ECOLOGICAL CHARACTERIZATION OF CYTOTYPES OF THE SIMULIUM VENUSTUM/VERECUNDUM COMPLEX (DIPTERA: SIMULIIDAE) FOUND ON THE AVALON PENINSULA, NEWFOUNDLAND

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by

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A Thesis submitted to the School of Graduate Studies in partial fulfilment of the requirements for the degree of Doctor of Philosophy

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ISBN 0-315-68264-7

ABSTRACT

The S. venustum/verecundum complex (Avalon Peninsula, Newfoundland) was examined to ascertain if individual cytotypes had unique and predictable ecological characteristics. Emphasis was placed on larval spatial-temporal distribution. Six cytotypes were found: EFG/C venustum, CC2-3 venustum (combined CC2 and CC3), AC(gb) venustum, AA verecundum and ACD verecundum.

Larval cytotypes selected different macrohabitats. EFG/C and ACD were sublacustrine species; CC2-3 and AC(gb) preferred downstream sites. Within downstream habitats CC3 usually occurred in large streams and AC(gb) in cool canopied sites with a bed of small stones or rubble; CC2 was ubiquitous. AA preferred outlets and trickles. Cytotype fauna changed in a continuous, directional and predictable manner with increased distance from outlets.

Abundance of CC2-3 increased with increasing seston and discharge, and decreasing conductivity. AC(gb) abundance increased with increasing stream width.

Larval cytotypes also selected different microhabitats. Optimal velocity for EFG/C and ACD was 0.36 and 0.71 m/s, respectively. EFG/C preferred shallow water but ACD showed no depth preference. These differences were not related to larval size. Maximum abundance of EFG/C occurred 10.5 - 16.0 m from outlets; ACD was most abundant just below outlets.

Proportionally more ACD and AA were found on vegetation than rocks; the reverse was true for EFG/C. The density of ACD and AA was higher on vegetation than rocks.

Seasonal occurrence of cytotypes overlapped but population peaks were typically asynchronous. EFG/C appeared 1 - 5 weeks earlier than other cytotypes. ACD and AA appeared later and remained longer than other cytotypes. EFG/C was univoltine. AC(gb) may have a limited second generation. CC2-3 was bi- or multivoltine. ACD and AA were multivoltine.

EFG/C complete larval development from 5 - 30°C with maximum survival between 15 - 20°C (68.0 - 73.5%). An ACD-AA mixed population completed development between 5 - 25°C with maximum survival between 15 - 25°C (55.3 - 61.0%). The temperature survival curve of EFG/C was significantly different from ACD-AA. Threshold temperature was 0°C. Degree-days to complete larval development varied with temperature, cytotype and sex. Temperature also affected larval size.

It was concluded that each cytotype examined was a distinct species with a unique and predictable ecological profile, supporting the hypothesis of widespread sibling speciation within the S. venustum/verecundum complex.

ACKNOWLEDGEMENTS

First and foremost I wish to express my sincere thanks to my supervisor, Dr. Murray H. Colbo. Not only did he provide the means to make this study possible, but more importantly he always found the time to help. I also wish to thank the other members of my supervisory committee, Drs. Allan Whittick and John 'Pick' Pickavance for their invaluable assistance throughout this project. My thanks are also extended to Ray Feraday and the late Dr. Klaus Rothfels, University of Toronto, for teaching me the art of cytotaxonomy.

I would also like to express my appreciation to the following individuals for their contribution to this project:

Dr. Graham Skanes for his statistical advice;

Roy Ficken for his photographic assistance;

Arthur Dent and Ford Perfect for their constant source of inspiration.

My sincere thanks are extended to Ludwig 'Papa' Gubler whose financial support during the writing phase of this project will always be remembered.

Finally, I wish to dedicate this theses to my wife Edith Gubler McCreadie for her unlimited encouragement, patience and support. This will never be forgotten.

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Science is the art of estimating the error of our opinions.

CHAPTER 1

GENERAL INTRODUCTION

Analyses of larval salivary gland polytene chromosomes show that most simuliid morphospecies (i.e., species designated on external morphology) are complexes of biologically distinct, morphologically similar, reproductively isolated sibling species, which are referred to as cytospecies or cytotypes (Rothfels 1979, 1981a b, 1987). Adler (1987) suggested 1 - 2 sibling species exist, on average, for every nominal species in any given area. Because of the difficulty in separating sibling species using conventional morphological characters, biological studies have lagged far behind the rapid designation of these cytotypes and have been largely descriptive in nature (Adler 1987). A few rigorous studies (e.g., Adler and Kim 1984; Ciborowski and Adler 1990) have shown ecological segregation among sibling species. Less than 10% of the world's morphospecies have been investigated cytologically (Crosskey 1987) and the necessity for a complete re-evaluation of behavioural, physiological, taxonomic and ecological data is evident.

Although a number of cytotypes in the Simulium venustum/verecundum complex have been described (Rothfels et al. 1978; Rothfels 1981a; Adler 1986), little is known about the larval ecology of individual cytotypes. In North America, Lake and

Burger (1983) examined the phenology of larval simuliids at lake outlets in New Hampshire, which included 5 cytotypes of the S. venustum/verecundum complex. Gordon and Cupp (1980) attempted to explain the occurrence of larval A/C verecundum (referred to as AA-AC verecundum), ACD verecundum and CC venustum among 4 sites in New York State on limnological grounds. Ciborowski and Adler (1990) provided evidence of habitat segregation among ACD verecundum, CC3 venustum and AA-A/C-CC verecundum (combined AA, A/C and CC verecundum), as did Hunter (1990) for EFG/C venustum, CC venustum, ACD verecundum and A/C verecundum. Adler (1986) reported ACD verecundum was ecologically distinct from venustum line cytotypes in Alberta. The few remaining studies contained limited ecological information, consisting primarily of collection records (Rothfels et al. 1978; Rothfels 1981a; Snyder 1982; Cupp and Gordon 1983; Pistrang and Burger 1984; Colbo 1985; Adler and Kim 1986; Currie and Adler 1986).

Presented here are the results of a 4 year broad-based ecological study of those cytotypes of the S. venustum/verecundum complex found on the Avalon Peninsula of Newfoundland. Emphasis was placed on the spatial and temporal distribution of larval cytotypes, though aspects of preimaginal physiology and morphology, as well as adult oviposition and dispersal, were investigated.

OBJECTIVES

The purpose of the present study was to describe comparatively the larval ecology of those cytotypes of the S. venustum/verecundum complex found on the Avalon Peninsula of Newfoundland. The hypothesis used to structure this project was as follows:

> H_0: Larval cytotypes of the S. venustum/verecundum complex do not exhibit ecological profiles distinct from each other.
>
> H_A: At least some larval cytotypes possesses distinct ecological profiles.

Ecological profiles were largely drawn from spatial and temporal distribution data. Characterization of cytotypes on spatial and temporal axes has 2 advantages: i) such data may be useful in determining species status in cases where cytological evidence is unclear and; ii) the ability to reliably identify cytotype assemblages and predict their occurrence on spatial (stream type) or temporal (seasonality) criteria would greatly reduce the dependence on cytotaxonomic identification.

As stream temperature plays a profound role in the life history of preimaginal simuliids and is of particular relevance to cytotype ecology (above), a detailed laboratory investigation of temperature was also undertaken.

Reliance on cytotaxonomic procedures results from the current inability to distinguish larvae on conventional

morphological criteria (e.g., Rothfels et al. 1978; Adler 1986; Adler and Kim 1986). Crosskey (1987) concluded that morphometric analysis of existing characters, rather than a search for new ones, would probably be required to separate sibling species. As recent attempts have met with limited success (Adler 1983; Snyder and Linton 1983), a preliminary morphometric analysis was conducted to determine how environmental conditions (temperature) may influence the taxonomic value of morphological characters.

REVIEW OF RELEVANT LITERATURE

Simuliid life history

Black flies (Simuliidae: Diptera) are holometabolous insects with 4 distinct stages of development, egg, larva, pupa and adult (Cupp and Gordon 1983; Currie 1986). General treatments of simuliid biology include Crosskey (1973, 1990), Laird (1981), Peterson (1981), and Kim and Merritt (1987). With rare exceptions (e.g., Crosskey 1973; Peterson 1981; Ladle et al. 1985), all preimaginal stages of the life cycle are restricted to lotic (running water) habitats ranging from temporary trickles to large rivers (Davies et al. 1962; Stone 1964; Stone and Snoddy 1969; Colbo and Wotton 1981). Adults are usually dimorphic, aerial and terrestrial (Davies et al. 1962; Stone 1964; Cupp and Gordon 1983).

Temperature, oxygen and photoperiod determine the onset and/or rate of embryonic development (Elsen 1979; Imhof and Smith 1979; Colbo and Wotton 1981; Shipp and Whitfield 1987). Upon hatching, early instars often move within a riffle from oviposition sites to locations more suitable for larval development (Stone and Snoddy 1969; Reisen 1977; Colbo and Moorhouse 1979; Colbo and Wotton 1981). Larvae attach to substrates by a posterior circlet of hooks embedded in a small pad of silk secreted from the salivary glands (Jamnback 1976; Wallace and Merritt 1980). Dispersion over a substrate is either "spaced", with a well defined area surrounding each larva, or "clumped", with each larva occupying only enough substrate to attach the silk pad (Colbo and Wotton 1981; Eymann and Friend 1988).

Most larvae possess highly modified labral fans used to passively filter suspended particles from stream water (Wallace and Merritt 1980; Colbo and Wotton 1981; Currie and Craig 1987). Attached larvae, with the head in a downstream direction, twist the body longitudinally (90 - 180°) to use the ventral surface of each head fan to filter water (Wallace and Merritt 1980). Facultative scraping, obligatory scraping, deposit feeding and opportunistic predation are alternative feeding strategies (Chance 1970; Colbo and Wotton 1981; Walsh 1985; Currie and Craig 1987). Larvae filter particles between 0.091 - 350 μm with the majority of particles ingested less than 100μm (Wotton 1976; Chance 1977; Wallace and Merritt

1980; Walsh 1985). Feeding efficiency is low, generally below 10% (Kurtak 1978).

The larval diet consists of detritus, bacteria, animal tissue and algae (Fredeen 1964; Wallace and Merritt 1980; Wotton 1980a; Walsh 1985; Schröder 1987). Gut contents are largely determined by particle size and availability in the seston (Anderson and Dicke 1960; Moore 1977a b; Kurtak 1978, 1979; Schröder 1983; Thompson 1987a). The size distribution of ingested particles may shift with larval age (Schröder 1981, 1983; Thompson 1987a). Recent reviews relevant to larval feeding include Wallace and Merritt (1980), Walsh (1985) and Currie and Craig (1987).

The larval stage can last from 2 weeks to several months depending on stream temperature, food availability and species (Lewis and Bennett 1974; Ross and Merritt 1978, 1987; Merritt et al. 1978, 1982; Colbo 1979, 1982; Colbo and Porter 1979, 1981). The number of larval instars varies between 6 - 11, depending on species and stream conditions (Ross and Merritt 1978; Colbo and Wotton 1981; Currie 1986; Colbo 1989). Mature last instars (i.e., pharate pupae or prepupae) will often move to calmer water before spinning a cocoon of silk in which to pupate (Maitland and Penny 1967; Crosskey 1973; Colbo and Moorhouse 1979). Pupal development varies from 2 days to 2 weeks depending on temperature (Stone 1964; Elsen 1979; Peterson 1981; deMoor 1982; Prügel 1988). Adults emerge diurnally from the pupal skin and rise to the stream surface

in an air bubble (Crosskey 1973; Peterson 1981; Wenk 1981).

Reproduction is usually sexual, with copulation occurring either in mating swarms or by individual males waiting for females near streams, hosts, or nectar sources (Davies et al. 1962; Stone 1964: Crosskey 1973; Wenk 1987). Triploid parthenogenetic forms also occur (Rothfels 1979, 1989). Adult females usually require a bloodmeal in order to develop each batch of eggs (anautogenous), although some species mature the first batch without a blood meal (autogenous) while others do not take blood (Davies et al. 1977; Currie 1986; Anderson 1987). Most blood sucking simuliids feed diurnally on mammals (mammalophilic) or birds (ornithophilic), though attacks on other vertebrates and invertebrate hosts are known (Davies et al. 1962; Stone 1964; Stone and Snoddy 1969; Crosskey 1973, 1990).

Adults take plant juices such as nectar to satisfy energy requirements (Peterson 1981; Currie 1986). Females can disperse 200 - 600 km from breeding sites in search of suitable hosts (Fredeen 1969; Walsh et al. 1981), but 15 km or less is probably the normal range of movement (Stone 1964; Bennett and Fallis 1971; Wenk 1981). Adult longevity is usually less than a month (Bennett and Fallis 1971; Crosskey 1973, 1990; Jamnback 1976).

Oviposition usually takes place in the evening, when females either deposit eggs freely into the stream or attach eggs in masses to substrates (e.g., rocks, trailing

vegetation, dam sluices) at or below the water line (Jamnback 1976; Colbo and Wotton 1981; Golini and Davies 1987). Other unique forms of oviposition have been recorded (Peterson 1981; Ladle et al. 1985). Most species deposit 100 - 600 eggs per gonotrophic cycle, although this can vary from 25 (Gymnopais Stone) to 800 (Simulium Latreille) (Davies et al. 1962; Crosskey 1973, 1990; Currie 1986). Depending on species or time of year, eggs can hatch in several days or enter diapause (Davies et al. 1962; Stone 1964; Jamnback 1976; Ross and Merritt 1987; Shipp 1987). Eggs cannot withstand desiccation (Imhof and Smith 1979), but some species may survive in the moist soil of dry stream beds for several months to several years (Fredeen et al. 1951; Anderson and Dicke 1960; Colbo and Moorhouse 1974; Adler and Kim 1986). Recent reviews on simuliid reproduction include Anderson (1987), Golini and Davies (1987) and Wenk (1987).

The seasonal distribution of Nearctic simuliids falls into 3 general categories (Stone 1964; Ross and Merritt 1987). These include: i) univoltine species (e.g., Prosimulium Rombaud) where eggs deposited in the spring or early summer hatch in late fall or early winter. Larval growth is slow over most of the winter and pupation starts by early March; ii) univoltine species (e.g., Simulium) where eggs deposited in the early summer hatch the following spring. Larval growth is rapid and pupation starts by April or May and; iii) bivoltine and multivoltine species (e.g., Simulium) with eggs often

hatching later than group i) or ii). Larvae, pupae and adults may be present all summer. Eggs laid by the last summer or fall generation overwinter until the following spring.

Spatial distribution patterns of preimaginal simuliids

Spatial distribution of preimaginal simuliids has been considered on 2 broad scales - macrodistribution (i.e., larval distribution between stream and sections thereof) and microdistribution (i.e., the distribution of larvae within a small section of the stream) (Colbo and Wotton 1981). The broadest macrodistribution studies have examined the distribution of simuliids among and within the 6 zoogeographic regions of the world (e.g., Crosskey 1981, 1987). Numerous studies have considered patterns of larval distribution within a large section of a zoogeographic region (e.g., Cupp and Gordon 1983; Corkum and Currie 1987). At the lowest scale, studies of macrodistribution have examined larval distribution between streams in highly localized areas (e.g., Colbo 1979) and within a single drainage basin (e.g., Maitland and Penny 1967). These latter studies have stressed the role of stream site characteristics (e.g., food supply, ovipositional cues, temperature, substrate) in determining preimaginal distribution.

Distribution within a stream reach (e.g., Ulfstrand 1967) or dispersion over individual substrates (e.g., Rühm and Pegel 1986) has customarily been referred to as microdistribution.

In recent years, laboratory studies have focused on the patterns of dispersion within groups and the factors responsible for these patterns (Craig and Chance 1982; Hart 1986; Chance and Craig 1986; Eymann and Friend 1988; Ciborowski and Craig 1989).

