### THE UNIVERSITY OF CALGARY

Population Dynamics and Life History Strategies of Hyalella azteca (Amphipoda) in a Prairie Pond

 $\mathbf{B}\mathbf{Y}$ 

Yuan Hua Wen

#### A THESIS

# SUBMITTED TO THE FACULTY OF GRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

# DEPARTMENT OF BIOLOGICAL SCIENCES

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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, "Population Dynamics and Life History Strategies of *Hyalella azteca* (Amphipoda) in a Prairie Pond", submitted by Yuan Hua Wen in partial fulfillment of the requirements for the degree of Master of Science in Aquatic Ecology.

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#### ABSTRACT

The population dynamics, spatial and temporal distribution, production and life history strategies of the amphipod *Hyalella azteca* (Saussure) in a prairie pond were investigated over a period of two years by integrating both laboratory experiments and field sampling.

Population density of H. azteca increased markedly in June and reached a maximum in July each year. The most rapid decline in density occurred during November - December and density reached a minimum during the winter and early spring (January to May). Biomass fluctuated in a similar pattern but with a two to three month time lag. Both density and biomass were much higher in the shallow zones than those in the deeper zone. However, they were not significantly different between shaded and unshaded zones, and between the west and east basins. The abundance of H. azteca in the water column was significantly higher than in the benthos. Density and biomass were highest at a depth of 0.9 to 1.2 m and lowest at 2.0 to 2.5 m, both showing negative correlations with water depth.

Substrate preference by *H. azteca* was examined in the laboratory using a 3x3 Latin Square experimental design. *H. azteca* preferred macrophyte and rock substrates over mud as a resting site, and preferred conditioned macrophytes over micro organism-free macrophytes presumably because of the additional food resources present.

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During the breeding season (May to September) male and female *H. azteca* formed precopula pairs. Positively size assortative pairing was observed in the laboratory with male:female ratios of 1:2 to 2:1. Beyond this ratio random pairing occurred. In the field, however, males and females did not match for size. Young were recruited to the population from late June to late August. Female fecundity was correlated to size with larger females more fecund than small ones. However, there were trade-offs between number of eggs brooded and mean egg weight and between number of young released and mean young weight.

Three cohorts were recognized during the sampling period. The growth rate was 0.086 mg/day from April to August 1985 for the first cohort; 0.043 mg/day for the second cohort, and 0.097 mg/day for the third cohort during the first two months after hatching (July and August). Laboratory experiments showed that the growth rate of H. azteca depended on temperature, and food quantity and quality.

Production was calculated by two methods giving values of 19.99 and 19.65 g dry weight/ $m^2$  in 1985 and 12.54 and 9.56 g dry weight/ $m^2$  in 1986. P/B ratios were 3.05 in 1985 and 3.23 in 1986. Production of *H. azteca* in Stephenson's Pond is the highest so far reported in the literature.

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## Chapter 1

### **GENERAL INTRODUCTION**

#### **1.1.** THE SPECIES STUDIED

#### 1.1.1. Taxonomy

First described by Saussure (1858) from specimens collected in Vera Cruz, (Mexico), *Hyalella azteca* belongs to the family Hyalellidae, superfamily Talitroidea, order Amphipoda in the class Crustacea (Holsinger, 1972). *H. azteca* has often been cited in the literature under the synonyms *Hyalella knickerbockeri* (Bate) or *Hyalella dentata* (Smith).

#### 1.1.2. Diagnosis and Description

The body of *Hyalella azteca* is elongated and laterally compressed, with the first thoracic segment fused to the head. The total length of mature animals ranges from 5.0 to 8.3 mm. The thorax is composed of seven segments and the abdomen of six; the telson is small and entire. Two eyes are sessile, compound, and round or nearly so. The first antenna is shorter than the second antenna and is without an accessory flagellum. The peduncle of first antenna consists of three joints with the first and second joints about equal in length and slightly longer than the third. The

accessary flagellum of the second antenna consists of seven to nine joints, and is about twice as long as the peduncle. The peduncle of second antenna consists of five joints, with the antennal gland located on the second joint. The two distal joints of the peduncle are elongated and nearly equal in length. The number of joints in the flagellum of the second antenna varies from 8 to 15.

The first two pairs of thoracic legs differ from the others in structure and are called gnathopods, the remaining five pairs are more or less similar in structure and are termed pereopods. All thoracic legs, except the first and the last pairs, bear gills on the inner side of the first joint. The gills are of two types: sternal and coxal. Small lateral sternal gills are located on the thoracic segments 3 to 7 inclusive, and the coxal gills project from the inner surface of the first joint of thoracic legs 2 to 6 inclusive.

Extending from the first three abdominal segments are three pairs of pleopods, each pleopod consisting of a long basal joint and two multiarticulated setose rami. Pleopods are used not only in swimming, but also in directing water to the gills. A dorsal tooth projects from the posterior edge of each of the first two abdominal segments. Each of the three posterior abdominal segments bears a pair of uropods. These are directed backward and fitted for springing.

#### **1.1.3.** Geographic Distribution

Hyalella azteca is widely distributed in North America, and also occurs in South America (Pennak, 1978). Bousfield (1958) and Pennak (1978) reported it in

lakes, ponds, sloughs, marshes, rivers, streams, ditches, spring streams and esturaries from Mexico north to the tree line in Canada and Alaska, and from the Atlantic to the Pacific, provided that the monthly mean summer water temperatures exceed  $10^{\circ}C$ .

# **1.2. DYNAMIC PATTERNS AND THE INFLUENCING FACTORS**

Population dynamics is the study and integration of critical rate functions of natality, mortality, immigration and emigration (Halls, 1964). Such analysis can provide accurate estimations of such ecological parameters as population stability and productivity, and also serves as a necessary prerequisite for the study of population regulation based on these field data.

Geisler (1944) cultured *H. azteca* in the laboratory, and made a comprehensive study of postembryonic development. She concluded that water temperature is the critical factor governing the rate of development. Similarly Bovee (1950) investigated the effects of temperature on the rates of embryonic, postembryonic and adult growth in *H. azteca* and concluded that all three processes were temperature dependent.

Cooper (1965) studied the dynamics of field populations of *H. azteca* in Sugarloaf Lake, Michigan (U.S.A.), and found that in the spring when water temperature rose to  $20^{\circ}C$ , *H. azteca* populations showed a synchronous onset of reproduction. He further reported that during the early summer high population growth and size frequency distribution were indicative of a rapidly expanding population. Size-specific mortality operated most heavily on large adults during the summer. However, with the decline of the water temperature in the autumn, mortality, reproduction and growth rates declined, and during the winter individuals grew very little (Cooper, 1965).

Since temperature influences growth, voltinism and length of life cycle, it is an important factor determining the secondary production of H. azteca (Waters, 1979). Lindeman and Momot (1983) studied the relationship between environmental factors and benthic production of H. azteca in a lake in northern Ontario, and found that H. azteca production was temperature dependent and increased with the length of the growing season.

Recent work has shown that lake acidification affects the distribution of H. azteca populations. Stephenson and Mackie (1987) found that H. azteca was present in 69 of 71 nonacidified lakes in Ontario, but absent from all eight lakes recently acidified. France and Stockes (1987) investigated the tolerance of H. azteca to low pH of different sizes and developmental stages, and found that exposure of adults to water below pH 5.0 during pulses of acid snowmelt, or of juveniles to below pH 5.5 through gradual lake acidification, resulted in population declines. France and LaZerte (1987) proposed a simple model to explain the restricted distribution and predicted the population changes of H. azteca .

Population density is another important factor influencing population dynamics. Wilder (1940), while examining the effects of population density upon growth and reproduction of H. azteca, found that in laboratory populations, growth rate

and fecundity were inversely related to population density. This was due to the accumulation of excretory products and the decrease of food and space availability.

Hargrave (1970a, b, c, 1971) showed that *H. azteca* is an omnivorous deposit-feeder, feeding largely on epibenthic algae, bacteria and epiphytic biota. Hargrave (1970b) found that the horizontal and vertical distribution of *H. azteca*, its daily growth rate and seasonal abundance are related to sediment microflora production and the standing stock of digestible sediment organic matter. Mathias (1971) also observed that *H. azteca* displayed poor growth when fed on lake sediments, but grew well on a diet of periphyton. From field data, Lindeman and Momot (1983) concluded that the quality and quantity of macrophytes also influence the distribution and standing stock of *H. azteca*.

The effects of predation on the population dynamics of H. azteca have been examined by a host of workers. Cooper (1965) found that H. azteca maintained a high turnover rates under high predation pressure by fish, yellow perch (Perca flavescens (Mitchill)) and bluegill (Lepomis macrochirus, Rafinesque). Hall et al. (1970) demonstrated a reduction in H. azteca densities associated with predation by bluegill sunfish and suggested that H. azteca is highly adapted to strong predation regimes and persists by maintaining high turnover rates. Milstead and Threlkeld (1986) testing effect the of darter. Etheostoma spectabile (Agassiz) (Percidae) predation on H. azteca population in the field observed a shift in H. azteca size structure and a slight decrease in population density.

The population dynamics of H. azteca are thus affected by many external and internal factors either directly or indirectly. To date some approaches to investigating this sort of problem have been established. One of the easiest ways is to study population dynamics in the field and correlate population variables with environmental variables. This approach is useful and fruitful, but the results are often difficult to interpret unless many of the normally varying environmental conditions are stabilized. An alternative method has also been used, namely looking at population dynamics in the laboratory, where most of the variables can be kept constant. However it is difficult to know how the results of experiments carried out under simplified conditions in the laboratory can be applied to the more variable and complex situations in the field. Unfortunately, accurate quantitative analyses of populations of H. azteca, both in the field and in the laboratory, are still relatively scare.

#### **1.3. LIFE HISTORY TRAITS**

Life history information is of fundamental importance for virtually all ecological studies of freshwater invertebrates. Oliver (1979) defined life history as events that govern the reproduction and survival of a species or a population, including fecundity, development, longevity, and behavior. This definition has been widely developed and applied to many ecological studies.

A great deal of the variation among life histories relates to aspects of reproduction. Therefore reproduction has been considered a very important component

in the studies of life history of H. azteca . Reproduction in H. azteca has been described by several authors (Wilder 1940; Kruschwitze 1972; Strong 1971 and De March 1977). It is obligately sexual, and during the eighth instar males amplex females for the first time. Amplexus terminates after the male fertilizes the ova produced at the end of the instar. Eggs are brooded by the female during the following molt cycle and young released from the brood pouch before the next ovulation. Amplexus, ovulation and fertilization can then be repeated. The juveniles grow through seven instars into adults.

Sex recognition by *H. azteca* and aspects of its sexual behaviour, such as precopulation (amplexus) and fertilization, were first described by Holmes (1902, 1903). The life history strategies of *H. azteca* were first studied by Jackson (1912) who described the distribution, habitat, colour, size, molting, breeding, food, feeding, predators, locomotion, and thigmotaxis. Gaylor (1922) further studied the life history strategies of *H. azteca*, particularly reproduction, including reproductive season, time between broods and distribution of brood size. Life history variation among populations was investigated by Strong (1971). He compared the reproductive features of three populations living along environmental gradients and found that the maturation time, egg volume, size of young, size of female at maturity, growth rate of adult female, and size of the first clutch varied with fish predation. The reproductive behavior patterns of *H. azteca*, such as carrying behaviour and mating behaviour, were briefly recorded by Kruschwitz (1972).

Many factors have been shown to influence the life history characteristics of H. azteca. Wilder (1940) examined the effect of population density upon reproduction and found that fecundity was generally inverse to numbers present. Bovee (1949) measured thermal death in H. azteca, and found that it occurs from 33. to  $50^{\circ}C$ , with the time required for thermal death ranging from more than 11 hr at  $33^{\circ}C$  to less than 1 sec at  $50^{\circ}C$ . Size-specific predation can select for small size and slow growth rate and reduce fecundity (Strong 1972). Strong (1973) also observed the effect of predation pressure on ecotypic variation in amplexus and found that the duration of amplexus is negatively correlated with an index of the intensity of predation by visually orienting fish. The effects of photoperiod and temperature on the induction and termination of the reproductive resting stage in H. azteca were analysed by De March (1978), who showed that photoperiod determined whether reproduction continued or discontinued. However, temperature influenced the rate of change. De March (1978) also looked at the effects of constant and variable temperatures on size, growth, and reproduction and noted that the time to maturity and embryonic development time were inversely correlated with temperature in animals reared at constant temperatures. However, the maturation times were longer than expected if early development occurred at low temperatures, and shorter if early development occurred at high temperatures. The abiotic and biotic environmental determinants of H. azteca preamplexus were presented by France (1987) who showed that the variation in time of preamplexus of H. azteca was positively correlated with water temperature but was not corre-

lated with other abiotic variables including pH, alkalinity, total phosphorus, or chlorophyll concentration. The effects of pH on the mortality of different life stages of *H. azteca* were reported by France and Stokes (1987) who found that acidification to pH < 5.0 for adults and to pH < 5.5 for juvenile caused increased mortality. Acute toxicity occurred below pH 4.2 for adults and below pH 4.5 for juveniles.

The usual ecological approach to the study of life history is to compare the life histories of two or more populations, and to try to understand the differences between them with respect to differences in their environments (Davies and Reynoldson, 1976). However, studies of the life history of *H. azteca* are very few, and insufficient to make valid comparisons. Thus, more research in various geographic areas needs to be undertaken. Recently attention has been given to the understanding of variation and covariation of life history traits and how they are related to resource tracking (Denno and Dingle, 1981). This type of investigation requires: assessment of the genetic structure, analysis of the environmental constraints and influences, and examination of a broad range of life history traits, especially those aspects of physiology and behaviour which confer phenotypic flexibility.

# **1.4. RESEARCH OBJECTIVES**

The objectives of this investigation were:

- (1) to describe the morphometric characteristics of H. azteca.
- (2) to quantify the population dynamics of *H. azteca* in the field by examining spatial and temporal abundance changes, size frequency distribution, individual growth rate and mortality.
- (3) to estimate production, seasonal biomass changes and turnover rates of the *H. azteca* population.
- (4) to examine sexual dimorphism, precopulatory mate guarding and mate choice.
- (5) to examime the environmental and biological cues used by *H. azteca* to choose a particular micro-habitat.

# Chapter 2

# SITE DESCRIPTION AND GENERAL METHODOLOGY

### 2.1. HABITAT CHARACTERISTICS

#### 2.1.1. Locality, Morphometry and Environment

Stephenson's Pond is located approximately 5 km northwest of Calgary, Alberta, Canada  $(51^{\circ}9'N, 114^{\circ}16'W)$  in the knob and kettle topography of the prairie-foothills transition zone (Legget 1969) (Figure 1. 1). Morphometric parameters are given in Table 1. 1. The southern shore line is covered by *Populus balsamifera* Linn. and *Salix* sp. while the remainder of the catchment basin is grass-covered, with some sedges and shrubs. The substrate of Stephenson's Pond consists of mud, allochthonous debris, and sand with large rocks in the dike area at the end of the east basin. Water enters the pond by surface runoff, including a small intermittent stream at the end of the west basin, and seepage. An earthstone dam at the east end of the pond prevents surficial runoff. The catchment basin is heavily utilized by grazing livestock which contributes a substantial nutrient loading to the pond.



#### 2.1.2. Climate

A dry, cold climate predominates with the average air temperature  $3.7^{\circ}C$  in 1985 and  $5.2^{\circ}C$  in 1986 (data from the University of Calgary weather research station). The highest air temperatures occurred from May to August (above  $10^{\circ}C$ ), and the lowest from November to February (below  $0^{\circ}C$ ). The maximum temperature was  $32^{\circ}C$  in June and July, and the minimum temperature was  $-34^{\circ}C$  recorded in November 1985 and February 1986.

The average annual precipitation was 414.2 mm in 1985 and 506.1 mm in 1986, of which rainfall contributed nearly 70% in 1985 and 80% in 1986. More than 90% of the rainfall occurred from May to September. Snowfall normally begins in September and ends in April, but there was an unusually severe snow storm (38.5 mm) in May 1986.

#### **2.1.3.** Physical Features

#### 2.1.3.1. Water Temperature

Water temperature curves for 1985 and 1986 are shown in Figure 2. 2. There was a steady rise in water temperature from April towards July, when the mean water temperature reached approximately  $20^{\circ}C$ . The maximum water temperature noted was  $23.8^{\circ}C$  on July 10 1985 and  $22.5^{\circ}C$  on May 30 and 31 1986. The seasonal decline in water temperature began in September, and by October the mean water temperature was below  $4^{\circ}C$ . During the ice covered winter months,

the water temperature was generally between  $1^{\circ}$  and  $4^{\circ}C$ , but sometimes fell below  $0^{\circ}C$ .

#### 2.1.3.2. Light Absorption

The light absorption coefficients (k) obtained from a light meter varied from 1.38 to 7.92 during the open water period, depending on the weather, season and water depth. In the deep region of Stephenson's Pond, no light was transmitted below 1.25 m during summer algal blooms.

#### 2.1.4. Water Chemistry

#### 2.1.4.1. Dissolved Oxygen

Dissolved oxygen concentration measured with Hydrolab remote datasondes varied drastically during the period of May to October (Figure 2. 3). A mean of 8.7 ppm dissolved oxygen occurred during this ice-free period. Dissolved oxygen concentration decreased rapidly during the first month following freeze-up, subsequently reaching a low and stable level close to anoxia. After ice-cover was broken up in April the oxygen content increased steadily (Baird *et al*, 1987).

### 2.1.4.2. pH and Conductivity

The mean pH changed from 6.2 during the winter months (January to March) to a maximum of 10.0 in August. During the summer, pH generally fluctuated



Figure 2.2. Mean Water Temperature at a Depth of 1.0 m from January 1985 to April 1986

between 8.0 and 9.0. Conductivity averaged  $453.67\mu^{\circ}S$  with a range from 300.0 to  $500.0\mu^{\circ}S$ .

#### 2.1.4.3. Macro- and Micro-nutrients

The concentrations of macro- and micro-nutrients in Stephenson's Pond during 1985 and 1986 (Table 2. 2) were similar to those reported by Rasmussen (1983), indicating similarities from year to year.

#### 2.1.5. Biological Limnology

#### 2.1.5.1. Plankton

The phytoplankton is mainly blue-green (Cyanophyta) algae, especially the species Aphanizomenon flos-aquae (L.) Ralfs. which is the dominant component of midsummer blooms. In addition, Chlamydomonas sp. and Haemal-coccus sp. are also abundant seasonally. The chlorophyll-a concentrations during the open water stage are shown in Table 2. 3. The monthly mean values of chlorophyll-a and the high total phosphorus concentration (1500 and 1400  $\mu g/l$  in summer and winter separately) (Baird *et al.* 1987) indicate that this pond is hypereutrophic (Wetzel 1983).

Many species of Cladocera, especially *Daphnia* sp. constituted an important part of the zooplankton community. In addition, some species of Protozoa, Rotifera and Copepoda also occurred.

Parameter	
Maximum length (m)	266
Maximum width (m)	145
Maximum depth (m)	2.50
Shore line (m)	705
Fetch (m)	253
Surface area $(m^2)$	20611
Basin volume (m <sup>3</sup> )	27896
Percent mean slope	3.08
Shoreline development	1.38
Volume development	1.62

Table 2. 1. Morphometric Parameters for Stephenson's Pond

Table 2. 2. Concentrations (mg  $l^{-1}$ ) of macro- and micronutrients in Stephenson's Pond during 1985 to 1986. (SE = standard error; N = sample size).

