may have, in some cases, resulted in very little growth during a particular growth interval. These factors in various combinations could have resulted in the high degree of scatter seen in Figure 14. Nonetheless, the relationship between growth ratio and size is statistically significant, and will be used in further calculations.

A number of methods are available for indirectly determining the number of instars a species passes through, many based on size-frequency distributions. Because initial inspection of the data indicated nearly uniform

distributions of head widths for both species, none of those methods was applicable here. Instead, a mean growth ratio (1.081 \pm 0.027) was used to approximate instar number based on a head width range from 0.2 mm to 1.4 mm over the life span of an average individual. The resultant estimate of 25 instars is well within the range of 10 to 50 reported by Brittain (1982).

Feeding Rate

Feeding time did not correlate with total gut length, total gut volume, or foregut volume when controlling for body size (n = 33, Partial Correlation Coefficients = 0.09, 0.40, -0.06, respectively; p>0.30, p>0.05, and p>0.40, respectively). This indicates that there was no appreciable distention or elongation of the alimentary tract with increased gut content. While it was not possible, then, to properly plot any of these variables against feeding time to asymptotically determine gut filling time, I was able to estimate the proportion of total gut space filled and relate that to feeding time. Given that no distention occurs, when no empty space was apparent the gut was assumed to be filled to capacity. For both species all sizes of animals tested, guts were full in 1.0 to 2.0 hr. Therefore, a gut-filling time of 1.5 hr was used to calculate feeding rates.

For an animal of a given size, its feeding rate (mg/hr) will equal the quotient of size-specific gut mass divided by gut-filling time (l.5 hr). There is a significant linear relationship between the log-transformed values of head width and total gut mass (n = 30, Linear Regression, $r^2 = 0.57$, p<0.001) (Figure 15). Therefore, a size-specific feeding rate is simply equal to the regressed total gut mass divided by l.5 hr (Figure 16).

Respirometry

For each temperature at which respiration rates were measured, a linear regression of oxygen consumption (uL/mg/hr) against log-transformed head width (mm) produced a regression line with a negative slope (Table 5). This indicates decreasing weight-specific respiration with increased body size at any temperature within the thermal range experienced by either species. With increasing temperature, y-intercepts of the regression lines increased and slopes became increasingly negative, although the lines for 6°C and 7°C cross and those for 11°C and 12°C are reversed (Figure 17). This trend suggests that while the weight-specific respiration of small <u>Ameletus</u> is greatly influenced by temperature, that of large larvae is much less affected. Indeed some regression lines converge or cross near the upper end of the head-width scale, and it could be inferred that the effect of temperature on

weight-specific respiration is nil or even reversed in very large larvae. I believe, however, that this is an artifact of the regressions themselves and of the high variation in respiration rates of large pre-reproductive, pre-emergent larvae. Specifically, the regression line forces weight-specific respiration to zero at some large body size. This is unrealistic, of course, as respiration must level off at some value greater than zero. Confounding the matter is the fact that respiration generally increases in late-instar females as reproductive tissues and eggs rapidly mature (Vannote, 1978; Ciancara, 1980). Atrophy of the digestive system and other physiological preparations for emergence probably also affect respiration. Some final-instar larvae died, presumably drowned, while confined to respiration chambers even though there was no external indication of imminent emergence. Apparently the switch from gill-mediated to spiracular respiration begins during the ultimate larval instar, and this may further confound the problem. In short, less than precise respiration rate estimates which ignore the physiological complications and variations of the final larval instars were necessary to construct a predictive energy budget. This was done in two respects.

In the construction of Figure 18, the regression equation parameters from Table 5 were used to plot oxygen

consumption values at the extremes of the head width axis for each temperature. A line was then fitted by eye along the temperature axis at each end of the head width scale, and these were then connected by a straight line at each temperature. The resultant three-dimensional figure graphically illustrates the relative effects of size and temperature on oxygen consumption.

For temperatures too low to test directly (<6 $^{\circ}$ C), a multiple regression equation was calculated from all available data and used to determine oxygen consumption: y = 0.69(t) - 2.1(h) - 3.05

where y is respiration rate (uL $O_2/mg/hr$), t is temperature (°C), and h is natural log-transformed head width (mm). Partial correlation coefficients for temperature and head width are 0.58 and -0.60, respectively (n = 280, p<0.001 for both). The r² value for the combination of head width and temperature is 0.70 (p<0.001).

Other factors which may contribute to the variance in oxygen consumption include body mass, movement, and ventilation behavior. While body mass has been shown with be closely correlated with head width for both species of <u>Ameletus</u>, variation does occur. This is especially true in larger larvae (Figures 10 and 11). Therefore, as head width increases greater variance in weight-specific oxygen consumption is expected.

Movement did not contribute significantly to oxygen consumption (n = 280, Partial Correlation Coefficient = 0.08, p>0.05). While this does not seem logical it is explainable within the context of this experiment. First, the extent of movement, based only on oscilloscope readings, is not easily translatable to actual types of movement or their energetic costs. Movement values were arbitrarily assigned according to their appearance on the oscilloscope screen. It is possible that movements which differed only slightly in their energy requirements resulted in grossly different oscilloscope displays. Continuous observation of each experimental animal would be required to eliminate this subjective element, and this was not possible. A solution may be to standardize movement and oscilloscope readings for a particular experimental species by preliminary observation. Second, movement was extremely limited in the respiration chambers, especially for larger larvae. Animals simply may not have had sufficient space to move to a degree where their increased oxygen consumption was detectable. One remedy may be to enlarge respiration chambers, but that would necessitate decreasing already very slow flow rates in order to get detectable levels of oxygen consumption. In any event, although movement was not significantly correlated with

34

oxygen consumption, it may still have contributed to variation in weight-specific respiration.

Unlike movement, ventilation behavior was readily interpretable from the rhythmic pulses it presented on the oscilloscope. Ventilation was significantly and positively correlated to oxygen consumption (n = 280, Partial Correlation Coefficient = 0.10, p<0.05).

Movement was significantly affected by temperature (Kruskal-Wallis ANOVA, n = 185, X^2 = 32.10, p<0.001). The gamma statistic, an index of the strength and direction of relationship between two variables, showed the relationship to be a "fairly strong" negative one (gamma = -0.39) (Nie <u>et al</u>., 1975). Movement was also significantly affected by size (Kruskal-Wallis ANOVA, n = 280, X^2 = 26.88, p<0.001). Movement generally decreased with increasing body size in <u>A</u>. <u>similior</u> (gamma = -0.31), but increased with body size in <u>A</u>. <u>celer</u> (gamma = 0.28).

Temperature had no effect on ventilation (Kruskal-Wallis ANOVA, n = 185, $X^2 = 6.80$, p>0.30). However, ventilation occurred significantly more frequently among larger size classes than among the smaller of both species (Kruskal-Wallis ANOVA, n = 280, $X^2 = 19.98$, p<0.001, gamma = -0.36). This behavior may also be a function of current velocity.

<u>Calorimetry</u>

The calorific value of whole-animal samples was significantly correlated to size class (Partial Correlation Coefficient = -0.31, p<0.01), but not to species (Partial Correlation Coefficient = 0.13, p>0.20) or to sex (Partial Correlation Coefficient = 0.17, p>0.50) (n = 65). Mean energy values for the eight size classes are shown in Table 6. By assigning each value to the mode head width size in its respective size class and plotting a linear regression, an equation which describes the approximate energy values of whole animals over the entire head width range was calculated:

y = 34.5 - 5.3x

where y = energy value of the whole mayfly (J/mg) and x = natural log-transformed head width (mm) ($r^2 = 0.68$, p<0.01) (Figure 19).

The mean energy content of <u>A</u>. <u>similior</u> eggs (30.1 J/mg +2.3) was higher than that of <u>A</u>. <u>celer</u> (21.8 J/mg ±0.2) (t-test, p<0.01) (Table 6). It should be noted, however, that due to the enormous number of eggs required to make a single calorimetry pellet of sufficient mass (at least 2 mg), sample sizes were small (<u>A</u>. <u>similior</u> = 5, <u>A</u>. <u>celer</u> = 2). Mean energy content of exuviae (42.0 J/mg ±4.7) was assumed to be the same for both populations (Table 6).

<u>Sex</u> <u>Ratio</u>

<u>A</u>. <u>similior</u> occurs in a female-biased sex ratio at the Elbow River study site. Deviations from 1:1 sex ratios in aquatic insects have been largely explained as the result of small sample size, differential mortality, or sexual differences in emergence timing (Butler, 1984). There is no evidence to date of any adaptive significance in deviations from 1:1 sex ratios in these animals (Williams, 1979; Butler, 1984).

The Elbow River population of <u>A</u>. similior, however, is susceptible to a high rate of mermithid nematode endoparasitization which almost always results in mortality for infected individuals. Because this appears to occur almost exclusively in females, it may represent a selective pressure for an overabundance of females to balance the high female mortality. If sexes cost the same to produce, then the expected sex ratio is 1:1 (Fisher, 1930), but if a sexually-specific source of high mortality persists in a population, natural selection may act to balance this by an increase in the production of the susceptible sex. This would result in an effective sex ratio approaching 1:1. It would also result in a higher percentage of an individual's genes being passed on in subsequent generations

(i. e. higher fitness). For example, if a female gives birth to ten males and ten females, but half the females are lost to parasitization, then half the males are also lost due to lack of mates, assuming random one-time mating which is probably fairly realistic in mayflies. Thus, a full 50 percent of the parent's genes are lost. However, if the same female produces ten males and twenty females, and still loses half the females to parasitization, all males may still mate with the remaining females. In this case, only 25 percent of the parent's genes are lost.

What complicates this matter is that endoparasitism can cause gynandromorphy (Hominick and Welch, 1980), and some parasitized <u>A</u>. <u>similior</u> larvae could have been mistakenly identified as true females. However, these animals exhibited not only female genitalia, but the smaller interocular distance peculiar to females as well. Whether parasitic infection can affect both characteristics is not known. Examination of gonads was not possible as parasitized animals were sterile with only vestiges of internal sex organs.

What would be the advantage to the parasite for infecting females only? Larvae were infected only in the final few instars. It is during this time that reproductive tissues rapidly mature. Because females almost certainly process more energy than males during this

period in order to produce eggs, the infection of females may ensure a larger nutrient supply for the parasite. In addition, using only a large, late-instar larva as host would provide more space within which the parasite could grow. Larger body size for the parasite, in turn, probably results in a higher fecundity.

This scenario, however, produces another possible explanation for the observed shortage of males. If females do in fact process more energy than males, males may simply succumb sooner and more frequently to the energetic stresses of parasitization. Females may be able to better support the parasite for a longer period of time. A higher male mortality, then, might explain the biased sex ratio. However, given that the parasitic stage of mermithid nematodes is extremely short (as littles as eight days, Hominick and Welch, 1980), it seems that at some point some infected males would have been collected.

A final explanation for the female-biased sex ratio may be the occurrence of parthenogenesis. Various species of <u>Ameletus</u> are know to exhibit parthenogenesis to some degree (Brittain, 1982; B. Sweeney, pers. comm.). In the <u>A. similior</u> population at Elbow River, males do occur so obligate parthenogenesis may be ruled out. But nonobligatory parthenogenesis may explain the occurrence of

excess females as well as why mating swarms were never observed.

Microdistribution

Among the conditions which affect the microdistribution of aquatic insects some such as temperature, oxygen concentration, and water chemistry are probably fairly homogeneous over localized areas (Rabeni and Minshall, 1977). While these factors may determine whether an area is at all habitable for a certain organism, they often do not fluctuate sufficiently over small distances to greatly impact microdistribution. Other conditions such as current velocity, substrate size, and silt or detritus deposition may be more important on a relatively small scale.

Silt, sand and detritus can fill substratum interstices, thereby decreasing the size and number of available refugia, and reducing the interstitial flow of water and so reducing oxygen tension (Eriksen, 1966). Fine particle accumulation may also make the substratum surface unstable by its movement, especially during periods of high current velocity such as spates and spring run-off (Dunne and Leopold, 1978; Vannote and Minshall, 1982). Heavy siltation may reduce population density except for burrowing species (Eriksen, 1968), whereas light siltation has differential effects depending on species (Cummins and

Lauff, 1969). Rabeni and Minshall (1977) showed that <u>Ameletus oreqonensis</u> and several other mayfly species preferred substrata without silt accumulation. Current velocity and substrate size interact in determining where and how silt and detritus accumulate. Generally, fine detritus does not settle in large-particle substrata with large interstices and high current velocity, but its propensity for accumulation elsewhere appears to be a positive influence on benthic invertebrate colonization (Rabeni and Minshall, 1977; Statzner, 1981; Culp <u>et al</u>., 1983; Erman and Erman, 1984).

In this study, neither silt nor detritus was examined except as one substrate size classification. How they might interact with other substrata or with current velocity to influence the microdistribution of <u>Ameletus</u> at either Elbow River or Ford Creek is unknown.

Apart from the role of the substratum as a collecting site for particulate matter, substrate particle size is important in benthic invertebrate microdistribution. Insects generally increase in numbers with increasing particle size (Brusven and Prather, 1974; Rabeni and Minshall, 1977; Culp <u>et al</u>., 1983; Erman and Erman, 1984), but numbers are often reduced on very large-particle substrata (Ward, 1975; Rabeni and Minshall, 1977). Fine-particle substrata may exclude large organisms because

of the lack of interstitial spaces usable as refugia. In addition, the stability of small particles is more easily affected by current velocity and turbulence (Dunne and Leopold, 1978; Newbury, 1984). Coarse substrata may not trap sufficient detritus for those organisms requiring it as food, and interstitial spaces may be too large to provide a reliable refuge from some predators. The size heterogeneity and surface irregularity of substrate particles may also be important in colonization, but this has not yet been conclusively shown (Erman and Erman, 1984; Minshall, 1984).

Current velocity may directly determine the portion of a habitat a given organism can inhabit. Where the current is rapid, drag on benthic animals may limit colonization to those with morphological adaptations such as dorso-ventral flattening or broad surfaces for attachment (Statzner and Holm, 1982). Size-related current preferences in insects may result from respiratory demands arising from the decrease in surface area to volume ratio with an increase in body size (Kovalak, 1978). In many species, water temperatures are generally lower during early growth and so respiratory demands are not as great. At these times growth rates may be higher in slow water because less energy is spent maintaining position in the current, and so these areas of slower current may be selected. During

later growth, temperatures are usually warmer and respiratory demands increase, so larger larvae may move to faster water to increase oxygen delivery.

Water current affects microdistribution not only by its influence directly on benthic insects, but also by its influence on substrate particles. At a constant current velocity substrate particles of some minimum size are stable and potentially colonizable. However, if because of spating or melt-off current velocity is increased, small particles may become unstable and force emigration.

Patterns of microdistribution can be disrupted for a number of reasons. Mayflies can be displaced by behavioral, catastrophic, or constant drift (Waters, 1965; Holt and Waters, 1967). Displaced animals may not return to their originally selected substratum (Lemkuhl and Anderson, 1972), or may do so under the influence of density-dependent factors (Hemphill and Cooper, 1983). Negative phototactic behavior may also alter diel microdistribution patterns (Hughes, 1966; Elliott, 1967; Waters, 1972).

The microdistribution of <u>A</u>. <u>celer</u> at Ford Creek appears to be at least partly structured by current velocity and substrate size. Over the entire collection period, the largest larvae were taken from intermediate-size substrate while smaller animals inhabited

small substrate. This suggests that interstitial size relative to the size of the animal was important either because of the size of the space it provided or the oxygen concentration supplied. Why small <u>Ameletus</u> also inhabit large substrate is not known, but it is conceivable that smaller animals may be occasionally displaced from preferred areas and temporarily occupy large substrate where the current velocity is generally slow.

Large animals were also found most often in intermediate and fast current, while small larvae usually inhabited slow current. How respiratory demands affect this distribution in not known for certain because size-specific substrate selectivity was not examined with respect to water temperature, but these data do fit the pattern of higher oxygen demands in large animals. Another explanation is that larger animals are simply better able to maintain their position in faster water than are smaller individuals.