Preimaginal habitat selection on any scale depends upon the interaction of numerous physical and biotic factors. Tables 1.1 and 1.2 provide comprehensive (though not exhaustive) lists of those factors thought to influence preimaginal macrodistribution and microdistribution respectively. Although some factors affect distribution over both scales of study (e.g., water velocity, substrate, biotic interactions), the influence of many factors can be ignored at the level of the microhabitat (Ulfstrand 1967; Rabeni and Minshall 1977). For example, water chemistry and temperature are usually considered homogeneous over localized areas of a stream. Consequently, microdistribution studies have focused on conditions which vary over a distance of a few centimeteres or meters, such as water depth and velocity. Minshall and Minshall (1977) have suggested that microdistribution of benthic insects is the product of a series of responses to a set of interacting variables imposed in a hierarchical fashion. These authors also emphasised that responses to stream conditions are species specific, hence the need to consider species individually.

Various factors may influence preimaginal distribution

via several pathways and different factors may interact. For example, velocity can influence larval microdistribution by its effects on filter feeding (Chance and Craig 1986; Craig and Galloway 1987; Morin and Peters 1988), substrate suitability (Ulfstrand 1967; deMarch 1976; Rabeni and Minshall 1977; Reice 1980; Minshall 1984), substrate stability (Newbury 1984), and the distribution of potential predators and competitors (Ulfstrand 1967; Minshall and Minshall 1977; Orth and Maughan 1983).

Reviews concerned wholly or in part with the spatial distribution of preimaginal simuliids include Carlsson (1967), Colbo and Wotton (1981), Grunewald (1981), Ross and Merritt (1987) and Wotton (1987).

Hydrodynamics of flow

Hydrodynamics are fundamental to larval habitat selection (Craig and Galloway 1987). General treatments on the consequences of flow to living organisms can be found in Vogel (1981, 1988), Lugt (1983), Nowell and Jumars (1984) and Statzner et al. (1988). Specific treatments for simuliids include Décamps et al. (1975), Craig and Chance (1982), Chance and Craig (1986), Craig and Galloway (1987). Stream hydrology was reviewed by Newbury (1984).

Velocity at the substrate-water interface is zero due to frictional forces, but increases with distance from the substrate until mainstream velocity is reached (Statzner et

al. 1988). The region where the water velocity is 90% (some authors use 99%, see Chance and Craig 1986; Statzner et al. 1988) or less than that of the mainstream velocity is known as the boundary layer (Craig and Galloway 1987). In streams most of the boundary layer is typically turbulent, but flow immediately above the substrate exhibits laminar flow and is termed the 'laminar' or 'viscous sublayer' (Craig and Galloway 1987; Statzner et al. 1988). It is in the boundary layer that simuliid larvae are found (Craig and Galloway 1987). Although it has often been assumed that lotic insects living within the boundary layer are protected from flow, recent studies have shown that lotic invertebrates negotiate the complicated patterns of flow and consequently deal with these forces (Statzner et al. 1988).

Simple hydraulic characters, such as depth, velocity and stream bottom substrates, correlate well with both the macrodistribution and microdistribution of preimaginal simuliids (Tables 1.1 and 1.2). Assuming uniform flow, measurements of these variables (mean values) allow calculations of complex hydraulic variables (Statzner et al. 1988). These include Froude number, Reynolds number, viscous sublayer thickness, shear stress, and boundary layer Reynolds number. Froude and Reynolds numbers describe general flow conditions; viscous sublayer thickness, shear stress, and boundary layer Reynolds number describe conditions near the stream bottom (Orth and Maughan 1983; Chance and Craig 1986;

Craig and Galloway 1987; Statzner et al. 1988). Both Froude number and viscous sublayer thickness are known to correlate with simuliid distribution (Statzner 1981a; Orth and Maughan 1983; Wetmore et al. 1990). At present it is not certain whether simple or complex hydraulic characters best describe the stream environment (Statzner et al. 1988).

Recently, Wetmore et al. (1990) pointed out that under conditions of chaotic flow (e.g., rapids) stream bed conditions such as shear stress and boundary layer thickness cannot be estimated using mean flow velocity, depth, slope and substrate size. Instead, a more detailed characterization of flow is required. Undoubtedly, future studies on stream ecology will place more emphasis on determining which hydraulic variables most influence insect distribution.

The hydrodynamics of flow at the substrate level also influence simuliid dispersion patterns and biotic interactions (Craig and Chance 1982; Chance and Craig 1986; Craig and Galloway 1987; Ciborowski and Craig 1989) which may in turn influence distribution over higher scales.

Temperature

Temperature plays a major role in the ecology and evolution of aquatic insects (Ward and Stanford 1982; Sweeney 1984). Stream temperature has a significant influence on the macrodistribution (Table 1.1) and population dynamics (Ross and Merritt 1987) of preimaginal simuliids as well as on:

embryonic development and eclosion (Elsen 1979; Imhof and Smith 1979; Shipp and Whitfield 1987), larval and pupal development (Mokry 1976; Elsen 1979; Colbo and Porter 1981; deMoor 1982; Merritt et al. 1982; Prügel 1988; Wirtz et al. 1990), larval survival (Davies and Smith 1958; Mokry 1976), growth rate (Hauer and Benke 1987; Morin et al. 1988a), feeding (Schröder 1981; Thompson 1987b), number of instars (Ross and Merritt 1978; Post 1983), larval size (Ross and Merritt 1978; Merritt et al. 1982; deMoor 1982; Post 1983), number of generations (Zahar 1951; Carlsson 1962), emergence (Wenk 1981) and fecundity and/or adult size (Chutter 1970; Neveu 1973; Neveu and Lapchin 1979; Colbo and Porter 1981). The seasonal abundance of preimaginal stages has also been correlated with stream temperature (Mohsen and Mulla 1982; Shipp and Procunier 1986). Temperature may also act as a means of partitioning species along a 'thermal gradient' (Merritt et al. 1982).

The Simulium venustum/verecundum s. l. complex

The S. venustum/verecundum complex has a long and confused taxonomic history. A detailed account of this holarctic complex can be found in Rothfels et al. (1978) and Gordon and Cupp (1980). This complex is characterized by a larva with a negative head pattern and a pupa with 6 respiratory filaments.

Simulium venustum was described by Say in 1823 from

adults collected by the Ohio River near Shippingsport (Rothfels et al. 1978). Stone and Jamnback (1955) showed that S. venustum in North America was a complex of 2 morphospecies, the second, named verecundum, was distinguishable from S. venustum Say on the basis of adult male genitalia. These authors also reported S. venustum as univoltine and anthrophilic, whereas S. verecundum Stone and Jamnback was multivoltine and did not attack man. Davies et al. (1962) described diagnostic differences in the female genitalia.

Regional faunistic surveys, ecological investigations and laboratory studies have produced a great deal of information regarding the S. venustum/verecundum s. l. complex (Stone and Jamnback 1955; Wolfe and Peterson 1959; Anderson and Dicke 1960; Bennett 1960; Davies et al. 1962; Stone 1964; Abdelnur 1968; Craig 1969; Stone and Snoddy 1969; Chance 1970; Moore 1977a b; Merritt et al. 1978; Bauer and Granett 1979; Bruder and Crans 1979; Imhof and Smith 1979; Kurtak 1979; LaScala 1979; Westwood and Brust 1981; Currie 1986; Shipp and Procunier 1986; Corkum and Currie 1987; Morin et al. 1988b). This is particularly true in Newfoundland where all stages of the life cycle have been studied (e.g., Pickavance et al. 1970; Davis 1971; Lewis and Bennett 1973, 1974, 1975; Ezenwa 1974; Mokry 1976; Colbo and Porter 1979, 1981; Colbo 1982; McCreadie et al. 1984, 1985, 1986; Thompson 1987a b c).

From the above studies a general life history for the S. venustum/verecundum complex can be constructed. In North

America, larvae are found as far north as Alaska and as far south as Louisiana. In temperate regions the egg is the overwintering stage. Larvae are found in streams from February to November, but are more commonly found between April and September. In more southern locations (e.g., Alabama) larvae may be present throughout most of the winter. Preimaginals have been found in almost every lotic habitat, from small temporary streams to large rivers. The pupal stage lasts approximately 1 week, depending on temperature.

Adults are dimorphic, anautogenous, mammalophilic blood feeders, which occasionally feed on birds. Due to their blood feeding habits, adults are one of the most important pests of man and domestic livestock in NE North America. Females either deposit eggs freely into the stream where they sink to the bottom (venustum line ?) or attach egg masses to substrates (e.g., rocks, trailing vegetation) at or below the water line (verecundum line ?).

Many studies have separated the larval S. venustum complex from S. verecundum complex on morphological grounds. However, recent studies have cast doubt on the ability of published morphological characters to separate larvae along these 2 lines (Rothfels et al. 1978; Adler 1986; Adler and Kim 1986; Currie 1986; Colbo pers. comm. 1987). Therefore, all references to larval S. venustum or S. verecundum based on conventional morphological criteria should be considered S. venustum/verecundum s. l. complex. Adults can usually be

separated into S. venustum complex and S. verecundum complex (Stone and Jamnback 1955; Davies et al. 1962). In central Ontario, Hunter (1990) was able to assign adults and pupae of 4 cytotypes to previously described species: EFG/C venustum = Simulium truncatum (Lundström); CC venustum = S. venustum; ACD verecundum = Simulium rostratum (Lundström); A/C verecundum= S. verecundum. Whether morphological separation is possible over a larger geographic area remains to be seen.

Simuliid taxonomy

Simuliid systematics have changed considerably over the past 30 years as cytological and biochemical approaches have become increasingly more important in species recognition (Crosskey 1987). Based on traditional morphological characters, approximately 1500 species are currently recognized globally; to this can be added at least 150 cytotaxonomically recognized sibling species (Crosskey 1987).

Most cytological studies of simuliids rely on salivary gland polytene chromosomes of late instars (Rothfels 1979; Rothfels 1987), although adult polytene chromosomes, especially those from Malpighian tubule cells, are occasionally used (e.g., Bedo 1976; Adler 1983; Procunier and Post 1986; Hunter 1990). In many simuliid tissues, repeated chromosome replication within a single nucleus (endopolyploidy), with replicates remaining tightly synapsed in parallel, results in very wide (Fig. 1.1) polytene

chromosomes (Farnsworth 1978). Simuliids almost invariably have 3 pairs (n = 3) of metacentric chromosomes (rarely n = 2) with homologues intimately paired (Rothfels 1979). Chromosomes are designated I, II, and III in descending order of length, with short and long arms of each chromosome labelled S and L respectively (Rothfels 1979).

Stained polytene chromosomes show very distinct light and dark banding patterns (Fig. 1.1), which allows visual detection of chromosomal rearrangements, such as inversions and interchanges (Rothfels 1979). Cytotypes commonly differ in fixed autosomal inversions, floating inversions (polymorphisms) or sex chromosomes. Other aspects of cytology, such as the presence/absence of B chromosomes and details of male meiosis can also vary among species (Rothfels et al. 1978; Rothfels 1979, 1981a b, 1987). Selection of which band sequence in a chromosome arm is considered standard or inverted is arbitrary, but by convention standard is selected for its 'centrality', i.e., the sequence which gives rise to the largest number of independent derivatives (Rothfels 1979).

Biochemical approaches to simuliid taxonomy include gas liquid chromatography of cuticle hydrocarbons (Carlson and Walsh 1981; Phillips et al. 1985), enzyme electrophoresis (May et al. 1977; Snyder 1982; Snyder and Linton 1983; Grams and Zillmann 1984) and DNA sequencing (Townson et al. 1987). To date most of these procedures have met with limited success. Reviews pertaining to simuliid taxonomy include morphological

(Crosskey 1981, 1987), biochemical (Townson and Meredith 1979, Townson et al. 1987) and cytological treatments (Rothfels 1979, 1981a b, 1987).

Cytology of the Simulium venustum/verecundum complex

Members of the S. venustum/verecundum complex have 3 pairs (n = 3) of metacentric chromosomes (Rothfels et al. 1978). Rothfles has numbered inversions in order of discovery, for example, IIL-1 refers to the first inversion described on the long arm of chromosome II (Rothfels et al. 1978; Rothfels 1981a). For purposes of the present study, I will use s (= standard) and i (= inverted) to indicate the homozygous or heterozygous condition. For example, for the IIL-4 autosomal inversion, IIL-4 ss, IIL-4 si, and IIL-4 ii denote the homozygous standard state (i.e., no inversion), the heterozygous state (i.e., one chromosome with inversion) and the homozygous inverted state (i.e., both chromosomes with inversion) respectively. If an inversion marks the sex chromosomes a slash is used (e.g., ♀ CC2 venustum = X:X = IIL-1 i/i and ♂ CC2 venustum = Y:X = IIL-1 s/i).

The only exception to the above designations is the IIS arm where Rothfels has labelled inversions using the letters A, C, D, E, F, G, H, and J (Rothfels et al. 1978; Rothfels 1981a). The band sequence C has been designated as standard for the IIS arm (Rothfels et al. 1978). Where there is only one sequence for a cytotype it is listed twice (e.g., CC

venustum) and where there are several they are all listed (e.g., ACD verecundum). As above, inversions marking the sex chromosomes are separated by a slash (e.g., EFG/C venustum) (Rothfels et al. 1978).

Fourteen cytotypes have been described on the basis of chromosomal banding patterns from larval salivary gland cells (Rothfels et al. 1978; Rothfels 1981a; Adler 1986). Ten of these belong to the venustum line (CC, CC1, CC2, CC3, CC4, EFG/C, A/C, AC(gb), JJ and H/C) and 4 to the verecundum line (ACD, AA, A/C, CC). The 2 lines are separated by at least 10 fixed inversions (Rothfels 1981a). Where larvae were sympatric, species status was assumed in the absence of chromosomally recognizable hybrids (i.e., inversions). Differences in sex chromosomes have also been used to separate cytotypes. In allopatric situations cytological data can be unclear; conventional morphological characters also suffer the same limitations (Rothfels et al. 1978). Cytotype designation has relied heavily on inversions found on chromosome II.

A cytological description and geographic range of all known cytotypes of the S. venustum/verecundum complex is given in Table 1.3. This is not a list of all known inversions, but rather a taxonomic aid for simuliid ecologists primarily interested in identifying local demes. B chromosomes have not been included due to their limited diagnostic value (Rothfels 1987).

EFG/C venustum and ACD verecundum conform cytologically

to the European species S. truncatum and S. rostratum (syn. = sublacustre Davies 1966 and groenlandicum Enderlein 1935) respectively (Rothfels et al. 1978, Crosskey 1987). This concurs with the work of Hunter (1990). Literature references to S. rostratum must be treated with caution as some authors have confused Simulium corbis Twinn with this species (Crosskey 1987). Crosskey (1987) suggested that AA verecundum is probably the morphospecies S. verecundum described by Stone and Jamnback (1955).

For purposes of this study, EFG/C and ACD refer to cytologically identified North American material and S. truncatum and S. rostratum to European material recognized by conventional morphological means. Remaining cytotypes of the S. venustum/verecundum complex will also be referred to by their IIS letter designation. The specific suffix venustum or verecundum is used in cases where the IIS mnemonic is insufficient (e.g., A/C venustum vs A/C verecundum).

Little is known about most cytotypes. What is known comes from a few preliminary ecological studies (Gordon and Cupp 1980; Lake and Burger 1983; Adler 1986; Ciborowski and Adler 1990; Hunter 1990) and numerous collection records (Rothfels et al. 1978; Rothfels 1981a; Snyder 1982; Cupp and Gordon 1983; Pistrang and Burger 1984; Colbo 1985; Adler and Kim 1986; Currie and Adler 1986). Based on these studies one can infer that voltinism, seasonal distribution and larval habitat selection varies among cytotypes.

THESIS FORMAT

Test formatting follows that of the Canadian Journal of Zoology, which follows the guidelines set forth by the Council of Biology Editors Inc., (Bethesda, MD., U.S.A., 20814). Due to the variety of topics covered (cytotype fauna, temperature, macrodistribution, microdistribution, phenology, and morphology) the thesis has been written in a chapter format. However, results were integrated between chapters when appropriate in order to present a unified body of knowledge with a logical progression of ideas, rather than a series of unconnected works. Chapter 2, General Methodology, provides a general description of the study area and details of procedures commonly used throughout the study (e.g., staining procedures, cytological identification, measurement of stream variables). Methods specific to any one chapter are given under the Materials and Methods section of that chapter.