Parameter	N	Mean	SE
Alkalinity	15	253.67	4.58
T-hardness	32	239.22	5.38
Ca-hardness	31	134.55	7.27
Mg-hardness	32	101.59	5.19
Na <sup>+</sup>	28	23.49	1.41
K+	32	24.86	0.72
$SO_4^{}$	31	74.94	13.71
Cl <sup>-1</sup>	31	34.98	4.62
$NO_3^-$	43	0.00035	0.00003
$NO_2^-$	72	0.0053	0.0006
$PO_4^{-3}$	61	0.164	0.005
Total dissolved P	39	0.173	0.014
Total P	46	0.278	0.029

#### 2.1.5.2. Macrophytes

Extensive macrophytes appear in the littoral zone over the summer. The dominant species is *Potamogeton richardsonii* (Benn.) Rydb., which makes up more than 90% of the macrophyte biomass. Other species, such as *Myriophyllum exalbescens* Fern. also occur in the pond. The biomass of macrophytes over the summer is given in Table 2. 4.

#### 2.1.5.3. Benthic Community

The most abundant benthic macro-invertebrate found in Stephenson's Pond are the leeches, *Nephelopsis obscura* Verrill and *Erpobdella punctata* (Leidy), the amphipod, *Hyalella azteca* (Saussure), chironomids, (*Chironomus riparius* Meigen and *Glyptotendipes paripes*), and oligochaetes, (*Tubif ex tubif ex* (L.)), which collectively make up of 99% of macro-invertebrate biomass (Davies *et al.*, 1987). 1987). *Hyalella azteca* comprises more than 90% of the amphipod biomass, while the sympatric species *Gammarus pulex* L. is much less abundant and forms only a small proportion of total amphipod biomass.

#### 2.2. SAMPLE COLLECTION

#### 2.2.1. Sampling Programs

Ice-cover on Stephenson's Pond usually lasts four to five months (November - March). To facilitate the randomization of sampling, different sampling programs

Table 2. 3. Average chlorophyll-a concentration  $(\mu g l^{-1})$  of phytoplankton in water column recorded in Stephenson's Pond during the open water period in 1985. Data are mean  $\pm$  standard error. N number of water strata sampled.

Date	N	Shallow Zone	N	Deep Zone	Mean
Apr. 12	4	$41.38 \pm 14.45$	7	$28.53 \pm 11.95$	$33.20 \pm 9.01$
Apr. 29	5	$261.62 \pm 6.99$	9	$271.17 \pm 11.72$	$267.76 \pm 7.84$
May 20	5	$10.20 \pm 0.62$	9	$9.90 \pm 0.68$	$10.10 \pm 0.48$
Jun. 9	5	$11.68 \pm 0.71$	_		$11.68 \pm 0.71$
Jul. 10	5	$82.79 \pm 13.18$	8	$65.81 \pm 13.11$	$72.34 \pm 9.49$
Jul. 24	5	$119.08 \pm 8.80$	8	$70.31 \pm 4.28$	$89.07 \pm 7.98$
Aug. 6	5	$80.78 \pm 26.76$	7	$33.60 \pm 6.51$	$53.38 \pm 13.12$

Table 2. 4. Dry biomass (g /625 cm <sup>2</sup>) of macrophytes collected in the shallow water zones of Stephenson's Pond. N is sample size, SE standard error.

<u> </u>	1			
Year	Date	Ν	Biomass	SE
1985	Jul. 2	6	9.10	1.41
•	Jul. 31	11	4.80	0.91
	Aug. 26	20	4.73	0.56
	Oct. 2	10	2.8	0.60
	Nov. 12	6	0.73	0.28
1986	Jun. 11	7	1.38	0.58
	Jun. 25	12	1.60	0.39
i	Jul. 17	10	3.62	0:67
	Jul. 30	10	3.26	0.61
	Aug. 14	11	3.61	0.93

were established for the periods of ice-cover and open water.

### 2.2.1.1. Open-water Sampling Program

Three habitats in Stephenson's Pond (Figure 2. 1) were chosen as the main sampling sites: one offshore station (deep zone), one shaded shore station (shaded shallow zone), and one unshaded shore station (unshaded shallow zone). Each station was sectioned into sampling grids so that samples were taken in order of increasing depth (Figure 2. 2), ranging from 0 to 1.5 m for the two shallow stations and 1.5 to 2.5 m for the deep station. The three sampling stations represented about 30% of the total pond area. In addition, some random samples were taken from other regions in the two basins.

Samples were taken using an integrated macrophyte-water-column and sediment sampler (Figure 2. 4) (Gates *et al.* 1987) twice a month during the *H. azteca* reproductive season of May to September, and once a month during the remainder of the open water period. Within each station, three replicate samples were taken, with the water depth measured. Both the water-column samples and sediment samples were seived separately into a bucket with a stainless steel mesh bottom ( $390\mu m$ ) *in situ* to remove fine particles, and the remainder preserved in 70% alcohol before being taken to the laboratory in plastic bags.



(INSIDE VIEW OF JAW)





#### 2.2.1.2. Ice-cover Sampling Program

During ice-cover, random samples were taken over the whole pond to increase sampling depth coverage probability. Once a month two replicate samples were taken using an Ekman dredge with the same size as the composite sampler. After sampling the depth was measured, the samples seived (mesh size  $390\mu m$ ), and preserved in alcohol.

#### **2.2.1.3.** Laboratory Samples Collection

The benthic macroinvertebrates found in the soft sediments and on the aquatic macrophytes were separately picked from the preserved samples. When the macroinvertebrate population densities were <200 individuals/sample, the total number of each species was counted, and individual weight measured directly. With densities >200 individuals/sample, the samples were subsampled using the Wrona *et al.* (1982) technique. All picked animals were preserved in 70% alcohol and kept for analysis.

#### 2.3. STATISTICS

For all statistical analyses significance testing was performed at the p=0.05level of probability unless specifically stated otherwise. If a null hypothesis was rejected the alternative hypothesis was assumed to be true for further data analysis.

The chi-square test was used to determine whether an observed distribution confirms with a theoretical distribution. The null hypothesis of no difference for two or more populations was always tested. When the degree of freedom was 1 or the expected frequency was less than 1.0, and more than 20% of the expected frequencies were less than 5.0, the Yates correction was employed (Zar, 1984).

When two populations were a deliberate or natural pairing, the difference between their means was tested using a paired sample t-test. The null hypothesis tested was that there was no difference between two population means. Otherwise, the difference between the two means was tested by a t-test after the test of a hypothesis concerning the equality of the variance of two population using an F test. When two populations had equal variances, a two sample t-test was performed. When two populations had unequal variances, the Mann-Whitney nonparametric statistical method was employed.

Analysis of variance (ANOVA) was used whenever three or more experimental groups were tested for differences in population means. When single-factor ANOVA rejects the null hypothesis of equality of population means, a Duncan multiple range test was used to determine which population means were significantly different.

Linear regression by least squares was used to quantify the relationship between two variables and to establish its statistical significance. The significance of a relationship between two variables was tested by correlation analysis (Zar, 1984), and also by an F-test using a null hypothesis that the slope = 0. Multifactorial comparisons were made by comparing regressions (slopes and intercepts) using analysis of covariance (Zar, 1984). The independent and dependent variables
of curvilinear data (power function) were log-transformed to obtain a linear regression.

A 3 X 3 Latin Square experimental design was utilized to test substrate preference by *Hyalella azteca*. In a Latin Square design, treatments are grouped into replicates, in which every row and column of any square is a complete replication. This double grouping minimizes the errors in differences among rows (row effects) and columns (column effects) (Cochrane and Cox, 1957). The dispersal patterns of *H. azteca* were determined by examination of the row and column effects. Because the substrate treatments were applied in sequence (both in row and column), there was a risk of residual or carry-over effects of one treatment onto the succeeding treatment. To solve this problem, the Latin Square statistical design was balanced with respect to residual effects by having each treatment proceeded twice by each of the other treatments and the 3 X 3 Latin Squares replicated four times within the apparatus. A computer program (Wrona, 1982) was used to calculate the direct effects of substrate treatment free from the influence of residual effects.

When population size was small (n<50), the general statistics, including measures of central tendency, dispersion and variability, testing for goodness of fit, two-sample and paired-sample hypotheses, were performed using comprehensive statistical packages such as Statistix, or Key Stat on a IBM-PC computer. Otherwise they were computed using MINITAB, version 5.1.3. on a Honeywell Multics. SPSS programs (Nie, Hull, Jenkins, Steinbrenner and Brent, 1975) were used to calculate linear regression and correlation, one-way and two-way ANOVA with multiple comparison analysis. SPSS scatergrams were also utilized for plotting ordination results.

# **Chapter 3**

#### **MORPHOMETRIC PROPERTIES**

#### **3.1. INTRODUCTION**

Biomass estimates used in production computations of *Hyalella azteca* (Cooper, 1965; Mathias, 1971) and many *Gammarus* species (Iversen and Jessen, 1977; Welton, 1979; LaFrance and Ruber, 1985) are often indirectly determined through the use of more directly obtainable length measurements. The length variables used include head length, first thoracic segment length, body length and total length. Head length is measured in profile as the arc between the tip of the rostrum and the dorsal margin of the head capsule (Lindeman and Momot 1983); first thoracic segment length from the posterior margin of the head to the anterior margin of the second thoracic segment (Iversen and Jessen, 1977); body length from the anterior margin of the telson (Welton, 1979); and total length from just in front of the eyes to the base of the telson (Marchart *et al* . 1981). Based on these measurements, the quantitative relationships between body size (length) and biomass can be established.

A number of studies have successfully developed equations to predict the biomass of some species of aquatic insects (Smock, 1980). This information is, however, not available for H. azteca, resulting in difficulties for population

studies. Since biomass can be estimated from several different length variables, it is necessary to determine the validity of the length parameter used to predict biomass accurately. In addition, because of the use of several length variables in the literature, it is very difficult to make comparisons. Therefore, it is necessary to examine the morphometric relationships of H. azteca before population studies are carried out. In this chapter, data on relationships between length measurements, between length and weight measurements and between weight measurements (*i.e.*, wet weight, dry weight and ash-free dry weight) are presented. The objectives were: (1) to examine the quantitative relationships between the morphometric variables and thus to derive equations that can be used to predict the value of one variable from another; (2) to evaluate each equation and to choose the best estimate a common variable for use in population studies; (3) to assess the effect of preservation in alcohol of H. azteca

### **3.2. MATERIALS AND METHODS**

All specimens of H. azteca were collected in open water from Stephenson's Pond in 1986 and 1987 using a hand net. Individuals representing the maximum length range were selected from the samples, washed in distilled water, then measured for head length, first thoracic segment length, body length and total length to the nearest 0.5 mm under a microscope (10X10) fitted with an ocular micrometer after straightening each specimen out with dissecting needles. The number of segments on the primary flagellae of the first and second pairs of the antennae were counted under 10X10 magnification.

Wet weight was measured to the nearest 0.1 mg immediately after removal of external water by blotting dry each specimen on absorbent paper three to five times. Dry weight was determined after each individual was oven-dried ( $60^{\circ}C$ ) for 48 hr and stored in a desiccator for 12 hr. Ash weight was measured after each individual *H. azteca* was held in a pre-ashed, pre-weighed foil boat in a muffle furnace and ignited at  $500^{\circ}C$  for 2 hr. Ash-free dry weight (AFDW) was computed by subtraction of ash weight from dry weight.

Data sets were plotted using Minitab on a Honeywell Multics computer. Appropriate programs were used to fit regressions to the points and calculate the parameters and correlation for each regression. Levels of significance shown are p>0.05 not significant, 0.05>p>0.01 significant, 0.01>p>0.001 very significant and p<0.001 highly significant.

The effects of preservation using alcohol on wet weight changes of H. azteca were assessed monthly. For each alcohol concentration (50, 65, 80 and 95%), 20 individuals covering all size classes were selected, and put into a 90 ml jar. The initial mean wet weight of individuals chosen in four jars were not significantly different (F=0.99, p>0.05). Twenty individuals were weighed to the nearest 0.1 mg after being blotted dry every month in the first six months of preservation and every two to three months thereafter. Two-way statistical analyses of wet weight changes with preservation time and alcohol concentrations were carried out using the SPSS (Nie *et al*. 1975) statistical package implementing

# standard Model I ANOVA.

#### 3.3. RESULTS

# 3.3.1. Length Measurements and Wet Weight

Correlation coefficients for the relationships between head length, first thoracic segment length, body length and total length are shown in Table 3. 1. An F-test showed that all pairs of variables are significantly correlated with the correlation coefficient between body length and head length the highest, and between head length and first thoracic length the lowest. Residual analysis also showed a significant relationship for each regression (Table 3. 1), indicating that the slope is signifiantly from 0.

There were significant correlations between the logarithms of length and wet weight (Table 3. 2). The highest r values were found in the correlations between log body length and log wet weight, and log total length and log wet weight. Thus, body length and total length are the best predictors of wet weight.

# 3.3.2. Flagellar Segments on Antennae and Length, Wet Weight

The mean number of flagellar segments was used to estimate the age of H. azteca by Cooper (1965), who assumed that growth rates are the same and constant for all individuals in a natural population. Therefore flagellar segments on antennae can be employed as correlates of the length and weight measurements

Table 3. 1. Regression and correlation coefficients (r) between head length (L1), first thoracic segment length (L2), body length (L3) and total length (L4) of *Hyalella azteca*. The coefficients a and b are the intercept and slope respectively of the regressions. All regressions and correlations are significant ( $F_{res}$ : ANOVA; F-test, N = 99).

Variable	a	b	Fres.	r	F	р
L1 on L2	0.360	0.795	19.96	0.412	2.40	$\leq 0.01$
L1 on L3	0.174	0.057	<sup>-</sup> 59.52	0.616	4.21	$\leq$ 0.01
L1 on L4	0.215	0.055	48.45	0.574	3.70	$\leq$ 0.01
L2 on L3	0.067	0.038	156.7	0.787	8.39	$\leq$ 0.01
L2 on L4	0.079	0.038	139.5	0.768	7.62	$\leq$ 0.01
L3 on L4	0.442	0.999	1376	0.946	54.56	≪0.01

Table 3. 2. Regression and correlation coefficients (r) between the log 10 head length (L1), log 10 first thoracic segment length (L2), log 10 body length (L3), log 10 total length (L4) and log 10 wet weight (WW) of *Hyalella azteca*. The coefficients a and b are the intercept and slope respectively of the regressions. All regressions and correlations are very significant ( $\mathbf{F}_{res}$ : ANOVA; F-test, N = 99).

Variable	a	b	Fres.	r	F	р
logL1 on logWW	-3.14	0.194	114	0.735	6.547	≤0.01
logL2 on logWW	-3.18	0.262	260	0.854	12.699	<u>≤</u> 0.01
logL3 on logWW	-0.74	0.276	604	0.927	26.397	≪0.01
logL4 on logWW	-0.81	0.284	587	0.927	26.397	≪0.01

based on the same assumption. From Table 3. 3, it can be seen that the mean numbers of flagellar segments are significantly correlated to length measurements (F < 0.05). Residual F also showed that each slope of the regression is significantly different from 0.

The  $\log_{10}$  body weight of *H.azteca* changes non-linearly with the mean numbers of segments on the flagellae of the first and second pairs of antennae (Figure 3. 1). When the mean numbers of flagellar segments in the first and second antennae are both less than ten,  $\log_{10}$  wet weight increases very rapidly with the additions of flagellar segments. However, when the mean number of flagellar segments in both antennae are more than ten,  $\log_{10}$  wet weight does not increase with the addition of flagellar segments.

#### 3.3.3. Wet Weight : Dry Weight Ratio

The significant correlations between fresh and dry weights of *H. azteca* are presented in Figure 3. 2. Adult males were significantly heavier (paired t = 4.81, n = 53, p<<0.01) than adult females. Adult males ranged from 10.7 mg to 17.1 mg (mean = 14.04 mg), and dry weight from 1.8 mg to 3.5 mg (mean = 2.44 mg); adult females ranged from 8.8 mg to 15.8 mg (mean = 11.84 mg), and dry weight from 1.1 mg to 3.3 mg (mean = 2.04 mg). Young ranged from 0.8 mg to 2.2 mg (mean = 1.57 mg), and dry weight from 0.1 mg to 0.6 mg (mean = 0.29 mg). On average, dry weight was 17.76% of the fresh weight of *H. azteca*.



Figure 3.1. Non-linear Relationship between Mean Number of Flagellar Segments and log 10 Wet Weight of Hyalella azteca

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#### 3.3.4. Ash-Free Dry Weight and Dry Weight

The relationship between ash-free dry weight (AFDW) and dry weight (DW) is shown in Figure 3. 4. There were significant linear relationships between AFDW and DW for male, female and young H. azteca.

#### **3.3.5.** Effects of Preservative on Wet Weight Biomass

Changes in wet weight of *H. azteca* after preservation in different alcohol concentrations are presented in Figure 3. 5. Two-way ANOVA analyses show that both alcohol concentrations and preservation time have significant effects on wet weight changes of *H. azteca*. However, no significant two-way interactions were found among wet weight variables (Table 3. 4). One way ANOVA showed that wet weight changes with time occurred within the first two months of preservation in all four alcohol concentrations (Table 3. 5). However, changes in wet weight in the lower alcohol concentrations were significantly higher than those in higher concentrations (Table 3. 6). After nine months preservation, the percentage wet weight losses in the three lower concentrations were very similar (mean = 18.54%). However, in 95% alcohol a 28.7% wet weight loss of initial total wet weight occurred.

#### 3.4. DISCUSSION

This study has shown that significant relationships exist between the head length, first thoracic segment length, body length and total length. Therefore, any length measurement can be predicted by one of the established predictive



Figure 3.2. Relationships between Wet Weight and Dry Weight for Males, Females and Young of *Hyalella azteca* 



Figure 3.3. Relationship between Dry Weight and Ash-Free Dry Weight of Adult Males and Females of Hyalella azteca.



Figure 3.4. Percentage Changes of Wet Weight of Hyalella azteca Remaining after Preservation in Four Alcohol Concentrations

Table 3. 3. Regression and correlation coefficients (r) between head length (L1), first thoracic segment length (L2), body length (L3), total length (L4) and the mean number of flagellar segments on the first (FIRST) and second (SECOND) pairs of antennae of *Hyalella azteca*. The coefficients a and b are the intercept and slope respectively of the regressions. All regressions and correlations are significant ( $\mathbf{F}_{res}$ : ANOVA; F-test, N = 99).

Variable	a	b	Fres.	r	F	р
L1 on FIRST	0.461	0.021	4.7	0.214	1.545	$\leq 0.05$
L1 on SECOND	0.371	0.022	8.19	0.279	1.744	≤0.01
L2 on FIRST	0.028	0.030	52.25	0.592	3.902	$\leq$ 0.01
L2 on SECOND	0.038	0.025	65.19	0.634	4.464	$\leq$ 0.01
L3 on FIRST	0.970	0.629	54.28	0.599	3.988	≤0.01
L3 on SECOND	2.100	0.471	45.69	0.566	3.608	≤0.01
L4 on FIRST	0.940	0.596	51.12	0.587	3.843	≤0.01
L4 on SECOND	1.730	0.466	48.94	0.579	3.750	$\leq 0.01$

Table 3. 4. Two-way analysis of variance comparing wet weight changes of *Hyalella azteca* with preservation time and alcohol concentration. Significance was tested by an F statistic (DF = degree of freedom, MS = mean square).