There is undoubtedly some interaction between current velocity and substrate size, as water speed appears to determine to some extent the particle sizes prevalent in a given area. Silt and sand occur mainly in backwaters and pools. Cobbles occur widely over areas of slow to moderate riffles, while large cobbles and boulders are confined mostly to areas of fast riffles. Small larvae, then, are

more common in areas of small substrate particles and slow current, while larger larvae most often inhabit intermediate-size substrata in intermediate speed current. Again, refugium availability and oxygen delivery are the most likely factors influencing this distribution. Among large substrate particles in fast water both large and small larvae are found. Large <u>A</u>. <u>celer</u> may occupy these areas because of their increased ability to maintain position in fast water, while smaller individuals may be temporarily displaced there. Alternatively, small larvae may simply be less influenced by current velocity or substrate size than larger larvae, which might explain the occurrence of smaller larvae over a wide range of conditions.

Results from the Elbow River site are quite straightforward with regard to substrate size. Almost all <u>Ameletus similior</u> larvae were taken from intermediate-size substrata. Larvae were probably restricted from small-particle substrate because it was generally situated in very deep and slow portions of the stream bed. Coarse substrata were also located chiefly in backwaters or in very fast riffles. Most obviously suitable sections of the site were largely covered with intermediate-size substrate

The distribution of A. similior at Elbow River with respect to current velocity is quite different from that of A. celer at Ford Creek. The smallest larvae were collected mainly from fast currents while largest larvae were taken from intermediate speed waters. An intermediate size group was collected in slow water. The fact that much of the fast water at Elbow River was covered with thick algae mats may have enabled small individuals to inhabit those areas. The collections of algae may have slowed the interstitial current velocity or reduced turbulence at the boundary layer. At both sites intermediate speed currents were the areas of highest colonization by large larvae, although they were also common in fast water at Ford Creek. At these current speeds the preferred intermediate-size substrate is probably fairly stable and oxygen is likely delivered in abundant supply. However, why an intermediate size class occupies the slowest currents is not known. These pools and backwater areas are typically deep with silt or sand substrata, and would not seem to provide favorable conditions. Some backwater sections do have accumulations of large cobbles, and pools are bordered by banks covered with very thick moss. Animals were undoubtedly occupying these substrata, but why they would fall into an intermediate size class remains unclear.

Current velocity-substrate size relationships at the Elbow River site are not the same as at Ford Creek. Sand and silt occur mainly in pools and backwaters, but sand and gravel, also considered a "small" substratum, also occur in some riffles. Animals are apparently restricted from small-particle substrata because of the lack of refugia and poor oxygen delivery in pools, and because of the lack of a stable gripping surface in riffles. Cobbles occur in all areas of the streambed, and this apparently preferred substrate size may have allowed the observed habitation of all current velocities. This does not explain, however, size distributions or the occupation of slow water if oxygen delivery is a factor there. Large cobbles and boulders occurred in some slow backwaters and pools as well as in fast riffles. Again, lack of refugia due to large interstitial space and poor oxygen delivery may have influenced the lack of colonization of these substrata in slow water. High current velocity within interstitial spaces may have prevented their utilization in faster-moving water.

Overall, the microdistribution of <u>A</u>. <u>similior</u> at Elbow River appears to be much more highly influenced by substrate size alone than the microdistribution of <u>A</u>. <u>celer</u> at Ford Creek. However, size-specific substrate selectivity at the Elbow River site may have been

complicated by seasonal respiratory demands which were not tested. Physical disturbance of the <u>A</u>. <u>similior</u> population during sampling may also have been a factor.

Feeding

Food selectivity has been demonstrated in tipulids (Hall and Pritchard, 1975), chironomids (Ward and Cummins, 1979), trichopterans (Otto, 1974; Iversen, 1974; Grafius and Anderson, 1979), as well as in ephemeropterans. Brown (1960) reported size-specific food habits in <u>Cloeon</u> <u>dipterum</u>. Brown (1961) observed that the guts of <u>Baetis</u> <u>rhodani</u> and <u>C</u>. <u>dipterum</u> always contained more detritus than any other food, and Shepard and Minshall (1984) reported that <u>Baetis tricaudatus</u> and <u>Ephemerella inermis</u> preferred fecal detritus over diatoms, filamentous algae, or other detritus types. Other authors, however, have found no food selectivity in other aquatic insect larvae (Mecom and Cummins, 1964).

Diatoms constituted a high percentage of the total gut contents in both <u>Ameletus celer</u> and <u>Ameletus similior</u> from the field. There were no obvious differences in gut contents either between species or over time, although these factors were not quantified. Neither were diatoms taxonomically identified, so it is not known whether there were any preferences for certain diatom species.

The diet of A. similior could be in part a function of restriction in its food alternatives at the Elbow River site. Due to the site's proximity to the spring source and the lack of substantial streamside vegetation, allochthonous input and fecal detritus may not be sufficient to provide a steady diet of these materials. Filamentous algae are present in large quantities, but Shepard and Minshall (1984) showed that Ameletus oregonensis was unable to survive on a diet of Cladophora alone and I never observed such material in the gut of A. similior. It seems unlikely, however, that A. similior is subject to any serious restrictions in food availability at the Elbow River site, although food availability was not empirically tested. A. celer is certainly not restricted by food type at Ford Creek. The site was far enough downstream from the source to allow the accumulation of all detritus types, and the herbaceous, deciduous, and coniferous input into the stream was substantial. The most likely explanation for both species seems to be a definite selectivity of diatoms over other available food types.

The nutritional significance of such selectivity for <u>Ameletus</u> is not known, but Chapman and Demory (1963) suggested that live algal cells are more nutritious than detritus which loses nutritional value with leaching and microbial activity. However, these authors neglected to

consider the nutritional value of the microbial biomass itself (G. Pritchard, pers. comm.). Obviously selection for food preference must be closely correlated with taxon-specific nutritional requirements. This is demonstrated by the different growth and survival responses of mayflies when reared on diets of various leaf types (Sweeney et al., 1986), leaves and diatoms (Sweeney and Vannote, 1984), filamentous algae and diatoms (Ciancara, 1979), or diatoms, detritus, fecal detritus, and filamentous algae (Shepard and Minshall, 1984). In these studies various species fared better on different diets. Of the five species they studied, Shepard and Minshall (1984) found that the daily weight gain was highest for two species on a diet of diatoms, while the other three species In contrast, each grew fastest on different food types. Ciancara (1979) reported that while the growth rate of Cloeon dipterum was much slower on a diet of filamentous algae than on one of diatoms, maximum size was not affected. Sweeney et al. (1986) and Sweeney and Vannote (1984) showed that not only are leaf types or the inclusion of diatoms important in growth, but that temperature may interact with diet and affect ultimate size. Further experimentation involving survivorship, growth rate, adult size and fecundity as a result of active food selectivity

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rather than diet restriction may serve to clarify these relationships.

Food consumption can be influenced by food concentration, temperature, and animal size (Monakov, 1972). In feeding experiments with Ameletus, food was provided ad libitum as concentration was not considered a factor at either field site. Ingestion rates for A. celer and A. similior were not tested over a range of temperatures, but rather at a single temperature which reflected the conditions at each site (5°C for <u>A</u>. similior, and 10°C for A. celer). Both species had similar ingestion rates, and gut-filling times were size-dependent to the same degree. Ladle et al. (1972) and Reisen (1974) found temperature-independent feeding rates for Simulium vittatum, as did Schindler (1968) for Daphnia magna. Most other authors, however, have noted a temperature effect on feeding rate in various aquatic invertebrates. Nilsson (1974) observed that consumption rate in Gammarus pulex increased with temperature. Lawton (1971) found a similar relationship in the damselfly Pyrrhosoma nymphula, and Otto (1974) reported that the trichopteran Potamophylax cingulatus ate three times as much at 15°C as at 2°C. Lacey and Mulla (1979) noted that the feeding rate of Simulium vittatum remained constant over intermediate temperatures, then increased at higher ones.

When gut-filling time is influenced by temperature it appears that the mechanism affected is the speed with which food is ingested rather than alimentary tract peristalsis. The movement of food in <u>Simulium vittatum</u> was not affected by peristalsis at all and was accomplished only by continual ingestion (Lacey and Mulla, 1979). This may explain why some mayflies apparently eat constantly (Zimmerman and Wissing, 1978) or are always found to have full guts in natural populations (pers. obs.). Therefore, food passage time may be equated with gut-filling time.

Food passage time in mayflies is typically short. Reported retention times vary from 30 minutes in <u>Baetis</u> <u>rhodani</u>, <u>Cloeon dipterum</u> (Brown, 1961), and <u>Tricorythodes</u> <u>minutus</u> (McCullough <u>et al</u>., 1979) to 5.5 hr in <u>Hexagenia</u> <u>limbata</u> (Zimmerman and Wissing, 1978). All sizes of <u>A. celer</u> and <u>A. similior</u> tested were able to fill their guts in from 1 to 2 hr at constant temperatures 10° C and 5° C, respectively.

The importance of ingestion rate was demonstrated by Hagstrum and Workman (1971) for the mosquito <u>Culex</u> <u>tarsalis</u>. They found that in order to complete development in a certain number of days, <u>Culex</u> had to ingest more food at 20^oC than at 30^oC. However, the difference in the amount of food necessary for development became greater as development time became shorter. It is interesting to

note, too, that at all development times (12 to 24 d) adult <u>Culex</u> were 12 to 18 per cent heavier when reared at 20°C. This suggests that previously discussed differences in adult mayfly size due to temperature regime may not be due solely to metabolic costs, but may be in part due to thermally induced differential feeding rates.

<u>Growth</u>

Growth is a fundamental aspect of any life history study. Size largely determines how an organism can interact with its environment during a given life stage or time of year. It may control or limit inter-individual behavior, useable food types, microdistribution, temperature tolerance, breeding opportunities, and predation susceptibility. In mayfly life history studies, growth is particularly important in terms of the ultimate body size of an individual. Mayflies are characterized as r-selected (MacArthur and Wilson, 1967) organisms occupying habitats in which mortality is generally random and minimally related to genotype (Alexander, 1982). In such r-selected populations fitness depends mainly on the intrinsic rate of increase (r), and so faster-multiplying genotypes should increase. Because a large body size in female mayflies is invariably correlated to a greater fecundity, it is also equated with a higher fitness. A large body size may also be beneficial to males if it

enables them to better actively compete for females or if it makes them more sexually attractive to selecting females. For Ameletus, therefore, maximization of body size (and so fecundity and fitness) should be an optimal strategy. Assuming high r-selection, this is true for all mayfly species except when the stimulus which induces emergence negates the benefits of growth maximization. Τf emergence is cued by temperature (Sweeney and Vannote, 1984) or photoperiod (Trimble and Smith, 1979), then emergence should be fairly synchronous and "extra" growth which an individual attains can be considered an increase in fitness. This appears to be the case for both species of interest here, particularly A. celer which emerges mainly during a single month. However, if the emergence stimulus is body size, early rapid growth may be catastrophic if the individual emerges in the absence of potential mates or into unfavorable weather conditions.

Invertebrate growth is affected by a number of factors including temperature, body size, and food quality and availability. In laboratory growth experiments, <u>Ameletus</u> was supplied diatoms <u>ad libitum</u>. As gut analyses indicated that diatoms are the food of choice of <u>Ameletus</u>, food availability and quality were not believed to be important considerations in these experiments. It was also assumed that diatom availability was not limiting at either study site. The full guts observed in nearly all larvae examined support this assumption. Although a small proportion of some gut contents was composed of detritus, I believe that it was probably incidental to the collection of diatoms by <u>Ameletus</u> and not dietetically important. Consequently, food quality was assumed to fairly constant and not an important factor in either field population. Temperature and body size, however, were both important in shaping the growth patterns of <u>Ameletus</u>.

The effect of temperature on aquatic insect growth rates is well-documented for populations in the field (Sweeney, 1978; Humpesch, 1979; Mackay, 1979) and for laboratory-reared animals (Sweeney and Schnack, 1977; Sweeney, 1978; Vannote and Sweeney, 1980). The negative correlation between temperature and moult interval has also been shown (Headlee, 1940; Bar-Zeev, 1958; Lutz, 1974). One way in which growth can be maximized (and therefore optimized, assuming strict r-selection in mayflies) is by shortening the interval between moults (Ross and Merritt, 1978) while holding constant or increasing the growth When this interval is sensitive to temperature, ratio. growth maximization would be expected to occur at some temperature intermediate to the extremes within a population's habitat range, but not necessarily at the same temperature over the entire life cycle. While increased

size compensates for the effect of high temperature on weight-specific respiration rate, high temperature is Thus instantaneous positively correlated with growth rate. growth maximization will occur at increasing temperatures over the life cycle, but at temperatures low enough that potential growth energy is not used in increased respiration. However, excessively high temperatures can also cause early maturation of adult tissues and emergence at a smaller size and lower fecundity (Sweeney and Vannote, 1984). Previous studies have shown that, in winter-spring mayfly species inhabiting warm water streams and rivers, early emerging adults which have experienced relatively cooler developmental regimes are the largest and most fecund of their cohort (Fremling, 1973; Sweeney, 1976, 1978; Vannote, 1978; Sweeney and Vannote, 1984). Mackey (1977) also reported that chironomids reared at lower temperatures were usually longer. In cold water streams, relatively late emergence may have a similar effect by exposing larvae to the warmest part of the annual thermoperiod. Data from Elbow River support this latter hypothesis. Final instar A. similior, identified by black wing pads, were larger during August (mean head width = 1.68 mm) than during September (mean head width = 1.39 mm). Assuming the emergence period immediately follows the black wing pad stage, emergence of the largest larvae occurs

immediately following peak summer temperatures. Data on <u>A. celer</u> from Ford Creek do not permit such a comparison as final instar larvae were not collected in sufficient numbers before or after July. It is interesting to note, however, that emergence of this population apparently occurs prior to the period of peak summer temperatures. If Ford Creek can be considered to be a warm water stream, at least in comparison to Elbow River, this does lend some support to the former hypothesis.

It is the negative relationship between temperature and moult interval which appears to be associated with accelerated summer growth in Ameletus. For all sizes of A. celer and A. similior tested, the moult interval at 6⁰ was slightly more than double that at 10°C. At 15°C the interval was longer than at 10°C, but not significantly so. From this it is estimated that the temperature which induces the shortest moult interval is near 10°C for both species. This estimate lends further support to the idea that optimal temperature regimes, in terms of maximal growth and fecundity, may occur later in the year in cold water habitats such as the Elbow River site, and early in warmer water such as Ford Creek. Although temperatures never reach 10^OC at the Elbow River site, they come closest immediately preceding the emergence of the largest larvae. Further sampling downstream of the cold springwater

influence may shed some light on this phenomenon. At Ford Creek, emergence is concentrated in July when the mean temperature is 9.3°C. These results cannot be considered conclusive, however. High mortality at the higher temperatures among experimental animals limited the amount of useable data, and a real difference between moult intervals at 10°C and 15°C is not obvious. Further investigation of rearing techniques for these species and additional growth experiments at other temperatures higher than 15° are needed to clarify this point.

A second way of maximizing growth is to increase the growth ratio while holding constant or decreasing the moult interval. For <u>Ameletus</u>, growth ratio was not sensitive to temperature but was negatively correlated to the size of the animal. The mean growth ratio for <u>Ameletus</u> (1.08) was low in comparison with the median growth ratio (1.27) among the 50 hemimetabolous insect species Cole (1980) reviewed. Only one species of Saldidae was as low. It must be noted, however, that Cole's review of 105 insect species included no Ephemeroptera. While only about ten percent of the variance in growth ratio in <u>Ameletus</u> was explainable by body size, this was statistically significant. Other factors contributing to the variance may have been differences in feeding rates at various temperatures or some other nonlinear temperature effect which did not show
in analysis. It is also likely that the nonspecific stress of experimental conditions which caused such high mortality played a large part in clouding the true extent of the body size-growth ratio relationship.

It should be possible to approximate a nearly optimal thermal regime for an individual from each study site, within the range of regimes which actually occur. First instar larvae appear to be of nearly uniform size. Using that starting size and the known mean water temperature during the month of hatching, moult intervals and growth ratios can be assigned through successive body sizes and monthly temperature regimes up to an estimated date of emergence. Fecundity is assumed to follow the log-linear relationship already established. By iteration an approximately optimal thermal regime, defined as a hatching date which ultimately results in the highest fecundity, can be identified. An example of this procedure is found in Chapter Two where it is used to construct approximate growth curves for <u>A</u>. <u>celer</u> and <u>A</u>. <u>similior</u>.