Finally, there has been a proliferation of jargon in simuliid taxonomy over the past 30 years. The terms cytospecies, cytotype, zymospecies, zymotype, forms, morphospecies, complex and group are commonly encountered in the literature (Rothfels et al. 1978; Rothfels 1979, 1981a, 1987; Crosskey 1981, 1987). Rothfels (1987) pointed out that 'species' implies reproductive isolation between populations, with prefixes (cyto, morpho, zymo) simply indicating the nature of the supporting evidence. 'Type' on the other hand,

is a more provisional designation. A 'complex' refers to an assemblage of cytospecies within a morphospecies (e.g., S. venustum/verecundum complex) and a 'group' refers to a number of closely related morphospecies (Crosskey 1981, 1987).

For simplicity and to avoid excessive jargon, I have elected to use the term cytotype for all members of the S. venustum/verecundum complex. For example, CC and CC1 are distinct from CC2 venustum. However, it is not certain whether CC is distinct from CC1 (Rothfels et al. 1978). Using the term cytotype when referring to CC and CC1, but cytospecies when comparing CC2 venustum to CC venustum or CC1 venustum or both, creates unnecessary confusion. Furthermore, larvae cannot always be unequivocally identified. For example, CC2 venustum and CC3 venustum females, in many cases cannot be separated chromosomally (Rothfels 1981a). Therefore in the present study they were grouped as CC2-3 venustum. Referring to CC2-3 venustum as a cytospecies is clearly incorrect, but the term cytotype is acceptable as female CC2-3 venustum were distinguishable on cytological grounds from other members of the complex in Newfoundland (Rothfels 1981a; see chapter 3). In order not to second guess other authors, I have used the terminology quoted by those authors when referring to other complexes.

TABLE 1.1. Stream factors associated with the macrodistribution of larval and pupal black flies†.

Stream factor	Reference
Larval substrate (availability, suitability)	Zahar 1951 Maitland and Penny 1967 Chutter 1968 Rühm 1970 Lewis and Bennett 1975 Carlsson et al. 1977 Reisen 1977 Colbo and Moorhouse 1979 Adler and Kim 1984, 1986 Hershey and Hiltner 1988 Morin and Peters 1988
Water velocity	Zahar 1951 Davies and Syme 1958 Anderson and Dicke 1960 Maitland and Penny 1967 Rühm 1970 Lewis and Bennett 1975 Williams and Hynes 1976 Boobar and Granett 1980 Grunewald 1981 Mohsen and Mulla 1982 Adler and Kim 1984 Morin and Peters 1988
Discharge (high, low, stable, fluctuating) and flow pattern (turbulent, calm)	Zahar 1951 Yakuba 1959 Chutter 1968 Reisen 1977 Mohsen and Mulla 1982 Townsend et al. 1983
Water temperature	Zahar 1951 Davies and Smith 1958 Davies and Syme 1958 Anderson and Dicke 1960 Maitland and Penny 1967 Thorup 1974 Ladle et al. 1977 Reisen 1977 LaScala 1979 Grunewald 1981 Mohsen and Mulla 1982

TABLE 1.1 continued.

Stream factor	Reference
Water temperature (continued)	Adler and Kim 1984, 1986 Adler 1986 Pistrang and Burger 1988
Water chemistry (pH, dissolved oxygen, ionic content, pollution etc.)	Carlsson 1967 Stone and Snoddy 1969 Glötzel 1973 Ali et al. 1974 Grunewald 1976, 1981 Quillévéré et al. 1976,1977 Grunewald et al. 1979 Gordon and Cupp 1980 Townsend et al. 1983 Adler and Kim 1984, 1986 Gordon 1984 Pistrang and Burger 1984 Corkum and Currie 1987
Stream size (width) order (1st, 2nd, etc.) type (temporary, permanent)	Zahar 1951 Davies and Syme 1958 Anderson and Dicke 1960 Stone and Snoddy 1969 Konurbayev 1978 Colbo 1979 Adler 1986 Adler and Kim 1986 Corkum and Currie 1987 Schröder 1988 Ciborowski and Adler 1990
Food quantity and/or quality	Carlsson 1967 Maitland and Penny 1967 Chutter 1968 Glötzel 1973 Thorup 1974 Carlsson et al. 1977 Ladle et al. 1977 Wotton 1979 Morin and Peters 1988 Schröder 1988
Terrain, canopy cover, riparian vegetation	Zahar 1951 Davies and Smith 1958 Anderson and Dicke 1960 Bishop 1973

TABLE 1.1 continued.

Stream factor	Reference
Terrain, canopy cover, riparian vegetation (continued)	Thorup 1974 Colbo and Moorhouse 1979 Mohsen and Mulla 1982 Adler and Kim 1984 Behemer and Hawkins 1986 Olejnicek 1986 Shipp 1986 Schröder 1988
Outlets, impoundments	Anderson and Dicke 1960 Carlsson 1967 Stone and Snoddy 1969 Ezenwa 1974 Thorup 1974 Carlsson et al. 1977 Sheldon and Oswood 1977 Carlsson and Mullar 1978 Glatthaar 1978 Colbo 1979 Wotton 1979, 1982, 1987 Adler and Kim 1986 Olejnicek 1986 Corkum and Currie 1987 Morin and Peters 1988 Pistrang and Burger 1988 Ciborowski and Adler 1990
Biotic interaction (predations, parasitism, competition)	Chutter 1968 Wotton 1982 Harding and Colbo 1981 Hershey and Hiltner 1988
Water depth	Lewis and Bennett 1975
Ovipositional behaviour	Zahar 1951 Rühm 1972 Carlsson et al. 1977 Ladle et al. 1977 Wotton 1979 deMoor et al. 1986

†Macrodistribution defined as the distribution of larvae between streams or sections thereof.

TABLE 1.2. Stream factors associated with the microdistribution of larval and pupal black flies[†].

Stream factor	Reference
Larval substrate (availability, suitability)	Ulfstrand 1967 Décamps et al. 1975 Hudson and Hays 1975 deMarch 1976 Rabeni and Minshall 1977 Colbo and Moorhouse 1979 Boobar and Granett 1980 Reice 1980 Gregg and Rose 1985 Rühm and Pegel 1986 Malmquist and Otto 1987 Morin and Peters 1988 Das et al. 1989 Pruess 1989
Water velocity	Phillipson 1956, 1957 Maitland and Penny 1967 Ulfstrand 1967 Chutter 1969 Décamps et al. 1975 Lewis and Bennett 1975 Minshall and Minshall 1977 Reisen 1977 Colbo and Moorhouse 1979 Gersabeck and Merritt 1979 Boobar and Granett 1980 Osbourne et al. 1985 Wotton 1985 Yamagata and Kanayama 1985 deMoor et al. 1986 Morin et al. 1986 Rühm and Pegel 1986 Morin and Peters 1988 Ciborowski and Craig 1989 Wetmore et al. 1990
Discharge	Yakuba 1959 Lewis and Bennett 1975 Reisen 1977 Gersabeck and Merritt 1979 Eymann and Friend 1987

TABLE 1.2 continued.

Stream factor	Reference
Flow pattern (laminar, turbulent, subcritical, critical, supercritical)	Maitland and Penny 1967 Décamps et al. 1975 Colbo 1979 Orth and Maughan 1983 Osbourne et al. 1985 Rühm and Pegel 1986 Wetmore et al. 1990
Food availability	Ciborowski and Craig 1989
Canopy cover	Towns 1981
Outlets	Morin et al. 1986
Biotic interaction (predation, competition, etc.)	Disney 1972 Reisen 1977 Colbo 1979 Colbo and Moorhouse 1979 Gersabeck and Merritt 1979 Wiley and Kohler 1981 Hemphill and Cooper 1983 Hart 1986 Rühm and Pegel 1986 Malmquist and Otto 1987 Eymann and Friend 1988 Fuller and deStaffan 1988 Hemphill 1988 Ciborowski and Craig 1989
Water depth	Ulfstrand 1967 Chutter 1969 Lewis and Bennett 1975 Reisen 1977 Gersabeck and Merritt 1979 Granett 1979 Yamagata and Kanayama 1985 Morin et al. 1986 Morin and Peters 1988 Pruess 1989
Photoperiod	Gersabeck and Merritt 1979

[+]Microdistribution defined as distribution of larvae within a small (< 100m) section of stream.

TABLE 1.3. Cytological descriptions of all presently known cytotypes in the Simulium venustum/verecundum complex. Chromosome maps showing all inversions tabled below are provided in Rothfels et al. (1978). Note inv. = inversion.[+]

CYTOTYPE[++]	IS	IL	IIS	IIL	IIIS	IIIL
EFG/C venustum (= S. truncatum)[++] Holarctic	ss	ss	♂ EFG/C ♀ EFG/EFG X:EFG Y:C	ss	ss	IIIL-5 ss
CC venustum (= S. venustum?) North America	ss	ss	CC	ss	ss	IIIL-5 ii (rarely ss)
CC1 venustum Ont. Mich.	ss	ss	CC	ss	ss	♂ IIIL-5 s/i IIIL-6 s/i ♀ IIIL-5 i/i IIIL-6 s/s X:IIIL-5 i Y:IIIL-6 i
CC2 venustum Ont. Nfld. N.H. Pa.	ss	ss	CC	♂ IIL-1 s/i ♀ IIL-1 i/i X:IIL-1 i Y:IIL-1 s	ss	IIIL-5 ii (most demes) IIIL-5 ss (some demes)

TABLE 1.3 continued.

CYTOTYPE	IS	IL	IIS	IIL	IIIS	IIIL
CC3 *venustum* Que. Man. Alta. Nfld. Sask.	ss	ss	CC	IIL-1 ss si ii	ss	♂ IIIL-5 s/i ♀ IIIL-5 i/i X:IIIL-5 i (2% = s, Que.) Y:IIIL-5 s plus several other inv.
CC4 *venustum* Alta.	ss	ss	CC	ss	ss	♂ IIIL-5 ss si ♀ IIIL-5 ss
AC(gb) *venustum* Nfld. Que. N.H. N.Y.	ss	ss	AA AC CC (rare)	IIL-1 ss si ii	ss	IIIL-5 ii
A/C *venustum* N.H. Mich. Alaska	ss	ss	♂ A/C ♀ A/A X:A Y:C	ss	ss	IIIL-5 ii

TABLE 1.3 continued.

CYTOTYPE	IS	IL	IIS	IIL	IIIS	IIIL
H/C *venustum* Que.	ss	ss	♂ H/C ♀ H/H X:H Y:C	IIL-1 ss si ii	ss	ss plus other inv.
JJ *venustum* Oregon	ss	ss	JJ	ss	ss	IIIL-5 ss
AA *verecundum* (= *S. verecundum*?) Pa. Nfld. N.Y., Sask. ?	ss	IL-1 ii	AA	IIL-1,2 ii IIL-4 ss si ii	ss	IIIL-1 ii IIIL-2 ii IIIL-3.4 ii plus 4 or more inv. (complex) IIIL-5 ss
A/C *verecundum* (= *S. verecundum*?) N.Y. N.H. *Que.* Ont. Sask. ?	ss	IL-1 ii	♂ A/C ♀ A/A X:A Y:C	PS3 ii PS3/IIL-1,2 IIL-1,2 ii PS3 = IIL-1,2.3	ss	IIIL-1 ii IIIL-2 ii IIIL-3.4 ii plus 4 or more inv. (complex) IIIL-5 ss

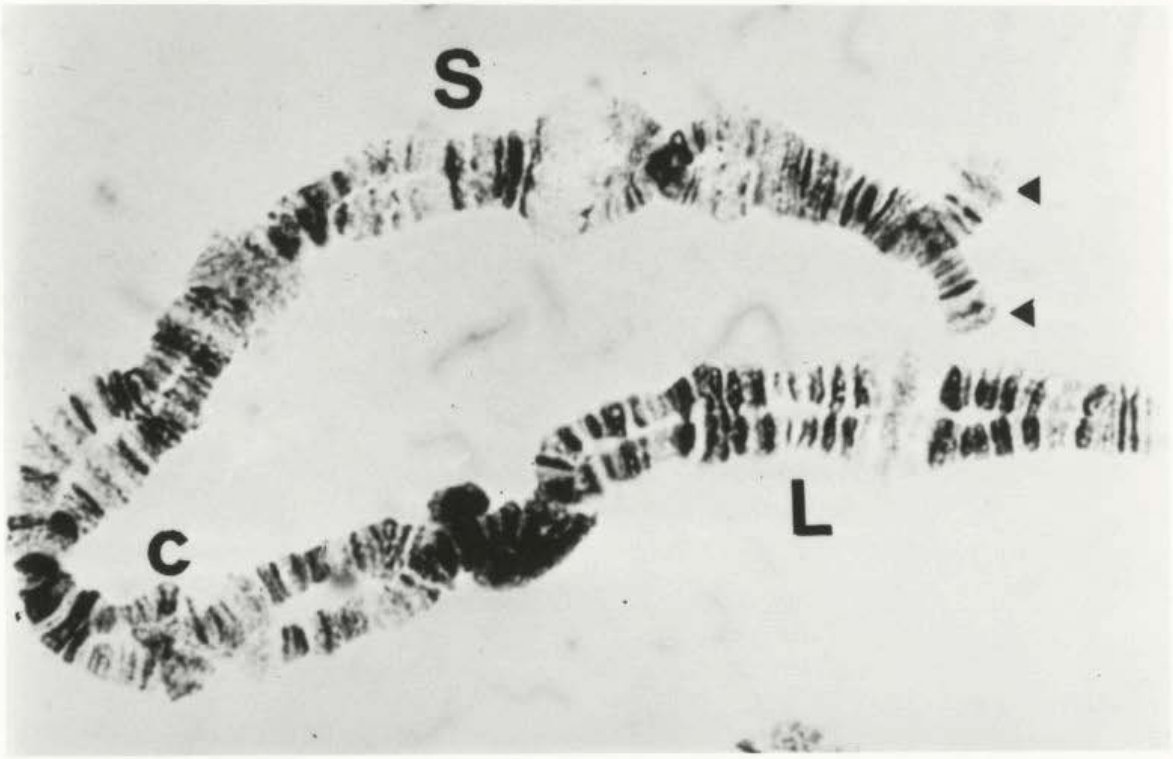
TABLE 1.3 continued.

CYTOTYPE	IS	IL	IIS	IIL	IIIS	IIIL
CC verecundum Que. Sask. ?	ss	IL-1 ii	CC	PS3 ii PS3/IIL-1,2 IIL-1,2 ii PS3 = IIL-1,2.3	ss	IIIL-1 ii IIIL-2 ii IIIL-3.4 ii plus 4 or more inv. (complex) IIIL-5 ss
ACD verecundum (= S. rostratum) Holarctic	ss	IL-1.2 ii	AA AC AD CC CD DD	♂ IIL-5.6+4/ IIL-1,2 ♀ IIL-5.6+4 i/i X:IIL-5.6+4 Y:IIL-1,2 plus other inv. very complex common sequences: X_E:IIL-7+5.6.4.9 X_u:IIL-(5,8).6.4.9 X_3:IIL-7+5.6,10.9.4 Y_E:IIL-1,2+14 Y_u:IIL-1,2 Y_3:IIL-1,2+15	ss	IIIL-1 ii IIIL-2 ii IIIL-3.4 ii plus other inv. IIIL-5 ss

+ Cytological descriptions taken from Rothfels et al. (1978), Rothfels (1981a), Adler (1986) and Feraday (pers. comm. 1986). Locality records from Rothfels et al. (1978), Gordon and Cupp (1980), Rothfels (1981a), Snyder (1982), Cupp and Gordon (1983), Lake and Burger (1983), Pistrang and Burger (1984), Colbo (1985), Adler (1986), Adler and Kim (1986), Currie and Adler (1986), Ciborowski and Adler (1990) and the present study. EFG (= Simulium morsitans) Edwards has been removed from the complex as pupae have 8 respiratory filaments (Rothfels 1979).