Source of variance	DF	MS	F	Significance
Time .	6	118.30	19.52	p ≪0.01
Concentration	. 3	119.87	19.78	p ≪0.01
Time X concentration	18	3.16	0.52	p ≥0.05
Error	532	6.06		

Table 3. 5. One-way analysis of variance and Duncan's multiple range test for seven wet weight variables of *Hyalella azteca* over preservation time (days). Preservation time are ranked according to mean wet weight from the lowest at left to the highest at right. Wet weight on days underscored by the same line do not differ significantly. Significance (p) was tested by an F statistic.

Alcohol			•
concentration	F	р	Duncan's multiple range test
95%	8.22	≤0.01	<u>290 190 90 70 130</u> 30 0
80%	3.46	≤0.01	<u>290 90 70 190 130 <u>30</u> 0</u>
65%	3.68	≤0.01	<u>290 90 190 130 70</u> 30 0
50%	5.59	≤0.01	<u>290 90 190 130 70</u> 30 0

Table 3. 6. One-way analysis of variance and Duncan's multiple range test for wet weight variables of *Hyalella azteca* among alcohol concentrations. Alcohol concentrations are ranked according to mean wet weight from the lowest at left to the highest at right. Wet weight in concentrations underscored by the same line do not differ significantly. Significance (p) was tested by an F statistic.

Preservation			· · · · · · · · · · · · · · · · · · ·
. time (days)	F	р	Duncan's multiple range test
30	4.70	$\leq 0.01$	<u>95% 80% 65% 50%</u>
70	4.44	≤0.01	95% 80% $65%$ 50%
90	1.88	$\geq 0.05$	95% 80% $65%$ 50%
130	2.17	$\geq 0.05$	<u>95% 80% 65% 50%</u>
190	5.31	≤0.01	95% 80% $65%$ 50%
290	4.20	≤0.01	95% 80% $65%$ 50%
	1		

equations. The lowest, but still significant, correlation coefficient was found between head length and first thoracic segment length. This is probably because the first thoracic segment is fused with the head (Holsinger, 1972) and consequently the boundary between the head and the first thoracic segment is difficult to determine. However, the highest correlation coefficient was found between body length and total length because they are almost identical measurements.

Strong (1972) used head length as the main size index of H. azteca . From three populations he found a significant relationship between body length and head length. All regressions had equal slopes, but different intercepts, indicating no allometry between body length and head length. This result is similar to that found in the present study. Unfortunately Strong failed to use body length or total length directly as body size measurements. This study has shown that both of these length measurements are much better predictors of wet weight than head length. A significant relationship between head length and body length was also found for related species of *Gammarus* (Skadsheim 1984).

Significant relationships were also found between wet weight and all length measurements. Generally the length to wet weight relationships of amphipods given in the literature are mathematically formulated in the same way as the regressions given above, *i.e.*  $\log_{10}W = b\log_{10}L + \log_{10}a$ . >From this equation, the highest correlation coefficients were found between wet weight and body length and between wet weight and head length. Furthermore there was a highly significant relationship between wet weight and dry weight. Thus, the dry weight of

H. azteca can be predicted directly from any length measurement. Mathias (1971) also found a significant relationship between head length and dry weight of H. azteca, but he did not calculate the relationship between dry weight and body length or total length. Iversen and Jessen (1977) recorded a correlation between dry weight and the length of the first thoracic segments of Gammarus pulex L., but no one has previously examined these relationships in H. azteca.

Wilder (1940) and Giesler (1944) found that, in H. azteca, 0 - 1 flagellar segments of antennae were added per molt. Therefore, some authors claim that flagellar segments are added in a regular or consistent manner and that it is possible to identify each instar and compare growth increments per instar within and between natural populations. Cooper (1965) used the mean number of flagellar segments to estimate the ages of H. azteca in a lake. This assumes that growth rates are the same and constant for all individuals in a natural population. In the present investigation, significant positive correlations between the mean number of segments on the primary flagellae of the first and second pairs of antennae and all length measurements were found, indicating that the antennal segment number increases with the size of H. azteca. However, the relationship between wet weight and number of flagellar segments was exponential on semi-logarithmic scales, demonstrating that the wet weight of H. azteca does not increase linearly with addition of number of flagellar segments.

The routine collection of large number of benthic samples usually requires preservation of the material at some stage prior to final analysis since sampling of

the benthos is a very time consuming activity. But these data can be used only if the effects of preservative and preservation time on wet weights are known. If this is known it is possible to estimate biomass from preserved samples by applying the appropriate correction factors (Slack, 1976; Anderson and Hooper 1965; Hynes and Coleman, 1968).

One commonly used preservative for *H. azteca* is alcohol. However, several different alcohol concentrations are used, *e.g.* Cooper (1965) used 100% alcohol while 70% was used by Mathias (1971). It is therefore necessary to know whether different concentrations cause different percentage weight losses. Results from this study show that different alcohol concentrations do have significant effects on weight changes. Weight loss is greater in high concentrations of alcohol than in low concentrations, probably because of the high solubility of some fats in high alcohol concentrations. The percentage weight loss in alcohol concentrations of 50, 65 and 80% were very similar, averaging 18.54% after nine months preservation, but in 95% alcohol the weight loss was 28.66%.

There was a significant loss in wet weight of *H. azteca* during the first month of preservation. This is similar to the results of Howmiller (1972) who found that wet weight loss of tubificid worms (mostly *Limnodrilus* spp.) ranged from 10 to 38% in the first 24 hr and 24 to 74% after 44 days in formalin, alcohol and isopropanol solutions, respectively. Stanford (1972) also recorded a similar pattern for some benthic insect larvae suggesting this was the result of dehydration in alcohol. However, Stanford found some species showed weight gains for up to

seven days in alcohol, as did Donald and Paterson (1977) for chironomid larvae.

# Chapter 4

# POPULATION DYNAMICS AND PRODUCTION

#### 4.1. INTRODUCTION

Numerous studies on population dynamics have been conducted either in the laboratory with controlled environmental variables or in the field where variables are uncontrolled (Frank, 1960; Cooper, 1965; Green, 1976; Wilkialis and Davies, 1980; Iversen and Thorup, 1987 and Hart, 1987). In laboratory experiments, the analyses are often much more powerful and yield knowledge on the fundamentals of population growth (Hall, 1964). However, they are limited to specific conditions sometimes seldom found in nature. It is possible to obtain reliable and detailed quantitative analysis of the dynamics of an animal population in the field, although the analyses are often limited to correlating population phenomena with environmental variables and frequently involve large errors in estimates of the inferred rates (Hall, 1964). The disadvantages of laboratory population studies can be partially overcome by manipulating laboratory populations in such a manner that information appropriate to an analysis of natural population is obtained. In the last twenty years, population ecologists have usually investigated population dynamics by integrating mathematical theory and experimental laboratory studies with quantitative analyses of natural populations. Therefore, more and more ecologists are

interested in the laboratory examination of the deterministic, mathematical models applicable to population studies. Consequently, accurate quantitative analyses of natural populations are relatively scarce. This scarcity can be attributed to the difficulties encountered when attempting quantitative studies because of the complexity and variability of natural populations (Cooper, 1965).

Hyalella azteca is an appropriate experimental organism with which to conduct both laboratory and field experiments, due to its short generation time, frequent high abundance and the ease of culturing it in the laboratory. Cooper (1965) first studied the population dynamics of *H. azteca* in Sugarloaf Lake (U.S.A), concentrating on population size and structure, natality, mortality, growth and productivity. Thereafter, Hargrave (1970) conducted a series of studies in Marion Lake (British Columbia) on distribution, growth, and the seasonal changes of *H. azteca* in relation to sediment food resources. At the same site Mathias (1971) conducted studies on the population dynamics, energy flow and secondary production. Production of *H. azteca* in a northern Ontario lake was estimated by Lindeman and Momot (1983), who calculated cohort production to biomass ratios, and compared these data with those from other geographic areas.

In this chapter, data are presented on the population density, annual weight frequency distribution, natality and mortality, growth rates and production of H. azteca in Stephenson's Pond. The objectives of this chapter were to obtain a detailed quantitative analysis of the dynamics of a natural population of H. azteca, and thereafter, to estimate related critical rate functions for this

species.

#### 4.2. MATERIALS AND METHODS

The sampling program, including sampling sites, field and laboratory procedures, sampling frequency and schedule, have been presented in detail in Chapter 2. The field samples were washed in a sieve (mesh size 0.20 mm) and sorted. Because of the large number of H. azteca in some samples, subsamples were taken using the Wrona *et al.* (1982) technique. As accurate length measurements frequently involve damage to the animals when straightening them out, weight was used as an estimate of size. This has excellent repeatability and precision and is significantly correlated with length (Chapter 3).

The fecundity of *H. azteca* was studied in females collected by hand net from Stephenson's Pond in the reproductive season (June to August). From each sample, the egg-carrying and/or young-carrying females were selected for analysis. The measurements taken included total length, body weight and weight of attached eggs or young. The absolute fecundity was determined in the laboratory by counting all the eggs or young on the female. Eggs or young were removed from the marsupiae by pressing them out through the interlocking hair of the oostegites. Ten eggs or young taken from each female randomly were weighed.

The reproduction experiments in the laboratory were carried out at constant temperature in illuminated incubators. Four temperatures (5, 10, 15 and  $20^{\circ}C$ ) represent the range found in Stephenson's Pond. The reproduction chamber

(modified from Welton and Clarke, 1980) consisted of one plastic pot placed inside another, each 80 mm high, having a basal diameter of 90 mm and a top diameter of 110 mm. The bottom of the inner pot was removed and replaced with a 12 mesh/cm monofilament mesh, to prevent young from passing through the net. Two 6 mesh/cm mesh covered holes on opposite sides of the outer pot allowed circulation of water. Chambers were cleaned by removing the inner pot containing the animals and placing it in fresh pond water at the appropriate temperature. A mature male and female in precopula were placed in each chamber and fed *ad libitum* on detritus collected from Stephenson's Pond. Young were removed and counted on release from the females.

The growth rates of *H. azteca* were estimated both in the laboratory and in the field. Field growth rates were determined by tracking recognizable cohorts with weight frequency distribution (2.0 mg) from successive sample dates. Growth rates of *H. azteca* were also examined under controlled laboratory conditions to assess the effects of temperature, and for comparison to field growth estimates. All sizes of *H. azteca* were collected from open water and brought to the laboratory. Four weight groups were arbitrarily defined and chosen from these samples: group 1 was less than 6.0 mg; group 2 from 6.0 to 9.9 mg; group 3 from 10.0 to 15.0 mg; and group 4 heavier than 15.0 mg. Experimental animals were kept in plastic pots containing 70 ml of filtered pond water at 10, 15 or  $20^{\circ}C$ , representing field conditions during the growing season. The food supply consisted of fresh leaves of *Potamogeton richardsonii, Salix* sp., conditioned  $(10^{\circ}C)$ , one month) plant

detritus or pond water. The water was changed weekly and fresh food added every three or four days. Fluorescent lighting was maintained at a 14 h light:10 h dark photoperiod.

Mean changes in body weight of animals fed on each food type at each temperature were expressed in two ways. The average daily gain in wet weight (DW, mg/day) was calculated from the following equation:

$$DW = \frac{w_t - w_0}{t}$$

where  $W_0$  is the initial weight (mg) and  $W_t$  is the weight at time t (days). Specific growth rates (G, mg/mg/day) were calculated by dividing DW by the initial weight (mg).

Production of amphipods has been usually calculated in several ways (Waters, 1981; Lindeman and Momot, 1983; LaFrance and Ruber, 1985). In this study, two commonly used methods; the size frequency method (Hynes and Coleman, 1968; Hamilton, 1969; Benke and Waide, 1977), and the cohort G method (Mathews, 1970; Hall *et al* ., 1980) were used. In single-species applications, the size-frequency method has been proven highly successful, and appears to be well accepted in secondary production research (Cushman *et al* ., 1978; Benke, 1979; Waters, 1979). However, this method calculates only total cohort production and cannot be used to obtain a seasonal pattern of production. The advantage of the G method is that it can estimate production for short intervals or parts of the cohort

life cycle. It is best used for populations that exist in discrete cohorts (Waters and Crawford, 1973, Benke and Waide, 1977), for which it is possible to determine growth rates by following progressive mean mass increases through the life cycle.

In the size-frequency method of estimating production, the mean annual weight distribution of the population is treated as an average cohort. The losses of the number (N) between two adjacent weight classes (j and j+1) are multiplied by the mean weight (w) of an individual between the two classes to determine interval loss (LaFrance and Ruber, 1985). Through the annual life cycle, there are several weight classes of animal (i), therefore, production data must be multiplied by (i). Since *H. azteca* is univoltine (Strong 1972; Lindeman and Momot, 1983), correction must be made for the true interval from hatching to death of the largest animal, *i.e.* cohort production interval (CPI) (Benke, 1979). Therefore, the complete calculation is:

$$P = i \sum_{j=1}^{i} [(\overline{N_j} - \overline{N_{j+1}})(W_j W_{j+1})^{1/2}] \frac{365}{CPI}$$

The production to mean biomass ratios were calculated by dividing the annual production estimate by the mean annual standing crop.

The equation for calculating a production by the cohort G method is:

$$P = G \cdot \vec{B}$$

where G is instantaneous growth rate for the time period and is determined for a whole cohort interval simply by  $G = \ln (max \ wt \ ind \ / \min \ wt \ ind)$ ; B is mean biomass during the time period, in weight/spatial unit and calculated for an interval between two sampling dates; P is production for a given period of time in weight/spatial unit/time period. Cohort production is taken as the sum of all intervals for the cohort and annual production as the sum of all the cohort.

#### 4.3. RESULTS

#### 4.3.1. Population Density and Biomass

Mean densities of H. azteca fluctuated considerably throughout the 18month study period (Figure 4. 1). Both density and biomass were lowest and most stable from January to May, as indicated by the flatness of the density and biomass curves. Density increased in June with the onset of reproduction and reached a maximum by the end of July. This was followed by a sharp fall in the succeeding months. However, the peak biomass of H. azteca ap-peared two or three months after peak density.

The coefficients of population growth rate (r) were calculated from measurements of population sizes (N) on two consecutive sampling dates, t1 and t2. When r is positive, the population is increasing, when negative, it is decreasing. Table 4. 1 shows that rates of population change fluctuated considerably. The only period when r was positive for more than two consecutive intervals was June and July. The longest periods of population decrease occurred in the winter and early spring (January to May).

Table 4. 1. Mean intrinsic rate of increase (r) for Hyalella azteca during time periods from 1985 to 1986.

Period	Duration		Period	Duration	
1985	days	r	1986	days	. r
Apr. 12 - Apr. 29	17	0.055	Jan. 9 - Feb. 10	32	-0.042
Apr. 30 - Jun. 26	58	0.016	Feb. 11 - Mar. 3	22	-0.007
Jun. 27 - Jul. 30	34	0.030	Mar. 4 - Apr. 8	26	0.020
Jul. 31 - Aug. 26	27	-0.015	Apr. 9 - May 22	34	-0.017
Aug. 27 - Oct. 2	37	0.002	May 23 - Jun. 11	20	0.052
Oct. 3 - Nov. 12	41	-0.010	Jun. 12 - Jun. 25	14	0.059
Nov. 13 - Dec. 4	22	-0.008	Jun. 26 - Jul. 17	22	0.066
Dec. 5 - Jan. 9	36	-0.025	Jul. 18 - Jul. 30	13	-0.020
			Jul. 31 - Aug. 14	15	0.009
			Aug. 15 - Sep. 25	42	-0.026

The amplitude of both the density and biomass fluctuations during the growing season in 1985 was significantly higher than that in 1986 (t-test, p < 0.01), indicating that the magnitude of population variation differs from year to year (Figure 4. 1). The length of the growing season in 1986 was much shorter than that in 1985. The time lag between peak density and peak biomass in 1985 was about two months longer than in 1986 (Figure 4. 1).

#### 4.3.2. Weight Frequency Distribution

Weight frequency distributions were analyzed for recognizable cohorts. A cohort is herein considered as a group of individuals all hatched over the entire summer (Hudson and Swanson, 1972). The weight frequency distributions of *H. azteca* collected from Stephenson's Pond in 1985 and 1986 (Figure 4. 2) show three recognizable cohorts. The first cohort recruited in June and July 1984, matured over winter, and disappeared from the population by January 9 or early February 1986. Cohort 2 first occurred in June 1985, became adults through the winter and spring, and declined to a minimum on August 14 1986. Cohort 3 began with its first occurrence in weight class 1 (0.5 to 1.5 mg) on June 25 1986.

The spring population consisted entirely of adults and subadults overwintering from the previous summer. After reproduction, the large adults rapidly declined in abundance and eventually disappeared from the population. Offspring composed approximately 50% of the population in the latter part of June and early July, but The proportion of small animals declined very rapidly in August and September,





due to the cessation of reproduction and growth into the heavier weight groups.

In most months, only one cohort occurs in the population and only after reproduction, do two cohorts co-occur until winter. The mean cohort duration for the 1985 generation was 414 days.

# 4.3.3. Mortality

Population density began to decline in late August and continued to decrease until February, with the most rapid decline occurring from October to December (Figure 4. 1). During this period mortality was relatively high.

The number of adults decreased from late July to January, but was particularly rapid from August to September due to post-reproductive mortality (Figure 4. 2).

The weight frequency distributions (Figure 4. 2) showed a high loss of two weight classes on February 10, indicating massive mortality at this time, probably related to anoxic winter-kill of H. azteca under ice cover.

#### 4.3.4. Reproduction

Weight frequency histograms of *H. azteca* (Figure 4. 2) indicate that the life cycle in Stephenson's Pond starts in July when juveniles are first released from the marsupium and recruited into the population. The population goes through one generation per year and breeds intensively only during the July to August period. The histograms from May and early June 1986 suggest that individual weight increases very rapidly during this period to reach maturity. During the breeding



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Figure 4.2. Monthly Percent Weight-Frequency Distributions for Hyalella azteca in Stephenson's Pond

season, the minimum weight at reproduction was around 8 mg for males and 6 mg for females.

Fecundity in each size group of females varied widely (Tables 4. 2 and 4. 3). For the entire population the number of eggs per female ranged from 4 to 48 and the number of young per female from 8 to 45. Smaller females usually laid fewer eggs or released fewer young than larger females. The wet weight of eggs or young carried by a female was significantly correlated with the wet weight of the female (Figures 4. 3 and 4. 4). There were negative correlations between egg number and total egg weight and between young number and young weight (Figures 4. 5 and 4. 6) for both sampling years.