A predictable growth ratio coupled with known temperature effects on moult interval also allows a unique approach to instar number determination in <u>Ameletus</u>. Typically, simple size-frequency, the Janetscheck (1967) method, the Cassie (1954) method, or fixed growth ratio method (Dyar, 1890) have been employed to estimate instar

number in a variety of species. Fink (1980, 1984) has criticized all of these methods as inaccurate and misleading, and reported as high as tenfold differences in instar number estimates from identical data. He argued that Dyar's law (fixed growth ratio) is useful only for insects with constant development and instar number because growth ratios should tend to vary with size and time of year. While this is true, simple growth experiments over large size and temperature ranges can eliminate the need for using a constant growth ratio or arbitrarily guessing at moult intervals. Mean minimum and maximum body sizes are easily determined from field samples. By cumulatively multiplying size-specific growth ratios and body sizes at intervals predicted by known temperature regimes, a reasonable instar number estimate can be attained. Certainly fluctuations in feeding rate, abnormal temperatures, and other stressors may complicate actual instar numbers in natural populations. Indeed growth is known to be indeterminate and plastic among the Ephemeroptera (Brittain, 1982). This does not preclude, however, the possibility of reasonable approximations based on well-founded data. This approximation and a comparison with the instar number estimate calculated from the mean growth ratio will also be included in the next chapter.

Some mention must be made of the use of constant temperature versus fluctuating temperature in life history By far most manipulative studies of the effect of studies. temperature on life history have subjected experimental animals to constant rather than fluctuating temperature regimes; but the magnitude and temporal patterns of a thermal regime may greatly influence the growth and reproduction of benthic invertebrates (Sweeney, 1976, 1978). Eqg development and larval growth of some species may be keyed to "spikes" of maximum temperature rather than to mean temperature (Sweeney, 1978; Sweeney and Schnack, 1977). Periods of rapid larval development occur during these thermal spikes, although prolonged exposure to the same high temperature reduces the degree of growth stimulation (Shelford, 1927; Headlee, 1940; Sweeney and Schnack, 1977). Thus, development time predictions based on mean temperatures may be greater than actual rates under natural regimes. In contrast, Humpesch (1979, 1982) found no difference in hatching time for Ecdyonurus venosus eggs reared under constant and fluctuating temperatures, nor any difference in hatching success for eggs of Ecdyonurus sp. or Rhithrogena hybrida. So, while the universal importance of fluctuating temperature in benthic invertebrate life histories is not conclusive, it must be considered a potential factor in most cases.

Degree-days have often been used to describe the number of thermal units required to complete all or part of larval development. Generally, degree-day requirements for eggs and larvae decrease as developmental temperature increases, up to some critical limit, then begin to increase as biochemical disruption occurs. Two main factors contribute to the different thermal requirements observed over a range of developmental temperatures.

First, the minimum temperature required to induce growth and development at various developmental stages is not necessarily constant. Mackey (1977) has shown that developmental zeroes among 25 species of chironomids range from $-4^{\circ}C$ to $12^{\circ}C$. Corbet (1957) proposed that in odonates the minimum temperature required to induce growth may also be different for different larval body sizes. In this way small larvae, which begin growing sooner (at colder temperatures) as the water warms during spring, may "catch up" to larger larvae which begin growing later at higher temperatures. This may explain the lack of size variation in adult odonates. Sweeney and Vannote (1981) argued that above a certain threshold temperature growth in all size classes of certain mayfly species is stimulated and proceeds to adulthood. This may account for the size variation often observed in a single adult mayfly cohort.

Second, growth rate is not usually constant over a range of temperatures above the growth threshold. High temperatures serve to speed enzymatic and hormonal actions, and so accelerate growth and development. Early stimulation of adult tissue maturation, however, can lead to early metamorphosis at a reduced adult size and fecundity.

Because thermal requirements often do not remain constant between temperature treatments the use of degree-days has been criticized (Shelford, 1929; Sweeney and Schnack, 1977). In addition, the use of degree-days is largely limited to the description of the thermal sums above some developmental zero at certain constant temperatures. When the developmental zero is not known or is not constant, the predictive value for natural populations is questionable. This is particularly true in fluctuating temperature regimes where it is possible to have similar heat-unit totals in two very different thermal environments. When growth is heavily influenced by daily thermal maxima (Sweeney and Schnack, 1977; Sweeney, 1978), degree-day calculations do not reflect that fact. Neither do degree-days include thermal energy accumulated at or below 0°C. Mackey (1977) showed that growth may indeed occur at these cold temperatures. Because heat energy is present down to a temperature of absolute zero (-273.15°C),

some scale other than Centigrade may be appropriate for such measurements.

The difficulties and shortcomings of the degree-day approach to optimal growth are demonstrated by data collected for A. celer and A. similior. Table 3 shows that the annual degree-hours at the Elbow River site are nearly as high (94%) as at the Ford Creek site. Yet A. similior requires twice as long as A. celer to develop to an adult of about the same size. This must be due to the differences in summer temperature regimes. Mean high temperatures at Ford Creek during June, July and August are nearly double those at Elbow River during the same period, and it is certainly this difference which allows A. celer to undergo a period of rapid growth and emerge in a single year (see Sweeney and Schnack, 1977). During winter, however, Elbow River is actually warmer than Ford Creek due to the influx of spring water. This could be misleading, however, as temperatures are probably too low to permit substantial growth at either site. Degree-hour calculations do show some thermal differences between sites during certain months, but specifically how regimes differ is not obvious. Statements about the mechanisms which promote faster growth at Ford Creek cannot be made in sufficient detail, and predictive potentials are lost.

The number of degree-days necessary for the completion of development has been used as an indicator of thermal adaptation in the eggs and/or larvae of stoneflies (Mutch and Pritchard, 1986), mosquitoes (Pritchard and Mutch, 1985), and dragonflies (Pritchard and Leggott, 1986). These authors argued that a slope of less than zero on a double-logarithmic temperature versus degree-days plot indicated an adaptation to higher temperatures, while a slope of more than zero indicated an adaptation to lower temperatures within some tolerable range. Assuming that an adaptation is the result of selection toward maximum fitness, this suggests that a temperature treatment which minimizes the thermal unit requirement for egg or larval development is optimal. However, neither the brevity of development nor thermal efficiency alone would seem to be a reliable measure of adaptation (i. e. optimality). Mortality and the effects on subsequent growth and ultimate body size must also be considered. Differences in slope may be a reflection of the ranges of temperatures over which enzymes and hormones related to growth are active. While one would expect a warm water species to be biochemically more active over a higher temperature range than a cold water species, faster or more thermally efficient development at higher temperatures within that range is not necessarily an indication of adaptation to

higher temperatures. The very fact that the temperature range is higher for one species would seem to be the evidence for thermal adaptation. Wright <u>et al</u>. (1982) showed that <u>Hexagenia bilineata</u> egg development and larval growth were more efficient at higher temperatures. However, larval survival and final-instar larval size were both decreased significantly. Similar results can be extrapolated for several species of <u>Ephemerella</u> (Sweeney and Vannote, 1981), for <u>Cloeon triangulifer</u> (Sweeney and Vannote, 1984), and for the stonefly <u>Capnia atra</u> (Brittain <u>et al</u>., 1984). It is evident, therefore, that temperatures which may meet one measure of optimality at a particular life stage often will not ultimately maximize fitness, and so cannot be considered truly optimal.

While an optimum number of degree-days (i. e. one which maximizes growth and fecundity) may be calculable for each growth stage under certain conditions, it again seems merely descriptive. What it says about an optimal temperature regime is not clear because of changing developmental zeroes and the effects of fluctuating temperatures on growth and heat-unit totals. With regards to mayflies specifically, degree-days provide no information on the ambient conditions which maximize the instantaneous growth rate by increased growth ratio and/or shortening the moult interval, nor do they provide

sufficient information to predict what those conditions might be. Certainly the weight of current evidence indicates that neither a short development time nor thermally efficient development is necessarily indicative of adaptation to that thermal regime.

<u>Respiration</u>

A significant portion of ingested energy is allocated to metabolism. The cost of maintenance over the lifetime of an aquatic invertebrate may be as high as 50 per cent of the total assimilated energy (Kamler, 1970). This is important not only in an individual's energy budget, but at the ecosystem level as well because energy expended for metabolism is irretrievably lost to other trophic levels.

A great deal has been written about the oxygen consumption of various animal taxa. Among aquatic invertebrates respiration has been shown to be sensitive to ambient temperature (Edwards, 1958; Otto, 1974; Sweeney and Schnack, 1977; Sweeney, 1978; Vannote, 1978; Vannote and Sweeney, 1980; Sweeney and Vannote, 1981), acclimation temperature (Laybourn-Parry and Tinson, 1985), body size (Olson and Rueger, 1968; Brown and Fitzpatrick, 1978; Sweeney and Schnack, 1977; Sweeney, 1978; Vannote, 1978; Vannote and Sweeney, 1980; Sweeney and Vannote, 1981), developmental stage (Vannote, 1978; Ciancara, 1980), current speed (Kovalak, 1978), oxygen concentration

(Nagell, 1973; Wrona and Davies, 1984), substrate size (Eriksen, 1963), light intensity (Ulanoski and McDiffett, 1972), water chemistry (Osborne <u>et al</u>., 1980; Linton <u>et</u> <u>al</u>., 1982), and sex (Laybourn-Parry and Tinson, 1985).

Although the importance of all these factors cannot be overlooked in natural populations, the interactive effects of ambient temperature and body size have been shown to be the most influential elements bearing on respiration rate. Typically, mayfly weight-specific respiration (amount of oxygen consumed per unit body size per unit time) increases with temperature for a given size, but decreases with increasing size at a fixed temperature. Increased size serves to compensate for the effect of high temperature on respiration; thus larval metabolic rates may remain within a well-defined "activity level" through the life cycle (Vannote, 1978; Vannote and Sweeney, 1980).

Body size affects metabolic rate in several ways. Generally, oxygen consumption is correlated to body mass as an allometric function. Bertalanffy (1951) proposed that insect larvae have metabolic rates proportional to body weight, but oxygen consumption tends to follow the increase in surface area rather than weight (Prosser, 1950). Few animals, however, show metabolic responses exactly proportional to weight or surface area (Prosser, 1973). The expected exponent would be 0.67 from an exact

relationship between oxygen consumption and surface area, or 1.00 if the relationship was with weight. In fact, most exponents are disposed around 0.75 (Kamler, 1970). Needham (1942) argued that this may be because surface area is not limited to external surfaces, but includes internal respiratory area as well. Prosser (1950, 1973) maintained that older and larger animals have relatively smaller amounts of "active protoplasmic tissue" due to the accumulation of low metabolic rate material such as fat, connective tissue, and exoskeleton. Enzyme production may also increase less in proportion to weight, or may even decrease in larger individuals (Prosser, 1973). Other factors which can affect the relationship between respiration rate and body size are metabolic adaptations (Prosser, 1950), the normal activity level of the taxon (Schmidt-Nielsen, 1970), surface area of external gills, and body shape (Kamler, 1970). Temperature can sharply affect this relationship, and expected exponent values may be obtained only at some intermediate temperature and pressure, if at all.

The metabolism of small poikilotherms is particularly affected by temperature, and this is reflected in the disproportionately high respiratory responses of small aquatic invertebrates to increased temperature (Edwards, 1958; Nilsson, 1974; Sweeney, 1978; Vannote and Sweeney,

1980). This phenomenon may also be related to relatively high enzyme production and the abundance of other highly metabolic tissue in younger animals, and is probably in part a function of the insulation efficiency of the body surface (Prosser, 1950).

In short, then, while an approximately exponential increase in respiration rate with body size, and a corresponding decrease in weight-specific respiration are expected, there is no <u>a priori</u> reason why those relationships should not show considerable variation in strength and slope among taxa, developmental stages, and temperatures.

In this study head width rather than weight was plotted on the abscissa against weight-specific respiration. This was done for the sake of consistency as other measurements which were used in the energy budget synthesis were highly correlated with head width, and because a linear measurement such as head width can be made without killing the experimental animal. Furthermore, the correlation between head width and weight-specific respiration rate was as high as when weight was used, and the variance was no greater.

Given the allometric relationships between head width and weight, and between weight and respiration, the expected relationship between head width and respiration is

a negative hyperbolic curve (L. Harder, pers. comm.). For Ameletus, however, this was not the case. Rather, a natural logarithmic transformation of head width against untransformed respiration values provided the best fit. The actual relationship, therefore, is a curvilinear one, but without the acute increase in respiration at the smaller head widths. Exactly why this difference occurs is not clear. Most importantly, the general trend of decreasing weight-specific respiration with increasing size does occur, as does the disproportionate response to increasing temperature of the smaller size classes. Given the complex interaction between size, temperature, and respiration rate, the fact that values do not require the precise transformations projected is not surprising. Indeed, such projections may be little more than an exercise in syllogism.

Over all temperatures and head widths, the weight-specific respiration of <u>Ameletus</u> was somewhat higher than rates reported for other mayflies (see Olson and Rueger, 1968; Kamler, 1969; Ulanoski and McDiffett, 1972; Nagell, 1973; Sweeney, 1978; Vannote, 1978; Sweeney and Vannote, 1981). This is probably due to a number of factors.

First, the flow-through design of the respirometer prevents a decrease in oxygen concentration during the

course of an experiment. This and the 100 per cent oxygen saturation used, are likely to result in respiration rates which are high in comparison with those reported using closed-bottle systems. Kamler (1970) stated that closed-bottle systems are often "charged with serious errors" and can cause false results. Perhaps the greatest source of error in closed-bottle respirometry is the decrease in oxygen tension within the flask during the course of the experiment (Wightman, 1977, 1981; McEvoy, 1985). Numerous authors have reported substantial drops in oxygen concentration in closed-bottle apparatus (Larimer and Gold, 1961; Kamler, 1969; Nagell, 1973; Otto, 1974; McCullough et al., 1979). Final oxygen concentrations as low as 40 per cent have been reported (McCullough et al., 1979), and such drastic drops in oxygen availability substantially decrease respiration rate in mayflies and other aquatic invertebrates. McCullough et al. (1979) noted a significant drop in the metabolism of Tricorythodes minutus below 60 per cent oxygen saturation. Cloeon dipterum respiration decreased by 28 per cent with a change in oxygen concentration from 100 to 50 per cent, and three species of stonefly exhibited declines in metabolism of 31, 34, and 50 per cent under the same conditions (Nagell, 1970). Similarly, the respiration rate of the odonate Anax imperator was halved when oxygen concentration fell from

100 to 50 per cent (Klekowski and Kamler, 1968). Remarkably, oxygen consumption in crayfish decreased by two-thirds in response to a drop in oxygen concentration of only 27 percent (Larimer and Gold, 1961), and Wrona and Davies (1984) found respiration in two leech species to be five times higher at an oxygen concentration of 100 percent than at 50 percent. In the majority of studies finishing oxygen tensions are not reported. However, it is evident that decreasing oxygen availability within closed-bottle respirometers will significantly affect aquatic insect respiration in many cases, and so bias mean respiration rates.

Temperatures to which experimental animals were naturally acclimatized may have played some part in the somewhat elevated respiration rates displayed by <u>Ameletus</u>. While most aquatic insects don't exhibit metabolic compensation to temperature (Keister and Buck, 1974; Sweeney, 1978), some do show different respiratory responses depending on acclimatization. Aquatic poikilotherms acclimated to cold temperatures can have a metabolic rate at those temperatures which is markedly higher than individuals acclimated to high temperatures (Prosser, 1973), and the reverse can also be true. Brown and Fitzpatrick (1978) demonstrated both of these phenomena in the dobson fly <u>Corydalus cornutus</u>. <u>Ameletus</u> which were

collected early in the summer while water temperatures were still fairly cold were subjected to respirometry experiments at temperatures as high as 14°C. Although animals were always acclimated overnight, this may not have been sufficient to counteract the effects of any in-stream acclimitization. Similarly, larvae collected late in the summer at high natural temperatures were tested at temperatures as low as $6^{\circ}C$, and this may also have complicated the situation. Unfortunately, there is no way to know for certain if thermal acclimitization had occurred, to what degree, or if experimental acclimation was in any way off-setting. The genus Ameletus, while not frequently studied, does appear to inhabit colder water (Ide, 1935) or develop during colder portions of the thermoperiod (Barton, 1980; Clifford, 1982; Vannote and Sweeney, 1981), and so might be expected to show a relatively high respiratory response to warmer temperatures.