++ Chromosomally, larval EFG/C and ACD are equivalent to the European species S. truncatum and S. rostratum respectively (Rothfels et al. 1978; Crosskey 1987). Crosskey (1987) suggested that AA verecundum is the S. verecundum first described by Stone and Jamnback (1955). In central Ontario Hunter (1990) has been able to assign adults and pupae of 4 cytotypes to previously described species: EFG/C venustum = S. truncatum; CC venustum = S. venustum; ACD verecundum = S. rostratum; A/C verecundum = S. verecundum.

FIG. 1.1. Typical appearance of a feulgen stained simuliid salivary gland cell polytene chromosome, showing the characteristic light (repeat DNA) and dark (areas of genetic activity) bands. This particular example is the II chromosome (S = short arm, L = long arm) taken from a female EFG/C. Sister homologues (arrows) and the centromere (C) are well illustrated. (Note that the last 10% of IIL is cut off in this photograph)


S
C
L

CHAPTER 2

GENERAL METHODOLOGY

This study is primarily of the last larval instar, although limited information is presented on oviposition (chapters 4 and 5), pupal distribution (chapter 7) and dispersal of adults (chapter 6). Emphasis is placed on last larval instars because: i) this stage produces the best chromosomal preparations (Feraday pers. comm. 1986; Hunter 1990) and; ii) data is standardized by being based on a single stage of the life cycle which is easily identified by the size, shape and color of the histoblast. Therefore, unless indicated otherwise, all results presented here pertain to the last larval instar.

Study area

The study area included streams occurring on Newfoundland's Avalon Peninsula (9000 km^2) which lies between 46°.35′ - 48°.11′ N and 54°.13′ - 52°.38′ W (see Fig. 3.1). The hydrology and water chemistry of Newfoundland streams has been reviewed by Jamieson (1974) and Larson and Colbo (1983). In brief, much of the island's land mass is a recently glaciated plateau, elevated sharply over much of the coast line. This results in many streams having a low gradient over most of their length and then descending rapidly into the sea.

Hence drainage basins do not show the 'typical' graded stream profile. Water flow is usually lowest from July to September, but seasonal variation in precipitation can produce relatively high water levels throughout the year. Stream temperatures are cool and only rarely exceed 25°C. Waters on the Avalon Peninsula are acidic and have low nutrient levels.

The climate of insular Newfoundland has been reviewed by Banfield (1983) and the following account is taken from this source. Island climate is greatly influenced by its proximity to the Labrador current and the extensive cold ocean surface of the North Atlantic. On the Avalon Peninsula annual precipitation varies between 1200 - 1700 mm. Winters are usually mild with less than half of the precipitation falling as snow. Summers are cool with frequent sea fog. Due to the cool climate, development of most insect species is a month or more behind mainland populations (e.g., Lewis and Bennett 1974).

Most of the Avalon Peninsula falls within the Maritime barren ecoregion (Damman 1983). The region is characterized by a mixture of barren areas (consisting largely of dwarf shrub heaths, bogs and shallow fens) and forested sections dominated by balsam fir (Abies balsamea). Other trees common to this area include white birch (Betula papyrifera), white pine (Pinus strobus), alder (Alnus crispa), black spruce (Picea mariana), white spruce (Picea glauca) and red maple (Acer rubrum) (Damman 1983).

Cytotaxonomic procedures

Unless stated, chromosome analysis was restricted to last larval instars (i.e., larvae with distinct histoblasts) as recommended by Feraday (pers. comm. 1986) (also see Lake and Burger 1983; Pistrang and Burger 1988; Hunter 1990). All larval field collections were held on ice for transportation to the laboratory. In the laboratory, larvae were fixed in 3 - 5 changes of acetic ethanol (1:3), depending on the amount of debris present in samples. Larvae were then stored in acetic ethanol at 4°C until needed. The polytene salivary gland chromosomes were stained using a modification of the Rothfels and Dunbar (1953) procedure. Changes to the procedure were as follows:

i) fixed larvae were soaked in distilled water for 40 min

ii) hydrolysis of larvae was for 20 min at 65 - 70°C

iii) the SO_2 rinse was eliminated

These changes to the staining procedure greatly improved chromosome preparation quality. Larvae so stained were identified using the chromosome maps and descriptions of Rothfels *et al*. (1978) and Rothfels (1981a) (see Table 1.3). Larval segregation was based on inversions found on the IIS, IIL and IIIL arms. Stained larvae could also be sexed.

For purposes of this study, CC2 and CC3 were grouped as CC2-3 because the IIL-1 inversion found as the sex arm segment

in female CC2 floats in CC3. Thus routine separation between these cytotypes was difficult, especially in mixed populations. The high sex exception rate of CC2 (≈ 10%) reported in Newfoundland (Rothfels et al. 1978) further confounded separation. Although males can be separated on the IIIL arm (♂ CC2 = IIIL-5 ii; ♂ CC3 = IIIL-5 s/i), this is a difficult inversion to identify in poor material. Where possible, CC2 and CC3 were treated separately. The grouping of these cytotypes is justified as the distinctiveness of various members of the 'CC group' (i.e., CC, CC1, CC2, CC3, and CC4) is uncertain (Adler pers. comm. 1987). Rothfels suggested (1981a, Fig. on p. 26) that CC2 and CC3 were closely related.

Measurements of stream conditions

Conductivity, pH and dissolved oxygen. Conductivity was measured with a Yellow Springs Instrument Co. (model 5890) conductivity-salinity meter. At the beginning of each field season the meter was calibrated with a solution of KCl (0.745g/L) over a temperature range of 0 - 25°C. Because stream conductivity varies with temperature (Reid and Wood 1976), water temperature was noted at each reading and conductivity expressed as μS/cm at 25°C. Stream pH was measured with a Lamotte Instrument pH meter that was calibrated before each reading with buffers of known pH. Dissolved oxygen (mg/L) was measured with either a dissolved

oxygen meter (Cole-Palmer DO meter, model 5513-60) or by field titrations (Hach chemical kit, model CA-10). The meter was calibrated weekly using a solution of sodium sulfite as recommended by the manufacturer.

Water temperature. Water temperature (°C) at the time of sampling was measured with alcohol or mercury filled hand-held thermometers. Each thermometer was calibrated for accuracy at 0, 37, and 100°C before being taken into the field. Weekly minimum and maximum water temperatures were recorded with maximum-minimum thermometers (Sybron Ltd., model 5460). These thermometers were calibrated against hand-held thermometers before being used.

Seston: Water was collected 1 - 3 m above a sampling station by placing a capped 1-L polyethylene bottle just above the stream bed. The cap was then removed allowing the bottle to fill. Bottle contents were strained through a 1 mm^2 plastic mesh to remove larger debris and then placed on ice for transport to the laboratory. Three water samples were taken at each sampling station. In the laboratory, the amount (mg/L) of suspended solids (diameter = 0.45 - 1000 μm) was determined by filtering the contents of each bottle through pre-weighed 0.45 μm Millipore™ membrane filters using a vacuum pump, drying at 60°C for 24 h and reweighing the filters to the nearest 0.01 mg. Samples from each site were averaged.

Stream bed, riparian vegetation and canopy cover. These were all estimated by visual inspection. A modification of the

Wentworth system (Table 2.1) was used to classify the stream bed as mud, sand, small stones, rubble or boulders, based on the size (diameter) of the predominant benthic particles. Visually estimating aspects of the stream bed is a common procedure (e.g., Statzner et al. 1988). Riparian vegetation was categorized as open, brush, or forest (Table 2.1). The extent of canopy cover was estimated as none, partial or complete.

Stream velocity, depth, width and order. Water velocity was measured with an Ott meter (2.5 cm diameter propeller) at various depths, depending on the particular investigation. For each reading the propeller was positioned in an upstream direction and velocity estimated from a 15 s reading. Usually a second reading was taken and results averaged. Depth was measured with either a metal wading rod or meter stick. In most cases depth was measured at 3 - 5 equidistant locations (depending on stream width) along a line running 90° to the stream bank. Usually 1 - 3 lines were measured at each site. For certain investigations (chapter 7) single point readings were required. Width was measured with a meter stick (small streams) or tape measure (large streams). Stream order was taken from 1:50000 topographical maps.

Larval rearing procedures

When required (chapters 4 and 5) larvae were reared in 1-L plastic containers (500 mL of water) using the stir-bar

rearing system of Colbo and Thompson (1978). Either dechlorinated (i.e., tap water allowed to stand for 24 - 48 h) or distilled water was used. Larvae were fed TETRA™ fish food (Tetra Werke, West Germany) prepared by blending food in distilled water for 1 min. Food dosage administered to larvae varied with the requirements of each study and is given in appropriate chapters.

Head capsule measurements

Head capsule measurements of last larval instars were used on several occasions to establish size-temperature, size-velocity and size-depth relationships and to detect simple allometry of size. In each case last instar larvae were cleared in a 10% KOH solution for 12 h and run through an ethanol (70 - 100%) series (Adler 1983). Head capsules were then removed from each carcass, placed in depression slides with glycerol and measured with an ocular micrometer fitted into a compound scope. Viewing each head capsule from above and using the terminology of Chance (1970) the following 4 linear head capsule measurements (Fig. 2.1) were made: i) CAW = cephalic apotome at the widest point; ii) HCW = head capsule at the widest point; iii) HCL = length between the lateral-dorsal aspect of the postantennal buttress to the lateral-dorsal aspect of the postocciput and; iv) PAW = distance between the dorsal aspect of the postantennal buttresses.

General statistical procedures

General statistical methodology followed Elliott (1977), Sokal and Rohlf (1981) and Zar (1984). Statistical and sampling procedures specific to a particular investigation (e.g., microdistribution, allometry) are detailed in the appropriate chapter under the Materials and Methods section. All statistical tests were considered significant at $p < 0.05$.

The coefficient of variation (CV) was used to compare the amount of variability among samples relative to sample means. CV was used when comparisons were among dissimilar groups (e.g., different head capsule characters) or among means of different orders of magnitude (e.g., larval abundance over time). CV, expressed as a percent, was calculated (Zar 1984) as:

[1] CV = (mean/standard deviation) x 100

Heterogeneity of variance was detected using the Bartlett and F_{max} tests (Sokal and Rohlf 1981; Zar 1984) and departures from normality following Ryan et al. (1985). In cases where heterogeneity of variance and/or non-normal distribution were encountered, data were either transformed or nonparametric procedures used, the particular course of action dependant on the peculiarities of each data set. When sample variance exceeded the mean and data were skewed to the right a $\log_{10}(y + 1)$ transformation was considered appropriate (Elliott 1977;

Allan 1984; Montgomery 1984). Other transformations unique to a particular data set (e.g., chapter 7) are detailed in the appropriate chapters.

Multiple sample hypotheses tests commonly used throughout this study were the parametric one way analysis of variance (= ANOVA) and the Kruskal-Wallis nonparametric analysis of variance. ANOVAS were used on raw or transformed continuous variables when the assumptions of normality and homogeneity of variance were approximated, otherwise the Kruskal-Wallis procedures were used. Ordinal data were also subjected to Kruskal-Wallis procedures.

Multiple comparisons among groups for significant ANOVAS were performed using the Tukey test. Significant Kruskal-Wallis tests were further analyzed with Tukey-type multiple comparisons based on either $q_{\alpha,\infty,k,}$ (equal sample size among groups) or $Q_{\alpha,k}$ (unequal sample size) where $\alpha = 0.05$, ∞ = df, and k = number of groups (Zar 1984).

TABLE 2.1. Classification of stream bed particles and riparian vegetation.

Stream bed particles			Riparian vegetation	
Category	Substrate diameter (mm)	Phi value	Category	Vegetation
mud	-	-	open	-pasture, grassland -barrens
sand	1 - 2	0 - 4		-bogs and fens
small stones	2 - 32	-1 - -4	brush	-alders -scattered trees
rubble	32 - 256	-5 - -7		-extensive herbaceous growth
boulders	> 256	≤ -8		
			forest	-continuous border of trees along stream bank

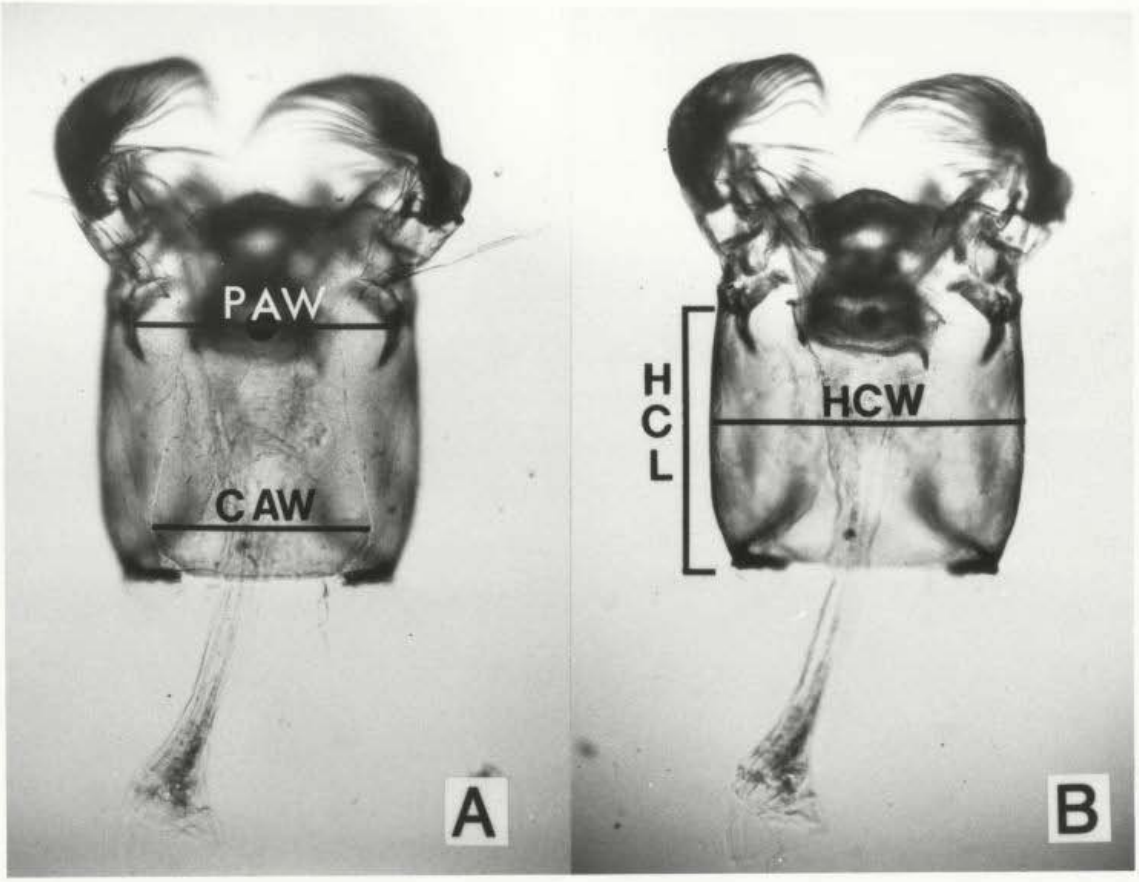
FIG. 2.1. Head capsule measurements of last larval instars used to examine size-temperature, size-velocity and size-depth relationships and to detect simple allometry of size.

CAW = cephalic apotome at the widest point

HCW = head capsule at the widest point

HCL = length between the lateral-dorsal aspect of the postantennal buttress to the lateral-dorsal aspect of the postocciput

PAW = distance between the dorsal aspect of the postantennal buttresses.


PAW
CAW
A
HCL
HCW
B

CHAPTER 3

CYTOTYPE FAUNA OF THE AVALON PENINSULA

INTRODUCTION

This chapter briefly summarizes cytological identifications made during the course of the present study. The intent here was to: i) establish which cytotypes occur on the Avalon Peninsula; ii) determine how reliably they can be distinguished and; iii) document the frequency of sex exceptions and autosomal polymorphisms. Because the present work was concerned primarily with cytotype ecology rather than with simuliid population genetics, emphasis was placed on taxonomic separation of study populations.