Reproduction in the laboratory experiments showed that temperature has a significant effect on the success of reproduction in *H. azteca*. At  $5^{\circ}C$ , no young were produced; at  $10^{\circ}C$ , a few young were produced; at  $15^{\circ}C$ , the average fecundity was 24.13 young per female ranging from 3 to 46; at  $20^{\circ}C$ , the average fecundity was 16.07 young per female ranging from 4 to 38. A two sample t-test showed no statistical difference between fecundity at  $15^{\circ}$  and  $20^{\circ}C$  but both of them were significantly higher than the fecundity at  $10^{\circ}C$ .

The mean egg number in each female size group of *H. azteca* was higher than the mean number of young (Tables 4. 2 and 4. 3), indicating that not all the eggs brooded successfully hatched. The estimated hatching success rate was 78.83% in 1986 and 71.14% in 1987. It was also noted that the embryonic development of *H. azteca* were not homogeneous for the same brood. Sometimes



Figure 4.3. Relationship between Female body Weight and Total Egg Weight of Hyalella azteca in 1986 and 1987



Figure 4.4. Relationship Between Female Weight and Total Young Weight of *Hyalella azteca* in 1986 and 1987



Figure 4.5. Relationship between Egg Number and Egg Weight in Hyalella azteca in 1986 and 1987



Figure 4.6. Relationship between Number of Young and Young Weight in *Hyalella azteca* in 1986 and 1987

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	Size group (mm)						
	≤€	3.5	≥'	7.6			
Variable	. 1986	1987	1986	1987	1986	1987	
N	13	9	40	46	3	10	
Range	3-34	14-35	4-40	13-52	9-42	20-48	
Mean	19.54	25.78	25.78	32.5	30.33	35.60	

Table 4. 2. Number of eggs in *Hyalella azteca* from Stephenson's Pond. (N = number of females investigated).

Table 4. 3. Number of young in *Hyalella azteca* from Stephenson's Pond. (N = number of females investigated).

	Size group (mm)							
	$\leq 5$	5.5	5.6 -	6.5	6.6 -	7.5	$\geq 2$	7.6
Variable	1986	1987	1986	1987	1986	1987	1986	1987
N	3		25	5	21	24	5	8
Range	8-17		8-33	6-34	8-33	4-18	14-36	11-45
Mean	12.33		18.88	19.2	20.95	22.7	27.4	24.88

eggs and juveniles were found in the same marsupium of the females.

### 4.3.5. Growth

Field growth rates were determined from the mean size of recognizable cohorts (Figure 4. 2). For the 1984 generation, the growth rate estimate of 0.086 mg/day was obtained from April through August 1985. An average growth rate of 0.043 mg/day was recorded for the 1985 generation from June 1985 through August 1986. For young hatched in 1986, the growth rate was 0.097 mg/day for the first two monthsafter hatching. The highest growth rates were found from May to June for adults before reproduction, and from July to September for new-born animals. No growth (or degrowth) occurred during winter.

Growth rates of individuals in the laboratory were determined by regression analyses (Figures 4. 7, 4. 8 and 4. 9). The growth rates of H. azteca fed on different food items followed a linear function. The regression coefficients for the relationships determined between wet weight (mg) and time (days) are shown in Table 4. 4.

With the exception of *H. azteca* fed on *P. richardsonii* leaves, the regression slopes (coefficients of growth) are all significantly different from zero (p <0.05), revealing that growth occurred during the experimental period at each of the three temperatures.

In terms of specific growth rates, multiple-factor ANOVA detected significant differences among the growth rates of H. azteca fed on different food items at



Figure 4.7. Growth Rates of Hyalella azteca when Fed on Fresh Macrophytes Potamogeton richardsonii under ad libitum Food Conditions at 10, 15 and 20°C



Figure 4.8. Growth Rates of Hyalella azteca when Fed on Salix sp. Leaves under ad libitum Food Conditions at 10, 15 and 20°C



Figure 4.9. Growth Rates of Hyalella azteca when Fed on Conditioned Plant Detritus under ad libitum Food Conditions at 10, 15 and 20°C

Table 4. 4. Regression coefficients for relationships between wet weight and growing time for *Hyalella azteca* fed on three food types (1: Salix sp. leaves; 2: Potamogeton richardsonii leaves and 3: conditioned plant detritus) at three temperatures (10, 15 and 20 °C). N: number of measurements; a: intercept; b: slope (growth rate); r: correlation coefficient; p: probability,  $p \leq 0.01$ , very significant;  $p \leq 0.05$ , significant,  $p \geq 0.05$ , not significant.

Food type	Temp (°C)	N`	a	b	r	р
	10	7	9.85	0.0195	0.979	$\leq$ 0.01
1	15	7	8.46	0.0291	0.974	$  \le 0.01  $
	20	7	8.49	0.0470	0.993	$\leq 0.01$
	10	5	10.55	-0.0014	-0.772	$\geq 0.05$
2	15	5	10.31	0.0097	0.792	$\geq 0.05$
	20	5	9.63	0.0100	0.784	$\geq 0.05$
	10	5	9.89	0.0152	0.941	$\leq 0.05$
3	15	5	10.53	0.0227	0.932	$\leq$ 0.05
	20	5	9.58	0.0260	0.910	$\leq 0.05$

different temperatures. One-way ANOVA showed that there were significant differences in growth rates of *H. azteca* fed on the three food items (F = 4.22, p <0.05). Growth rates of *H. azteca* fed on *P. richardsonii* leaves were significantly lower than those fed on *Salix* sp. leaves or conditioned plant detritus. However, no differences were found between the growth rates of *H. azteca* fed on *Salix* sp. and conditioned plant detritus. The effects of temperature on specific growth rates are also significant (F = 3.85, p <0.05). Growth rates at  $10^{\circ}C$  were significantly lower than those at  $20^{\circ}C$ , but not significantly different from those at  $15^{\circ}C$ .

One-way ANOVA only showed significant differences among specific growth rates in the four weight groups for *H. azteca* fed on *P. richardsonii* leaves at 10 and  $15^{\circ}C$  (Table 4. 5). The smallest weight group grew much faster than groups 3 or 4, in which degrowth occurred. However, group 2 showed no differences in specific growth rate from groups 1, 3 and 4.

*Hyalella azteca* provided with pond water survived for two months at  $10^{\circ}C$  and one month at 15 and  $20^{\circ}C$ , during which periods, no growth occurred ( $10^{\circ}C$ , one-way ANOVA, F = 0.01, p >0.05;  $15^{\circ}C$ , paired t = 0.49, p = 0.66;  $20^{\circ}C$ , paired t = 0.24, p = 0.83).

### 4.3.6. Production

The mean population densities of *H. azteca* in 1985 and 1986 are presented in Table 4. 6. Since body weight was measured on preserved samples, the mean weight of each individual was adjusted to the normal weight by using a correction factor of 1.185, and dry weight was calculated by multiplying wet weight by 17.76% (Chapter 3).

The size-frequency method applied without correction for multiple cohorts (CPI) yielded an annual production value (P) as dry weight of 22.67  $g/m^2$  from a mean standing biomass of 6.69  $g/m^2$  in 1985 and 14.23  $g/m^2$  from a mean standing biomass of 4.41  $g/m^2$  in 1986. The CPI correction factor was obtained by dividing the mean cohort interval into the number of days in a year (365/414). If CPI is assumed to be equal in both years, the corrected production value is 19.99  $g/m^2$  in 1985 and 12.54  $g/m^2$  in 1986 (Table 4. 7).

The mean weight of the largest *H. azteca* in Stephenson's Pond was 22.5 mg (wet weight) in 1985 and 1986, while the mean weight of individuals at hatching was 0.322 mg (wet weight). Thus the cohort G was 4.247. The daily G was computed as the cohort G divided by the length of the growing season. Population histograms (Figure 4. 2) showed that the growing season in Stephenson's Pond was about 180 days (from May to October) in 1985 and 220 days in 1986 when the average daily G was 0.0193.

The daily production fell to a winter minimum of about 0.01 g dry  $wt/m^2$  in February, March and April, and then increased slowly until mid summer. From Table 4. 5. One-way analysis of variance and Duncan's multiple range test for growth rates of four weight groups of Hyalella azteca fed on three food types (1: Salix sp. leaves; 2: Potamogeton richardsonii leaves and 3: conditioned plant detritus) at three temperatures (10, 15 and 20 °C). Weight groups are ranked according to growth rate from the lowest at left to the highest at right. Growth rates of weight groups underscored by the same line do not differ significantly. Significance (p) is tested by an F-test.

Food type	T°C	F	р	Duncan's multiple range test
	10	1.5	$\geq 0.05$	<u>4 2 3 1</u>
1	15	0.98	$\geq 0.05$	<u>4 3 1 2</u>
	20	0.12	$\geq 0.05$	<u>3214</u>
	10	5.52	≤0.05	<u>3 4 2 1</u>
2	15	4.19	$\leq 0.05$	4 2 3 1
	20	2.23	≥0.05	<u>4 3 2 1</u>
	10	0.44	≥0.05	<u>4321</u>
3	15	2.95	≥0.05	<u>3421</u>
	20	1.83	$\geq 0.05$	<u>3421</u>

Table 4. 7. Annual production and mean annual standing stock  $(g/m^2, dry weight)$ , mean annual density  $(No./m^2, and annual P:B ratio for$ *Hyalella azteca*in Stephenson's Pond.

<u> </u>	Calculated	adjusted	Standing	Mean	P:B
Year	production	production	stock	density	ratio
1985	22.67	19.99	6.69	5814.63	3.05
1986	14.23	12.54	4.41	3641.71	3.23

Table 4. 6. Mean density  $(No./m^2)$  and estimated mean dry weight per individual (mg) in each weight class (mg, wet weight) of *Hyalella azteca* in Stephenson's Pond.

		Mean	density
Weight class	Mean dry wt	1985	1986
$\leq 2.00$	0.178	785 <sup>.</sup>	448
2.00 - 4.00	0.533	747	506
4.00 - 6.00	. 0.888	1206	727
6.00 - 8.00	1.243	1364	681
8.00 - 10.00	1.598	892	478
10.00 - 12.00	1.954	361	351
12.00 - 14.00	2.309	169	278
14.00 - 16.00	2.664	129	123
16.00 - 18.00	3.091	98	30
18.00 - 20.00	3.374	40	8
$\geq 20.00$	3.734	24	5

July in both years, the daily production increased very rapidly reaching over 0.1 g dry  $wt/m^2$  from August to December. The annual production was 19.65 g dry  $wt/m^2$  in 1985 and 9.54 g dry  $wt/m^2$  in 1986 (Table 4. 8), both of which are very close to the production estimate using the size-frequency method.

# 4.4. DISCUSSION

# 4.4.1. Seasonal Abundance and Mortality

The variation in seasonal abundance of the *H. azteca* population in Stephenson's Pond demonstrated a typical pattern of interaction between the environmental properties (mainly temperature) and population parameters (growth, reproduction and mortality). The population density of *H. azteca* in Stephenson's Pond showed a more than 20-fold increase between the minimum and the maximum (Figure 4. 1). The most abrupt changes in abundance occurred during July and August. The overwintering population however, was relatively constant. The high summer density can be due to the exploitation of favourable conditions for reproduction and growth. The low abundance during the winter can be contributed to low temperatures, low oxygen conditions due to the ice-cover, and low food availability. Similar changes were noted by Cooper (1965) and Hargrave (1970) who also found numerical maxima in the summer and numerical minima in the winter.

Table 4. 8. Production of Hyalella azteca (g dry weight/ $m^2$ ) between sampling dates in 1985 and 1986, calculated by cohort G method.

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	Prod	luction	•	Proc	luction
1985	daily	interval	1986	daily	interval
Apr. 12			Jan. 9		
	0.034	0.577		0.024	0.784
Apr. 29			Feb. 10		
	0.063	3.683		0.009	0.186
Jun. 26		0.100	Mar. 3	0.010	0.400
T. 1. 20	0.062	2.106	A	0.012	0.439
Jul. 30	0.076	9.064	Apr. 8	0.014	0.401
Aug 26	0.076	2.004	Mar 22	0.014	0.481
Aug. 20	0 145	5 363	Widy 22	0.020	0 4 0 4
Oct 2	0.140	0.000	Jun 11	0.020	
0000. 2	0.155	6.184	buint 11	0.047	0.651
Nov. 12			Jun. 25		
	0.105	2.314		0.045	0.996
Dec. 4			Jul. 17		
				0.056	0.724
			Jul. 30		
				0.115	1.722
			Aug. 14		,
				0.106	4.432
			Sep. 25		
		22.201			10,810
		19 65			9.54
ک Annual		19.65			9.54

Size-specific mortality operated most heavily after reproduction, *i.e.*, high post-reproductive mortality. In the laboratory, it was frequently observed that adults died soon after reproduction. The mortality risk of male adults was relatively higher than for females. However, the final period of mortality, at an average weight of 20 mg, probably resulted from ageing at the end of the life cycle. Some of the mortality over winter could be due to the oxygen conditions. Davies *et al* (1987) found that in Stephenson's Pond, over winter the water column is highly hyperoxic or anoxic for 120-160 days with anoxia at the water-substrate interface. Starvation could also be a factor causing mortality during the winter because metabolism could be very low.

# 4.4.2. Life Cycle and Reproduction

The general pattern for the life cycle of H. *azteca* in Stephenson's Pond can be summarized as follows. A new generation appears in late June and July which grows rapidly over the rest of the summer but more slowly or not at all over winter. Sexual maturity is reached after further rapid growth in May and early June. A proportion of adults die after breeding, but a proportion survive until the onset of winter. The maximum life span for *H. azteca* in Stephenson's Pond is about 414 days, which is very close to the life span of 412 days found by Lindeman and Momot (1983) in northern Ontario.

Hyalella azteca reproduces once a year in Stephenson's Pond, and is therefore semelparous. Begon et al. (1986) pointed out that a semelparous organism should put maximal investment into its offspring to increase fitness. The field data (Tables 4. 2 and 4. 3) indicated that females tend to increase fecundity with size. Therefore larger females lay more eggs and produce more offspring. This phenomenon has been noted in many aquatic crustaceans (Ridley and Thompson, 1985).

Hyalella azteca can allocate its energy resources spent on eggs into either producing many small or a few large eggs. This principle is also applied to young, because *H. azteca* broods young in the marsupium before they are released. Kolding and Fenchel (1981) presented a model for the development of an evolutionarily stable egg size based on the assumption of a fixed reproductive effort (egg size times egg number is constant) balanced between two opposing selective forces: to increase fecundity (total egg number) at the expense of egg size or increase juvenile survival and decrease fecundity by increasing egg size. The model predicts that the most favoured alternative is the one in which the offspring have the greatest summed reproductive value. According to this model, *H. azteca* in Stephenson's Pond take the first alternative way to increase fitness *i.e.* increase total egg number rather than egg size.

The reproductive season for *H. azteca* in the literature varies in different geographical areas. Gaylor (1921) recorded that the reproductive season in Indiana (U.S.A.) was from the end of June to the middle of August. In contrast, Mathias (1971) reported that females in Marion Lake (B.C.) were carrying eggs from late May until early September, and young were recruited into population from middle

of June to early September. These breeding periods overlap the reproductive season in Stephenson's Pond. Embody (1911), however, found the earliest date of breeding in New York (U.S.A.) was April and breeding continued for 152 days until the end of September. Jackson (1912) reported *H. azteca* in Wisconsin (U.S.A.) bred more or less continuously during the entire year, but with the period of greatest reproduction during the summer months. The onset of reproduction reported by Cooper (1965) in Michigan (U.S.A.) occurred in the middle or late May, and continued until September. Strong (1972) studied three populations in Oregon (U.S.A.) and found that the breeding season varied among populations: in Hunter Hot Springs the population reproduced year round, while the mountain and coastal lake populations only bred during the summer months. Lindeman and Mornot (1983) noted that the reproductive season of *H. azteca* in northern Ontario lasted from late May to late August. The geographic variation in breeding periods is summarized in Table 4. 9.

Temperature could be the main factor causing the geographic variations in breeding period. Comparison of data from Embody (1911), Cooper (1965), Mathias (1971) and Strong (1972) shows the onset of reproduction, as indicated by the first appearance of eggs in marsupiae, corresponds closely with  $16^{\circ}C$  (Table 4. 9). De March (1978) found in a study done on laboratory population of *H. azteca* the size at maturity of both male and female is dependent on temperature during development and the time to reach maturity is inversely correlated with temperature. Therefore the maturation time is longer than expected if early development

occurred at lower temperatures, and shorter if early development occurred at higher temperatures. Thus, it can be predicted that the breeding season should commence earlier in warm areas than in cold areas.

Cooper (1965) and Strong (1972) reported reproduction of *H. azteca* to be independent of photoperiod. However, De March (1977) found that in the laboratory photoperiod determines whether reproduction is continued or discontinued. He also noted that although photoperiod is the main cue to the induction and termination of reproduction, active reproduction takes place only at temperatures between 20 to  $26^{\circ}C$ . Therefore, the influence of photoperiod also depends on temperature. However, many *H. azteca* populations do not respond to photoperiod in the field probably because photoperiod changes are very small with season at those latitudes (De March, 1977).

Young *H. azteca* hatched in Stephenson's Pond in both 1985 and 1986 matured and bred in their second summer reproducing extensively over the optimal growth season. Similar results were recorded by Gaylor (1921) and Mathias (1971). This is somewhat different from the results of Lindeman and Momot (1983) who found that the first young produced can mature and breed in the same summer. This difference could be contributed to the delay in reproduction in Stephenson's Pond due to the low water temperatures over winter.

The fecundity of H. azteca in Stephenson's Pond falls into the range reported in the literature (Table 4. 9), but the average fecundity is much higher than in some geographic areas. A regression analysis of total egg weight or total

young weight against female body weight, fitting the line by the method of least squares, indicated a linear relationship with a very significant correlation coefficients for both years. Strong (1972) showed a correlation between marsupial clutch size and head length of the females for the three *H. azteca* populations he studied in Oregon (U.S.A.). This has also been demonstrated for other amphipods (Hynes, 1954,1955; Steele and Steele, 1975; Welton, 1979; Kolding and Fenchel, 1981).

### 4.4.3. Growth

Evidence from both field and laboratory data indicates that H. azteca has a great potential for growth. Field-derived growth rates from the three cohorts are much greater than would be predicted from the laboratory experiments at similar temperatures. This discrepancy probably results from some laboratory conditions which were sub-optimal for growth. The most likely explanation is that the quality of the food produced in the laboratory was not as high as is actually in the field. Growth rates during the winter slowed in the field and as de March (1978) showed that growth of H. azteca is highly dependent on temperature, this was at least partly due to the low water temperatures. Shortly before reproduction, the weights of individuals increased very rapidly, partially because the adults grew fast to reach maturation, and partially because the females were brooding eggs or young.

Laboratory growth experiments showed that temperature has an important effect on body size of *H. azteca*, which grew much faster at  $20^{\circ}C$  than at

 $10^{\circ}C$  with an optimum growth temperatures between 15 and  $20^{\circ}C$ . Numerous workers have also observed a positive correlation between growth rates of amphipods and water temperature (Nilsson, 1977; Welton and Clarke, 1980; Sutcliffe *et al.*, 1981), probably because temperature affects the bioenergetics of *H. azteca* as well as the quantity and (or) quality of its food. Temperature often directly affects the growth of amphipods by influencing the rate of feeding, assimilation and respiration, food conversion efficiencies, enzymatic kinetics and endocrine process (Sweeney and Vannote, 1981, 1984), or indirectly by altering the quantity (*e.g.* density and/or productivity of periphytic algae) and quality (*e.g.* microbial populations associated with detritus) of available food materials (Cummins and Klug, 1979).