The natural activity level of <u>Ameletus</u> may also have played a part in producing relatively high respiration rates. Streamlined mayfly larvae such as baetids are known to be more active than the more sturdily-built ephemerellids or the dorso-ventrally flattened heptageniids (Elliott, 1968). Ulanoski and McDiffett (1972) found that the night time respiration rate of the baetid <u>Isonychia</u>

<u>sp</u>. was significantly higher than that of the heptageniid <u>Stenonema fuscum</u>. This higher metabolic rate may be necessary for the rapid, darting movements the more streamlined mayfly larvae are capable of. <u>Ameletus</u> is just such a streamlined larva. Because of this and the fact that respiratory chambers were contained within a lightproof container, respiration rates recorded for <u>Ameletus</u> were higher than might have been recorded for a slower-moving animal or under daylight conditions.

Finally, mean respiration rates for <u>Ameletus</u> include individuals which exhibited ventilation behavior (see below). Because the majority of animals demonstrated this behavior I was not able to analyze those that did not separately without seriously reducing the sample size. Wrona and Davies (1984) showed that ventilating leeches consumed more than twice as much oxygen at 100 per cent saturation than did those which did not ventilate. In <u>Ameletus</u> a significant amount (10%) of the variance in respiration rate was due to ventilation.

Most species of mayfly associated with lotic habitats are unable to physiologically regulate their oxygen consumption over concentration gradients (Brittain, 1982). Those species with immovable gills are often restricted to areas with high current velocities, and respiration rates can be directly related to current speed (Hyne, 1970).

Benthic invertebrates may compensate for oxygen availability by selecting microhabitats according to current velocity and oxygen requirements (Kovalak, 1978), by varying position in the water current (Wiley and Kohler, 1980), or by respiratory movements (Kamler, 1970; Nagell, 1973). Respiratory or ventilation behaviors have been shown to increase with decreasing oxygen tension (Kamler, 1970; Nagell, 1973).

In respiration experiments Ameletus exhibited ventilation behavior which appears to have been in response to low oxygen availability as a function of water flow rate. Although water entering respiratory chambers was 100 per cent oxygen-saturated, and the flow-through design allowed water replacement, flow rates were likely too slow to move water over the gills of Ameletus at sufficient speed. Behavioral ventilation involves moving the gas exchange surface, the overlying water, or both (Wiley and Kohler, 1984). Because respiration is dependent upon the passive diffusion of oxygen through the gills, movement of the gills speeds the replacement of boundary layer water and increases the rate of oxygen uptake. This is supported by the fact that the variation in respiration rate of ventilating Ameletus significantly increased by approximately ten per cent. It is interesting to note that ventilation was rarely observed during predation

experiments (see Chapter 3) which were conducted in artificial streams with rapidly flowing water.

Ventilation behavior occurred more frequently in large <u>Ameletus</u> larvae. This is most likely due to both a lower surface area to volume ratio and a higher oxygen requirement. Kovalak (1978) found that large larvae of several mayfly species occupy faster water currents than do smaller individuals due to these increased respiratory demands.

<u>Calorimetry</u>

Energy-content values for whole-body samples of Ameletus larvae decreased with increasing body size. This is consistent with the findings of Hagvar (1975) and Svensson (1977). Svensson (1977) found that decreasing energy values were correlated to decreasing fat content in larger Ephemera danica. MacKay (1985) found the same fat content to energy value correlation in the ant Pogonomyrmex Fat content in mayfly larvae may decrease for four sp. digestion of the residual yolk cell in very early reasons: larvae (Petr, 1970; Chapman, 1982), digestion of fat bodies during periods of accelerated growth, digestion of fat bodies during the formation of gonads (Svensson, 1977), and digestion of fat for all energetic needs as the alimentary tract atrophies and feeding ceases. Some decrease of energy content in late-instar larvae may also be due to the

use of fat bodies in egg production, but much fat body catabolism is simply replaced as fat in the eggs. In fact, Svensson (1977) found that energy values increased in gravid animals as a result of energy accumulation in eggs. Such an increase was not seen in <u>Ameletus</u>.

In constructing energy budgets, other authors have generally used a single mean energy content value for all sizes of larvae (Sweeney and Schnack, 1977; Sweeney, 1978; McCullough et al., 1979; Sweeney and Vannote, 1981), although some researchers using holometabolous insects have tested prepupae and pupae separately (Hagvar, 1975; Brown and Fitzpatrick, 1978). In previous studies mean larval energy content estimates ranged from 18.3 J/mg (Svensson, 1977) to 25.0 J/mg (Cummins and Wuycheck, 1971) for various mayfly species. My estimates for Ameletus were consistently higher, although values for the largest size class of Ameletus are comparable to Cummins and Wuycheck's (1971) values for <u>Stenonema</u> pulchellum. There is no reason to suspect that the calorimetry method used here is less accurate than previous methods. Indeed, the opposite should be true. If large larvae were used in other calorimetry experiments, then mean energy content estimates would be biased due to the relatively low energy values of larger larvae.

In two studies in which male and female mayflies were tested separately, males had a higher energy content (Svensson, 1977; Sweeney and Vannote, 1981). One might expect a higher value for females as reproductive tissues and eggs mature, although the difference should show only in late instars. No difference was found between the sexes in <u>Ameletus</u>.

Due to the short supply of adult <u>Ameletus</u>, no calorimetry was done on them. Previous studies have shown that the energy content of adult mayflies is slightly higher than that of larvae (Svensson, 1977; Sweeney and Vannote, 1981). When energetic differences between sexes existed in larvae, they were usually carried over into the adult stage. However, Brown and Fitzpatrick (1978) reported no difference between sexes in adult dobsonflies.

Reports of mayfly egg energy content are rare. Due to the enormous number of eggs required to form a calorimetry pellet of sufficient size, this is not surprising. Sweeney and Vannote (1981) reported a range of 23.6 J/mg to 27.3 J/mg for eggs of six species of Ephemerella. The eggs of other insect families are comparable: 25.8 J/mg for <u>Melasoma collaris</u> (Coleoptera) (Hagvar, 1975) and 27.6 J/mg for <u>Corydalus cornutus</u> (Megaloptera) (Brown and Fitzpatrick, 1978). The eggs of <u>A</u>. <u>celer</u> and <u>A</u>. <u>similior</u> are also similar (21.8 J/mg and 30.5 J/mg, respectively).

Based on previous discussion, the difference in eqq energy content between A. celer and A. similior should be dependent on fat content. The reason for this difference is unclear. Estimated adult emergence and egg-hatching periods indicate that eqgs of both populations undergo a similar development time, so there appears to be no reason why a higher energy store is necessary at the Elbow River site. The difference may simply be a function of species-specific physiology. Alternatively, early instar larvae at Elbow River may be exposed to lower food availability during the winter than those at Ford Creek, and the higher egg energy content there reflects a larger volk cell. The small sample size for eqq calorimetry may also have exaggerated the real difference between species, although the error terms are not large. Further investigation of microhabitat differences and the roles of biotic and abiotic forces within those microhabitats may provide a more definitive explanation for this apparent difference in reproductive strategies (see Chapter Two for discussion).

Only a few studies of bioenergetics include original estimates of the energy content of exuviae. Others have used a value reported by Stockner (1971), or an ash-free derivative thereof (McCullough <u>et al</u>., 1979). While the chemical composition of insect exoskeleton would seem to be

similar in all insect orders (Chapman, 1982), there is some discrepancy in the literature on its true energetic value. Values reported include 17.9 J/mg (Brown and Fitzpatrick, 1978), 21.6 J/mg (Stockner, 1971), and a range of from 20.3 J/mg to 21.9 J/mg for six species of <u>Ephemerella</u> (Sweeney and Vannote, 1981). Overall, it is evident that inconsistencies in energy estimates have the potential to alter substantially the synthesis of a complete energy budget. For energy budget calculations Stockner's (1971) estimate will be used.

CHAPTER TWO

SYNTHESIS

INTRODUCTION

In this chapter, life-history data and energy budget components from Chapter One are used to estimate instar number, construct instar-by-instar growth curves, and generate complete energy budgets for the subject species.

In the first section, I show how predictable growth ratios may be used to calculate instar number based on average first-instar and last-instar head widths, and how this estimate compares with one calculated from a single mean growth ratio. I also demonstrate how predictable growth ratios and moult intervals can be combined to develop growth curves which closely follow growth patterns estimated from size-frequency data. This method may also be employed to determine the optimal available temperature regime, based on egg hatching date, when the ranges of first-instar and final-instar head widths are known.

In the second section, I assemble an in-depth energy budget based on the growth, respiration, calorimetry, and feeding experiments described in Chapter One. Daily energy intake (consumption) and expenditures (respiration, growth, exuviae, egestion) are calculated. Proportions of assimilated energy allocated to respiration, growth, exuviae, and reproduction are also shown. Finally, assimilation efficiency and reproductive effort are reported. I discuss each facet of the energy budget and how it may be affected by temperature and body size. Energy budgets for <u>A</u>. <u>celer</u> and <u>A</u>. <u>similior</u> are compared, and possible differences in the effects of temperature and body size, as well as possible differences in life-history strategies are noted.

A. INSTAR NUMBER AND GROWTH CURVES

METHODS

In the initial chapter an instar number of 25 was estimated using a mean growth ratio of 1.08 and a head width range which represented that of an average individual over its life span. I showed, however, that growth ratio was negatively correlated to body size, and here I examine how that correlation affects the instar number estimate.

I also demonstrated in the first chapter that moult interval was negatively correlated to temperature. While this will not affect instar number <u>per se</u>, it does determine how moults, and therefore growth, are distributed over the life cycle, and so influences the date of

emergence if instar number is fixed. Here I show how the temperature-dependent moult interval and the size-dependent growth ratio can be combined to approximate the growth and emergence of a typical <u>Ameletus</u> larva from each study site.

The head width range in this instar number estimate was the same one used in the first chapter. Starting head width was always 0.2 mm, and growth ratio was determined for each successive instar by using the regression equation (Figure 15) from Chapter One. Final-instar head width was assumed always to be 1.4 mm. Instar number was approximated by the number of iterative multiplications of head width and size-specific growth ratio required to reach the maximum head width. Using this method the instar number estimate will remain constant regardless of the thermal regime. This reflects the results of growth experiments with Ameletus which suggest that moult interval, but not growth ratio, is temperature-sensitive. Instar number in odonates is known to be sensitive to temperature (e. g. Leggott and Pritchard, 1985), but while instar number in mayflies is known to be flexible (Brittain, 1982) the influence of temperature is not fully understood.

To estimate moult and emergence dates, the first instar head width size was plotted on a theoretical hatching date. The second instar head width, already

determined by the head width-growth ratio product described above, was then plotted by first calculating the moult interval based on the mean temperature for that date, then adding that interval to the first instar hatching date. This procedure was repeated for each subsequent instar, and the final date calculated was the estimated emergence date.

In the calculation of temperature-dependent moult intervals, two different approaches were taken. In the first a simple temperature-moult interval regression equation was used based on growth experiment data: y = 14.9 - 0.64x

Where x is monthly mean temperature (^{O}C) and y is the moult interval (d). This, of course, assumes a linear relationship between all temperatures and moult interval, and allows substantial growth even at $0^{O}C$.

A more realistic scenario may be a linear relationship between moult interval and temperature of the same y-intercept and slope for temperatures at or above 5°C, but one with a more negative slope below 5°C as the developmental zero is approached. Because growth experiments were not carried out at temperatures colder than 5°C, two assumptions had to be made about the moult interval-temperature relationship at these temperatures. First, developmental zero was assumed to be 0°C. Second, some maximum moult interval had to be assigned to a temperature approaching zero in order to obtain a regression equation. Assigning a growth rate of zero at 0°C results in an infinite moult interval, and the method becomes untenable. Rather, moult interval values of 30, 60 and 90 days at a near-zero temperature were used. 30 days represents the longest moult interval observed at 5°C. Because this was twice the longest moult interval observed at 10°C, 60 days was projected as a reasonable second estimate for a near-0°C moult interval. The resulting growth and emergence curves were then compared to a typical life cycle, determined from field data, for each population.

Typical hatching and emergence dates for the two populations, as estimated from size-frequency data, were substantially different. For <u>A</u>. <u>celer</u> at Ford Creek, the average individual was assumed to hatch on 1 August and emerge as an adult on 1 August of the following year - a larval life span of 365 days. <u>A</u>. <u>similior</u> at Elbow River was assumed to hatch on 15 October and emerge on 15 September in the second year following - a larval life span of 695 days. Egg development time, based on low water temperatures and the appearance of first instar larvae in monthly samples, is probably one year for <u>A</u>. <u>celer</u> and 13 months for <u>A</u>. <u>similior</u>. The only published data on egg development in siphlonurid mayflies (<u>Isonychia bicolor</u>:

Sweeney, 1978) predict incubation periods of 394 d for <u>A</u>. <u>celer</u> at Ford Creek and 506 d for <u>A</u>. <u>similior</u> at Elbow River. These estimates are based on mean annual temperatures $(4.0^{\circ}C \text{ at Ford Creek}, 3.5^{\circ}C \text{ at Elbow River}$ using the equation:

 $y = 5198^{-1.86}x$

where y is egg development time (d) and x is temperature ($^{\circ}C$) (Pritchard, unpub. , from data in Sweeney, 1978).

RESULTS

Because growth ratio is size-specific but temperature-independent, the number of instars estimated for <u>Ameletus</u> was the same under all temperature regimes. Using this method, <u>Ameletus</u> was estimated to undergo 21 instars as opposed to the 25 calculated using a mean growth ratio. The reason for the difference between the two estimates lies in the second method which produced growth ratios over the first 14 instars which were higher than the calculated mean of 1.08. This estimate is still well within the range of 10 to 50 commonly reported for the Ephemeroptera (Brittain, 1982).

Using a single temperature-moult interval regression equation resulted in a linear to asymptotic growth curve, and emergence dates which were 3.5 months and 13.5 months premature for <u>A</u>. <u>celer</u> and <u>A</u>. <u>similior</u>, respectively

(Figures 20 and 21). By assigning a moult interval of 30 d to a temperature approaching zero, a different regression equation for temperatures at and below $5^{\circ}C$ was calculated: y = 30 - 3.42x

where y is moult interval (d) and x is temperature (^{O}C). Using both equations in moult interval calculations, a slightly sigmoidal growth curve was produced, and the calculated emergence date for <u>A</u>. <u>celer</u> (14 July) was quite close to the assumed average one (1 August) (Figure 20). For <u>A</u>. <u>similior</u>, however, the calculated emergence date was still 11 months premature (Figure 21). A third and a fourth moult interval-temperature regression equation were calculated by assigning moult intervals of 60 and 90 days to a temperature approaching 0^OC. The regression equations produced were:

y = 60.0 - 9.42x

and

y = 90.0 - 15.42x

The resultant growth curves were increasingly sigmoidal. The first gave an emergence date which was 5.5 months premature, but the second produced an emergence date which was within 1 month of the estimated average (Figure 21).

DISCUSSION

The application of results from growth experiments suggest that the instar number of an individual is approximately 25. While instar number is known to be flexible among the Ephemeroptera (Brittain, 1982), in <u>Ameletus</u> it may fluctuate around some mode in response to stressors (e. g. temperature, food availability and quality, oxygen concentration, physical disturbance). Some species have been reported to display temperature-independent growth (Maxwell and Benson, 1963; Minshall, 1967; Waters and Crawford, 1973), and these may represent cases in which instar number is relatively constant.