MATERIALS AND METHODS

Cytotaxonomic identifications followed procedures outlined in chapter 2. Specimens were not necessarily scored for all inversions not required for identification, therefore the number of larvae scored for a particular inversion will not necessarily equal the number of larvae identified. G-tests were employed for statistical analysis of polymorphic frequencies.

RESULTS

A total of 4758 larvae were successfully cytotyped. At least 6 cytotypes were found to occur on the Avalon Peninsula which included EFG/C venustum, CC2-3 venustum (combined CC2 and CC3), AC(gb) venustum, AA verecundum and ACD verecundum (Table 3.1).

EFG/C venustum

EFG/C is distinguished by the EFG inversion complex on the IIS arm and standard sequences on all others. The IIS is differentiated as the sex determining arm (♀ = EFG/EFG and ♂ = EFG/C, Table 1.3). Sex exceptions (♂ = EFG/EFG and C/C; ♀ = EFG/C) were occasionally found on the Avalon Peninsula (Table 3.2). In addition, 1 A/C (♂), 3 A/EFG (♀), 5 IIL-1 = si (3♂, 1♀, 1?) and 10 IIIL-5 = si (8♂, 2♀) exceptional heterozygotes were also found (Table 3.2).

CC2 venustum and CC3 venustum

Table 3.3 summarizes the cytological identifications of all larvae where IIS = CC, in addition to having the IIL-1 and IIIL-5 inversions (fixed or polymorphic). Males where IIIL-5 = ii (n = 511) were identified as CC2; individuals with IIL-1 = s/i were considered typical and those in the homozygous states sex exceptions. Males with IIIL-5 = s/i (n = 135) were identified as CC3. As previously noted (chapter 2) CC2 and CC3

females are difficult to separate, accordingly females with the homozygous inverted IIIL-5 sequence (n = 709) were grouped as CC2-3. Males and unsexed larvae polymorphic for the IIL-1 inversion but not scored for the IIIL-5 inversion, as well as unsexed larvae found to be homozygous inverted for the IIIL-5 inversion (n = 103) were also designated as CC2-3.

Based on male identifications (n = 646) CC2-3 consisted of 79.1% CC2 (CI = 75.8 - 82.2%) and 20.9% CC3 (CI = 17.8 - 24.2). The distribution of the IIL-1 inversion was significantly different ($G_{adj.}$ = 189.0, df = 5, $p < 0.001$) in male CC2 and CC3. The heterozygous state was most frequently found in CC2 males (83.0%) and homozygous standard the most common sequence in CC3 (63.1%) (Table 3.3).

Other than CC3 males, larvae were almost invariably homozygous inverted for the IIIL-5 inversion. Only 1 individual out of 1330 scored larvae was found to be homozygous standard for IIIL-5. Of the 639 females scored for the IIIL-5 inversion, none were homozygous standard, 4 were heterozygous and 634 were homozygous inverted.

AC(gb) <u>venustum</u>

Larvae with IIS = AA or AC as well as a floating IIL-1 inversion and IIIL-5 = ii were identified as AC(gb) (Table 3.4). Distribution of the A sequence was partially sex linked ($G_{adj.}$ = 139.9, df = 1, $p < 0.0001$) with males almost exclusively AC (AC = 96.5%; AA = 3.5%) and females

preferentially AA (AA = 69.7%; AC = 30.3%) (Table 3.4). The IIL-1 polymorphism was independent of sex ($G_{adj.}$ = 3.05, df = 1, p > 0.05) (Table 3.4). Of the 264 larvae scored for the IIIL-5 inversion, only 5 exceptional heterozygotes (IIIL-5 = si) were found (Table 3.4).

AA verecundum

AA is characterized by the AA sequence on the IIS arm, the IIL-1,2 (fixed) and IIL-4 (floating) inversions on the IIL arm and the IIIL-5 = ss (Table 1.3). Of the 1093 larvae scored for the IIS arm few exceptional AC heterozygotes (2♂, 3♀) were found (Table 3.5). The IIL-4 inversion was carried (either heterozygotes or homozygotes) in 30.3% of larvae scored (n = 942). The frequency of this inversion was independent of sex ($G_{adj.}$ = 3.27, df = 2, p > 0.05). All 802 larvae scored for IIIL-5 were homozygous standard.

ACD verecundum

One of the basic diagnostic features of ACD is the IIL-5.6+4 sequence on the X chromosome (♀ = IIL-5.6+4/IIL-5.6+4) and the IIL-1,2 sequence on the Y chromosome (♂ = IIL-5.6+4/IIL-1,2) (Table 1.3). The IIIL-5 inversion is standard. A, C, and D inversions occur on IIS and all 6 combinations were found in Newfoundland (Table 3.6). Frequency of the A, C, and D inversions was independent of sex ($G_{adj.}$ = 8.30, df = 5, p > 0.05).

Since diagnostic inversions on the IIL arm occur heterozygously in males but are often homozygous in females (Table 1.3), detailed analysis of this arm was restricted to the latter sex where inversion recognition is easier. The X chromosome sequence appeared to be X_E (IIL-7+5.6.4.9) with 35.7% of all females examined (n = 395) showing additional polymorphism superimposed on this inversion complex. The most frequent polymorphism (95 of 314 scored) was a small inversion superimposed on the limits of the IIL-6 inversion. The IIL-4 inversion was consistently homozygous inverted in the 372 females examined. All 488 larvae (♂ and ♀) scored for IIIL-5 were homozygous standard.

DISCUSSION

EFG/C *venustum*

Frequencies of EFG/C sex exceptions in Newfoundland (♂ = 1.7%, ♀ = 2.1%) were similar to those found by Rothfels *et al.* (1978) (♂ = 0.0%, ♀ ≈ 2%) in Ontario. Rothfels *et al.* (1978) suggested that the exceptional female heterozygotes (X:C) were the product of crossover. If the sex locus was located just outside the EFG inversion complex, then crossover in intervening sections would produce X chromosomes that appeared 'cytologically' as Y (i.e., X:C) and vice versa. The discovery of the reciprocal crossover types (i.e., ♂ = C/C and EFG/EFG)

in Newfoundland supports a crossover mechanism. The rare occurrence of the A, IIL-1 = i, and IIIL-5 = i inversions (Table 3.2) probably represents ancestral relicts that have not been completely eliminated from the population (Rothfels 1981). Rothfels et al. (1978) also noted the occurrence of the A and IIL-1 sequences in Newfoundland populations.

CC2 venustum and CC3 venustum

Seventeen percent of CC2 males scored for the IIL-1 inversion were sex exceptions. A previous preliminary survey found 7 (♂ and ♀) of the 77 larvae examined to be sex exceptions (Rothfels et al. 1978). In contrast, only 4 (all ♀) out of 131 scored larvae in Ontario were sex exceptions and in Pennsylvania both males and females were homozygous inverted (Adler and Kim 1986). As with EFG/C, Rothfels et al. (1978) argued that CC2 sex exceptions were most likely the product of crossover and again the recovery of reciprocal crossover males (and presumably females, Table 3.3) supports this conclusion. Crossover would produce both X:IIL-1 = s and Y:IIL-1 = i polymorphisms. Disparity in the frequency of chromosomes carrying these polymorphisms would account for the geographic variability in sex exception rates. The IIL-1 inversion is an autosomal polymorphism in CC3 (Table 1.3). In Newfoundland 37% of male larvae carried this inversion either homozygously or heterozygously; presumably it occurred with a similar frequency in females. Tne IIL-1 inversion was rarely found in

western Canada CC3 populations (Adler 1986; Ciborowski and Adler 1990).

Some CC2 populations have retained the standard sequence on IIIL-5 (Rothfels 1981a). Male heterozygotes can therefore occur in both CC2 and CC3 which would make some males indistinguishable. As well, CC3 carries an X chromosome polymorphism (i.e., X:IIIL-5 = s) in both northern Quebec (≈ 2%) and Saskatchewan (≈ 12%) (Rothfels 1981a; Ciborowski and Adler 1990). The virtual absence of the standard IIIL-5 sequence in CC2-3 females and the lack of male standard homozygotes CC2 or CC3 demonstrates that in Newfoundland a standard sequence rarely, if ever, occurs as an autosomal polymorphism in CC2 or an X chromosome polymorphism in CC3. Males scored IIIL-5 = ii can be assigned to CC2 and those scored IIIL-5 = s/i to CC3 with a high degree of confidence.

Ninety-seven larvae met the classical criteria for the CC venustum cytotype (IIS = CC, IIL = ss, IIIL-5 = ii) and the possibility that some of these larvae belong to this taxon can not be unequivocally rejected. At least some of these larvae are undoubtedly CC3 (♀) and CC2 (♂ and ♀ sex exceptions). If CC does indeed occur on the Avalon it would do so in very low numbers and be a very minor part of the total cytotype fauna.

AC(gb) venustum

AC(gb) was first described from larvae (n = 104) collected on the Avalon Peninsula. In the original collections

neither the A nor IIL-1 sequences were strongly related to sex, though females tended to be AA and male to be AC. Results presented here also found the IIL-1 sequence was independent of sex. A and C sequences in the IIS arm were clearly related to sex as most males were AC and most females AA, though linkage was only partial as AA males and AC females also occurred. Because linkage was not complete, a low number of larvae with the CC sequence can be expected to occur (Rothfels et al. 1978; Rothfels pers. comm. 1986) which would be indistinguishable from CC2 males and CC2-3 females. Based on the frequencies of the A and C inversions given in Table 3.3, approximately 7.0% of AC(gb) venustum could be expected to have IIS = CC. Due to the relative scarcity of AC(gb) venustum it is extremely unlikely that the few IIS = CC individuals which may have been assigned to CC2-3 would compromise any analysis.

Almost 27% of larvae scored fit the classical criteria for the A/C venustum cytotype (IIS = ♂ A/C, ♀ A/A; IIL = ss; IIIL-5 = ii). Although all those larvae can be interpreted as belonging to AC(gb) venustum, the possibility that A/C venustum occurs sporadically on the Avalon cannot be excluded. The fact that the 3 largest collections of AC(gb) (n = 30, 20, 20) were all in Hardy-Weinberg equilibrium suggests that larvae have been correctly assigned to the AC(gb) cytotype.

As with EFG/C, the rare occurrence (1.9%) of the standard sequence of the IIIL-5 inversion can most easily be explained

as an ancestral relict.

AA *verecundum*

It is unclear whether AA *verecundum*, A/C *verecundum* and CC *verecundum* are distinct or a single polymorphic species (Rothfels 1981a). The Newfoundland population conforms strictly to the AA cytotype (IIS = AA, IIL-1,2 = ii, IlL-4 = ss, si or ii). Exceptional IIS heterozygotes (AC) were exceedingly rare (0.5%) and most easily explained as relicts. The IIL-1,2.3 sequence, common to A/C and CC *verecundum*, was never found.

ACD *verecundum*

ACD is chromosomally extremely variable and complex (Rothfels *et al*. 1978) but it is this complexity that makes identification relatively simple. Due to the extensive number of unique inversions on the IIL sex arm (Table 1.3) it is easily distinguished from other cytotypes and in almost all instances larvae can be unequivocally assigned to ACD.

In both males and females the A inversion was most common on the IIS arm. Typically the A and C inversions are most common in eastern North American populations and C and D most common in the west (Rothfels *et al*. 1978; Gordon and Cupp 1980; Adler 1986; Currie and Adler 1986). The IIL sex arm was examined in detail only in females where the basic sequence appeared to be X_E, though a few larvae may have had the X_W and

X_3 sequences. The X_E is typically eastern, X_W typically western and the X_3 sequence generally distributed (Rothfels et al. 1978). All 3 sequences can be found in eastern North America (Gordon and Cupp 1980).

SUMMARY

1. Based on 4758 cytological identifications of larvae taken from 72 sites during a 4 year period (1986 - 1989) it was found that at least 6 cytotypes occurred on Newfoundland's Avalon Peninsula. These were EFG/C venustum, CC2-3 venustum (combined CC2 and CC3), AC(gb) venustum, AA verecundum and ACD verecundum.

2. The possibility that low numbers of A/C venustum and CC venustum exist on the Avalon cannot be excluded, although this seems unlikely.

3. The discovery of EFG/C (X:C, Y:EFG) and CC2 (X:C, Y:IIL-1 = i) reciprocal sex exceptions supports the hypothesis that exceptional individuals are the product of crossover.

TABLE 3.1. Total number of individuals of each cytotype identified from the Avalon Peninsula from 1986 - 1989.

Cytotype	Number identified	% of Total
EFG/C *venustum*	1065	22.4
CC2-3 *venustum*[+]	1465	30.8
AC(gb) *venustum*	284	5.9
AA *verecundum*	1098	23.1
ACD *verecundum*	846	17.8
	4758	

[+]Based on 646 male identifications, CC2-3 consisted of 79.1% CC2 (CI = 75.8 - 82.2%) and 20.9% CC3 (CI = 17.8 - 24.2%).

TABLE 3.2. Summary of inversions identified on the IIS, IIL and IIIL arms of the EFG/C venustum cytotype.

Inversion		Frequency		
		Males	Females	Unsexed*
IIS	EFG/EFG	0.006	0.979	0.362
	EFG/C	0.983	0.016	0.638
	EFG/A	0.000	0.005	0.000
	C/C	0.008	0.000	0.000
	A/C	0.003	0.000	0.000
	n**	361	631	47
IIL-1	ss	0.991	0.998	0.975
	si	0.009	0.002	0.025
	ii	0.000	0.000	0.000
	n	339	403	40
IIIL-5	ss	0.964	0.990	1.000
	si	0.036	0.010	0.000
	ii	0.000	0.000	0.000
	n	222	203	32

*Larvae in which sex could not be determined.

**Number of individuals scored for a particular inversion.

TABLE 3.3. Summary of inversions identified on the IIL and IIIL arms of the CC2 and CC3 venustum cytotypes.

Inversion		Frequency			
		CC2 Males	CC3 Males	CC2-3 Females	Other+
IIL-1	ss (s/s)	0.071	0.631	0.099	0.155
	si (s/i)	0.830	0.246	0.073	0.534
	ii (i/i)	0.099	0.123	0.828	0.311
	n**	476	130	659	103
IIIL-5	ss (s/s)	1.000	0.000	0.000	0.022
	si (s/i)	0.000	1.000	0.006	0.022
	ii (i/i)	0.000	0.000	0.994	0.956
	n	511	135	639	49

+Larvae that could not be sexed and males that could not be scored for the IIIL-5 inversion.

**Number of individuals scored for a particular inversion.

TABLE 3.4. Summary of inversions identified on the IIS, IIL and IIIL arms of the AC(gb) venustum cytotype.

Inversion		Frequency		
		Males	Females	Unsexed[+]
IIS	AA	0.035	0.697	0.750
	AC	0.965	0.303	0.250
	n[++]	115	152	8
IIL-1	ss	0.312	0.396	0.429
	si	0.541	0.431	0.357
	ii	0.147	0.173	0.214
	n	109	144	14
IIIL-5	ss	0.000	0.000	0.000
	si	0.010	0.021	0.071
	ii	0.990	0.979	0.929
	n	104	146	14

[+]Larvae in which sex could not be determined.

[++]Number of individuals scored for a particular inversion.

TABLE 3.5. Summary of inversions identified on the IIS, IIL and IIIL arms of the AA verecundum cytotype.

Inversion		Frequency		
		Males	Females	Unsexed[+]
IIS	AA	0.995	0.005	1.000
	AC	0.005	0.995	0.000
	n[++]	430	595	68
IIL-4	ss	0.718	0.684	0.691
	si	0.242	0.289	0.255
	ii	0.040	0.027	0.054
	n	372	144	55
IIIL-5	ss	1.000	1.000	1.000
	si	0.000	0.000	0.000
	ii	0.000	0.000	0.000
	n	309	448	45

[+]Larvae in which sex could not be determined.

[++]Number of individuals scored for a particular inversion.

TABLE 3.6. Summary of inversions identified on the IIS and IIIL arms of the ACD verecundum cytotype.