The effects of food quality on the growth of H. azteca were demonstrated in the laboratory. Fast growth rates were recorded with H. azteca fed on fresh Salix sp. leaves and conditioned plant detritus, however no growth was found in H. azteca fed on P. richardsonii leaves, indicating that the growth rate of H. azteca depends on the type of food provided. The high growth rates exhibited by H. azteca fed Salix sp. leaves and conditioned plant detritus may be related to the degree of colonization by microbes. Anderson and Cummins (1979) and Ward and Cummins (1979) also found that there is a positive correlation between selective feeding and density of microbial flora, nitrogen content and respiration rate and ATP content per unit mass of food items. On the other hand, the physical conditions of the food type could also be important for the growth of H. azteca .

Sutcliffe *et al* . (1981) found that the thickness of leaf is important in determining palatability, perhaps because thin leaves soften more quickly when submerged in water and are therefore more easily bitten or scraped by the animals (Martin, 1964, 1966), perhaps also because of differences in chemical constituents, *e.g.*, polyphenols and tannins (Lyford, 1943).

The daily increase in weight of H. azteca in Stephenson's Pond were linear, similar to the results of Lindeman and Momot (1983). However, when growth rate of H. azteca in Stephenson's Pond was expressed as specific growth rate, nonlinear growth was found in H. azteca fed on fresh P. richardsonii leaves. This is probably because large individuals degrew when fed on this type of food, and small individuals appeared to grow relatively much faster.

### 4.4.4. Production Estimates

The estimates of production (199.9 Kg/ha/year, dry wt in 1985 and 125.4 Kg/ha/year, dry wt in 1986) for *H. azteca* in Stephenson's Pond are ten times higher than than the range of 11.5 - 19.3 Kg/ha/year quoted by Waters (1977) in his review of secondary production. The P/B ratios for *H. azteca* in Stephenson's Pond of 3.05 in 1985 and 3.23 in 1986 are lower than the range of 4.2-4.8 quoted by Waters (1977). Production of *H. azteca* in Stephenson's Pond is the highest recorded (Table 4. 10).

The size-frequency technique, when applied to production as a whole, yields accurate estimates of annual production (Waters, 1981). Therefore, the use of the

Table 4. 9. Geographic variation in breeding period and fecundity of *Hyalella azteca* in North America. (AR = Authority; PB = Breeding Period; BT = Breeding Temperature (°C) and FT = Fecundity (No./female)).

AR	BP	BT	FT
Embody (1911)	AprSpt.	14-20	6-32
Gaylor (1912)	Jun-Aug	_	18-44
Jackson (1912)	whole year	_	_
Cooper (1965)	May-Sept.	$\geq 20$	1-50
Mathias (1971)	JunSept.	-	40
Strong (1972)	year round	12-40	3-27
Strong (1972)	summer months	$\geq 12$	2-25
Lindeman (1983)	May-Aug.	16-20	_
Present study	JunAug.	15-20	4-48

Table 4. 10. Comparison of annual production (P,  $g/m^2/year$ . dry weight), annual biomass (B,  $g/m^2$  and annual P/B ratios for five populations of *Hyalella azteca*. (Annual data from Stephenson's Pond were calculated based on samples taken from Apr. 1985 to Mar. 1986).

Locality	P	B.	P/B	Authority
Sugarloaf Lake	1.93	0.40	3.9	Cooper (1965)
Michigan				
West Blue Lake	1.20	0.26	4.6	Biette (1969)
Manitoba				
Marion Lake	1.15	0.27	4.2	Mathias (1971)
British Columbia				, , ,
Dock Lake	1.23	0.32	3.9	Lindeman and
Ontario	-			Momot (1983)
Stephenson's Pond	16.26	5.55	3.1	This study
Alberta				v

size-frequency method in this study is appropriate and the advantage of this method is that it permits the estimation of production for populations that, as result of their particular life history, cannot be estimated with other traditional methods. Hynes (1961) and Hynes and Coleman (1968) presented the method to provide estimates only within an order of magnitude and they intended it to be used on entire fauna or groups of species. In practice, however, the method has been more commonly used on single species. Many investigations on *Gammarus* production, comparing estimates from the size-frequency method and other methods, yielded comparable results (Welton, 1979; Marchant and Hynes, 1981; Waters, 1981; LaFrance and Ruber, 1985). Therefore Waters (1981) suggested that this method can be well accepted as a standard in secondary production research.

There are, however, a number of shortcomings in the estimate of production of *H. azteca* in Stephenson's Pond. Firstly, production lost through molting has not been calculated. This will result in a cumulative underestimate of dry weight which is most serious for the bigger size classes which have molted the most. However, because these size groups contribute least to production this error is probably not serious. Mathias (1971) showed that molting only accounted for about 2.26% of the annual energy utilization.

Secondly, the production estimate did not include the production of eggs lost by females, which could underestimate production. Mathias (1971) estimated the eggs lost with females to be 15.2% in shallow water, 29.1% in medium water and 30.6% in the deep water of Marion Lake (B.C.). However, no data on egg loss is

available from Stephenson's Pond. The eggs accounted for only a small percentage of total production, and thus increase in egg number is unlikely to alter the total significantly. The last potential error in the production calculating is in the estimate of population density. Samples were taken for only seven months in 1985 and nine months in 1986. Data for other months, especially winter months, are not available and therefore the production is probably overestimated by a small amount.

Waters (1979) emphasized that temperature is an important factor in the life history of benthic invertebrates, because it influences features such as growth, voltinism, and length of aquatic life, all critical to secondary production. Lindeman and Momot (1983) compared annual production, biomass and P/B ratios of four populations of *H. azteca*, and found that production and biomass were significantly correlated with the water temperature (number of days above  $10^{\circ}C$ and above  $20^{\circ}C$ ). If the temperature data from Stephenson's Pond are included, both biomass and production do not fit the regression line (Figure 4. 10), indicating that other key factors affect the biomass and production of *H. azteca* in Stephenson's Pond. Comparing the data with those in the literature, it is clear that the differences in annual production by *H. azteca* are primarily due to the differences in population densities rather than to differences in the growth rates of individuals. The high population density level in this hypereutrophic pond reflects its high carrying capacity.



Figure 4.10. Comparison of Production of Hyalella azteca in North America

# Chapter 5

# HABITAT SELECTION

### 5.1. INTRODUCTION

Microhabitat utilization by benthic fauna depends upon the interaction of numerous physical and biological factors (Rabeni and Minshall, 1977; Adams, *et al.*, 1987). The main physical factors include temperature, disturbance, water velocity, water chemistry, substrate type, food quality and food abundance. The important biological factors are reproduction, crowding, predation, competition, parasites and the uneven dispersion pattern of the organisms (Chutter, 1969; Cummins and Lauff, 1969; Bovbjerg, 1970; Barber and Kevern, 1973; Ambrose, 1984 and 1986). These factors can act singly or in combination (Hynes, 1970), and either directly or indirectly (Wetzel, 1983).

Over the evolutionary process, organisms have developed adaptations to respond to these factors in a preferential manner. Therefore, the optimum conditions will be different for each species, and sometimes even for different life stages of the same species. Natural selection favours individuals that use habitats in which most progeny can be produced most successfully (Krebs, 1985). Individuals that choose poorer, marginal habitats will not produce as many progeny and will likely be selected against.

Considerable work on habitat selection of amphipods has been conducted by examining the factors influencing the microdistribution under laboratory conditions, then predicting the microdistribution in the field, e.g. Rees (1972) observed the distribution of Gammarus pseudolimnaeus Bousfield as influenced by oxygen concentration, substratum and water velocity and then extrapolated his findings to field populations. Williams and Moore (1982) examined the activity response of G. pseudolimnaeus to substrate size, current velocity and light regime in the laboratory, and based on these data predicted the distribution of G. pseudolimnaeus in the field. Similarly Thompson and Moule (1983) and Adams et al. (1987) studied the habitat choice behaviour of Gammarus pulex (L.) in an artificial stream, and found that the microdistribution is size assortative: larger animals were associated with larger substrate particles, small animals with smaller substrate particles. DeWitt (1987) looked at the effects of food availability, sediment thickness, and topographic relief on the microdistribution of Microdeutopus gryllotalpa Costa, and found that higher food concentration, deeper sediment, and greater topographic relief affected habitat selection.

Comparatively, little work has been done on patch selection by amphipods in the field. A simple approach to investigating habitat selection in the field is to determine correlations between population variables and measured environmental variables, and then see if these can explain the general distribution patterns observed. Gee (1982) used multiple linear regression to relate the number of *G. pulex* with certain organic and physical characteristics of the stream

substratum. Pringle (1982) and Williams and Moore (1986) working with G. pulex and G. pseudolimnaeus respectivity analyzed their field data and found very significant correlations between substrate particle size and body size.

In this chapter, habitat utilization by *H. azteca* was examined by comparing seasonal and spatial distribution patterns of in the field. Some phenomena observed in the field were tested by laboratory experiments. The objectives of this chapter were:

- to determine the spatial distribution patterns of *H. azteca* in Stephenson's Pond;
- (2) to understand whether these patterns are stable or liable to change with season and between years;
- (3) to determine if the distribution of *H. azteca* is homogeneous or heterogeneous horizontally and vertically; and
- (4) to determine whether there is sufficient evidence to indicate the primary factors resulting in differential habitat utilization.

### 5.2. MATERIALS AND METHODS

The field sampling program was described in detail in Chapter 2. Macrophytes were oven-dried ( $60^{\circ}C$ ) to a constant weight and weighed to the nearest 0.1 mg.

The animals used in the substrate preference tests were collected from Stephenson's Pond using a hand net, transported to the laboratory in plastic buckets. They were acclimatized in aerated water at  $20^{\circ}C$  with a supply of fresh macrophytes for one week and starved for one day before the start of the experiments.

The experiments were conducted in a circular (75 cm diameter), plexi-glass apparatus (Figure 5. 1), which contains 36 compartment (each 7 cm in diameter and 8 cm deep) of equal area and volume. Each compartment was filled with one substrate type. The advantage of the circular shape used in the experiment over the rectangular trough used by Hargrave (1970) is that there is no edge effect, and *H. azteca* do not congregate in the corners. This allows dispersal tendencies (row and column effects) to be measured simultaneously with substrate selection (treatment differences) (Gale, 1971; Wrona, 1985).

The substrates types used were: mud (0.5 - 2.0 mm in diameter), rocks (15 - 40 mm in diameter) and macrophytes (*Potamogeton richardsonii*) (30 - 180 mm in length), which represent the main substrate types in Stephenson's Pond. The substrates were arranged in the apparatus with four replicates of a modified 3X3 Latin Square statistical design, permitting each substrate to be preceded four times by the other two substrates, and enabling measurement of residual or carryover effects (Gale, 1971).

During the experimentation, temperature was maintained at  $20^{\circ}C$  and lighting was maintained on a diurnal, sideral clock geared to a 14 hr light: 10 hr dark photoperiod. Two replicate apparati were placed in a water table filled with pond water covering the substrates to a depth of about 20 cm. At the start of each



Figure 5.1. Distribution of Three Substrates in the Apparatus

experiment, 150 *H. azteca* in different size groups were placed in the center of each replicate apparatus, and allowed to disperse into the various substrates. Each experiment lasted four days. At the end of each experiment, the substrates were removed and the animals present in each substrate type counted with the column and row noted. Animals not found within a substrate compartment were captured using small hand net and enumerated. The counting was carried out in day or night.

To investigate whether H. azteca use macrophytes as a food resource as well as a shelter, plastic macrophytes were used to replace P. richardsonii in the apparati. Two differently treated plastic macrophytes were used, 1), conditioned for one week in the apparatus and 2), soaked in 90% alcohol for 24 hr to kill the epiflora and fauna and then thoroughly washed in distilled water. If amphipods chose conditioned plastic macrophytes and fresh macrophytes equally, this indicates that H. azteca feeds on the epiflora and/or epifauna as well as using them for shelter; if, however, H. azteca selected fresh macrophytes and conditioned plastic macrophytes over alcohol-treated plastic macrophytes, this indicate that H. azteca chooses macrophytes primary as a food resource rather than as a shelter. Based on these observations, several related hypotheses were tested. The data analyses of the Latin Square experimental design were carried out by a computer program written by Wrona (1982).

### 5.3. RESULTS

# 5.3.1. Spatial Heterogeneity

The monthly fluctuations of density and biomass of H. azteca in the water column and substrate during open water period are shown in Figures 5. 2 and 5. 3. Both density and biomass of H. azteca in the benthic samples showed similar trends. High densities and biomasses occurred from October to December in 1985, and from August to September in 1986. However, maximum density and biomass in the water column samples occurred in July and August in 1985 and 1986.

Significant correlations occurred between density and biomass in the benthic samples and those in the corresponding water column samples (for density, r=0.546; for biomass, r=0.599; N=200, P<<0.01) An F-test showed that the slopes of both regression lines were significantly different from zero (for density, F = 84.68; for biomass, F = 91.13, p << 0.01), indicating that the abundance of *H. azteca* in the water column was much higher than in the benthic samples during the open water period (Figures 5. 2 and 5. 3).

Most of the juveniles, especially newly hatched animals, stayed in the water column rather than settled in or on the substrate (Figure 5. 4). This was clearly shown by the higher percentage distribution of the first weight class in late June and July of both years. After growing larger *H. azteca* tended to move on to the substrate, although the largest mature animals also remained in the water column.





Figure 5.2. Density Changes of Hyalella azteca in Water Column and Benthos during Open Water Period in 1985 and 1986





Figure 5.3. Biomass Changes of Hyalella azteca. in Water Column and Benthos during Open Water Period in 1985 and 1986










Figure 5.4. Proportional Distribution of Hyalella azteca Body Weights in Benthos (B) and Water Column (W) in 1985 and 1986

The distributions of both density and biomass of H. azteca with depth during the sampling period are shown in Figure 5.5 and 5.6. The highest abundance was recorded from a water depth of 0.9 to 1.2 m. Abundance decreased as depth increases with the lowest abundance in the deeper zone. Both density and biomass were negatively correlated to depth, with significant correlations in all sampling months except April and May in the benthic samples and July, 1985 in the water column samples (Tables 5. 1 and 5. 2).

### **5.3.2.** Horizontal Variations

The annual average density and biomass of *H. azteca* in the three sampling zones (Figure 2. 1 in Chapter 2) showed very significant differences over the study period (Figure 5. 7 and 5. 8, one way ANOVA, for density, F = 32.77, p << 0.01; for biomass, F = 46.45, p << 0.01). Multiple comparison indicated that the total (combined) density and biomass in the deep zone was significantly lower than those in the two shallow zones, but there were no significant differences in density and biomass between the two shallow zones (shaded and unshaded).

In the deep water zone, both density and biomass in the water column were not significant different from those in the benthos (paired t-test, for density, t = 0.42, p = 0.68; for biomass, t = 0.56, p = 0.58; df = 227). In the two shallow zones, the differences in density and biomass in the water column and the benthos were very significant. In the shallow shaded zone the mean density and biomass in the water column were almost four times higher than those on the substrate (for



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Figure 5.5. Density Distribution of Hyalella azteca with Depth in 1985 and 1986



Figure 5.6. Biomass Distribution of Hyalella azteca with Depth in 1985 and 1986



Sampling Zone



.



Sampling Zone

Figure 5.8. Mean Biomass of Hyalella azteca in Three Sampling Zones in 1985 and 1986 (D: Deeper Zone; SS: Shallow Shaded Zone and SU: Shallow Unshaded Zone)

Table 5. 1. Correlation coefficients (r) of density and biomass of *Hyalella azteca* vs. depth for benthic samples. VS = very significant ( $p \le 0.01$ ), S = significant ( $0.05 \ge p \le 0.01$ ), NS = not significant ( $p \ge 0.05$ ). N = sampling size.

Year	Month	N	Density	p	Biomass	p
	Apr.	37	-0.144	NS	-0.147	NS
	Jun.	24	-0.504	S	-0.574	VS
	Jul.	24	-0.846	VS	-0.675	VS
1985	Aug.	22	-0.762	VS	-0.639	VS
	Oct.	24	-0.849	VS	-0.814	VS
	Nov.	24	-0.746	VS	-0.708	VS
	Dec.	24	-0.723	VS	-0.717	VS
	Jan.	24	-0.895	VS	-0.898	VS
	Feb.	22	-0.795	VS	-0.834	VS
	Mar.	24	-0.745	VS	-0.754	VS
	Apr.	23	-0.762	VS	-0.758	VS
1986	May	18	-0.294	NS	-0.279	NS
	Jun.	36	-0.833	VS	-0.886	VS
	Jul.	36	-0.928	VS	-0.929	VS
	Aug.	18	-0.582	VS	-0.596	VS
	Sep.	18	-0.832	VS	-0.767	VS

density, t = 3.14, p <0.01; for biomass, t = 4.99, p <<0.01; df =71). However, in the shallow unshaded zone the difference was only three times (for density, t = 3.53, p <0.01; for biomass, t = 3.25, p <0.01; df = 77).

Both the density and biomass of *H. azteca* in the east and west basins were not significantly different from each other (Figure 5. 9 and 5.10). A t-test showed no significant differences in density (t =0.69, p = 0.49, df = 335) and biomass (t = 1.34, p = 0.18, df = 335) in benthic samples and no differences in density (t = 1.55, p = 0.12, df = 231) and biomass (t = 1.41, p = 0.16, df = 231) in the water column samples.

### 5.3.3. Association With Macrophytes

The association of H. azteca with macrophytes was studied in the summer of 1986 (Table 5. 3). Both density and biomass before and at the end of reproduction were significantly correlated with the dry biomass of macrophytes. However, during the breeding period no significant correlation between the abundance of H. azteca and macrophytes was found.

#### 5.3.4. Substrate Preference

### 5.3.4.1. Experiments 1 and 2

The first hypothesis for substrate preference was that H. azteca choose macrophytes mainly as a substrate to attach to or rest on rather than as a food



Basin

Figure 5.9. Mean Density of Hyalella azteca in Two Basins in 1985 and 1986 (EAST: East Basin and WEST: West Basin)



Figure 5.10. Mean Biomass of Hyalella azteca in Two Basins in 1985 and 1986 (EAST: East Basin and WEST: West Basin)

Table 5. 2. Correlation coefficients (r) of density and biomass of *Hyalella azteca* vs. depth for water column samples. VS = very significant ( $p \le 0.01$ ), S = significant ( $p \le 0.05$ ), NS = not significant ( $p \ge 0.05$ ). N = sampling size.

Year	Month	N	Density	p	Biomass	р
	Jun.	21	-0.712	VS	-0.755	VS
	Jul.	35	-0.285	$\mathbf{NS}$	-0.264	NS
1985	Aug.	36	-0.405	S	-0.361	S
	Oct.	24	-0.840	VS	-0.840	VS
	Nov.	24	-0.825	VS	-0.841	VS
	May	18	-0.464	S	-0.552	VS
	Jun.	36	-0.846	VS	-0.881	VS
1986	Jul.	36	-0.942	VS	-0.947	VS
	Aug.	18	-0.922	VS	-0.924	VS
	Sep.	6	-0.768	S	-0.760	S

Table 5. 3. Correlation coefficients (r) between density and biomass of *Hyalella azteca* and dry biomass of macrophytes in 1986. VS = correlation coefficient is very significant from 0 (p  $\leq 0.01$ ); S = significant (p  $\leq 0.05$ ); NS = correlation coefficient is not significant from 0 (p  $\geq 0.05$ ) by an F-test. N = sampling size.