Growth curves for ephemeropterans are commonly reported as linear, asymptotic or sigmoidal. The shape of the curve for a particular cohort or species largely depends upon the thermal regime. Mayflies which hatch and emerge in the same year generally develop quickly with little deviation in the growth rate. Growth curves for these animals range from nearly linear to asymptotic (Humpesch, 1979; Sweeney and Vannote, 1981, 1984). Those which overwinter in the larval stage typically exhibit a period of very slow or no winter growth which results in a more sigmoidal growth curve (Sweeney, 1978; Humpesch, 1979;

Vannote and Sweeney, 1980; Sweeney and Vannote, 1981, 1984). Species which have two- or three-year life cycles undergo long periods of no growth during the coldest months, and their growth curves are decidedly sigmoidal or logistic in shape (Svensson, 1977).

Using a single regression equation to describe the temperature-moult interval relationship results in a linear to asymptotic growth curve (Figures 20 and 21). For animals which complete their life cycles at temperatures well above the developmental threshold (spring-summer species and late summer cohorts), this rapid succession of moults rather than differences in growth ratio may explain the growth patterns they commonly display. However, when portions of the life cycle occur during periods of cold temperatures, especially near the developmental threshold, moult intervals may become quite long. This was simulated by using separate regression equations of steeper slope for temperatures at and below 5°C. With increasing slope values in the cold temperature range the resultant growth curves were increasingly sigmoidal.

It was possible to create growth curves which approximately fit proposed hatching and emergence dates of typical individuals from both sites. However, in order to do this it was necessary to use a different temperature-moult interval regression for each population

for monthly mean temperatures at or below 5°C. In other words, this test of the model assumed that the growth responses to cold temperatures in terms of moult interval are different for A. celer and A. similior. While such different responses to cold temperatures among congeners is not unheard of (Sweeney and Vannote, 1981), there is another explanation. It is possible that under natural conditions A. celer and A. similior respond similarly to cold temperatures. A. celer, however, because of the relatively high daily temperature pulses it experiences during the summer, may undergo shorts periods of very rapid growth which allow it to emerge a full year sooner than A. similior at Elbow River. Thus, an actual instar-by-instar growth curve for A. similior might appear similar to or slightly less sigmoidal than the estimated curve, while the true growth curve of A. celer might be more sigmoidal due to accelerated summer growth.

If this is so, why was it not apparent in the growth experiments? The principal cause may have been the constant temperature regimes to which experimental animals were subjected. The relationship between temperature spikes and rapid growth, and the growth-retarding effects of long exposure to high temperature have been previously discussed. If <u>Ameletus</u> indeed responds in such a manner, the experimental results would reflect the slow growth

brought on by prolonged exposure to high-temperature treatments. Further experiments at low temperatures in order to determine the threshold of development, and rearing under fluctuating temperature regimes which more closely simulate field conditions may clarify precisely how moult interval influences growth patterns. Nevertheless, the growth curves as constructed do in fact largely follow actual growth as estimated from size-frequency data, and do demonstrate a new viable approach to the description and prediction of growth patterns.

B. ENERGY BUDGET

METHODS

The basic energy budget equation used was: C = P + R + F + U

where C is consumption, P is production, R is respiration, F is egestion (feces), and U is excretion (urine). U is generally considered to be insignificant in larval insects (Winberg, 1956; Krueger <u>et al</u>., 1968) and it was not considered in calculating the energy budget. Production includes growth (G), exuviae (M), and reproduction (E): P = G + M + EGrowth, exuviae and respiration can be combined to

calculate assimilation (A):

A = G + M + R

G, M, R and A were expressed as both daily weight-specific energy expenditures (J/mg/d) and as total energy expenditures (J). All components of the energy budget and subsequent efficiency measures were calculated for each of the estimated 21 instars, as well as for the entire life cycle. In the following discussion, all daily energy budget measurements (R, M, G, A, and C) are in J/mg/d. All total measurements (total R, M, G, A, C, and F) are in J.

Insect energy budgets are unlikely to balance satisfactorily (see Wightman, 1981; McEvoy, 1985 for reviews). To avoid adjusting more than one energy budget component, I began by calculating A and P because G, M, R, and E were considered to be reliably measured. Where necessary to attain some semblance of balance, adjustments away from the initial assumptions of constant and constant-rate feeding over the entire life cycle were made to C, based on physiological, environmental, and behavioral factors.

To determine G, the mass of each instar was first determined from its head width (Figures 10 and 11). The mass of the exuvium for that instar, equal to 8.5% of the entire body mass (Sweeney, 1978) (Figure 22), was then substracted The size-specific energy value for that instar was then calculated (Figure 19), and multiplied by the body

mass to determine the whole-body energy value. M for each instar was estimated as the product of the exuvium mass and the previously determined energetic value for exuvia (21.6 J/mg). Both G and M were converted to daily energetic expenditures for each instar by dividing values by the number of days in that instar. To estimate R for each instar, it was first necessary to determine instar duration (Figures 20 and 21) and the mean temperature(s) to which each instar was subjected (Tables 1 and 2). Hourly weight-specific respiration rates were calculated, then converted to daily R and total R for each instar. These values were converted into energetic terms using the conversion factor 2.019 x 10^{-2} J/uL O₂ (Brody, 1945). G, R, and M were totalled to estimate A.

From these data net growth efficiency (NGE) and production:respiration ratio (P:R) were calculated: NGE = (G + M)/A

P:R = (G + M)/R

E was estimated by calculating the size-specific fecundity for the final instar (Figure 13), and multiplying by the mean egg mass (1.39 ug) and the species-specific egg energy content (Table 6). The proportion of A devoted to E, or net reproductive efficiency (NRE) was estimated: NRE = E/A
Finally, C was initially estimated for each instar (Figure 23) by first calculating the size-specific ingestion rate for each instar (Figure 16). Hourly ingestion rates were then multiplied by 24 to get daily consumption, or by instar duration to get total ingested mass for that instar. These values were then summed for the entire life cycle, and multiplied by a mean energy content for mixed diatoms (15.25 J/mg) (Cummins and Wuycheck, 1971). From this, assimilation efficiency (AE), reproductive effort (RE), and F were determined: AE = A/C

RE = E/C

F = C-A

RESULTS AND DISCUSSION

The following desciptions and discussion pertain to Tables 7 and 8 which show complete energy budget data for <u>A. celer</u> and <u>A. similior</u>, respectively.

R ranged from 0.08 to 2.5 for <u>A</u>. <u>similior</u> and from 0.07 to 2.9 for <u>A</u>. <u>celer</u>. These ranges are consistent with values previously reported for other mayfly species: 0.90 for <u>Stenonema pulchellum</u> (Trama, 1957), 0.72-0.98 for <u>Isonychia bicolor</u> (Sweeney, 1978), 0.38-1.36 for <u>Ephemerella dorothea</u> (Vannote, 1978), 1.22 for Tricorythodes minutus (McCullough et al., 1979), 0.29-0.92 for Ephemerella subvaria (Vannote and Sweeney, 1980), and 0.06-0.97, 0.34-0.99, 0.46-0.92, and 0.65-1.19 for E. funeralis, E. verisimilis, E. deficiens, and E. serrata, respectively, (Sweeney and Vannote, 1981). Instar-specific R values tended to be higher at Elbow River, and mean R was greater at Elbow River due to the warmer winter temperatures and lack of diel temperature fluctuations at that site. R decreased with body size throughout the life span of both species, except in the final four instars of A. similior, and the final two instars of <u>A</u>. <u>celer</u>. R remained within a narrow range (approximately 0.5 to 1.5) for A. similior over a large part of its life cycle, deviating only in the initial few instars which experience relatively warm temperatures, and in late instars during the winter. A. celer exhibited a similar range of R values, but varied more during middle instars which experienced winter conditions. This pattern may be compared to the one described by Vannote (1978) in which the weight-specific metabolism of Ephemerella dorothea remained nearly constant over its life cycle due to the compensating effects on metabolism of increased temperature and large body size. Figures 24 and 25 show that while neither total respiration nor weight-specific metabolism in Ameletus were constant, the latter did largely remain

within an activity range of 0.5-3.0 J/mg/hr. The principal difference between this pattern and that of E. dorothea appears to be a consequence of hatching time. Hatching of E. dorothea at White Clay Creek occurred quite late in the year, and early instars were exposed to fairly cold temperatures. As larvae increased in size, they were subject to increasingly warm temperatures with body size metabolically compensating for the effects of increasing temperature. At both Ford Creek and Elbow River, Ameletus hatched earlier in the year and small larvae were initially exposed to elevated temperatures. So rather than a direct relationship between size and temperature over the life cycle, middle instars experienced the coldest portion of the thermoperiod at Ford Creek, as did very late instars at Elbow River. Thus, very young larvae appeared to be somewhat metabolically stressed, while periods of metabolic depression occurred later. How this pattern reflects a lack of selection for an exact balance between growth and temperature regimes (Vannote, 1978) is open to speculation. It may be that a general adaptation to extremely cold water constrains Ameletus to early hatching in order to complete the life cycle in an amount of time that offsets the risks of developing for an additional year. If selection does indeed occur for genotypes which code for size-temperature balance, it may proceed more quickly or select more

strictly in habitats where temperature regimes are more constant and/or predictable. This would explain the somewhat more uniform weight-specific respiration rates (uL $O_2/mq/d$) and R values for <u>A</u>. <u>similior</u> which inhabits the nearly stenothermic habitat of the Elbow River headwater In contrast, Ford Creek is a more variable and area. probably less predictable habitat. The synchrony of growth rate and temperature regime may be under less selective pressure in A. celer, and this may be reflected in more fluctuating R values. Increased inter-individual variability for this trait would ensure a high fitness in some individuals in all years. The chances of a small adult body size and low fecundity in all individuals of a given clutch due to abnormal abiotic conditions would therefore be minimized.

A better measure of the relative effects of body size and temperature on R may be R/A. This gives the proportion of assimilated energy which is allocated to R and therefore lost to P. While R showed a strong negative relationship to body size, R/A appeared to be influenced by both temperature and body size to varying degrees. For <u>A. celer</u>, R/A was fairly constant (0.52-0.64) over the first 9 and final 4 instars, even though this developmental period occurred over a temperature range of 1.0° C to 9.3° C. It was only during prolonged periods of cold temperatures,

specifically instars 10-17, that R/A was depressed. While the absolute values of A and G were low during these middle instars, G was actually more efficient. This could represent periods during which fat was stored for later use during egg development, although calorimetry data did not indicate an increase in whole-body energy content. Such a trend of constant R/A may be another indicator of the compensating effect of large body size. Only extremely cold temperatures suppressed R/A values of <u>A</u>. <u>celer</u>, undoubtedly because of the inhibiting effects on respiration rate.

At Elbow River, two periods of cold temperatures occurred during the life cycle of <u>A</u>. <u>similior</u>. The first was during instars 2 through 5. During this period, R/A was quite high. Small body size and slow growth appeared to heavily influence R/A despite prevailing low temperatures. However, during the second winter instars 17 to 19 exhibited low R/A (0.34-0.48). At those stages of development, temperature or temperature plus size apparently kept R/A low. During periods of decreased temperature, which occurred mainly during the middle instars, R/A remained fairly constant at values similar to those of <u>A</u>. <u>celer</u> at high temperature (0.54-0.62).

There is clearly a difference between <u>A</u>. <u>celer</u> and <u>A</u>. <u>similior</u> in R/A values relative to temperature. Instars

6 and 18 of <u>A</u>. <u>celer</u> have the same R/A, even though temperatures are higher during instar 18. Similarly, instars 5 and 6 experience much the same temperatures as do instars 16 and 17 (4.6° C), but the R/A of instars 5 and 6 is only 1.2 times that of instars 16 and 17. This compares to instars 3 and 17 of <u>A</u>. <u>similior</u>. Despite similar thermal experiences, the R/A was 2.4 times higher in instar 3. Instars 6 and 20 also experience similar temperatures, but R/A was 1.3 times greater in instar 6. Thus, R/A values generally increase more in <u>A</u>. <u>similior</u> over a given increase in size.

The compensating effect of body size would not appear to be more efficient in <u>A</u>. <u>celer</u>, based on the previously discussed R value trends. More likely, the slow growth of <u>A</u>. <u>similior</u> contributes considerably less per day to assimilation, and so the proportion of assimilation allocated to respiration is "automatically" higher.

Because no instar occurred at the same temperature at both sites, neither R/A nor NGE could be compared directly. However, NGE appears to be somewhat higher in <u>A</u>. <u>celer</u> larvae of approximately the same size which experience similar mean temperatures (see below). Because respiration rates <u>per se</u> were not affected disproportionately between species with respect to temperature, it is difficult to pinpoint the physiological mechanisms which might be

responsible. It may be that hormones or enzymes related to growth are more efficient at some temperatures in <u>A</u>. <u>celer</u>. More likely, daily fluctuations in temperature regimes, which were not taken into account in this analysis, induced bursts of growth disproportionate to growth which would occur at constant temperatures of the same mean. In any event, higher overall temperatures at Ford Creek are clearly related to more efficient, as well as faster, growth in <u>Ameletus</u>.

NGE is a measure of the balance between catabolic (respiration and excretion) and anabolic (growth) processes (Johannsson, 1980). Maximum NGE values should range from 0.70 to 0.80 (Calow, 1979), although values as high as 0.88 have been reported for mayflies (Zimmerman <u>et al.</u>, 1975).

NGE varies considerably among aquatic invertebrates. Values reported include 0.0-0.835 for chironomids (Johannsson, 1980), 0.24-0.39 for simuliids (Stockner, 1971), 0.61-0.66 for corixids (Sweeney and Schnack, 1977), 0.34 for stoneflies (McDiffett, 1970), 0.12-0.71 for caddisflies (Otto, 1974), and 0.20-0.90 among other invertebrate groups (Healy, 1967; Welch, 1968; Winberg, 1971; Testerink, 1982; Wolters, 1985).

Values of NGE vary almost as widely among ephemeropterans: 0.20-0.45 in <u>Stenonema pulchellum</u> (Trama, 1957), 0.88 in <u>Hexagenia limbata</u> (Zimmerman <u>et al</u>., 1975),

0.32-0.43, 0.14-0.75, and 0.32-0.73 for <u>Ameletus ludens</u>, <u>Isonychia bicolor</u>, and <u>Tricorythodes minutus</u>, respectively (Sweeney, 1976 cited in Sweeney and Schnack, 1977), 0.58 in <u>Tricorythodes minutus</u> (McCullough <u>et al</u>., 1979), and 0.0 to 0.82 for several <u>Ephemerella</u> species (Sweeney and Vannote, 1981). NGE ranges for <u>Ameletus</u> are well within previously reported values for other ephemopterans: 0.36-0.71 for A. <u>celer</u> and 0.16-0.66 for <u>A. similior</u>.

NGE is often highest in early instars. Young animals frequently devote a greater fraction of available energy to growth, while older animals slow growth as reproduction takes over (Pianka, 1981). This was not the case with Ameletus. Highest NGE values occurred in middle or late instars, during which the lowest proportions of A were allocated to R. At Elbow River these instars were This is associated with relatively warm temperatures. consistent with the other studies which have reported decreased NGE during summer as respiration rates increase (Stockner, 1971; Sweeney and Schnack, 1977; Sweeney, 1978). However, at Ford Creek these instars were subject to the very coldest temperatures of the thermoperiod. In the case of A. similior, it appears that the compensating effect of increased body size on metabolic rate held respiratory energy losses fairly constant. In fact, weight specific respiration decreased during that period. This allowed

relatively more assimilated energy to be allocated toward growth. At Ford Creek, although the body size-temperature compensation mechanism was not as efficient at this stage of development, very low temperatures kept weight-specific metabolism low, and the effect on growth was essentially the same.

NGE may be expected to fall in final instars for reasons other than temperature. Mayflies generally do not feed in their final larval instars. At this time the gut atrophies in preparation for the adult stage. In addition, eggs develop rapidly in the final instar (Needham <u>et al</u>., 1935) and must take up a large proportion of stored energy upon which the mature larva subsists. Due to a decrease in C and an increase in E, G would be expected to decline. Indeed, both species of <u>Ameletus</u> showed a measurable drop in G in their final few instars.