Inversion		Frequency	
		Males	Females
IIS	AA	0.761	0.695
	AC	0.171	0.226
	AD	0.014	0.039
	CC	0.039	0.026
	CD	0.000	0.003
	DD	0.014	0.011
	n*	280	380
IIIL-5	ss	1.000	1.000
	si	0.000	0.000
	ii	0.000	0.000
	n	211	277

*Number of individuals scored for a particular inversion.

CHAPTER 4

THE INFLUENCE OF TEMPERATURE ON LARVAL SURVIVAL, DEVELOPMENT, GROWTH AND CHROMOSOME PREPARATION QUALITY

INTRODUCTION

Temperature is a primary factor controlling simuliid preimaginal macrodistribution (Table 1.1) and population dynamics (Ross and Merritt 1987). The role of temperature is critical in understanding cytotype ecology and differentiating life histories within a complex. In addition, data on appropriate rearing conditions for individual cytotypes and the consequences of changing these conditions will greatly facilitate the establishment of laboratory populations.

This chapter examines the effect of temperature on larval survival, development, growth (size) and chromosome preparation quality of EFG/C, ACD, and AA. The study was restricted to these cytotypes because: i) while the distinctiveness of some cytotypes is uncertain, EFG/C, AA and ACD are accepted as distinct from one another (Rothfels 1981a, 1987; Adler pers. comm. 1988); ii) EFG/C exists as a distinct population from ACD and AA for part of its development (chapter 8), allowing the establishment of separate laboratory cultures; iii) both the venustum line (EFG/C) and the

verecundum line (ACD, AA) of the complex are represented (Rothfels et al. 1978); iv) with rare exceptions all 3 cytotypes can be unequivocally identified cytologically in Newfoundland (Table 1.3) and; v) these cytotypes are found in the same habitat (lake outlets, see chapters 5 and 6).

MATERIALS AND METHODS

Survival and development time

Preimaginal black flies were collected from the outflow at Hughs Pond (see chapter 8 for details of this site) which is approximately 11 km W of St. John's, Newfoundland. Black flies were collected within 20 m of the pond outlet.

Larval EFG/C were collected as first instars in early April (water temperature ≈ 4°C), just after the ice cover on the pond had melted. Larvae were placed in a plastic container with a small amount of water and held on ice during transportation to the laboratory. To determine survival and development time in the laboratory, larvae were reared in 1-L plastic containers (500 mL of water) using the rearing system of Colbo and Thompson (1978). Fifty larvae were pipetted into each container. Four replications were used at each of the test temperatures of 5, 10, 15, 20, 25 and 30 ±1°C with containers of larvae randomly assigned to each temperature. To decrease the possibility of thermal shock, the

starting water temperature for all containers was ≈ 4°C, allowing a gradual rise to the assigned rearing temperature.

Egg masses containing both ACD and AA (see Colbo and Porter 1981) were collected from grasses in late June and held on ice until needed. At the time of the experiment, eggs were transferred from storage to 0.5-L deep well dishes containing 250 mL of distilled water and 10 mL of blended TETRA (4.0 g/L solution). Aeration was supplied by aquarium pumps through 1-mL pipettes. Dishes so equipped were held for 4 days at 20°C, at which time maximum hatching occurred. First instars were then handled in the same manner as EFG/C with the exception that starting water temperature of all containers was 20°C to reduce thermal shock. Thus 2 laboratory populations were established, one of pure EFG/C (venustum lineage) and another of mixed ACD-AA (verecundum lineage).

Additional replicates of EFG/C and ACD-AA were reared at 20°C to the last larval instar. These larvae were then transferred to new containers and held until emergence to estimate development time from the prepupa (i.e., final larval instar with completely black histoblast) to adult.

Larvae were fed a diet of TETRA at a dosage of 0.3 mg/larva/day. Water was changed every 3 days and larvae were checked daily. An entirely black histoblast indicated the completion of larval development.

Developmental time was expressed as both the number of days and degree-days (D°C) required to complete larval

development. Degree-days to complete development were computed for each last instar using the formula:

$$[1]\quad D°C = d(T - t)$$

where D°C is developmental time in degree-days, d is the number of days to complete larval development, T is the rearing temperature (°C) and t is the threshold temperature. Threshold temperature was estimated following Shipp and Whitfield (1987). In brief, the reciprocal of development time in days expresses development rate (%development/day). Least squares regression of development rate on temperature estimates threshold temperature (t) as the x intercept, i.e., the temperature at which the development rate (y), equals zero. Regression analysis followed Zar (1984).

In order to compare laboratory determined D°C requirements to field conditions, weekly collections were made from Beachy Cove Brook from March 5 to July 5, 1989. At each collection the presence of first and last instars was noted and weekly temperatures monitored with a maximum-minimum thermometer that was standardized against a laboratory-calibrated thermometer (chapter 2). Accumulated D°C were calculated using equation 1.

Growth

Head capsule measurements of EFG/C and ACD were used to

determine the influence of temperature on the size of last instars. Following the methods given in chapter 2, CAW, HCW, HCL and PAW lengths were measured (Fig 2.1). Because egg masses of the verecundum line contained both ACD and AA, only cytotyped ACD larvae were used. A random sample of larvae verified the homogeneity of the EFG/C population. Details of cytological identifications are given in the results.

Chromosome preparation quality

Early instars of wild ACD were used to determine the influence of water temperature on chromosome preparation quality. Three replicates of 20 larvae were reared to the last instars at 10, 15, 20, 25 ±1°C, in 0.5-L deep well dishes with 250 mL of distilled water. Water was changed every 3 days and larvae were fed TETRA (0.3 mg/larva/day). Aeration was supplied by aquarium pumps.

Two lots of last larval instars were selected from each temperature, stained following the procedure of Rothfels and Dunbar (1953), and the lot with superior preparation quality chosen for analysis. Selection of the best lot from each temperature ensured that poor chromosome preparation quality was not due to errors in staining. As part of a project separate from this study, EFG/C reared in the Colbo and Thompson (1978) system at 2 temperatures (15 and 21°C) and 2 food dosages (0.6 and 0.06 mg/larva/day) were available for rating as well. As with ACD, 2 lots of larvae from each

treatment were selected for staining and the best lot used in the analysis. Chromosome preparations were rated on 4 criteria:

i) chromosome size,

ii) the intensity of chromosome staining,

iii) the number of nuclei,

iv) the degree of chromosome spread after squashing.

Each criterion was rated on a scale of 0 to 3 (0 = poor, 1 = fair, 2 = good, and 3 = excellent), with an excellent preparation consisting of many large, well spread, darkly stained chromosomes. The sum of all 4 criteria was used as the datum for each preparation. Chromosome quality is therefore a measure of band discrimination and the ease of inversion recognition.

Data analysis

Preliminary analysis of development data indicated both heterogeneity of variance and departures from normality. Because these conditions were further complicated by unequal sample sizes between temperatures, differences in development time between treatment groups were detected with Kruskal-Wallis analysis ($H_{adj.}$) and Tukey-type multiple comparisons ($Q_{\alpha,k}$). Two sample hypotheses were tested with the Mann-Whitney U test and where sample sizes warranted it, the z approximation was used. Data on chromosome quality were

ordinal and subjective, therefore also suited to Kruskal-Wallis and Tukey-type ($Q_{\alpha,k}$) procedures.

Frequency of survival among temperatures, expressed as percents, was subjected to Chi square analysis; multiple comparisons were performed on the arcsine transformed percentages, using Tukey-type multiple comparisons ($q_{\alpha,\infty,k}$).

Initial analysis of head capsule data showed slight departures from homogeneity of variance with some measurements, but no indications of departures from normality. Since treatments were of equal sample size the analysis of variance is sufficiently robust (Glass and Stanley 1970). ANOVAS with significant results were further analyzed using the Tukey test.

RESULTS

Cytological identifications

Cytological identification of all surviving ACD-AA larvae (n = 422) was not possible, however, of 188 cytotyped larvae 75.5% were ACD (CI = 68.7 - 81.5%) and 24.5% AA (CI = 18.5 - 31.3%). Although data on survival and development time refer to a mixed ACD-AA population, cytotypes were treated separately when possible. Identification of a random sample (n = 91) of reared larvae confirmed the homogeneity of the EFG/C culture. Identification (n = 73) of reared early instars

collected in April as part of a separate study, as well as the results of chapter 8, show the early spring population in the section of Beachy Cove Brook below Hughs Pond to be pure EFG/C.

Survival

Temperature was found to have a profound effect on mortality. Both EFG/C and ACD-AA showed significant differences in survival over the range of experimental temperatures (Table 4.1). The survival curve of EFG/C was also significantly different ($X^2 = 141.2$, $df = 5$, $p < 0.001$) from that of ACD-AA. Maximum survival of EFG/C occurred at 15 and 20°C and for ACD-AA over the range of 15 - 25°C. Interestingly, EFG/C had a modest survival rate at 30°C (30.5%) whereas ACD-AA was unable to complete development at this temperature. Both ACD and AA were able to complete larval development at temperatures of 10 - 25°C. All 4 ACD-AA larvae which reached the last instar at 5°C were ACD. Due to the low survival at 5°C (EFG/C n = 2, ACD-AA n = 4) data from this temperature were excluded from further analysis.

Development

The Kruskal-Wallis, Tukey-type multiple comparison and Mann-Whitney procedures used to analyze development data employ ranks rather than population parameters in statements of hypothesis and test calculations. However, for purposes of

comparison with other published work, means have also been included in Tables 4.2, 4.3 and 4.4. Interestingly, ANOVAS, Tukey tests and t-tests of development data yielded virtually identical conclusions to those reached using nonparametric procedures.

The number of days to complete development steadily decreased with increasing temperatures for both EFG/C and ACD-AA (Table 4.2, Fig. 4.1). Development was relatively synchronous for temperatures $\geq$ 15°C where 90% of larvae reared at the same temperature completed development within 3 - 6 days of each other (Table 4.2). In contrast, at 10°C the time between the first appearance of final instar larvae and 90% of larvae reaching the final instar was between 11 - 13 days (Table 4.2).

Regressions of development rate (%development/day) on temperature were significant (EFG/C, $F_{1,3} = 83.11$, $p < 0.01$; ACD, $F_{1,2} = 70.21$, $p < 0.05$). The y intercepts were not significant (EFG/C, $t = -0.82$, $df = 3$, $p > 0.05$; ACD, $t = -0.95$, $df = 2$, $p > 0.05$) and therefore the null hypotheses that the regression lines passed through the origin (i.e., y and x intercepts = 0) could not be rejected (Snedecor and Cochran 1980). Consequently, regressions were recalculated assuming y intercepts (and therefore threshold temperatures) were equal to zero. The relationship between development and temperature ($p < 0.001$) for EFG/C and ACD-AA, repectively, was as follows:

[2] $\%D = 0.405(\pm 0.016)T$, $(R^2 = 95.4\%, F_{1,3} = 681.28)$

[3] $\%D = 0.380(\pm 0.015)T$, $(R^2 = 95.8\%, F_{1,2} = 625.89)$

where %D is the mean %development/day and T is the rearing temperature (°C). Slopes of the recalculated regressions did not differ significantly from each other ($t = -0.978$, $df = 5$, $p > 0.05$) and can be replaced by the single equation:

[4] $\%D = 0.396T$

where 0.396 is the common regression coefficient.

Mean degree-days required to complete larval development for EFG/C and ACD-AA varied at different rearing temperatures (Table 4.2). Accumulated D°C to reach the final instar were greater at 10°C than at any other temperature (Table 4.2) and the greatest decrease in days to complete development occurred between 10 and 15°C (Table 4.2). The Mann-Whitney U test found no significant difference in development time (days or D°C) between EFG/C and ACD-AA at 10°C ($z = 0.56$, $p > 0.05$). Contrary to this, EFG/C required significantly less time ($p < 0.001$) than ACD-AA to complete development at 20°C ($z = 4.42$) and 25°C ($z = 5.20$). At 15°C it was possible to score all ACD-AA larvae and demonstrate significant differences in development time between EFG/C, ACD and AA (Table 4.3).

Calculations of development time in the laboratory agreed

well with those in the field. At an average stream temperature of 8.5°C (maximum - minimum thermometer) the time between the appearance of first and last larval instars of EFG/C was estimated to be 28 days (238 D°C). In the laboratory at a constant temperature of 10°C, last instars began to occur 25 days (250 D°C) after the experiment started. At a mean stream temperature of 20°C the time between the appearance of first and last instars of ACD-AA was 9 days (180 D°C), whereas in the laboratory at a constant 20°C, last larval instars were first observed at day 10 (200 D°C).

As stained larvae could be sexed, sexual differences in development time (days or D°C) could be ascertained. Sex did not affect development time for either ACD or AA at 15°C (Table 4.4, Fig. 4.2a). Results for AA were based on a small sample size (n = 26). Male EFG/C reared at either 15 or 20°C required significantly less time to reach the last instar than ♀ EFG/C (Table 4.4, Figs. 4.2b and 4.2c). Development time from the prepupal stage to adult emergence did not differ between the sexes for either EFG/C or ACD-AA (Table 4.4).

Growth

Morphometric analysis of 4 head capsule measurements is given in Table 4.5. Rearing temperatures had a significant effect on larval size as judged by head capsule size. Means of measurements differed significantly between rearing temperatures (10 - 30°C) for EFG/C. Means of 3 of these

characters showed differences between temperatures (10 - 25°C) for ACD. The mean size for each character was consistently largest at 10°C (Table 4.5). With the exception of PAW ($t = 2.98$, $df = 125$, $p < 0.01$) no significant differences ($p > 0.05$) in mean head capsule measurements were found between EFG/C and ACD (data 10 - 25°C combined) indicating these cytotypes were of similar size.

Chromosome quality

ACD larvae reared at 10 and 15°C produced better quality chromosome preparations than those reared at the same food dosage but at temperatures of 20 and 25°C (Table 4.6). Mann-Whitney tests showed no significant differences ($p > 0.05$) in chromosome quality between EFG/C larvae reared at the same food dosage but different temperatures (15 vs 21°C at 0.6 mg/larva/day, $U = 116.5_{19,12}$; 15 vs 21°C at 0.06 mg/larva/day, $U = 192.5_{22,3}$). Consequently, data from larvae reared at the same food dosage were combined. Larvae fed at 0.6 mg/day ($n = 31$) showed significantly greater chromosome quality ($z = 4.16$, $p < 0.001$) than larvae fed 0.06 mg/day ($n = 37$), regardless of rearing temperature (15 or 21°C).

DISCUSSION

Mansingh and Steele (1973) found that between 4 - 20°C, larval Prosimulium mysticum Peterson had the lowest survival at 20°C. Stegopterna mutata Malloch is also sensitive to high temperature, with an optimum of 9 - 10°C (Mokry 1978). Davies and Smith (1958) found that P. hirtipes Fries suffered 50 - 100% mortality within 3 days at temperatures of 16 - 22.5°C, whereas larvae held at 4 - 5°C had a maximum mortality of 15%. The above simuliids are stenothermic 'winter species', and poor survival at warm temperatures is not surprising.

Employing the same rearing system as used in the present study, Colbo and Porter (1981, Table 2) presented survival data from first instar to pupation for Simulium vittatum Zetterstedt and S. verecundum at 3 temperatures (15, 20 and 25°C) and 4 feeding dosages (0.05, 0.1, 0.2 and 0.5 mg/larva/day). Although Colbo and Porter (1981) did not confirm the identity of the S. verecundum larvae chromosomally, their designation was probably correct because these larvae were reared from Newfoundland egg masses deposited on grasses. To date, all members of the S. venustum/verecundum complex reared from such egg masses in Newfoundland have been ACD or AA. (Rothfels et al. 1978; present study). Chi square analysis of complete data sets presented by Colbo and Porter (1981) showed no significant

differences ($p > 0.05$) in survival between temperatures of 15 - 25 °C for S. verecundum, which concurs with the present findings. In contrast, S. vittatum showed highly significant differences ($p < 0.001$) in survival between temperatures, the highest percent of successful pupation occurring at 25°C. Becker (1973), starting with 5 day old S. vittatum, found no evidence of temperature induced mortality between 17 and 27°C. Bernardo's et al. (1986) conclusion that the greatest larval attrition of S. vittatum occurs in the first 2 stadia may account for Becker's (1973) findings.