[		Density			Biomass		
Date	N	r	F	p	i r	F	р
Jun. 6	7	0.95	43.84	VS	0.88	17.94	VS
Jun. 25	12	0.89	36.07	VS	0.29	0.94	NS
Jul. 17	10	0.34	1.02	NS	0.48	<sup>·</sup> 2.39	ŃS
Jul. 30	10	0.14	0.15	NS	0.24	0.47	NS
Aug. 14	11	0.94	65.91	VS	0.95	67.52	VS

resource. To test this hypothesis, two experiments were conducted in the laboratory.

In the first experiment, conditioned (20 days,  $10^{\circ}C$ ) macrophytes (*Potamogeton richardsonii*), conditioned plastic macrophytes (one week in apparati) and alcohol-treated plastic macrophytes were used as substrates. The experimental results (Figure 5. 11) show that there was no difference in preference between conditioned macrophytes and conditioned plastic macrophytes during the day (chi-square = 0.03, p >0.10). This indicates that conditioned plastic macro-phytes and conditioned macrophytes are equally utilized by *H. azteca*. The significantly lower numbers (chi-square = 33.43, df = 2, p < 0.001) on alcohol-treated plastic plants indicates that while equally suitable as resting sites the absence of epiflora was important. The data collected during the night showed that *H. azteca* has no preference for any of the three substrate types provided (Figures 5. 13, chi-square = 4.1, df = 2, P < 0.001), suggesting that *H. azteca* selected resting site at night without reference to feeding potential.

In the second experiment, fresh macrophytes were substituted for alcoholtreated plastic macrophytes. The results (Figure 5. 12 and table 5. 4) showed that *H. azteca* preferred conditioned plastic macrophytes and conditioned macrophytes over fresh macrophytes during the day. This indicates that fresh macrophytes are not a suitable food resource compared to the other two types of substrates, and supported by the results of the growth experiments (Chapter 4) where *H. azteca* was shown to grow very well on a diet of detritus, but poorly when fed fresh

Table 5. 4. Comparisons of substrate preferences of Hyalella azteca for conditioned plastic macrophytes (CPP), conditioned macrophytes (CP) and fresh macrophytes (FP).

r	Day			Night			
Substrate	Chi-square	df	р	Chi-square	df	р	
CPP/CP/FP	39.12	2	$\leq 0.001$	25.62	2	$\leq 0.001$	
CPP/CP	0.004	1	$\geq 0.10$	24.17	1	$\leq 0.001$	
CPP/FP	32.90	1	$\leq 0.001$	6.59	1	$\leq 0.05$	
CP/FP	32.90	1	$\leq$ 0.001	5.50	1	$\leq 0.05$	



Figure 5.11. Substrate Preference of Hyalella azteca for Conditioned Plastic Macrophytes (CPP), Alcohol-Soaked Macrophytes (APP) and Conditioned Macrophytes (CP)





Figure 5.12. Substrate Preference of Hyalella azteca for Conditioned Plastic Macrophytes (CPP), Fresh Macrophytes (FP) and Conditioned Macrophytes (CP)

macrophytes. However, *H. azteca* preferred fresh macrophytes over conditioned macrophytes at night, indicating that fresh macrophytes was a more suitable shelter than the conditioned macrophytes.

Chi-square analysis also showed that in each experimental run, the number of animals found in the substrates were significantly higher than those not found in the substrates (chi-square test, p < 0.001). These experimental results are identical with those obtained from analysis of variance. No significant row and column effects were detected (p >0.05), indicating that animals dispersed equally in all directions in all experimental trials. However, analysis of variance showed significant differences in the effects of substrate treatments (p < 0.01) except for the first experimental run at night (p > 0.05).

## 5.3.4.2. Experiment 3

In this experiment, the preference of H. azteca for three substrates: fresh macrophytes, rocks and mud was examined. Since substrate preference may change in different life stages or between sexes, experiments were carried out for combined size group of non-reproductive individuals, single reproductive males and females, and precopulatory pairs of H. azteca . The hypothesis tested was that H. azteca does not discriminate among the three substrates.

Chi-square test showed that the number of animals recorded in the substrates was significantly higher than recorded in the water both during the day and at night for each experiment (p<0.001), indicating that the animals showed preference for

substrates during the experiments. The analysis of variance showed no statistical difference in the column and row effects (p > 0.05), confirming that the animals distributed randomly in all directions in the apparati. A significant difference in treatment effects (p < 0.01) was recorded for each experiment except on the combined size group at night (p > 0.05), indicating that *H. azteca* preferred some substrate types.

The comparison of preference displayed by *H. azteca* for the three substrates displayed by *H. azteca* is illustrated in Figures 5. 13, 5. 14, 5. 15 and 5. 16, and the results of the chi-square analyses are shown in Table 5. 5. *H. azteca* selected for macrophytes and rocks equally but the mud substrate was not usually utilized. However, equal preference for all three substrates was showed by combined size group of non-reproductive animals at night.

## 5.4. DISCUSSION

During the open water period in Stephenson's Pond both the density and biomass of *Hyalella azteca* in the water column were higher than in the benthos. This vertical pattern is comparable to that of some species of *Gammarus* (Marchant and Hynes, 1981; Gee, 1982, Williams and Moore, 1982, 1986) and other invertebrates (Davies and Everett, 1977). The significant correlation between abundance in the water column and in the benthos (Figure 5. 2 and 5. 3) indicates that *H. azteca* frequently migrate vertically in the summer, and Hargrave (1970) showed that *H. azteca* can move extensively by swimming.

Table 5. 5. Comparisons of substrate preference displayed by *Hyalella azteca*. Substrate types are macrophytes (P), rocks (R) and mud (M). The test animals are all size group of nonreproductives (All), single reproductives (Male and Female) and precopulatory pairs (Pair). The relative magnitude of preference is ranked from the lowest at left to the highest at right. Preferences underlined by the same line are not significantly different  $(x^2-test)$ .

		Day		Night			
Group	x <sup>2</sup>	р	p Preference		р	Preference	
All	11.22	≤0.01	<u>M</u> <u>R</u> <u>P</u>	1.09	$\geq 0.10$	MRP	
Male	32.84	$\leq 0.001$	<u>M R P</u>	14.66	$\leq$ 0.001	<u>M R P</u>	
Female	28.67	$\leq 0.001$	<u>M R P</u>	24.13	$\leq 0.001$	MRP	
Pair	31.85	≤0.001	MRP	30.93	$\leq$ 0.001	<u>M R P</u>	





Substrate Type

Figure 5.13. Substrate Preference of Non-reproductives for Fresh Macrophytes (P), Rocks (R) and Mud (M)



Substrate Type

Figure 5.14. Substrate Preference of Reproductive Males for Fresh Macrophytes (P), Rocks (R) and Mud (M)



Figure 5.15. Substrate Preference of Reproductive Females for Fresh Macrophytes (P), Rocks (R) and Mud (M)



Figure 5.16. Substrate Preference of Precopula Pairs for Fresh Macrophytes (P), Rocks (R) and Mud (M)

The density and biomass of H. azteca were not significantly different between the two basins of Stephenson's Pond in two successive years. This is probably due to the similar morphological features and the habitat homogeneity within the two basins. The maximum depth in the two basins is about the same (2.5 m), and macrophytes cover both shallow areas. Although the west bank of the west basin is weedy and the east bank of the east basin is rocky, laboratory experiments showed that H. azteca does not discriminate between macrophyte and rock substrates.

Laboratory experiments also indicate that H. azteca discriminates between macrophyte and mud substrate. This is consistent with field results. In the three sampling zones, both density and biomass in the two shallow macrophytes zones were not significantly different, but they are significantly higher than in the deep water, mud zone. In addition, the absence of differences in density and biomass between the two shallow zones suggests that shading has no significant effects on the distribution patterns of H. azteca . Thus the presence of allochthonous leaves and submerged tree trunks do not drastically influence the distribution of H. azteca .

The depth distribution of H. azteca is probably related to the food distribution in Stephenson's Pond. The highest abundance of H. azteca occurred from 0.9 to 1.2 m (Figure 5. 5 and 5. 6), overlapping the macrophyte zone. Deeper than 1.2 m there are no macrophytes because light transmission is low, and food availability is low. Hargrave (1970) also found that food resource is an important factor

determining H. azteca distribution with significant correlations between epibenthic primary production and number of H. azteca both in the laboratory and the field. This suggest that the depth distribution of H. azteca is closely correlated to the attenuation of light and decrease in epibenthic algal production with depth.

The substrate preference experiments also showed that *H. azteca* selected conditioned macrophytes and conditioned plastic macrophytes equally during the day, but rarely used alcohol-treated plastic macrophytes as resting sites. This suggests that *H. azteca* feeds mainly on the macrophyte epiflora. When fresh macrophytes were provided, *H. azteca* also fed and this feeding behaviour was also observed in the field by Wagner and Blinn (1987). Given the choice of conditioned macrophytes, conditioned plastic macrophytes and fresh macrophytes, *H. azteca* less frequently selected fresh macrophytes probably due to the low abundance of epiphytic algae. Fresh macrophytes were, however, suitable as shelter for *H. azteca* during the night, perhaps as a refuge from predation (France and LaZerte, 1987).

The mechanism involved in substrate preference decision making is also possibly related to the particle size of the substrates. Numerous authors have reported that particle size is the most important variable determining the numerical distribution of *Gammarus* (Gee, 1979; Pringle, 1982; Thompson and Moule, 1983; Williams and Moore, 1986; Adams and Greenwood, 1987). Generally the greatest number of amphipods settle in microhabitats with large substrate particles. In

substrate preference experiments, the particle size of macrophytes and rocks was larger than of mud. Thus, in the experiments H. azteca also displayed a size assortative pattern.

Gates and Davies (1987) reported that temperature has a major influence on the depth distribution of *Nephelopsis obscura* Verrill and *Erpobdella punctata* (Leidy). This might explain the correlation of seasonal changes on the depth distribution of *H. azteca* in both the benthos and the water column (Tables 5. 1 and 5. 2). No significant correlations between depth and *H. azteca* abundance were recorded in April and May in the benthos. In these months water temperature was increasing, and the amphipods became very active and moved from the bottom into the water column. The low correlation coefficients between depth and the numbers in the water column from August to September could be due to the drop in water temperature resulting in *H. azteca* leaving the water column and settling on the bottom substrate. The reason for the low correlation recorded in July 1985, in the water column samples is unknown.

The high association of H. azteca with macrophytes before breeding could be predicted by the substrate experiment which showed that pairs in precopula preferred macrophytes over rocks and mud. This oviposition habit has been reported for many animal species (Wetzel, 1983). Shortly after onset of reproduction, the density of H. azteca was significantly correlated with the dry biomass of the macrophytes. For H. azteca biomass, the correlation with dry biomass of macrophyte was not significant, probably because of the greater heterogeneity of individual size of the offspring and the adults at this time. During the breeding period, because most young are free swimming and stay in the water column (Figure 5. 4), no significant correlation was found. After growing larger, *H. azteca* displayed substrate preference, again resulting in a high correlation with macro-phyte biomass. A close association of *H. azteca* with macrophytes was also found by Cooper (1965), Lindeman and Momot (1983), Wagner and Blinn (1985) and France and LaZerte (1987).

# Chapter 6

# SEXUAL DIMORPHISM AND PRECOPULATORY MATE GUARDING

# 6.1. INTRODUCTION

Sexual size dimorphism in a species implies that the two sexes experience somewhat different ecological or reproductive demands (Howard, 1981). The reasons for this can be very complex, but can be often explained in terms of parental investment and mating effort (Howard, 1981). In Crustacea, females are usually larger than males, and this is thought to be due to increasing fecundity with increasing female body size. However, in many species of Malacostraca, males are larger than the females, probably because combat between males for females has resulted in selection against small males. In most species of Gammarus, the average male/female body length ratios vary from 1.19 to 1.50 (Adams and Greenwood, 1987) and the mean weight ratio is about 2.0 (Greenwood and Adams, 1984), varying with geographical region and habitat. In Hyalella azteca, Bousfield (1973) reported the male/female body length ratio in North American streams as 1.33, and Adams and Greenwood (1987) recorded a ratio of 1.22 in ponds and lakes. Sexual size dimorphism in amphipods has been demonstrated to relate strongly with the type of mating strategy shown (Adams and Greenwood, 1983; Naylor and Adams, 1987; Ward, 1983; 1987 and 1988).

Precopulatory mate guarding (amplexus) is a sexual selection strategy in which male and female pair together briefly prior to copulation. In many species of Crustacea, females only produce eggs just after a molt (Patel and Crisp, 1961; Thompson and Manning, 1981), the males guard the females at these times, as only then can the male copulate and fertilize the eggs produced by the female. This often leads to competition among the males for access to receptive females. Studies on a number of species of *Gammarus* indicate that mates are apparently selected on the basis of a variety of characters, such as large size, high fecundity and optimal age, *etc.* (Ward, 1985 and 1988; Elwood *et al.*, 1987).

In the studies of *Gammarus* species, three main hypotheses have been proposed to account for mate choice decision:

(1) Birkhead and Clarkson (1980) suggested that males select females on the basis of time before moult and size assortative mating (homogamy) resulted from spatial heterogeneity in the environment leading to clumping of like-sized individuals. This hypothesis predicts that large individuals occupy different microhabitats than small individuals, and within a microhabitat ran-dom mating with respect to size may occur. However, when large samples were taken from streams, assortative mating is frequently found. Birkhead and Clarkson's hypothesis has been rejected by Thompson and Moule (1983) who found that size assortative mating occurred in artificial environments of uniform particle size.

- (2) It has been proposed that there is a loading constraint which determines an optimum male/female size ratio (Adams and Greenwood, 1983). Since female size and fecundity are positively correlated with each other, a male would be expected to increase his fitness by acquiring as large a female as possible. However, during the precopula phase, the male carries the female under and parallel to his body and performs most of the swimming movements. The female impedes the male's locomotion (Adams and Greenwood, 1983; Greenwood and Adams, 1984), so that the possession of a large female exacts a higher energy cost for locomotion than does a smaller one. There may thus be opposing selection forces: selection for a large female to increase fecundity but selection for a small female to decrease locomotion energy cost. In lotic ecosystems as water velocity increases the more difficult it is for a male to swim with a large female suggesting that the optimal male:female size ratio should be influenced by water velocity. However, Elwood et al. (1987) examined the male:female size ratio in adjacent fast and slow sections of a stream and found no difference in size ratio dependent upon water velocity.
- (3) Ward (1983) presented data consistent with assortative mating resulting from direct competition between males for large females. There is also evidence that males are more tenacious in guarding large females (Ward, 1983; 1984). Females may exercise some effects on the size ratio as they struggle when males attempt to enter precopula, resulting in large females preventing copulation attempts by small males (Ward, 1984, 1988).

Amplexus and mate choice of H. azteca were studied by Holmes (1903) and Strong (1972, 1973). Holmes (1903) placed sexually attractive females in wire gauze enclosures and reproductive males outside of the enclosures so that mates could contact each other visually. He noted that males outside the enclosures paid no attention to the females, suggesting that males had no tendency to seize all other conspecifics. Strong (1972; 1973) also found that there was no attraction of males by females in field populations. Amplexus was initiated only after the male and female collided. Males attempt to amplexus all H. azteca encountered regardless of sex and phase of molt cycle, however they succeed only with willing females. This suggest that only tactile communication precipitates amplexus and phermone attraction of males by females recorded in *Gammarus* (Dahl *et al*., 1970; Dunham, 1978) does not occur.

## **Objectives**

Examination of the sexual size dimorphism and mate guarding strategy of H. azteca in the laboratory and in the field was to determine:

- (1) whether sexual dimorphism occur in *H. azteca* by comparing the weight distribution of males and females in reproductive seasons.
- (2) the differences in performance in precopulatory mate guarding behaviour between males and females:
- (3) the effect of the sex ratio on mating decisions for H. azteca.

# 6.2. MATERIALS AND METHODS

Adult *H. azteca* were collected with a hand net from several open water sites in Stephenson's Pond during June to August 1986 and 1987. Pairs were immediately extracted from the samples and maintained in the laboratory in a large aerated stock tank with pond water and small pieces of macrophytes for food and cover. All animals were subject to fluorescent light (14 hr light and 10 hr dark) and  $20^{\circ}C$  and allowed to acclimatize to these conditions for at least 12 hr. Pairs of *H. azteca* already in precopula were removed from the stock tank and each sex was weighed ( $\pm$  0.1 mg) and the body length measured ( $\pm$  0.1 mm) under 10X10 magnification.

Mate choice was examined in the laboratory using pairs of H. azteca in precopula with the null hypothesis that assortative mating does not occur. Thus, giving a male a choice of two or three females of different sizes, there will be no significant tendency for males to choose on a size basis. To test whether females pair preferentially with larger males, two experiments were carried out.

The first experiment to examine the male's choice was carried out in three groups. In order to ensure that each animal was physiologically available for pairing, the males and females were all selected from precopula pairs. Immediately after collection, each male was separated from his partner, weighed and placed in a plastic cup with approximately 300 ml aerated pond water. For the first group, one big female and one small female which had previously been in precopula with other males were added simultaneously into each cup. For the second group, two big females and one small female were added and for the third group, two small females and one big female were added (number of replicates was > 30 for all groups). The males usually entered precopula with one of the females within 1 to 2 hr. At this time the rejected female(s) was (were) removed and weighed. Unpaired animals were left for an additional 20 hr for pairing to occur. In the same way, the rejected female(s) was (were) removed and weighed. Finally the paired male and female were removed, separated and weighed.

The second experiment to examine female's choice was conducted in a similar manner. In the first group, one big male and one small male which had previously been in precopula with other females were placed in each cup. In the second group, two big and one small males were placed and in the third group two small and one big males were placed. After the precopulatory pairs were formed, each individual was removed and weighed separately (number of replicates was > 30 for all groups).

## 6.3. RESULTS

### 6.3.1. Sexual Size Dimorphism

The mean length of males was significantly longer than that of females in precopula pairs of *H. azteca* in Stephenson's Pond in both 1986 and 1987 (Table 6. 1, for 1986 data, paired t = 8.34, p << 0.01; for 1987 data, paired t = 3.78, p << 0.01). Between years, there were significant differences in body length in

Table 6. 1. Mean body length (mm) and length range of precopulatory male and female *Hyalella azteca* collected from Stephenson's Pond during 1986 and 1987. (N =sampling size).

[		Male t	ody length	Female	e body length	Male:female	
Year	N	mean	range	mean	range	length ratio	
1986	53	7.02	6.2-7.8	6.40	5.5-7.7	1.10	
1987	48	8.12	7.4-8.8	7.09	5.8-8.5	1.16	

precopulatory males and females (for males, t=10.18, p < 0.01, for females t = 4.6, p < 0.01). Male:female length ratio was 1.10 in 1986 and 1.16 in 1987 (Table 6. 1). While the average male/female body weight ratio was 1.21 in 1986 and 1.48 in 1987.