Values for M were dependent solely on larval body size and instar duration. Because the life cycle of <u>A</u>. <u>similior</u> is considerably longer than that of <u>A</u>. <u>celer</u>, but instar number is assumed to be the same, the daily cost of M is lower in <u>A</u>. <u>similior</u>. This also explains the lower mean M value for <u>A</u>. <u>similior</u> despite its slightly larger body size. Sweeney (1978) found that M accounted for 45% of all energy allocated to P, but only 5.6% of assimilated energy in <u>Isonychia bicolor</u>, while McCullough <u>et al</u>. , (1979)

reported 31% and 2.8%, respectively. M accounted for identical proportions of P (0.25) and A (0.11) in the two species of <u>Ameletus</u>.

G values for <u>A</u>. <u>similior</u> ranged from 0.08 to 0.97, excluding the initial instar. G was most sensitive to temperature, and lowest values were during winter. Values for <u>A</u>. <u>celer</u> were similarly affected by temperature, but were much more uniform overall (0.30-0.90, excluding instars 1 through 4). The difference appears to be because at Elbow River intermediate instars were exposed to relatively warm temperatures which increased G, and later instars were exposed to quite cold temperatures which lowered G substantially. At Ford Creek, intermediate and late instars generally experienced increasing temperatures throughout the life cycle, and G values showed less fluctuation.

Assimilation value ranges for <u>A</u>. <u>similior</u> and <u>A</u>. <u>celer</u> are somewhat different, and again this appears to be due to the thermal regimes to which various instars are subject. The two-year life cycle of <u>A</u>. <u>similior</u> at Elbow River exposes middle instars to summer conditions, while at Ford Creek these instars experience near-freezing temperatures. During those stages, and during very early instars, assimilation values show the greatest differences.

Mean A values, 1.52 for <u>A</u>. similior and 1.92 for

<u>A. celer</u>, are well within the ranges of values reported for other mayfly species: 1.10-1.31 for <u>Stenonema pulchellum</u> (Trama, 1957), 3.72 for <u>Tricorythodes minutus</u> (McCullough <u>et al.</u>, 1979), 0.49-1.11 for <u>Ameletus ludens</u>, 0.67-2.90 for <u>Isonychia bicolor</u>, 0.79-2.90 for <u>Tricoythodes atratus</u> (Sweeney, 1976 cited in Sweeney and Schnack, 1977), and 0.34-3.22 for a complex of <u>Ephemerella</u> species from White Clay Creek (Sweeney and Vannote, 1981).

P:R is an indicator similar to NGE, but which compares the amount of anabolic energy directly to catabolic energy rather than to the total amount assimilated. Sweeney (1978) reported relatively high P:R values (1.59-2.74) for small larvae of Isonychia bicolor, and lower values (0.35-1.04) for larger larvae. Other reported values for mayflies generally fall within these ranges (Trama, 1957; Sweeney, 1976 cited in Sweeney and Schnack, 1977). P:R values for Ameletus follow the same pattern as did the NGE values. The amount of energy allocated toward production is equal to or greater than that allocated toward maintenance in the middle instars of A. celer, but less in early and late instars. Again, maximum allocation to P occurs during periods of very cold water temperature, suggesting that ingestion may be not necessarily inhibited under these conditions, but respiration may be reduced allowing relatively efficient production. It must be

noted, however, that in absolute terms production is quite low. At Elbow River, too, the pattern for P:R is similar to that of NGE. High values are associated with middle instars which experience elevated temperatures. Body size appears to compensate for the elevated temperatures to keep maintenance costs minimized, allowing more energy to be allocated toward production. Several subsequent instars (17 to 19) exhibit even higher P:R values. During these stages the effect appears to be the same as that described for <u>A</u>. <u>celer</u> at Ford Creek. Low temperatures reduce respiratory losses to such a degree that the relative amount available for production is high. Here again, however, the absolute amount of energy available for production is low.

Because C was measured at only one temperature for each species, the thermal effects on energy intake and processing are not known. Animals collected in the field exhibit full guts almost all of the time. However, behavioral observations in the laboratory suggest that \underline{A} . <u>similior</u> may not feed constantly as it spends approximately 50% of its time moving in laboratory microcosms (see Chapter Three). Assuming that <u>Ameletus</u> does not feed as it moves, estimates of C based on constant, size-specific ingestion rates were halved to reflect observed locomotory behavior. Even with this

correction, <u>ad libitum</u> feeding can cause overestimates of 20% to 79% (Sweeney, 1978). How this may have affected these feeding experiments is not known.

Consumption values for Ameletus were considerably higher than the mean reported by Trama (1957) (2.9), but more consistent with the value given by McCullough et al. (1979) (13.0). Total daily consumption in aquatic insects is known to often exceed body mass. Although Cummins (1974) gave a maximum of 1.6 times body weight for daily aquatic insect consumption, Zimmerman and Wissing (1978) reported that Hexagenia limbata consumed 4.1 times its weight every day. Johannsson (1980) estimated that Chironomus plumosus ingested nearly five times its weight in the same period. Estimates for Ameletus show a consumption rate of from 0.4 times body mass in small instars to over 25 times in the largest. These estimates for late-instar larvae appear high, but if my calculations of gut size relative to body size are accurate, only food availability and handling time should be limiting factors. Barring effects of temperature, there is no apparent reason why C could not increase markedly with size. Randolph et al. (1975) showed that C in pea aphids increased exponentially over the entire growth portion of its life cycle.

AE shows the proportion of ingested energy an organism

has to divide between maintenance and growth. It may be affected by temperature as respiration and feeding rate change with thermal regime (Johannsson, 1980), although some researchers have found that AE is not sensitive to feeding rate (Conover, 1966; Lawton, 1970; Otto, 1974). AE among insect larvae ranges from nearly zero to nearly unity. High AE is usually associated with low ingestion rates and long retention times (Johannsson, 1980; but see McCullough et al., 1979). High AE is also often associated with carnivorous insects: 0.80-0.98 has been reported for predaceous stoneflies and caddisflies (Lawton, 1970; Heiman and Knight, 1975; Brown and Fitzpatrick, 1978). Herbivores usually exhibit an AE of less than 0.50 (Trama, 1957; Nilsson, 1974; Hagvar, 1975; Wolters, 1985), and often less than 0.15 (Hargrave, 1970; McDiffett, 1970; Otto, 1974; Calow, 1975; Zimmerman and Wissing, 1978; Johannsson, 1980). Ameletus falls into this latter category with an AE of about 0.09 for both species. AE appears to be affected mainly by temperature. A. similior exhibited highest AE during early and middle instars when temperatures were highest. A. celer AE was highest during initial and final instars, again when temperatures were highest. Mean AE is slightly higher in A. celer, but because the difference is so small (0.004), it is difficult to pinpoint the reason.

Interestingly, A. celer's reproductive effort is 80% higher than that of A. similior, but its NRE is 13% less. This suggests that while <u>A</u>. <u>celer</u>'s higher NGE and P:R enable it to grow to maturity in a single year, the metabolic demands of higher temperatures and the channeling of energy into more rapid growth leave less for egg production. A. similior grows much more slowly and allocates a smaller proportion of ingested energy to E. However, slower growth apparently also allows more assimilated energy to be devoted to egg production (or diversion of more energy to egg production contributes to slower growth, depending on whether soma or reproduction is the stronger "competitor" (sensu Calow, 1979) for resources). Surprisingly, it is not fecundity or egg size which is affected. Rather, the energetic value per egg of A. similior is increased. How this affects the survivorship of the early larvae is not known, but it should enable the early instars of <u>A</u>. <u>similior</u> to endure longer periods of low food quality or availability (see also Benton, 1983; Benton and Uetz, 1986). Such food-poor periods have not been substantiated at Elbow River, but spring spates there are considerably longer in duration and greater in intensity than at Ford Creek: This may scour the stream bed of some diatoms and allochthonous food sources. Alternatively, high egg energy content may serve

as a bet hedge against years when diatom blooms may be suppressed due to atypical thermal or chemical fluctuations. Small larvae with high energy reserves may also need to spend less time foraging and therefore be less vulnerable to invertebrate predators (see Chapter Three).

In any event, this adds another dimension to the definition of fitness as traditionally applied to such short-lived, semelparous organisms as mayflies. Mayflies as a group typically exhibit many r-selected life-history characteristics: annual recolonization, short life span, small body size, semelparous reproduction, and density-independent adult mortality (MacArthur and Wilson, 1967; Pianka, 1970). Because r-selection is generally believed to be associated with high production rather than efficiency (Pianka, 1970, 1974), fitness in these organisms is commonly thought to be increased with an increase in fecundity. However, r- and K-selection are only the extremes on a continuum of life-history strategies, and strategies may be considered relatively r- or K-selected depending on the organisms being compared. Ameletus certainly fits the definition of an r-selected organism as a member of the Ephemeroptera. A comparison of energy budgets between <u>A</u>. <u>celer</u> and <u>A</u>. <u>similior</u>, however, indicates that an increase in net reproductive effort by A. similior is not necessarily associated with higher

fecundity, and therefore an increase in mayfly fitness may not be synonymous with higher fecundity in all habitats. A. similior, whether by adaptation (a genetic change to higher NRE by natural selection) or exaptation (a higher NRE by virtue of slow growth and more efficient energy storage at lower temperatures, for example) is able to channel substantially more energy than its congener into each offspring. This tendency toward reproductive efficiency as opposed to productivity is usually considered a K-selected characteristic (Pianka, 1970, 1974). In addition, A. similior exhibits a longer life cycle, a larger body size, slower development, and inhabits a more constant (and probably more predictable) habitat than A. celer. All these traits are also correlated to K-selection. Certainly the impact of abiotic forces, particularly temperature, on these traits cannot be ignored. Life histories and energy allocations of both species appear to be largely dictated by long-term adaptations and the metabolic demands imposed by their respective thermal regimes. The energy available for growth and reproduction may be considered "leftovers" from the demands of somatic maintenance. Yet the observed differences in NRE and, in particular, egg energy content may represent two distinct and measurable evolutionary responses to two very different habitats. Constant and

predictable thermal conditions, more limited refugia, and substantial invertebrate predation (see Chapter Three) may all have selected for more efficient and more competitively able offspring rather than for an increase in fecundity. Further investigation into the variance between years of NRE and fecundity in these two species, carefully controlled laboratory experiments which manipulate C, and an examination of the biotic interactions prevailing in each population should give further important insight into the selective forces which shape their local life-history and reproductive strategies.

SUMMARY

Predictable growth ratios and moult intervals made it possible to make a second, possibly more accurate estimate of instar number. It also made possible the construction of representative growth curves for both <u>A</u>. <u>celer</u> and <u>A</u>. <u>similior</u>. Despite some necessary assumptions regarding growth responses to temperatures below 5° C, these growth curve estimates were similar to growth patterns projected from field size-frequency data.

Complete energy budgets for both species were calculated based on the extensive experimentation described in Chapter One. Energy expended on respiration was not constant over the life cycle as reported in some other

species, largely due to relatively early egg hatching which exposed small larvae to somewhat elevated temperatures. A. similior exhibited slightly more uniform R values, and this was possibly a reflection of stricter selection for growth-temperature synchrony in the more thermally constant habitat. Conversely, R/A was more constant in A. celer. A. similior demonstrated higher R/A in smaller larvae than in large ones at a similar temperature. This greater influence of body size on R/A in A. similior may be a function of its relatively slow growth which contributes less per day to assimilation compared to that of A. celer. This difference in growth rates is also reflected in assimilation, growth, and net growth efficiency values which are generally higher in <u>A</u>. <u>celer</u>. Consumption estimates, even after being adjusted for projected nonfeeding time, were still quite high. However, unless food availability or handling time is limiting, the feeding rates of Ameletus relative to its body mass appear to be considerably higher than values previously reported for aquatic insect larvae. Assimilation efficiencies were low but comparable to those for other herbivorous aquatic insects. Although <u>A</u>. <u>celer</u>'s reproductive effort was higher than A. similior's, its net reproductive effort was lower. This may be a consequence of faster, more efficient growth by A. celer, or may represent greater "competition"

for resources by reproductive processes in <u>A</u>. <u>similior</u>. The higher energy expenditure on reproduction by <u>A</u>. <u>similior</u> was not by way of increased fecundity, however. Rather, egg energy content was higher. This and other life-history traits suggest that <u>A</u>. <u>similior</u> displays more K-selected traits than <u>A</u>. <u>celer</u>, and indicates that higher fecundity may not necessarily be synonymous with higher fitness in all mayflies in all habitats.

CHAPTER THREE

MAYFLY LOCOMOTORY RESPONSES TO PREDATOR PRESENCE AND ENDOPARASITIC INFECTION: THE EFFECTS ON PREDATOR ENCOUNTER RATE

INTRODUCTION

The behavioral responses of benthic prey insects to detection of predators include flight, seeking refuge, aggregation, and warning (Peckarsky, 1982). Mayflies are known to employ three of these responses. Some exhibit flight by entering the water column as drift (Baetidae: Peckarsky, 1980; Siphlonuridae: Edmunds et al., 1976), or by crawling (Heptageniidae: Peckarsky, 1980). Some Siphlonuridae move under loose cobble and boulders when disturbed, and very small larvae cling upside down to the water's surface, supported by surface tension, in response to the presence of a predaceous stonefly (pers. obs.). Others may seek temporal refuge from visual predators by increasing their activity (as evidenced by their occurrence in drift samples) at night (Allan, 1978). Peckarsky (1980) has reported warning behavior in some ephemerellids which exhibit "scorpion" posturing upon perceiving a predator.

A predation event has the following components: encounter, recognition, attack, capture, and ingestion. Therefore, the frequency of a predator's success is directly dependent on the frequency of encounters. For prey animals, then, an effective antipredation strategy should include the minimization of encounters with predators. An encounter is defined here as an instance when predator and prey are close enough that the predator can recognize the prey.

Regardless of whether the predator is an ambush or a cruising hunter, its prey can minimize encounters by maximizing the amount of time it spends stationary. By definition an ambush predator cannot encounter a stationary prey, and a cruising predator will encounter a stationary prey less often than its moving counterparts, assuming random predator movement (Koopman, 1956; Gerritsen and Strickler, 1977). But most animals, including mayflies, must move in order to feed. Therefore, in the presence of a predator it is most advantageous to move at a velocity which minimizes the likelihood of an encounter while still allowing for sufficient foraging and reproduction.

Parasitic infection is of major significance in the vulnerability to predation in some host populations (Holmes, 1979; Jenkins <u>et al.</u>, 1964). To date, the role of endoparasitism in insect populations has been treated

largely as an area of applied ecology. Interest has centered mainly around the possible role of endoparasites (principally mermithid nematodes) in the control of agricultural pests (Gordon and Webster, 1971) and insect-borne diseases (see Hominick and Tingley, 1984 for review). Mermithid nematodes are obligate parasites of invertebrates (Hominick and Welch, 1980). They are known to infect spiders (Poinar, 1985), mosquitoes (Hominick and Tingley, 1984), and black flies (Hominick and Tingley, 1984), as well as mayflies (Hominick and Welch, 1980). These infections may occur in as much as 95% of a population (Service, 1981). The mayfly Ameletus similior, when infected by a mermithid nematode, responds with increased respiration and feeding rates, and is almost always killed when the nematode emerges from the host's body cavity (Hominick and Welch, 1980; pers. obs.). Mayflies which do survive to adulthood are sterile from nutrient depletion (Hominick and Welch, 1980; pers. obs.), or may exhibit gynandromorphy (Hominick and Welch, 1980). By altering host behavior or by depleting energy reserves, endoparasites can also make a prey animal less able to evade or escape a predator (Anderson, 1981). Therefore, although the mention of endoparasites in the ecology literature has been largely anecdotal, they can be important factors influencing population size, temporal and spatial dynamics, and competitive interactions of many host insect species (Price, 1980).

Because of the nearly one hundred percent mortality rate among parasitized <u>A</u>. <u>similior</u> larvae, the measurement of a representative energy budget for that group is not really relevant, except perhaps to show how energy flow through the population and community is affected, or how parasitization might contribute to competition for food. However, the occurrence of endoparasitization did allow an empirical test of the effects of parasite-altered energetics as measured by changes in locomotory behavior and susceptibility to predation. At the same time, I was able to investigate the locomotory responses of nonparasitized <u>A</u>. <u>similior</u> to the presence of a predatory stonefly which also inhabits the Elbow River study site, and examine how such responses might influence susceptibility to predation.