At comparable dosages of TETRA, the combined survival of S. verecundum (15 - 25°C) in the Colbo and Porter (1981) study was significantly ($X^2 = 55.0$, $df = 1$, $p < 0.001$) higher (76.4%) than in the present study (59.5%). The difference in survival was most likely the result of water quality. In the present study rearing water was changed every third day which may have allowed a build up of excretory products, whereas Colbo and Porter (1981) changed the water daily. Larval black flies are sensitive to autotoxicity (Edman and Simmons 1985). In contrast to the results presented here and those of Colbo and Porter (1981), Mokry (1976) achieved poor survival for S. verecundum (referred to as Simulium venustum, see Colbo and Porter 1979) at temperatures of 15 - 21°C (5.7% - 20.4%) and larval development could not be completed between 5 - 12°C. This poor survival rate, as well as a prolonged development time reported by Mokry (1976) (500 - 525 D°C), indicates a

stressed population, possibly as a consequence of the high density of larvae (100 - 250 larvae/250 mL container). Bernardo et al. (1986) showed that development time is inversely related to larval density. In a review of simuliid colonization methods, Edman and Simmons (1985) concluded that 'spring/summer' Simulium species develop well at 20°C which agrees with my results.

The ability of EFG/C, a univoltine, early spring simuliid (Cupp and Gordon 1983; chapter 8), to complete development at 30°C, and the inability of the summer generation of ACD-AA to do so was unexpected. The broad range of temperatures tolerated by EFG/C might be an adaptation to the unpredictable and wide temperature fluctuations which characterize the spring climate on the Avalon Peninsula of Newfoundland. The remaining venustum cytotypes (AC(gb), CC2 and CC3) are spring simuliids, whereas the verecundum line of the complex comprises multivoltine cytotypes which occur somewhat later in the season and remain in streams for longer periods (chapter 8). It would be interesting to know if these 'spring' venustum cytotypes also have a wider temperature tolerance than verecundum cytotypes. Although EFG/C can be successfully reared over a wider range of temperatures than ACD-AA, both should be considered eurythermal in light of their modest to high survival over a broad range of temperatures (Table 4.1).

Plots of the development time (days) against constant rearing temperatures for EFG/C and ACD-AA (Fig. 4.1) produced

the classic development curve seen in most insects (Wagner et al. 1984). If the reciprocals of development time ($days^{-1}$) are plotted as a rate over the full range of temperatures at which development can occur, a normal curve truncated on the right is produced. At the lower thermal limit the development rate curve asymptotically approaches the x axis. As temperature increases from the lower thermal limit the development rate becomes proportional to temperature and a linear curve is found at intermediate temperatures. Finally, the rate of development decreases past some 'optimal' temperature due to the lethal effects of high temperature (Taylor 1981; Wagner et al. 1984). Many models have been proposed to describe the development rate response curve including a 6 variable model (Schoolfield et al. 1981), which has recently been used to describe simuliid embryonation (Shipp and Whitfield 1987). The high R^2 values obtained in my study by plotting development rate against temperature (equations 2 and 3) indicated that development was investigated over intermediate temperatures (i.e., the linear section of the response curve) and there was no need to invoke any variables other than temperature to describe this response. Other studies have shown the dependence of simuliid development on temperature (e.g., Mokry 1976; Colbo and Porter 1981; deMoor 1982; Merritt et al. 1982; Prügel 1988; Wirtz et al. 1990)

Threshold temperature was estimated to be zero for both EFG/C and ACD-AA. The only other laboratory derived estimates

of threshold temperature are those of Brenner et al. (1981) and Bernardo et al. (1986). Brenner et al. (1981) estimated the threshold temperature of a northern and southern strain of Simulium decorum Walker to be 7.25 and 6.47°C, respectively. Bernardo et al. (1986) calculated a threshold temperature of -1.85°C for S. vittatum (IS-7), but for purposes of D°C calculations used 0°C. Shippp and Procunier (1986) were unable to calculate threshold temperature under field conditions and therefore used 0°C when calculating D°C for Simulium arcticum Malloch (IIL-3), S. defoliarti Stone and Peterson (IIS-14.15) and S. tuberosum Lundström (FG). Other authors in North America (Table 4.7) have based D°C calculations on an assumed threshold temperature of 0°C.

The number of accumulated D°C needed to complete larval development was not constant between experimental temperatures, but these differences were minimal when temperatures exceeded 10°C (Table 4.2). Differences in D°C requirements at different temperatures have been reported for simuliids (Becker 1973) and other aquatic insects (Sweeney and Schnack 1977). This underscores the importance of stating the temperature(s) on which a D°C calculation is based. This also emphasises that rearing temperatures should approximate normal stream temperatures if laboratory results are used for predictions in the field. For example, at Beachy Cove Brook, with a mean weekly (spring) temperature of 8.5°C, 238 D°C had accumulated between the appearance of first and last instar

EFG/C. In the laboratory, the D°C calculation at 10°C (250 D°C) is a much better estimate of D°C requirements for wild larvae than calculations based on higher laboratory temperatures (175 - 210 D°C).

Field records of larval development and temperature at Beachy Cove Brook were taken on a weekly basis. However, the similarity of laboratory derived D°C requirements to those based on stream records clearly showed that laboratory determined computations approximate D°C required under natural conditions. Ross and Merritt (1987) concluded that temperature and food (quantity and quality) are 2 of the most important factors controlling population dynamics of preimaginal simuliids. Knowledge of the interactions between food and temperature could increase the ability to predict larval occurrence and development in the field and should receive further attention in the future. Food appeared to have a strong influence on the abundance of CC2-3 and AC(gb) in the Piccos drainage basin (chapter 6).

Differences in D°C were also sex related in EFG/C, where males required fewer D°C than females. No differences were found between male and female D°C requirements for ACD and AA larvae. The number of D°C needed to complete development from the prepupal stage to adult emergence was also independent of sex for both EFG/C and ACD-AA. Differences in development time between male and female EFG/C larvae could lead to protandrous emergence under natural conditions. Protandry is common in

black flies (Colbo and Thompson 1978; Colbo and Porter 1979; Brenner et al. 1981; Simmons and Edman 1981; Bernardo et al. 1986).

Table 4.7 gives examples of some D°C calculations for North American simuliids estimated by either field or laboratory studies. With one exception (Davies and Syme 1958), simuliids required approximately 200 - 500 D°C to complete larval or larval-pupal development, which is similar to the range of values reported here. In light of more recent studies, especially that of Ross and Merritt (1978), the estimate of 1110 D°C for the preimaginal development of P. mixtum/fuscum Davies and Syme (Table 4.7) is probably erroneous, or at the very least atypical. Differences in D°C values given in Table 4.7 might be attributable to species differences, rearing conditions (e.g., see Ross and Merritt 1987) and the lack of reliable threshold temperature estimates (see above).

It has been generally assumed that larval head capsule size is positively correlated with overall body size, e.g., Post (1983) and Reisen (1975) showed it increases with larval weight. Head capsules, and by extrapolation larvae, were largest at 10 - 15°C. Field studies of simuliids with more than one generation, or cohort per year, consistently show the largest larvae are produced during cooler temperatures (Thorup 1974; Ladle et al. 1977; Ross and Merritt 1978; deMoor 1982; Merritt et al. 1982; Post 1983). It is a reasonable conjecture

that larger last larval instars of EFG/C or ACD would produce larger adults (e.g., deMoor 1982). Many authors have shown a positive correlation between adult size and fecundity (Chutter 1970; Colbo and Porter 1981; Simmons and Edman 1981; Post 1983). Therefore, it is concluded that EFG/C and ACD would produce the largest and most fecund females at ≤ 15°C.

Vannote and Sweeney (1980) have proposed a 'thermal optimum' for each species of aquatic insect at which adult size and fecundity are maximized. Temperatures above and below this optimum suppress larval tissue growth more than adult tissue development, resulting in smaller, less fecund adults. Based on this concept of 'thermal optimum' the ideal temperature regime for EFG/C and ACD would be ≤ 15°C. Using the criterion that fitness is maximized by rapid development, Taylor (1981) proposed that 'optimal temperature' is that at which development rate is maximized. For EFG/C and ACD-AA this would be at ≥ 25°C. However, both survival (Table 4.1) and fecundity (Table 4.5; Colbo and Porter 1981) may be reduced at these temperatures. Any future considerations of 'optimal temperature' as applied to simuliids must take into account the joint effects of temperature on survival, development rate and fecundity, all of which contribute to fitness. For example, size (and presumably fecundity), survival and development time (days) for EFG/C were maximized at 10 - 15°C, 15 - 20°C, and 25 - 30°C respectively. Interestingly, Vannote and Sweeney (1980) found that maximum adult size was usually

not associated with maximum larval growth rate.

Superior chromosome preparations were obtained from larvae reared at cool temperatures and high food supply. Larvae collected on the Avalon Peninsula in May and June generally had large chromosomes which greatly facilitates identification. Collections in July and August, when water temperatures are highest (Larson and Colbo 1983, p. 601), often produced larvae with small, poorly staining chromosomes, which frequently did not spread when squashed. In many cases, chromosome quality was so poor at this time that many collections could not be cytotyped. Lake and Burger (1983) reported poor chromosome quality for S. venustum/verecundum in July and August and attributed this to high stream temperatures. Adler (1983) reported that low temperature and abundant food produced superior chromosome quality in S. vittatum. In contrast, Tarrant et al. (1987), employing a gravity-trough rearing system with recirculating water, were able to produce acceptable larval chromosome preparations of S. vittatum at temperatures of 23 - 26°C.

It has been my experience that holding wild larvae in the laboratory for several days (4 - 7) at 10 - 15°C on a diet of TETRA before fixation in acetic ethanol greatly improved chromosome quality. For cytological studies of S. venustum/ verecundum I recommend a temperature of 15°C and a feeding rate of ≥ 0.3 mg/larva/day of TETRA. This will ensure chromosome preparations of good quality, moderate larval

survival and a reasonable turnover time (≈ 2 - 3 wk from first to last instar).

SUMMARY

1. EFG/C completed larval development from 5 - 30°C, with maximum survival between 15 - 20°C (68.0 - 73.5%). ACD-AA completed development between 5 - 25°C with maximum survival between 15 - 25°C (55.3 - 61.0%). The temperature-survival curve of EFG/C was significantly different from ACD-AA.

2. Threshold temperature was determined to be 0°C. Mean degree-days required to complete larval development varied depending on temperature, cytotype and sex. Degree-day determination in the laboratory agreed well with field data.

3. Final larval instars of EFG/C and ACD were largest at 10 - 15°C. By extrapolation these larvae would give rise to adults with maximum fecundity.

4. The size and band distinction of the salivary gland polytene chromosomes used to identify the above cytotypes were found to vary with rearing conditions. Larvae reared at 15°C and fed TETRA will provide superior chromosome preparations.

TABLE 4.1. Chi square and Tukey-type multiple comparison analysis of EFG/C and ACD-AA larval survival at temperatures of 5 - 30°C.

Temperature (°C)	% Survival[+]	
	EFG/C	ACD-AA[++]
5	1.0 (0.1 - 3.6)[a]	2.0 (0.5 - 5.0)[a]
10	41.5 (34.6 - 48.7)[b]	45.5 (38.5 - 52.7)[b]
15	73.5 (66.8 - 79.5)[c]	55.3 (47.0 - 63.4)[c]
20	68.0 (61.1 - 74.4)[c]	61.0 (53.9 - 67.8)[c]
25	36.5 (29.8 - 43.6)[d]	61.0 (53.9 - 67.8)[c]
30	30.5 (24.2 - 37.4)[d]	0.0
X^2	288.7***	350.4***

*** $p < 0.001$.

[+]For EFG/C and ACD-AA percents with different letters were significantly different at $p < 0.05$. The 95% confidence limits are given in parentheses.

[++]ACD-AA consisted of 75.5% ACD and 24.5% AA. Data at 15°C based on 3 replicates.

TABLE 4.2. Kruskal-Wallis and Tukey-type multiple comparison analysis of days and D°C required by EFG/C and ACD-AA to complete larval development at different temperatures.

	Temperature (°C)	n	Days			D°C	
			Mean (±SD)	Mean* rank	90%** Range	Mean (±SD)	Mean* rank
EFG/C							
	10	82	33.4 ±3.3	458.5[a]	13	334.[illegible] ±32.7	446.9[a]
	15	147	15.4 ±1.5	339.6[b]	4	245.2 ±22.7	226.2[b]
	20	136	12.1 ±1.7	199.6[c]	5	241.9 ±33.2	205.0[b]
	25	73	9.1 ±1.2	83.1[d]	4	227.1 ±29.7	150.1[c]
	30	61	8.6 ±1.2	65.9[d]	3	259.2 ±36.4	262.7[b]
	$H_{adj.}$		444.6***			208.2***	
ACD-AA§							
	10	91	34.0 ±3.9	373.3[a]	11	340.2 ±38.8	352.0[a]
	15	83	16.3 ±1.9	272.2[b]	5	244.5 ±29.1	135.1[b]
	20	122	13.0 ±1.6	173.0[c]	5	260.7 ±32.9	183.2[c]
	25	122	10.5 ±1.9	81.4[d]	6	261.7 ±47.0	180.2[c]
	$H_{adj.}$		339.4***			172.0***	

*** $p < 0.001$.

*For EFG/C and ACD-AA mean ranks with different letters were significantly different at $p < 0.05$.

**Number of days between the appearance of the first mature larvae and 90% of larvae completing development.

§ACD-AA consisted of 75.5% ACD and 24.5% AA. Data for 15°C based on 3 replications.

TABLE 4.3. Kruskal-Wallis and Tukey-type multiple comparison analysis of larval development time (D°C and days) between EFG/C, ACD and AA at 15°C.

	n	Development time (±SD)		Mean† Rank	$H_{adj.}$
		Mean D°C	Mean Days		
ACD	57	235.0 ±22.2	15.7 ±1.5	85.5[a]	23.6***
EFG/C	147	245.2 ±22.7	16.3 ±1.5	119.7[b]	
AA	26	265.4 ±32.0	17.7 ±2.1	157.7[c]	

*** $p < 0.001$.

†Mean ranks with differing letters were significantly different at $p < 0.05$.

TABLE 4.4. Mann-Whitney U tests for sexual differences in development time.

			Development variable	Development time (±SD)		n	Rank sum	z*
				Mean D°C	Mean Days			
EFG/C	15°C	♂	larval	237.8 ±22.0	15.9 ±1.5	76	4432	4.62***
		♀	larval	253.1 ±20.8	16.9 ±1.4	71	6447	
	20°C	♂	larval	228.6 ±32.2	11.4 ±1.6	65	3314	4.96***
		♀	larval	254.1 ±32.2	12.7 ±1.5	71	6002	
	20°C	♂	prepupal - adult	102.7 ± 9.0	5.1 ±0.5	96	7426	0.31
		♀	prepupal - adult	102.1 ± 7.3	5.1 ±0.4	56	4203	
ACD	15°C	♂	larval	231.6 ±23.4	15.4 ±1.6	34	866	1.94
		♀	larval	240.0 ±19.7	16.0 ±1.3	23	787	
AA	15°C	♂	larval	257.3 ±36.1	17.2 ±2.4	13	147	113.50**
		♀	larval	273.5 ±26.1	18.2 ±1.7	13	205	
ACD-AA§	20°C	♂	prepupal - adult	107.6 ± 9.8	5.4 ±0.5	42	1808	0.61
		♀	prepupal - adult	105.5 ±12.0	5.3 ±0.6	42	1595	

*** $p < 0.001$.

*z approximation for the Mann-Whitney U test.

**U statistic was used for AA at 20°C due to a small sample size.

§ACD-AA consisted of 75.5% ACD and 24.5% AA.

TABLE 4.5. ANOVA and Tukey test multiple comparison analysis of EFG/C and ACD head capsule characters.