There is some overlap in the weight frequency distributions of precopulatory males and females of *H. azteca* in 1986 and 1987 (Figures 6. 1 and 6. 2), but the two groups are distinct. In 1986, the highest male weight frequency distribution occurred in the range of 12 to 20 mg, however, in females from 10 to 14 mg. In 1987 the highest male weight frequency distribution was recorded in the range of 14 to 22 mg, while in females from 10 to 26 mg. Among these females, a small proportion carried eggs or young.

### 6.3.2. Mate Choice in Pair Formation

The field data shows no significant correlation between the body lengths of males and females in pairs over the two year sampling period. In 1986, r = -0.043, n = 53, p > 0.05; in 1987, r = -0.104, n = 48, p > 0.05. Some males were larger than the females they carried in precopula, but some were much smaller and some similar in size. Thus, size assortative pairing was not observed in the field.

In the laboratory, the mate choice experiments showed that the success or failure of pair formation in H. azteca depended on the sex ratios of the physiologically mature males and females (Figure 6. 3). In tests with one male and several females, the males always entered precopula with one of the females (chi-



Figure 6.1. The Weight Distributions of Male and Female Adults in Stephenson's Pond in 1986






Male:Female Ratio



square tests, df = 1, p < 0.001). However, in tests with only one female available, only half of the females in all replicates entered into precopula (chi-square tests, df = 1, p > 0.05). Thus, pair formation by males was less successful at high male:female ratios.

In the first experiment, size assortative mating was only detected in tests with one male offered two females (Figure 6. 4) Most males preferentially chose big females (chi-square = 9.30, df = 1, p < 0.001), although some guarding males entered precopula with females weighing more than themselves (Figure 6. 4). However, in the two experiments with lower male:female ratios, the males paired equally with large and small females (Figure 6. 4, chi-square test, df = 1, p > 0.10), indicating that pair formation occurred randomly rather than matched for size. Even in the experiments where large females were twice as abundant as small females, size assortative pairing was not found (chi-square = 1.10, df = 1, p > 0.10). The weight difference between rejected females and those taken into precopula was not significant (Mann-Whitney U test, p > 0.05).

In the second experiment similar results were obtained (Figure 6. 5). In trials with one female and two males, size assortative mating was found (chi-square = 5.50, df = 1, p < 0.05). However, this trend was not found in trials with one female and three males ( chi-square tests, df = 1, p > 0.05), or in those trials in which more large males than small males occurred (chi-square = 2.84, df = 1, p > 0.05). In these two groups, there was also no weight difference between rejected males and those taken in precopula (Mann-Whitney U test, p > 0.05).



























Body Weight

## 6.4. DISCUSSION

Birkhead and Clarkson (1980) suggested that sexual dimorphism might be a product of intra-sexual competition, since larger males are more likely to breed than smaller ones. Supporting evidence has been found in many species (Greenwood and Adams, 1987; Ward, 1987, 1988). When males are larger (*e.g.*, most birds and mammals), this is usually attributed to sexual selection because larger size is assumed to give a competitive advantage to males competing for access to larger and more fecund females. In *H. azteca*, the average male size is significantly larger than the average female size in Stephenson's Pond and similar results were recorded by Bousfield (1973) and Adams and Greenwood (1987).

The performance difference in precopula between males and females has been observed experimentally. In trials with one male, the male successfully formed pairs with one of the females. However, in trials with one female half of the females did not enter precopula. This might be because females adopt a passive role in precopula formation or resist the males. Similar results were reported for *Gammarus pulex* by Birkhead and Clarkson (1980) and Ward (1984).

Darwin (1871) pointed out that sexual selection accounts for the evolution of characteristics which confer an advantage to some individuals over others of the same sex and species in relation to reproduction. In many species of *Gammarus* males are typically longer than, or twice as heavy as the female they carry (Birkhead and Clarkson 1980; Adams and greenwood, 1983; Naylor and Adams, 1987). However, in precopulatory pairs of *H. azteca* in both the field and laboratory, the

males are not always larger than the females with which they pair (Figures 6. 4 and 6. 5). As female fecundity is proportional to size, males might compete for the larger females to maximize the number of their offspring. An alternative reason may be the low loading constraints suggested by Adams and Greenwood (1984, 1987). No trend was found to indicate that males preferentially select females close to moulting in precopula. If so, males should always choose big females because females close to moulting are big individuals (Figures 6. 4 and 6. 5).

The mate choice experiments demonstrated that the sex ratio can influence mate choice decision making. The results from trials with one male and two females and with one female and two males clearly show size assortative pairing. This non-random pairing was found in many species of *Gammarus* by Birkhead and Clarkson (1980), Ward (1985), Adams and Greenwood (1987) and Elwood *et al* . (1987) and in some species of *Asellus* by Ridley and Thompson (1979), Thompson and Manning (1981), Verspoor (1982) and Adams *et al* . (1984). However, they did not conduct any experiment to look at how mate choice decision changes when more females or males are encountered. With *H. azteca* random pairing occurred in these trials, which are probably related to the effect of time investment on mating decision (Hunte *et al* ., 1985; Dunham *et al* . 1986; Elwood *et al* . 1987).

Precopulatory mate guarding by males is expensive both in terms of energy, since the male carries the passive female when swimming (Adams and Greenwood, 1983, 1987), and time as during this period the males can not mate with other

females. In Crustacea, mating is often restricted to a brief period of time following the female moult, since females cannot store sperm. Both male and female reproductive states should regulate mate guarding behaviour to avoid or minimize the wastage of time investment in pair formation. The time investment in males includes searching for mates, mating decision making and mate guarding. Usually a male attempts to monopolize a female, preventing other males gaining access, to ensure paternity of the offspring. Therefore, males should try to minimize time investment in searching and uncertainty in order to increase their chances of mating.

In the laboratory, the following activities of H. azteca were recorded in experimental trials after introduction of male(s) and female(s):

(1) contacts --- a male moves his antennae over the female;

(2) palpations --- male grabs, holds and palpates the female;

(3) precopula --- the male rotates the female into the precopula position and swims with her in this position.

These processes are all time-consuming. Sometimes a male palpates a female many times before taking her into precopula. This is probably the time the male invests to gather information about the female which allows him to assess her reproductive state. Hunte *et al* . (1985) found that the longer the females has been amplexing, the more certain the male may be of the female's physiological state. There is, however, a trade-off in time investment between mate guarding decision

processes and mate guarding when both male and female reproductive state and investment time are limited. To increase the guarding time, the male should minimize contact and palpation time. Therefore, Hunte *et al*. (1985) suggested that time spent in contact with the female is a critical variable because it permits the male to gather information about the female's reproductive state. This suggestion was confirmed in *H. azteca* experiments. The time spent in searching for mate in trials with three mates could be much shorter than that in trials with four mates. Therefore, in trials with three mates, it may be advantageous for the male to guard a female through size assortative pairing to increase reproductive success. However, in the trials with more mates, random mating may be energetically much cheaper than size assortative mating in getting the same success on the basis of future energy returns and the cost of completing the task (Dawkin and Carlisle, 1976; Boucher, 1977).

## Chapter 7

## CONCLUSIONS

The freshwater amphipod *Hyalella azteca* is widely distributed in North America. Bousfield (1958) reported it from lakes, ponds, sloughs, marshes, rivers, streams, ditches, spring streams and estuaries. However, only a few ecological studies in these ecosystems have been conducted. The main objectives of this study was to examine the population dynamics, seasonal distribution, production and life history strategies of *H. azteca* in a small prairie pond (Stephenson' Pond) in Alberta.

Standing stock of *H. azteca* fluctuated rather irregularly throughout the 18month study period. Numbers were lowest and constant throughout the winter and early spring (January to May) and increased in June. The annual density was higher during summer (June to August) with peak density in July. Population density declined markedly in November and December. Biomass of *H. azteca* fluctuated in a similar way, but peak biomass did not co-occur with peak density as there was a time lag of two or three months. The mean density ( $\pm$  SE) of *H. azteca* was 5814.63 ( $\pm$  1320.6) individuals/ $m^2$  in 1985 and 3641.71 ( $\pm$ 1215.8) individuals/ $m^2$  in 1986. The mean wet weight biomass ( $\pm$  SE) in 1985 was 25.25 ( $\pm$  5.37) mg/ $m^2$ , and in 1986 13.29 ( $\pm$  3.68) mg/ $m^2$ . The density and biomass of *H. azteca* in Stephenson's Pond were considerably higher than those reported in the literature for other water bodies (Cooper, 1965; Mathias, 1971 and Lindeman and Momot, 1983).

The mean density and biomass in two shallow zones (shaded and unshaded) were not significantly different, but they were significantly higher than those in the deeper water zone. However, no differences in density and biomass were found between west and east basins of Stephenson's Pond. Both the density and the biomass of H. azteca in the water column were significantly higher than those in the benthos. Significant correlations between density in the water column and in the benthos, and between biomass in the water column and in the benthos were existed.

Significant correlations between H. azteca density and macrophyte dry biomass and between H. azteca wet biomass and macrophyte dry biomass were found before and after the breeding period. However, these relationships did not occur during the breeding period. Laboratory experiments showed that H. azteca mainly chose macrophytes for shelter and for epiphytic algae attached on macrophytes, which serve as an important food for H. azteca . Among three substrates: macrophytes, rocks and mud, H. azteca preferentially chose macrophytes and rocks equally, but rarely chose mud.

Sexual size dimorphism of H. azteca in Stephenson's Pond occurred. Males were usually bigger and heavier than females. Male:female length ratio was about 1.1, while male:female weight ratio ranged from 1.2 to 1.5. Precopulatory mate

guarding started in late April or early May, and normally ended in late August. Size assortative mating was not recorded in the field. However, in the laboratory mating decisions depended on the sex ratio. When male:female ratio <1, pair formation was highly successful(more than 50% of males entered precopula). However, when male:female ratio >1, only half of the females succeeded in pair formation. Positive assortative mating was only detected in male:female ratio of 1:2 to 2:1. Outside this range, random mating occurred.

Reproduction of *H. azteca* in Stephenson's Pond began in late June and ended in September or October. Since each female produced one generation before death, *H. azteca* is semelparous. Fecundity varied with female size. Generally large females laid more eggs or released more young than smaller females. For the entire population the number of eggs per female ranged from 4 to 48 and the number of young per female from 8 to 45. There was a trade-off between the number of eggs brooded and the mean egg weight and between number of young released and mean young weight. The number of eggs carried or the number of young released by a female was significantly correlated to her body weight. It was found that reproductive success was mainly dependent on water temperature with an optimal range from 15 to  $20^{\circ}C$ . No reproduction occurred at  $5^{\circ}C$ .

The average size of *H. azteca* peaked early in the breeding season (May to middle June), then declined abruptly after recruitment of young into the population in late June. Over the winter months (January to March), *H. azteca* tremendously slowed down its growth and therefore, its size remained relatively constant. Small-

sized individuals (young and juveniles) were usually found in the water column along with the large-sized animals (old adults), while medium-sized individuals (juveniles and adults) were frequently recorded in the benthos.

By tracking the weight frequency distribution histograms, three recognizable cohorts were observed. The first cohort recruited in June 1984, matured over spring (April to early June), and disappeared from the population by January or early February. The second cohort appeared in late June 1985, grew through the spring (April to early June), and disappeared in August 1986. The third cohort appeared in the population on June 25 1986. However, no data are available on the maturation and disappearance of the third cohort. The mean duration of the second cohort was 414 days.

The growth rate of *H. azteca* for the 1984 generation in Stephenson's Pond was estimated to be 0.086 mg/day from April to August 1985, 0.043 mg/day for the 1985 generation, and 0.097 mg/day over the first two months after hatching for the 1986 generation. Laboratory experiments showed that growth of *H. azteca* was linear and its magnitude was influenced by temperature and food type. The optimal temperature for growth was 15 to  $20^{\circ}C$ . *H. azteca* grew much faster when fed on fresh *Salix* sp. leaves and conditioned macrophytes than on fresh *Potamogeton richardsonii* leaves.

Annual production of *H. azteca* was 19.99 (size-frequency method) or 19.65 g dry weight/ $m^2$  (the cohort G method) in 1985 and 12.54 (size-frequency method) or 9.54 g dry weight/ $m^2$  (the cohort G method) in 1986. Over the whole sampling

period, daily production varied from 0.009 to 0.155 g dry weight/ $m^2$ /day (the cohort G method). It was higher in summer (July to September) and fall (October to December) than in winter (January to March). P/B ratio (size-frequency method) was 3.05 for 1985 and 3.23 for 1986.

The morphometric ratio characteristics of H. azteca were examined. Significant relationships were obtained between and among the head length, first thoracic segment length, body length and total length. The relation between the log of these length measurements and log of wet weight were also significant and predictive equations were established. The best predictors of biomass of H. azteca are total length and body length. The mean number of flagellar segments in the first and second antennae is also a good predictor of length, but not of biomass. On average dry weight of H. azteca is about 17.76% of its fresh weight.

Preservation of *H.azteca* in four concentrations of ethanol (50, 65, 80 and 95%) caused changes in the preserved wet weight expressed as a percentage of the original live wet weight. About 18% and 29% loss of wet weight was recorded after three months in 50 to 80% and 95% ethanol, respectively. The magnitude of the changes varied with preservative concentration and preservation time. Failure to take these factors into account can lead to errors in wet weight measurements.

## **Bibliography**

- [1] Adams, J., Gee, J., Greenwood, P., Mckelvey, S. and Perry, R. 1987. Factors affecting the microdistribution of *Gammarus pulex* (Amphipoda): an experimental study. Freshwater Biol. 17: 307-316.
- [2] Adams, J. and Greenwood, P. J. 1983. Why are males bigger than females in precopula pairs of *Gammarus pulex*? Behav. Ecol. Sociobiol. 13: 239-241.
- [3] Adams, J. and Greenwood, P. J. 1985. Environmental constraints on mate choice in *Gammarus pulex* (Amphipoda). Crustaceana 50: 45-52.
- [4] Adams, J. and Greenwood, P. J. 1987. Loading constraints sexual selection and assortative mating in peracarid Crustacea. J. Zool., Lond. 211: 35-46.
- [5] Adams, J., Greenwood, P. J., Pollitt, R. and Yonow, T. 1985. Loading constraints and sexual size dimorphism in *Sellus aquaticus*. Behaviour 92: 277-287.
- [6] Ambrose, W. G. Jr. 1984. Increased emigration of the amphipod *Rhepoxynius abronius* (Barnard) and the polychaete *Nephtys caeca* (Fabricius) in the presence of predators. J. exp. mar. Biol. Ecol. 80: 67-75.
- [7] Ambrose, W. G. Jr. 1986. Experimental analysis of density dependent emigration of the amphipod *Rhepoxynius abronius*. Mar. Behav. Physiol. 12:

209-216.

- [8] Anderson, N. H. and K. W. Cummins. 1979. Influences of diet on the life histories of aquatic insects. J. Fish. Res. Bd. Can. 36: 335-342.
- [9] Anderson, R. O. and F. F. Hooper. 1956. Seasonal abundance and production of littoral bottom fauna in a southern Michigan lake. Trans. Amer. Micros. Soc. 75: 259-270.
- [10] Baird, D. J., T. E. Gates and R. W. Davies. 1987. Oxygen conditions in two prairie pothole lakes during winter ice cover. Can. J. Fish. Aquat. Sci. 44: 1092-1095.
- [11] Barber, W. E. and Kevern, N. R. 1973. Ecological factors influencing macro-invertebrate standing crop distribution. Hydrobiologia 43:53-75.
- [12] Begon, M., J. L. Harper and C. R. Townsend. 1986. Ecology: individuals, populations, and communities. Sinauer Associates, Inc. Publishers.
- [13] Benke, A. C. 1979. A modification of the Hynes method for estimating secondary production with particular significance for multivoltine populations. Limnol. Oceanogr. 24: 168-171.
- [14] Benke, A. C., and J. B. Waide. 1977. In defense of average cohorts. Freshwater Biol. 7: 61-63.
- [15] Birkhead, T. R., and K. Clarkson. 1980. Mate selection and precopulatory guarding in *Gammarus pulex*. Z. Tierpsychol. 52: 365-380.

- [16] Boucher, D. H. 1977. On wasting parental investment. Am. Nat. 111: 786-788.
- [17] Bousfield, E. L. 1958. Fresh-water amphipod crustaceans of glaciated North America. Can. Field-Nat. 72: 55-113.
- [18] Bousfield, E. L. 1973. Shallow-water gammaridean amphipoda of New England. Ithaca: Comstock Publishing Assoc.
- [19] Bovbjerg, R. V. 1970. Ecological isolation and competitive exclusion in two crayfish (Orconectes virilis and Orconectes immunis). Ecology 51: 225-236.
- [20] Bovee, E. C. 1949. Studies on the thermal death of Hyalella azteca (Saussure). Biol. Bull. 96: 123-128.
- Bovee, E. C. 1950. Some effects of temperature on the rates of embryonic, postembryonic and adult growth in *Hyalella azteca*. Iowa Academy of Science 57: 439-444.
- [22] Chutter, F. M. 1969. The distribution of some stream invertebrates in relation to current speed. Int. Rev. ges. Hydrobiol. 54: 413-422.
- [23] Cochran, W. E. and G. M. Cox. 1957. Experimental designs. John Wiley and Sons, Inc., New York.
- [24] Cooper, W. E. 1965. Dynamics and production of a natural population of a fresh-water amphipod, *Hyalella azteca*. Ecol. Monogr. 35: 377-394.

- [25] Cummins, K. W. and Klug, M. J. 1979. Feeding ecology of stream invertebrates. Ann. Rev. Ecol. Syst. 10: 147-172.
- [26] Cummins, K. W. and G. H. Lauff. 1969. The influence of substrate particle size on the microdistribution of stream macrobenthos. Hydrobiologia 34: 145-181.
- [27] Cushman, R. M., H. H. Shugart, Jr., S. G. Hildebrand and J. W. Elwood. 1978. The effects of growth curve and sampling regime on instantaneousgrowth, removal-summation, and Hynes/Hamilton estimates of aquatic insect production: a computer simulation. Limnol. Oceanogr. 33: 184-189.
- [28] Dahl, E., H. Emanuelsson and V. Mecklenburg. 1970. Pheromone reception in the males of the amphipod Gammarus duebeni Lilljeborg. Oikos 31: 42-47.
- [29] Darwin, C. R. 1871. The descent of man and selection in relation to sex.London: John Murray.
- [30] Davies, R. W. and Everett, R. P. 1977. The life history, growth and age structure of Nephelopsis obscura Verrill, 1872 (Hirudinoidea) in Alberta.
  Can. J. Zool. 55: 620-627.
- [31] Davies, R. W., and T. B. Reynoldson. 1976. A comparison of the life-cycle of *Helobdella stagnalis* (Linn. 1758)(Hirudinoidea) in two different geographic areas in Canada. J. Anim. Ecol. 45: 457-470.