The purpose of this experiment was twofold: 1. To test the hypothesis that potential prey animals (mayflies) reduce their mean velocity or alter their mode of locomotion in the presence of a predator in order to decrease encounter rate, and increase their mean velocity when infected by endoparasitic mermithid nematodes in order to increase its foraging rate in response to energetic stresses imposed by the parasite.

2. To test the hypothesis that changes in locomotory behavior or mean velocity affect predator encounter rate, attack rate, or capture rate.

METHODS

Stonefly and mayfly larvae were collected at the Elbow River study site. The prey in this experiment was <u>Ameletus</u> <u>similior</u>. The occurrence of mermithid nematode endoparasitization in this population is described in Chapter One. Both parasitized and nonparasitized larvae were collected for this experiment from June to September, 1986.

The predator was the large perlid stonefly, <u>Claassenia</u> <u>sabulosa</u> (Banks). It is a semivoltine or merovoltine (Pritchard, 1983) species with a two or three-year life cycle (Allan, 1982; Fuller and Stewart, 1977, 1979). Large <u>C. sabulosa</u> larvae prey mainly on mayflies (Allan, 1982; Fuller and Stewart, 1977; 1979), and this was verified by examining foregut contents of several individuals before beginning the experiment. <u>C. sabulosa</u> were collected for this experiment from July to September, 1986.

Experiments were carried out in a refrigerated room which kept water temperature at a constant 5^oC. A natural summer photoperiod (14 hr light/10 hr dark) was maintained. Circular plexiglas artificial streams (depth = 7.0 cm, diameter = 8.8 cm) were used as experimental enclosures (Figure 26). Mean surface current velocity in the artificial streams (15 cm/sec) was within the range found at the collection site. Stream bottoms were covered with approximately 1.5 cm of pebble substrate. Baseline locomotion velocities were determined by placing a single stonefly or mayfly in the artificial stream. Velocity of the predator in the presence of prey, and the velocity of prey in the presence of a predator, were determined by placing one stonefly and one mayfly in the artificial stream. After a 24-hour acclimation period, 10-minute direct observations were made of the mode, direction and distance of all movements. These observations were augmented by 3-hour videotapes shot from directly above the artificial streams, and which allowed for all movements by the predator or prey to be measured. All movements were measured relative to the circumference of the artificial Each movement, regardless of mode, was estimated stream. to the nearest one guarter of the circumference and later converted to actual distance. Very slight movements (e. g. stationary position changes, cross-stream movements) were ignored. At the end of the observation period, distances travelled in each mode and total distance moved were calculated. Mean velocities were calculated simply as the average distance (cm) moved per hour. Movements were

categorized into three modes: walking, swimming, and drifting. Walking and swimming were further categorized as either with or against the water current. Percent activity in a given mode or direction was calculated by dividing the distance travelled in that mode or direction by the total. distance moved during the entire observation period. Predator-prey interactions were recorded at the same time. Because stoneflies are normally considered to be tactile rather than visual predators, an encounter was recorded any time contact was made with Ameletus, or whenever Ameletus came within the predator's encounter hemisphere. The encounter hemisphere was defined as that volume around Claassenia's head capsule with a diameter equal to the distance between the tips of the laterally-extended antennae. Any movement of the predator toward the prey immediately following an encounter was considered to be an attack. This usually consisted of a single lunge by the stonefly. Capture was the actual grasping of the prey in the mouthparts of the predator. The experimental design allowed for four treatments: nonparasitized prey without predator, nonparasitized prey with predator, parasitized prey without predator, parasitized prey with predator. То test for the effects of predator presence and parasitization on mean velocity (total distance moved per hour), a four-way ANOVA was used on untransformed data. То

test for the effects on percent activity of modes and directions, four-way ANCOVA's were used with total distance moved and distance moved in the mode or direction as covariates. For percent activities, reciprocal transformations (1/n + 1.0) were employed. Data from all observations in each treatment were pooled for statistical analysis.

RESULTS

Predator Movement

Mean predator velocity was similar in both the presence and absence of prey (260.0 cm/hr \pm 13.48) (n = 101, ANOVA, F_{1,100} = 4.08, p>0.05). All locomotion was by walking; neither swimming nor drifting was ever observed. Virtually all movement (96%) was in the same direction as the current.

Prey Movement

<u>Mode</u>

The proportions of time spent walking, swimming and drifting by nonparasitized <u>Ameletus</u> was unchanged with the addition of <u>Claassenia</u> (n = 149, ANCOVA, $F_{1,144} = 1.58$, 1.32, 0.62, respectively, p>0.20 for all). The frequency of walking increased in parasitized animals with no predator present (ANCOVA, $F_{1,144} = 5.18$, p<0.05), but was

markedly decreased when a predator was added (see Figure 27) (ANCOVA, $F_{1,144} = 15.04$, p<0.001). Swimming behavior was proportionately decreased in parasitized mayflies both with and without a stonefly present (see Figure 27) (ANCOVA, $F_{1,144} = 6.78$, p<0.05).

Mode-Direction

Percent activity spent walking with the current mirrored the changes in walking overall. It was unchanged in nonparasitized mayflies, but increased in parasitized individuals with no predator present (n = 149, ANCOVA, $F_{1,144} = 15.08$, p<0.001). With the addition of a stonefly, walking with the current sharply decreased in the parasitized group (see Figure 27) (ANCOVA, $F_{1,144} = 13.91$, p<0.001). Parasitized <u>Ameletus</u> did not swim against the current at all in the absence of <u>Claassenia</u>, and did so only with very low frequency when the predator was present (see Figure 27) (ANCOVA, $F_{1,144} = 4.85$, p<0.05). Other mode-directions were not significantly affected.

Direction

The proportion of total activity spent moving against the current decreased in parasitized mayflies (see Figure 28) (n = 149, ANCOVA, $F_{1,144} = 9.80$, p<0.01). There was no predator effect on direction of movement in either parasitized or nonparasitized animals.

Mean Velocity and Time Spent Stationary

Mean velocity increased significantly when mayflies were parasitized and no stonefly was present (Figure 29) (n = 149, ANOVA, $F_{1,145} = 49.38$, p<0.001). Nonparasitized animals showed decreased mean speed with a predator present (ANOVA, $F_{1,145} = 10.34$, p<0.01). Parasitized <u>Ameletus</u> slightly (but significantly) reduced mean speed in the presence of a predator (ANOVA, $F_{1,145} = 17.75$, p<0.001) (Figure 29). Nonparasitized <u>Ameletus</u> spent over half (51%) of their time remaining stationary, while parasitized individuals remained stationary only 9% of the time. Predator-Prey Encounter Rate

Mean observed encounter rates were 5.8/hr for nonparasitized individuals and 14.7/hr for those parasitized.

Encounters, Attacks and Captures

Of 33 encounters observed between stoneflies and nonparasitized <u>A</u>. <u>similior</u>, 4 attacks and 0 captures were recorded. Of 103 encounters between <u>Claassenia</u> and parasitized mayflies, 21 attacks and 1 capture resulted. There was no significant difference in the frequency of attack per encounter between parasitized and nonparasitized <u>Ameletus</u> (n = 136, G-test, $G_{(1)} = 1.22$, p>0.20).

DISCUSSION

Effects of Predator and Parasitism on Mayfly Activity

Both healthy and infected Ameletus similior respond to the presence of <u>Claassenia</u> <u>sabulosa</u> by reducing their overall movement, although this response is considerably more pronounced in nonparasitized individuals. Walking, swimming and drifting behaviors were each reduced by a similar proportion, although the proportion of total downstream movement increased somewhat. Apparently the reduction of overall activity is a reaction to some long-range chemical cue (see Peckarsky, 1980), and serves to reduce predator encounter rate. Decreases in mean velocity by a prey animal should decrease the frequency of predator encounters (Gerritsen and Strickler, 1977). For nonparasitized and parasitized <u>A</u>. <u>similior</u> moving at two different mean velocities this was indeed the case. Α decrease in mean velocity of 68% resulted in a 61% decrease in the predator encounter rate.

Of the six species Peckarsky (1980) tested, two (both Ephemerellidae) exhibited a decrease in activity in the presence of a stonefly predator, and this was accompanied by an increase in the presumably defensive "scorpion" posturing. Four others (two Heptageniidae, two Baetidae) increased their activity levels, but the pre-emergent

condition of the <u>Baetis</u> larvae may have somehow contributed to their activity. Peckarsky (1980) also noted a qualitative observation in which <u>Baetis</u> larvae became less active in the presence of <u>Koqotus modestus</u> (Banks). This dichotomy of mayfly responses to the presence of stonefly predators suggests two strategies, based on locomotory and defensive behavior capabilities, for avoiding and escaping contact encounters or attacks.

One strategy is to decrease mean velocity when long-range chemical cues from predators are detected. This would reduce the chance of a predator encounter per unit time, but still allow locomotion necessary to forage. Such a lack of any immediate flight response to the presence of a predator may reflect the ability to escape contact encounters and attacks. Indeed, healthy Ameletus were able to escape all observed attacks (although possible experimental effects regarding this are discussed later). Like Baetis, Ameletus is streamlined and a strong swimmer. Both are able to flee quickly when approached closely or touched by a predator. Ameletus usually jumps or swims quickly into the water column, then passively drifts away. This conforms to the argument that drift is a response to invertebrate predators (Andersson et al., 1986). Ephemerellids, on the other hand, may rely on defensive posturing rather than flight when immediately threatened by

a predator. Posturing mayflies may be perceived as too large for consumption by the tactually hunting stonefly.

In the <u>Ameletus</u> - <u>Claassenia</u> system a strategy of reduced activity probably would not put the mayfly at any additional risk of being detected by some other means. While there is evidence that some predaceous aquatic insects use visual (Pritchard, 1966) or chemical (Peckarsky, 1980) cues to locate prey, stoneflies are generally believed to be tactile predators (Kovalak, 1978). I observed no evidence that <u>Claassenia</u> could perceive its prey prior to physically contacting it (but see Sjostrom, 1985). Stoneflies utilizing chemical cues would be expected to hunt in an upstream direction to locate prey, but in artificial streams <u>Claassenia</u> moved in a downstream direction 96% of the time, strong evidence that they are not following chemical trails.

A second strategy, used by slower-moving or relatively defenseless mayflies, would be to separate themselves from the vicinity of a predator immediately upon detection. If an animal is unable to effectively escape a contact encounter or attack, it may be safer for it to move out of the area where a long-range chemical cue occurs. If indeed some stoneflies move predominantly downstream, as observations of <u>Claassenia</u> indicate, simple lateral

movement out of the chemical cue stream may allow a potential prey to avoid them.

Infection by endoparasitic mermithid nematodes altered mayfly movement in a way that increased predator encounter rate. Total distance moved per unit time in all parasitized animals and the proportion of total activity spent walking when no predator was present increased significantly. There are at least three possible explanations for the increased activity of these infected animals. First, parasitized animals are under a significant nutrient deficit. Mermithids absorb nútrients directly from the haemolymph of their hosts (Ittycheriah et al., 1977) as glucose or trehalose (a glucose precursor) (Chapman, 1980), and both glycogen stores (Chapman, 1980) and fat bodies (Phelps, 1962) are depleted. Increased walking behavior, therefore, may indicate an increase in food-searching to combat the nutrient deficit. Since food was unavailable to experimental animals, increased drifting when no predator was present may represent attempts to locate new food patches. Second, mayflies may be responding to tactile stimulation from the endoparasite. A. similior reacts to external tactile stimulation with escape responses (pers. obs.). It is possible that the presence oor movement of the mermithid nematode elicits similar responses. Third, large nematodes may well inflict

some dearee of discomfort on their hosts. Some drifting might be explained as an attempt by the mayfly to escape the source of discomfort. Parasitized Ameletus showed very little or no swimming behavior. Percentage of activity spent moving against the current was similarly reduced. Since these are the more energetically expensive activities, their decrease probably reflects the nutrient and energetic stresses to which the infected mayfly is subjected. When Ameletus was subjected to both parasitic infection and a predator, overall activity, total downstream movement, and swimming behavior were similar to those shown when no predator was present. However, the proportion of activity spent walking decreased while drifting behavior increased. This suggests that parasitized Ameletus probably attempt to forage in response to their nutrient deficit in spite of the presence of Claassenia. The simultaneous decrease in walking and increase in drifting is most likely due to the high number of predator encounters they experience and represent escape responses.

Effect of Parasitism on Encounter, Attack and Capture Rates

Infection by mermithid nematodes causes <u>Ameletus</u> to increase its overall activity even in the presence of <u>Claassenia</u>. As predicted, this increase in activity appears to be the direct cause of increased predator-prey

encounters. Apparently the stimulus causing the increased activity (probably hunger) is more powerful or persistent than the stimulus which would normally lessen activity when a predator is present.

Under experimental conditions neither nonparasitized nor parasitized Ameletus were very vulnerable to capture by Claassenia. Only one parasitized Ameletus was observed being captured. Gut content analyses showed that Claassenia do indeed take A. similior in their natural habitat. Why, then, was this not borne out in the laboratory? A lack of substrate heterogeneity may have been important in allowing Ameletus to escape into the water column in the artificial streams. Ameletus is usually found under cobbles and boulders where such quick movement into the water column may not always be possible. Many benthic invertebrates have been shown to preferentially colonize substrate with more interstitial space (Flecker and Allan, 1984) and higher heterogeneity (Erman and Erman, 1984). While this may be adaptive toward avoiding fish predators, it probably does not always provide refugia from stoneflies. Positions in small blind crevices may even prohibit escape. In one study, Peckarsky (1980) reported that stoneflies were able to capture <u>Baetis</u> larvae only by cornering them. Ameletus may also be more susceptible to capture at night when activity levels may be
higher. Walde and Davies (1986) have found that the foreguts of some stoneflies contain more mayflies at night and during the early morning. In addition, night drift typically contains more mayflies than day drift, and this may be indicative of higher invertebrate predator encounters (Andersson <u>et al.</u>, 1986).

SUMMARY

Responses of mayflies to the presence of stonefly predators suggest two possible prey strategies to avoid contact encounters with predators. Quick, strong-swimming mayflies or those utilizing defensive behavior may rely more on their ability to escape attacks, and so reduce their mean velocity when long-range chemical cues are perceived. This lessens the probability of contact encounters with tactile predaceous stoneflies. Slower-moving and defenseless mayflies may immediately vacate areas of chemical cues to lessen their risk of attack.

Changes in normal locomotory patterns due to the presence of a predator may affect the energy budget. If activity is decreased, an animal may spend more time in a poor food patch or take longer to travel to a new patch, and so decrease the amount of energy consumed per unit time. Reduced activity would also reduce the amount of

energy expended toward locomotion per unit time. Mayflies which retreat upon detecting predators may be displaced prematurely from good food patches, and be faced with the increased energy demands of escape and relocating suitable areas for foraging. For those mayflies which reduce their activity upon detecting a predator, such changes in locomotory behavior and the consequent effects on the energy budget may be sensitive to predator density. This research is limited to the idea that mayflies are capable of detecting only predator presence or absence. If this is true, then a single optimal mean velocity which reflects mean predator density would be expected. However, if mayflies are able to distinguish predator densities, perhaps as a function of the strength of a chemical cue, then locomotory responses may be graded accordingly since encounter rates are expected to increase with predator density at a given mean prey velocity. Future research in this area must address the energetic ramifications of predator avoidance and escape strategies, as well as the effects of predator density if questions of optimal behavior are to be answered.

Endoparasitic infection increases the activity of mayflies whether or not a predator is present. This increased activity, probably due to the nutrient deficit the animal is under, increases the probability of predator

encounter and may make the mayfly more susceptible to attack and capture. Because almost all parasitized larvae die before reaching the adult stage, the energetics of parasitized <u>A</u>. <u>similior</u> are not directly relevant to life-history strategies. However, they are indirectly important in community energetics with respect to competition for food and energy processing (e.g. the production of fecal detritus). Parasitization may also be important in the predator-prey dynamics of the community because of the increased activity of infected individuals and the resultant increase in predator encounter rate. The possible effects of endoparasitization on the sex ratio in the subject population is discussed in Chapter One. Table 1. Monthly and annual mean high and low temperatures at the Elbow River study site. See Figure 3 for standard deviations.

MONTH	MEAN HIGH	MEAN LOW	GRAND MEAN
JAN	2.0 ⁻⁵	2.0	2.0
FEB	2.0	2.0	2.0
MAR	2.0	2.0	2.0
APR	2.5	2.5	2.5
MAY	4.0	3.0	3.5
JUN	5.0	4.0	4.5
JUL	6.4	4.7	5.6
AUG	5.9	5.0	5.5
SEP	5.2	4.4	4.8
OCT	4.5	4.0	4.3
NOV	3.7	3.7	3.7
DEC	2.5	2.5	2.5
ANNUAL	3.8	3.0	3.5

Table 2. Monthly and annual mean high and low temperatures at the Ford Creek study site. See Figure 5 for standard deviations.

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MONTH	MEAN HIGH	MEAN LOW	GRAND MEAN
JAN	1.0	1.0	1.0
FEB	1.0 ⁻	1.0	1.0
MAR	2.5	2.0	2.3
APR	4.0	2.5	3.3
MAY	5.9	3.2	4.6
JUN	8.0	4.1	6.1
JUL	12.1	6.4	9.3
AUG	10.8	5.6	8.2
SEP	6.1	3.1	4.6
OCT	4.0	2.5	3.8
NOV	2.5	2.0	2.3
DEC	1.0	1.0	1.0
ANNUAL	4.9	2.9	4.0

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Table 3. Monthly and annual degree-hours at the Ford Creek and Elbow River study sites.

MONTH	FORD CREEK	ELBOW RIVER
JAN	744	1488
FEB	672	1344
MAR	1674	1489
APR	2340	1800
MAY	2395	2604
JUN	4366	3240
JUL	6882	4129
AUG	6101	4055
SEP	3312	3456
ОСТ	2418	3162
NOV	1620	2664
DEC	744	1860
ANNUAL TOTAL	33268	31291

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Table 4. Relative mean larval sizes associated with current speeds and substrate particle sizes at the Ford Creek and Elbow River study sites.

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	CURRENT SPEED	RELATIVE LARVAL SIZE	SUBSTRATE PARTICLE SIZE	RELATIVE LARVAL SIZE
ELBOW RIVER	SLOW	INTERMEDIATE	SMALL	N/A
	MODERATE	LARGE	MEDIUM	ALL
	FAST	SMALL	LARGE	N/A
FORD CREEK	SLOW	SMALL	SMALL	SMALL
	MODERATE	LARGE	MEDIUM	LARGE
	FAST	LARGE	LARGE	SMALL
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Table 5. Statistics of natural log-transformed head width versus weight-specific respiration regressions at experimental temperatures.

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TEMP (^o C)	N	Y-INTERCEPT	SLOPE	STD. ERROR OF ESTIMATE	r^2	ъ р
6.0	25	2.2	-1.0	0.43	0.66	0.05
7.0	10	2.5	-0.5	0.26	0.76	0.30
8.0	50	2.6	-0.9	0.51	0.68	0.01
9.0	35	2.9	-1.2	0 <i>.5</i> 8	0.67	0.01
10.0	8	2.9	-1.9	0.17	0.87	0.001
11.0	25	4.8	-3.0	0.25	0.81	0.01
12.0	18	3.6	-3.6	0.34	0.61	0.05
14.0	10	7.1	-5.8	0.07	0.94	0.001

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HEAD WIDTH CLASS (mm)	ENER CONT (J/mg)	GY ENT (s.e.)	
0.24-0.42	34.1	4.1	
0.43-0.62	30.0	2.7	
0.63-0.82	29.3	4.2	
0.83-1.02	29.0	4.2	
1.03-1.22	31.8	1.9	
1.23-1.42	27.4	2.8	
1.43-1.63	27.0	2.5	
>1.64	24.1	1.7	
EGGS			
<u>A. celer</u>	21.8	0.2	
<u>A</u> . <u>similior</u>	30.5	2.3	,
EXUVIAE	42.0	4.7	
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Table 6. Whole body, egg, and exuvia calorific values for <u>Ameletus</u>.

Table 7. Complete energy budget for <u>A</u>. <u>celer</u> at the Ford Creek study site (continued next page).

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	Duration	Mean	R	Total		M	Total		G
Instar	(d)	Temp (°C)	(J/mg/d)	R(J)	<u></u>	(J/mg/d)	M(J)	M/A	(J/mg/d)
1	10	8.2	2.862	0.228	0.415	0.184	0.015	0.027	3,836
ź	ĩŏ	8.2	2,709	0.378	0.607	0.184	0.026	0.042	1.565
3	10	8.2	2.565	0.591	0.619	0.184	0.042	0.044	1.396
4	10	5.0	1.785	0.641	0.553	0.184	0,066	0.057	1.258
5	14	4.6	1.584	1.262	0.605	0.131	0.105	0.050	0.902
6	14	4.2	1.382	1.683	0.590	0.131	0.160	0.056	0.828
7	17	3.8	. 1.188	2.629	0.613	0.108	0.239	0.056	0.642
8	17	2.9	0.903	2.919	0.560	0.108	0.349	0.067	0.602
9	22	2.2	0.653	3.907	0.557	0.083	0.499	0.071	0.436
10	27	1.0	0.330	3.403	0.450	0.068	0.701	0.093	0.334
11	28	1.0	0.238	3,502	0.394	0.066	0.964	0.109	0.300
12	27	1.0	0.151	2.906	0.294	0.068	1.306	0.132	0.295
13	27	2.0	0.264	6.710	0.436	0.068	1.732	0.113	0.273
14	22	2.8	0.350	9.491	0.466	0.083	2.268	0.111	0.318
15	19	3.4	0.381	11.500	0.464	0.097	2.921	0.118	0.343
16	14	4.6	0.557	15.730	0.496	0.131	3.703	0.117	0.435
17	14	4.6	0.492	17.400	0.477	0.131	4.634	0.127	0.408
18	15	5.9	0.690	32.263	0.590	0.122	5.725	0.105	0.358
19	11	6.1	0.673	28.137	0.520	0.167	6.981	0.129	0.455
20	10	7.7	0.765	34.997	0.541	0.184	8.402	0.130	0.466
21	9	9.3	1.205	59.113	0.636	0.204	10.012	0.108	0,486
							50 047		
		TOTAL:		239.389			50.84/		
		MEAN:	1.397		0.536	0.128		0.114	0.760

TOTAL E = 47.483 RE = 0.009 NRE = 0.106

Instar	Total G(J)	NGE	A (J/mg/d)	Total A(J)	P:R	C (J/mg/d)	Total C(J)	Total F(J)	AE
1	0.307	0.587	6.882	0.549	1.414	35.150	2.812	2.263	0.195
2	0.219	0.393	4.458	0.623	0.648	31.564	4.419	3.796	0.141
3	0.321	0.380	4.145	0.955	0.614	29.417	6.766	5.811	0.141
4	0.453	0.447	3.870	1.160	0.809	28.172	10.142	8.982	0.114
5	0.720	0.395	2.617	2.087	0.654	25.400	20.269	18.182	0.103
6	1.008	0.410	2.420	2.851	0.694	22.328	27.196	24.345	0.105
7	1.419	0.387	1.938	4.287	0.631	22.229	49.127	44.840	0.087
8	1.945	0.440	1.788	5.212	0.786	19.851	64.118	58.906	0.081
9	2.612	0.443	1.195	7.018	0.796	18.878	112.966	105.948	0.062
10	3.449	0.549	0.732	7.554	1.220	18.592	191.756	184.202	0.039
11	4.412	0.606	0.604	8.878	1.535	17.105	251.445	242.567	0.035
12	5.657	0.706	0.514	9.868	2.396	16.348	313.833	303.965	0.031
13	6:952	0.564	0.411	15.393	1.294	15.850	399.357	383.964	0.039
14	8.630	0:535	0.642	20.388	1.148	14.389	390.937	370.052	0.052
15	10.373	0.536	0.807	24.793	1.156	14.522	438.982	414.189	0.056
16	12.279	0.504	1.123	31.712	1.016	15.020	424.144	392.432	0.075
17	14.417	0.523	1.031	36.451	1.095	14.529	513.408	476.957	0.071
18	16.722	0.410	0.911	54.710	0.696	13.142	614.661	559.951	0.089
19	19.010	0.480	1.295	54.128	0.924	13.923	582.267	528.139	0.093
20	21.322	0.459	1.088	64.721	0.849	12.419	568.293	503.572	0.129
21	23.838	0.364	1.940	92.963	0.573	12.108	594.224	501.261	0.156
TOTAL:	156.064			446.301			5581.122	5134.821	
MEAN:		0.482	1.924		0.998	19.568			0.090

Table 8. Complete energy budget for <u>A</u>. similior at the Elbow River study site (continued next page).

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Instar	Duration (d)	Mean Temp (°C)	R (J/ma/d)	Total R(J)	R/A	M (J/mg/d)	Total M(1)	M/A	G (.1/mg/d)
1	24	4.1	2.397	0.571	0.587`	0.075	0.018	0.018	1.600
2	33	3.3	1.743	0.980	0.774	0.055	0.031	0.024	0.455
3	51	2.2	1.374	1.965	0.816	0.036	0.051	0.021	0.275
4	59	2.0	1.196	1.638	0.719	0.031	0.081	0.036	0.215
5	59	3.0	/ 1.259	5.121	0.839	0.031	0.127	0.021	0.210
6	36	4.5	1.444	5.504	0.793	0.051	0.195	0.028	0.326
7	11	5.6	1.545	2.671	0.575	0.167	0.288	0.062	0.974
8	11	5.6	1.436	3.615	0.568	0.167	0.421	0.066	0.926
9	11	5.6	1.332	4.820	0.560	0.167	0.604	0.070	0.881
10	11	5.5	1.215	6.132	0.555	0.167	0.843	0.076	0.807
11	11	5.5	1.123	7.785	0.548	0.161	1.157	0.081	0.762
12	11	5.1	1.036	9.699	0.539	0.167	1.563	0.087	0.718
13	16	4.8	0.816	14.713	0.587	0.115	2.069	0.083	0.459
14	16	4.5	0.682	16.078	0.554	0.115	2.706	0.093	0.435
15	24	4.3	0.556	25.292	0.616	0.076	3.481	0.085	0.270
16	33	3.5	0.342	27.096	0.588	0.056	4.408	0.096	0.184
17	51	2.3	0.075	11.495	0.338	0.036	5.508	0.162	0.113
18	60	2.0	0.075	16.752	0.387	0.031	6.795	0.157	0.089
19	59	2.9	, 0.108	28.739	0.484	0.031	8.275	0.139	0.084
20	36	4.3	0.263	51.271	0.594	0.051	9.947	0.115	0.129
21	21	5.5	0.459	, 62.149	0.609	0.087	11.842	0.116	0.208
		TOTAL:		304.086			60.410		
		MEAN:	1.682		0.554	0.090		0.109	0.482
			100						

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TOTAL E = 66.433 RE = 0.005 NRE = 0.121

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Instar	Total G(J)	NGE	A (J/mg/d)	Total A(J)	P:R	C (J/mg/d)	Total C(J)	Total F(J)	AE
1	0.384	0.413	4.054	0.973	0.704	51.130	6.136	5.163	0.159
2	0.255	0.226	2.257	1.266	0.292	22.738	12.756	11.490	0.099
3	0.392	0.184	1.686	2.408	0.226	21.967	31.369	28.961	0.077
4	0.558	0.281	0.877	2.277	0.390	11.012	28.586	26.309	0.080
5	0.856	0.161	1.508	6.104	0.192	19.584	79.727	73.623	0.077
6	1.244	0.207	1.819	6.943	0.261	10.816	41.272	34.329	0.167
7	1.682	0.424	2.688	4.642	0.738	18.964	32.751	28.109	0.142
8	2.333	0.432	2.529	6.370	0.762	17.977	45.284	38.914	0.141
9	3.187	0.440	2.379	8.611	0.785	17.026	61.618	53.007	0.140
10	4.073	0.445	2.188	11.048	0.802	16.277	82.181	71.133	0.134
11	5.275	0.452	2.051	14.216	0.826	15.756	109.186	94.970	0.130
12	6.718	0.461	1.921	17.980	0.854	14.900	139.481	121.501	0.129
13	8.269	0.413	1.389	25.050	0.703	13.944	251.447	226.397	0.100
14	10.252	0.446	1.231	29.037	0.806	12.630	297.857	268.820	0.097
15	12.292	0.384	0.915	41.065	0.624	12.186	554.503	513.438	0.080
16	14.552	0.412	0.581	46.057	0.700	11.777	933.117	887.060	0.049
17	17.027	0.662	0.222	34.031	1.960	10.291	1574.450	1540.419	0.022
18	19.727	0.613	0.195	43.274	1.583	11.063	2456.660	2413.386	0.018
19	22.394	0.516	0.223	59.408	1.067	10.766	2862.810	2803.402	0.021
20	25.087	0.406	0.442	86.305	0.683	10.489	2045.859	1959.554	0.042
21	28.109	0.391	0.753	102.101	0.643	10.237	1386.601	1284.5	0.074
TOTAL:	184.666			549.166		·	13033.651	12484.485	
MEAN:		0.399	1.519		0.744	16.263			0.094

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MONTH

Figure 3. Monthly mean high (broken line) and low (solid line) temperatures at the Elbow River study site. Vertical bars are 1 standard deviation.



Figure 4. Map of Ford Creek study site.



MONTH

Figure 5. Monthly mean high (broken line) and low (solid line) temperatures at the Ford Creek study site. Vertical bars are 1 standard deviation.



Figure 6. Diagram of mayfly enclosures used in growth experiments and current created by bubbling air under slanted styrofoam base.







Figure 8. Monthly size-frequency distributions of <u>A</u>. <u>celer</u> at the Ford Creek study site.



Figure 9. Monthly size-frequency distributions of <u>A</u>. similior at the Elbow River study site.



In HEAD WIDTH (mm)









In HEAD WIDTH (mm)









Figure 14. Relationship of growth ratio to natural log-transformed head width in <u>Ameletus</u>.







In HEAD WIDTH (mm)

Figure 16. Relationship of feeding rate to natural log-transformed head width in <u>Ameletus</u>. See Figure 16 for scatterplot.



Figure 17. Relationship of weight-specific respiration to natural log-transformed head width in <u>Ameletus</u> at experimental temperatures.







In HEAD WIDTH (mm)





Figure 20. Approximate growth curves for <u>A</u>. <u>celer</u> using a constant temperature-moult interval regression (solid line), and 30-day moult interval for near-zero temprature (broken line). H = average hatching date, E = average emergence date. Open triangles = estimated emergence dates.



Figure 21. Approximate growth curves for <u>A</u>. <u>similior</u> using a constant temperature-moult interval regression (solid line), and 30-day (broken line), 60-day (dotted line), and 90-day (dot-dash line) moult intervals for near-zero temperature. H = average hatching date, E = average emergence date. Open triangles = estimated emergence dates.



Figure 22. Comparison of instar-specific exuvium dry mass for <u>A</u>. <u>celer</u> and <u>A</u>. <u>similior</u>.



Figure 23. Instar-specific ingestion rates for Ameletus.


Figure 24. Instar-specific respiration rates for <u>A</u>. <u>celer</u> and <u>A</u>. <u>similior</u>. Vertical lines indicate mean temperature (and so respiration) change during instar.



Figure 25. Instar-specific weight-specific respiration rates for <u>A</u>. <u>celer</u> and <u>A</u>. <u>similior</u>. Vertical lines indicate mean temperature (and so respiration) change during instar.

168



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Figure 26. Plexiglas artificial stream.

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Figure 27. Percent activity spent walking (w), swimming (s), and drifting (d) with (w) and against (a) the current by <u>Ameletus</u>. No Pa = nonparasitized, Pa = parasitized, No Pr = predator absent, Pr = predator present.

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Figure 28. Percent activity spent moving with and against the current by <u>Ameletus</u>. No Pa = nonparasitized, Pa = parasitized, No Pr = predator absent, Pr = predator present.

171



Figure 29. Mean velocity of nonparasitized (No Pa) and parasitized (Pa) <u>Ameletus</u> in the absence (No Pr) and presence (Pr) of a stonefly predator. Bars = 1 standard error.

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