- [32] Davies, R. W., T. Yang and F. Wrona. 1987. Inter- and intra-specific differences in the effects of anoxia on erpobdellid leeches using static and flow-through systems. Holarct. Ecol. 10: 149-153.
- [33] Dawkins, R. and T. R. Carlisle. 1976. Parental investment, mate desertion and a fallacy. Nature, Lond. 262: 131-133.
- [34] De March, B. G. E. 1977. The effects of photoperiod and temperature on the induction and termination of reproductive stage in the freshwater amphipod Hyalella azteca (Saussure). Can. J. Zool. 55: 1595-1600.
- [35] De March, B. G. E. 1978. The effects of constant and variable temperatures on the size, growth, and reproduction of the freshwater amphipod *Hyalella azteca* (Saussure). Can. J. Zool. 56: 1801-1806.
- [36] Denno, R. F. and H. Dingle. 1981. Insect life history patterns: habitat and geographic variation. Springer-Verlag New York Inc.
- [37] Dewitt, T. H. 1987. Microhabitat selection and colonization rates of a benthic amphipod. Mar. Ecol. Prog. Ser. 36: 237-250.
- [38] Donald, G. L. and Paterson, C. G. 1977. Effect of preservative on wet biomass of chironomid larvae. Hydrobiologia 53: 75-80.
- [39] Dunham, P. J. 1978. Sex pheromones in Crustacea. Biol. Rev. 53: 555-583.
- [40] Dunham, P., T. Alexander and A. Hurshman. 1986. Precopulatory mate guarding in an amphipod, *Gammarus lawrencianus* Bousfield. Anim. Behav. 34: 1680-1686.

- [41] Elwood, R., J. Gibson, and S. Neil. 1987. The amorous *Gammarus* : size assortative mating in *G. pulex*. Anim. Behav. 35: 1-6.
- [42] Embody, G. C. 1912. A preliminary study of the distribution, food and reproductive capacity of some freshwater amphipods. Intern. Revue d. ges.
   Hydrobiol. u. Hydrog. (Supplement) 4: 1-33.
- [43] France, R. L. 1987. Test of biotic and abiotic environmental determinants of amphipod (*Hyalella azteca*) preamplexus. Can. J. Fish. Aquat. Sci. 44:478-482.
- [44] France, R. L. and B. D. LaZerte. 1987. Empirical hypothesis to explain the restricted distribution of *Hyalella azteca* (Amphipoda) in anthropogenically acidified lakes. Can. J. Fish. Aquat. Sci. 44: 1112-1121.
- [45] France, R. L. and P. M. Stokes. 1987. Life stage and population variation in resistance and tolerance of *Hyalella azteca* (Amphipoda) to low pH. Can. J. Fish. Aquat.Sci. 44: 1102-1111.
- [46] Frank, P. W. 1960. Prediction of population growth form in Daphinia pulex cultures. Am. Nat. 94: 357-372.
- [47] Gale, W. F. 1971. An experiment to determine substrate preference of the Fingernail clam, Sphaerium transversum (Say). Ecology 52: 367-370.
- [48] Gates, T. E. and R. W. Davies. 1987. The influence of temperature on the depth distribution of sympatric Erpobdellidae (Hirudinoidea). Can. J. Zool. 65: 1243-1246.

- [49] Gates, T. E., D. J. Baird, F. J. Wrona and R. W. Davies. 1987. A device for sampling macroinvertebrates in weedy ponds. J. N. Am. Benthol. Soc. 6: 133-139.
- [50] Gaylor, D. 1922. A study of the life history and productivity of *Hyalella knickerbockeri* (Bate). (Contrb. Zool. Lab. Indiana Univ. No. 187.) Proc. Indiana Acad. Sci., (1922): 239-250.
- [51] Gee, J. H. R. 1982. Resource utilization by Gammarus pulex (Amphipoda) in a Cotswold stream: a microdistribution study. J. Anim. Ecol. 51: 817-832.
- [52] Geisler, F. S. 1944. Studies on the postembryonic development of *Hyalella azteca* (Saussure). Biol. Bull. 86: 6-22.
- [53] Grafen, A and Ridley, M. 1983. A model of mate-guarding. J. Theor. Biol. 102: 549-567.
- [54] Green, J. D. 1976. Population dynamics and production of the calanoid copepod Calamoecia lucasi in a northern New Zealand lake. Arch. Hydrobiol. (Suppl.). 50: 313-400.
- [55] Greenwood, P. J., and J. Adams. 1984. Sexual dimorphism in Gammarus pulex : the effect of current flow on pre-copula pair formation.
   Freshwater Biol. 14: 203-209.
- [56] Greenwood, P. J. and J. Adams. 1987. Sexual selection, size dimorphism and a fallacy. Oikos 48: 106-108.

- [57] Hall, D. J. 1964. An experimental approach to the dynamics of a natural population of Daphnia *Galeata mendota*. Ecol. 45: 94-110.
- [58] Hall, D. J., W. E. Cooper, and E. E. Werner. 1970. An experimental approach to the production dynamics and structure of freshwater animal communities. Limnol. Oceanogr. 15: 839-928.
- [59] Hall, D. J., T. F. Waters and E. F. Cook. 1980. The role of drift dispersal in production ecology of a stream mayfly. Ecology 61: 37-43.
- [60] Hamilton, A. L. 1969. On estimating annual production. Limnol. Oceanogr. 14: 771-782.
- [61] Hargrave, B. T. 1969. Epibenthic algal production and community respiration in the sediments of Marion Lake. J. Fish Res. Bd. Canada 27: 685-699.
- [62] Hargrave, B. T. 1970a. The utilization of benthic microflora by *Hyalella azteca* (Amphipoda). J. Anim. Ecol. 39: 427-437.
- [63] Hargrave, B. T. 1970b. Distrbution, growth, and seasonal abundance of Hyalella azteca (Amphipoda) in relation to sediment microflora. J. Fish.
   Res. Bd. Canada 27: 685-699.
- [64] Hargrave, B. T. 1970c. The effect of a deposit-feeding amphipod on the metabolism of benthic microflora. Limnol. Oceanogr. 15: 21-30.
- [65] Hargrave, B. T. 1971. An energy budget for a deposit-feeding amphipod. Limnol. Oceanogr. 16: 99-103.

- [66] Hart, R. C. 1987. Population dynamics and production of five crustacean zooplankters in a subtropical reservoir during years of contrasting turbidity. Freshwater Biol. 18: 287-318.
- [67] Holmes, S. J. 1902. Observation on the habits of Hyalella dentata. Science N. S. Vol. 5.
- [68] Holmes, S. J. 1903. Sex recognition among amphipods. Biol. Bull. 5: 288-292.
- [69] Holsinger, J. R. 1972. The freshwater amphipod crustaceans (Gammaridae) of North America. Biota of Freshwater Ecosystems. Identification Manual 5: 1-89.
- [70] Howard, R. D. 1981. Sexual dimorphism in bullfrogs. Ecology 62: 303-310.
- [71] Howmiller, R. P. 1972. Effects of preservatives on weights of some common macrobenthic invertebrates. Trans. Amer. Fish. Soc. 4: 743-746.
- [72] Hudson, P. L., and G. A. Swanson. 1972. Production and standing crop of *Hexagenia* (Ephemeroptera) in a large reservoir. Stud. Nat. Sci. No. 1.
- [73] Hunte, W., Myers, R. A. and Doyle, R. W. 1985a. Bayesian mating decisions in an amphipod, *Gammarus lawrencianus* Bousfield. Anim. Behav. 33: 366-372.
- [74] Hunte, W., Myers, R. A. and Doyle, R. W. 1985b. The effect of past investment on mating decisions in an amphipod: implications for models of decision making. Anim. Behav. 33: 366-372.

- [75] Hynes, H. B. N. 1954. The ecology of *Gammarus duebeni* Lilljeberg and its occurrence in fresh water in western Britain. J. Anim. Ecol. 23: 38-84.
- [76] Hynes, H. B. N. 1955. The reproductive cycle of some British freshwater Gammaridae. J. Anim. Ecol. 24: 352-387.
- [77] Hynes, H. B. N. 1961. The invertebrate fauna of a Welsh mountain stream.Arch. Hydrobiol. 57: 344-388.
- [78] Hynes, H. B. N. 1970. The ecology of running waters. University of Toronto Press.
- [79] Hynes, H. B. N., and M. J. Coleman. 1968. A simple method of assessing the annual production of stream benthos. Limnol. Oceanogr. 13: 569-573.
- [80] Iversen, T. M., and J. Jessen. 1977. Life cycle, drift and production of Gammarus pulex L. (Amphipoda) in a Danish spring. Freshwat. Biol. 7: 287-296.
- [81] Iversen, T. M. and J. Thorup. 1987. Population dynamics and production of Sialis lutaria L. (Megaloptera) in the Danish River Susa. Freshwater Biol. 17: 461-469.
- [82] Jackson, H. H. T. 1912. A contribution to the natural history of the amphipod, Hyalella knickerbockeri (Bate). Bull. Wisconsin Nat. Hist. Soc. 10: 49-60.
- [83] Kolding, S. and T. M. Fenchel. 1981. Patterns of reproduction in different populations of five species of the amphipod genus *Gammarus*. Oikos 37:

167-172.

- [84] Krebs, C. J. 1985. Ecology: the experimental analysis of distribution and abundance. 3rd ed. Harper and Row, New York.
- [85] Kruschwitz, L. G. 1972. An analysis of reproductive behavior patterns of the amphipod, Hyalella azteca . Am. Zool. 12: 658-659.
- [86] LaFrance, K. and E. Ruber. 1985. The life cycle and productivity of amphipod Gammarus mucronatus on a northern Massachusetts salt marsh. Limnol. Oceanogr. 30: 1067-1077.
- [87] Legget, R. F. 1961. Soils in Canada, geological, pedological and engineering studies. University of Toronto Press.
- [88] Lindeman, D. H. and W. T. Momot. 1983. Production of the amphipod Hyalella azteca (Saussure) in a northern Ontario lake. Can. J. Zool. 61: 2051-2059.
- [89] Lyford, W. H. 1943. The palatability of freshly fallen forest tree leaves to millipedes. Ecology 24: 252-261.
- [90] Manning, J. T. 1975. Male discrimination and investment in Asellus aquaticus (L.) and A. meridianus Racovitsza (Crustacea, Isopoda). Behaviour 55: 1-14.
- [91] Marchant, R. and H. B. N. Hynes. 1981. The distribution of Gammarus pseudolimnaeus (Crustacea: Amphipoda) along a reach of the Credit River, Ontario. Freshwater Biol. 11: 169-182.

- [91] Martin, A. L. 1964. The alimentary canal of *Marinogammarus obtusatus* (Crustacea: Amphipoda). Proc. Zool. Soc. Lond. 143: 525-544.
- [92] Martin, A. L. 1966. Feeding and digestion in two intertidal gammarids: Marinogammarus obtusatus and M. pirloti . J. Zool. 148: 515-525.
- [93] Mathews, C. P. 1970. Estimates of production with reference to general surveys. Oikos 21: 129-133.
- [94] Mathias, J. A. 1971. Energy flow and secondary production of the amphipods Hyalella azteca and Crangonyx richmondensis occidentalis in Marion Lake, British Columbia. J. Fish. Res. Bd. Canada 238: 711-726.
- [95] Milstead, B. and S. T. Threlkeld. 1986. An experimental analysis of darter predation on *Hyalella azteca* using semipermeable enclosures. J. N. Am. Benthol. Soc. 5: 311-317.
- [96] Naylor, C., and J. Adams. 1987. Sexual dimorphism, drag constraints and male performance in *Gammarus duebeni* (Amphipoda). Oikos 48: 23-27.
- [97] Nie, N. H., C. H. Hull, J. G. Jenkins, K. Steinbrenner, and D. H. Brent.
  1975. SPSS statistical package for the social sciences. 2nd ed. McGraw-Hill
  Book Co., New York.
- [98] Nilsson, L. M. 1977. Incubation time, growth and mortality of the amphipod, *Gammarus pulex* under laboratory conditions. Oikos 29: 93-98.
- [99] Oliver, D. R. 1979. Contribution of life history information to taxonomy of aquatic insects. J. Fish. Res. Bd. Canada 36: 318-321.

- [100] Patel, B., and D. J. Crisp. 1961. Relation between the brooding and moulting cycles in cirripeds. Crustaceana 2: 89-107.
- [101] Pennak, R. W. 1978. Fresh-water invertebrates of the United States. 2nd ed.
  A Wiley-Interscience Publication.
- [102] Pringle, S. 1982. Factors affecting the microdistribution of different sizes of the amphipod Gammarus pulex. Oikos 38: 369-373.
- [103] Rabeni, C. F. and Minshall G. W. 1977. Factors affecting microdistribution of stream benthic insects. Oikos 29: 33-43.
- [104] Rasmussen, J. B. 1983. An experimental analysis of competition and predation and their effects on growth and coexistence of chironomid larvae in a small pond. Ph. D. Thesis. University of Calgary, Alta.
- [105] Rees, C. P. 1972. The distribution of the amphipod Gammarus pseudolimnaeus Bousfield as influenced by oxygen concentration, substratum and current velocity. Trans. Am. Microscop. Soc. 91: 514-529.
- [106] Ridley, M. and D. J. Thompson. 1979. Size and mating in Asellus aquaticus (Crustacea: Isopoda). Z. Tierpsychol. 51: 380-397.
- [107] Ridley, M. and D. J. Thompson. 1985. Sexual selection of population dynamics in aquatic Crustacea. In Behavioural ecology: ecological consequences of adaptive behaviour: 409-422. Sibly, R. M. and Smith, R. H. (Eds). Oxford: Blackwells.

- [108] Skadsheim, A. 1984. Coexistance and reproductive adaptions of amphipods: the role of environmental heterogeneity. Oikos 43: 94-103.
- [109] Slack, H. D. 1967. A brief survey of the profundal benthic fauna of lakes in Manitoba. J. Fish. Res. Bd. Canada 24: 1017-1033.
- [110] Smock, L. A. 1980. Relationships between body size and biomass of aquatic insects. Freshwater Biol. 10: 375-385.
- [111] Stanford, J. A. 1972. A centrifuge method for determining live weights of insect larvae, with a note on weight loss in preservative. Ecology 54: 449-451.
- [112] Steele, D. H. and V. J. Steele. 1975. The biology of Gammarus (Crustacea, Amphipoda) in the northwestern Alantic. 11. Comparison and discussion. Can. J. Zool. 53: 1116-1126.
- [113] Stephenson, M., and G. L. Mackie. 1986. Lake acidification as a limiting factor in the distribution of the freshwater amphipod *Hyalella azteca*.
  Can. J. Fish. Aquat. Sci. 43: 288-292.
- [114] Strong, D. R. 1972. Life history variation among populations of an amphipod (*Hyalella azteca*). Ecology 53: 1103-1111.
- [115] Strong, D. R. 1973. Amphipod amplexus: the significance of ecotypic variation. Ecology 54: 1383-1388.
- [116] Sutcliffe, D. W., T. R. Carrick and G. Willoughby. 1981. Effects of diet, body size, age and temperature on growth rates in the amphipod

Gammarus pulex . Freshwater Biol. 11: 183-214.

- [117] Sweeney, B. W. and R. L. Vannote. 1981. Ephemerella mayflies of White Clay Creek: bioenergetic and ecological relationships among six coexisting species. Ecology 62: 1353-1369.
- [118] Sweeney, B. W. and R. L. Vannote. 1984. Influence of food quality and temperature on life history characteristics of the parthenogenetic mayfly, *Cloeon triangulifer*. Freshwater Biol. 14: 621-630.
- [119] Thompson, D. J. and J. T. Manning. 1981. Mate selection by Asellus (Crustacea: Isopoda). Behaviour 78: 178-187.
- [120] Thompson, D. J. and Moule, S. J. 1983. Substrate selection and assortative mating in *Gammarus pulex* L. Hydrobiologia 99: 3-6.
- [121] Verspoor, E. 1982. Assortative mating for size in Asellus aquaticus (L.)(Isopoda). Crustaceana 43: 216-219.
- [122] Wagner, V. T. and D. W. Blinn. 1987. A comparative study of the maxillary setae for two coexisting species of *Hyalella* (Amphipoda), a filter feeder and a detritus feeder. Arch. Hydrobiol. 109: 409-419.
- [123] Ward, G. M., and K. W. Cummins. 1979. Effects of food quality on growth of a stream detrtivore, *Paratendipes albimanus* (Meigen)(Diptera: Chironomidae). Ecology 60: 57-64.
- [124] Ward, P. I. 1983. Advantages and disadvantages of large size for male Gammarus pulex . Behav. Ecol. Sociobiol. 14: 174-184.

- [125] Ward, P. I. 1984. The effects of size on the mating decisions of Gammarus pulex (Crustacea: Amphipoda). Z. Tierpsychol. 64: 174-184.
- [126] Ward, P. I. 1986. A comparative field study of the breeding behaviour of a stream and a pond population of *Gammarus pulex* (Amphipoda). Oikos 46: 29-36.
- [127] Ward, P. I. 1987. Sexual selection and body size in *Gammarus pulex* : a reply to Greenwood and Adams. Oikos 48: 108-109.
- [128] Ward, P. I. 1988. Sexual selection, natural selection, and body size in Gammarus pulex (Amphipoda). Am. Nat. 131: 348-359.
- [129] Waters, T. F. 1977. Secondary production in inland waters. Adv. Ecol. Res. 10: 91-164.
- [130] Waters, T. F. 1979. Influence of benthos life history upon the estimation of secondary production. J. Fish. Res. Bd. Can. 36: 1425-1430.
- [131] Waters, T. F. 1981. Seasonal patterns in the production and drift of Gammarus pseudolimnaeus in Valley Creek, Minnesota. Ecology 62: 1458-1466.
- [132] Waters, T. F. and G. W. Crawford. 1973. Annual production of a stream mayfly population: a comparison of methods. Limnol. Oceanogr. 18: 286-296.
- [133] Welton, J. S. 1979. Life history and production of the amphipod *Gammarus pulex* in a Dorset chalk stream. Freshwater Biol. 9: 263-275.

- [134] Welton, J. S. and R. T. Clarke. 1980. Laboratory studies on the reproduction and growth of the amphipod, *Gammarus pulex* (L.). J. Anim. Ecol. 49: 581-592.
- [135] Wetzel, R. G. 1983. Limnology. 2nd ed. CBS College Publishing, New York.
- [136] Wetzel, R. G. and G. E. Likens. 1979. Limnological analyses. W. B. Saunders Co., Philadephia.
- [137] Wilder, J. 1940. The effects of population density upon growth, reproduction and survival in *Hyalella azteca*. Physiol. Zool. 13: 439-461.
- [138] Wilkialis, J. and R. W. Davies. 1980. The population ecology of the leech (Hirudinoidea : Glossiphoniidae) Theromyzon tessulatum. Can. J. Zool. 58: 906-912.
- [139] Williams, D. D. and K. A. More. 1982. The effect of environmental factors on the activity of *Gammarus pseudolimnaeus* (Amphipod). Hydrobiologia 96: 137-147.
- [140] Wrona, F. J. 1982. The influence of biotic and abiotic parameters on the distribution and abundance of two sympatric species of Hirudinodea. Ph. D. Thesis. University of Calgary, Calgary, Alta.
- [141] Wrona, F. J., Culp, J. M. and Davies, R. W. 1982. Macroinvertebrate subsampling: a simplified apparatus and approach. Can. J. Fish. Aquat. Scci. 39: 1051-1054.

[142] Zar, J. H. 1984. Biostatistical analysis. Prentice-Hall Inc., Englewood Cliffs, N.J.