	Mean size* (mm ±SD)			
	CAW	HCW	PAW	HCL
EFG/C				
10°C	0.415 ±0.019[a]	0.624 ±0.022[a]	0.494 ±0.018[a]	0.528 ±0.018[a]
15°C	0.411 ±0.024[a]	0.609 ±0.026[a]	0.489 ±0.018[a]	0.516 ±0.016[a]
20°C	0.387 ±0.020[b]	0.588 ±0.020[b]	0.467 ±0.025[b]	0.498 ±0.018[b]
25°C	0.383 ±0.026[b]	0.585 ±0.014[b]	0.464 ±0.015[b]	0.485 ±0.012[cb]
30°C	0.380 ±0.029[b]	0.583 ±0.029[b]	0.463 ±0.026[b]	0.479 ±0.022[c]
$F_{4,120}$	12.25***	15.11***	11.70***	36.62***
ACD				
10°C	0.406 ±0.027	0.624 ±0.033[a]	0.487 ±0.021[a]	0.523 ±0.021[a]
15°C	0.407 ±0.030	0.605 ±0.028[ab]	0.467 ±0.027[ab]	0.509 ±0.019[ab]
20°C	0.405 ±0.021	0.594 ±0.032[b]	0.462 ±0.026[b]	0.494 ±0.019[bc]
25°C	0.398 ±0.013	0.591 ±0.020[b]	0.452 ±0.014[b]	0.488 ±0.015[c]
$F_{3,64}$	0.66	5.12**	8.03***	11.77***

** $p < 0.01$, *** $p < 0.001$.

*Means for each character and cytotype with different letters were significantly different at $p < 0.05$ ($n = 24$ and 16 per treatment for EFG/C and ACD respectively).

NOTE: CAW = cephalic apotome width. HCW = head capsule width, PAW = postantennal buttress width, HCL = head capsule length.

TABLE 4.6. Kruskal-Wallis and Tukey-type multiple comparison analysis of ACD larval chromosome preparation quality reared at temperatures of 10 - 25°C.

Temperature (°C)	n	Mean Rank[+]	$H_{adj.}$
10	8	30.4[a]	27.2***
15	8	23.8[a]	
20	10	17.1[b]	
25	10	6.1[b]	

*** $p < 0.001$.

[+]Mean ranks with different letters were significantly different at $p < 0.05$.

TABLE 4.7. Degree-day requirements for some North American simuliids.

Reference	Species, species complex, or cytotype	Development variable	Rearing temp. (°C)	D°C*
Davies and Syme 1958	Prosimulium mixtum/fuscum	larval-pupal	5	1110
Davies et al. 1962	Simulium venustum	larval	9	275
Becker 1973	Simulium vittatum	larval-pupal	17	228
Mokry 1976	Simulium verecundum	larval	15-20	500-525
Ross and Merritt 1978	Prosimulium mixtum/fuscum	larval-pupal	--	240
	Stegopterna mutata	larval-pupal	--	250-275
	Cnephia dacotensis	larval-pupal	--	475
Brenner et al. 1981	Simulium decorum	larval-pupal	13-21	190-271
Shipp and Procunier 1985	Simulium arcticum (IIL-3)	larval	--	246-309
	Simulium defoliarti (IIS-14.15)	larval	--	302
	Simulium tuberosum (FG)	larval	--	475
Bernardo et al. 1986	Simulium vittatum (IS-7)	larval-pupal	12.5-20	460
present study	EFG/C	larval	20	242
	EFG/C	larval-pupal	20	344
	ACD-AA	larval	20	267
	ACD-AA	larval-pupal	20	374

*All D°C calculations were based on a threshold temperature of zero expect that of Brenner et al. (1981) which used a threshold temperature of ≈ 7°C.

FIG. 4.1. Mean number of days to complete larval development for EFG/C and ACD-AA (verecundum) at temperatures of 5 - 30°C.

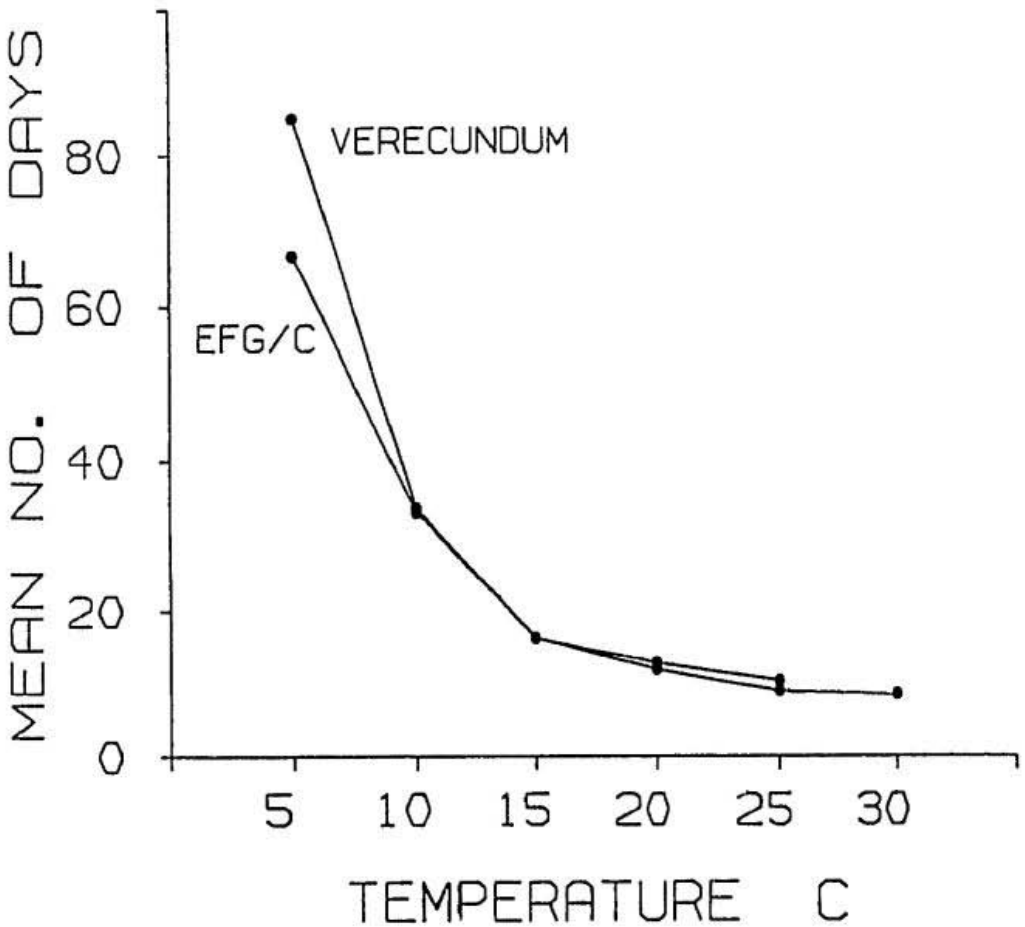
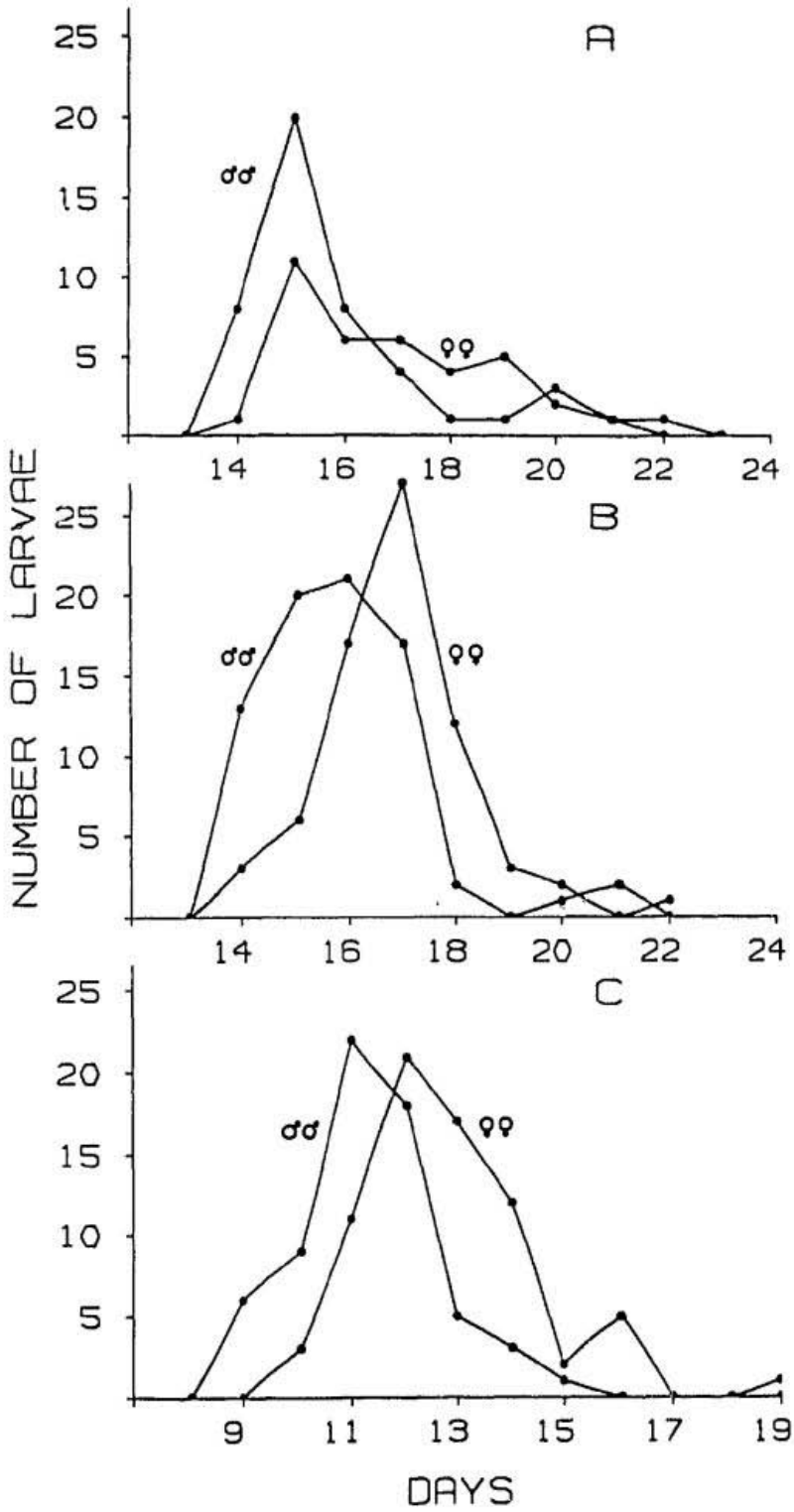

MEAN NO. OF DAYS
0
20
40
60
80
VERECUNDUM
EFG/C
5
10
15
20
25
30
TEMPERATURE C

FIG. 4.2. Number of days to complete larval development for male and female larvae of (A) ACD at 15°C, (B) EFG/C at 15°C, and (C) EFG/C at 20°C.


A
B
C
♂♂
♀♀
NUMBER OF LARVAE
DAYS
25
20
15
10
5
14
16
18
20
22
24
9
11
13
15
17
19

CHAPTER 5

MACRODISTRIBUTION PATTERNS OF LARVAE. I. FACTORS ASSOCIATED WITH CYTOTYPE OCCURRENCE

INTRODUCTION

Macrodistributional studies have examined the distribution of simuliids among 6 zoogeographic regions of the world (e.g., Crosskey 1981), within a large section of a zoogeographic region (e.g., Corkum and Currie 1987), among streams in highly localized areas (e.g., Colbo 1979) and within a single drainage basin (e.g., Maitland and Penny 1967). The latter 2 scales of investigation, examined here and in the following chapter, have stressed the role of stream site characteristics (e.g., food supply, ovipositional cues, temperature, substrate) in determining preimaginal distribution.

The objective of the present investigation was to test the hypothesis that the occurrence (presence / absence) of each cytotype among different stream sites over a limited geographic area (Avalon Peninsula) is nonrandom and to some extent predictable. Stream variables that might account for observed patterns of distribution were also examined. Data from 60 stream sites sampled over a 4 year period were used to test this hypothesis.

MATERIALS AND METHODS

Sampling procedures

The study area includes streams occurring on the Avalon Peninsula, Newfoundland. Details of the hydrology, water chemistry, and geomorphology of this area can be found in chapter 2. Based on a synthesis of the literature (Colbo and Wotton 1981; Adler 1987; Burger 1987; Wotton 1987; Table 1.1) stream sites were classified into 2 broad categories. Collections taken within 50 m of a pond outlet (regardless of stream width) were classified as outlet sites (O). Collections ≥ 500 m from the nearest outlet were considered downstream sites and further classified by stream width into trickle (T) ≤ 1 m wide; small (S) = 2 - 5 m wide; and large (L) = 12 - 30 m. Stream width at downstream sites was measured at riffles. Collection sites were intentionally classified into 4 discrete, easily identified, nonoverlapping types (O, T, S, L) to prevent error in site classification.

Potential collection sites were preselected from 1:50000 topographic maps. Sites were then visited and final site selection was based on matching the above criteria with accessibility. Fifteen sites from each stream type (O, T, S, L) were sampled for a total of 60 sites (Fig. 5.1). No 2 collection sites were further than 120 km apart. As far as I am aware all streams sampled were permanent. As seasonal changes in the S. venustum/verecundum complex are known to

occur in Newfoundland (Lewis and Bennett 1974; Rothfels et al. 1978), each site was sampled twice, once in the spring (mid May to mid June) and again in the summer (July to early August), for a total of 120 collections.

The contiguous distribution of larval S. venustum/ verecundum complex precluded a strictly random sampling procedure as insufficient larvae would have been collected for chromosomal analysis. For example, at one site used to study cytotype phenology (BP, chapter 8), weekly rock collections (n = 10) from early May to mid September, 1988, yielded only 23 last larval instars. Small dense clumps of larvae were, at times, missed by chance as a result of enforcing the random sampling regime. To ensure routine sampling of such clumps would have required an unmanageable sample size (e.g., Elliott 1977; Allan 1984). Therefore, each site was qualitatively sampled by walking a swath from bank to bank while hand collecting larvae from all available natural substrates, thus ensuring sufficient larvae for chromosomal analysis. Larvae and substrate were placed in plastic containers with a small amount of water and held on ice for transportation to the laboratory. As in other studies of this type (e.g., Corkum and Currie 1987), it was assumed that cytotypes found in the swath sample from each site were representative of local occurrences. Collections were made from 1986 to 1989 with 82% of samples taken in 1988 and 1989.

Stream width, depth (along collection swath), water

temperature, larval substrate, size of stream bed particles, riparian vegetation and extent of canopy cover were noted at time of collection (see chapter 2 for details). Larval substrates were classified as vegetation (trailing and emergent grasses, aquatic macrophytes, branches, leaves, sticks, etc.), rocks (gravel to boulders), or mixed (vegetation and rock). Single point measurements of conductivity, pH and dissolved oxygen were measured at time of sampling for most collections (n = 106), as outlined in chapter 2.

Cytological identification

Cytological identifications of the spring collections were based on procedures outlined in chapter 2. Frequently, larvae collected during the summer could not be cytotyped, presumably because of higher stream temperatures at this time (Lake and Burger 1983). Good chromosome preparation quality can be induced by rearing larvae at moderate temperatures and high food dosages (see chapter 4), hence larvae from summer collections were often held at 10 - 15°C for 3 - 7 days and fed a diet of blended TETRA (≥ 0.3 mg/larva/day), before fixation. Such treatment permitted cytological identification of ultimate, and if required, penultimate instars which otherwise would have been impossible.

Data analysis

Cytotaxonomic identification of all larvae in the swath sample from each site would have required a prohibitive amount of time; accordingly swath samples were subsampled. Subsample size was a compromise between the probability of missing a cytotype present in a swath sample versus identification time. The probability of a subsample not containing a cytotype which occurred in the swath sample depends on cytotype frequency in the sample as well as sample and subsample size. Given these constraints, attention was focused on the occurrence of dominant cytotypes, defined here as any cytotype which comprised > 15% of a sample. Restricting attention to dominant taxa is a common approach in benthic studies (e.g., Kownacki 1985; Schröder 1988). The selected subsample size of 14 gives a maximum theoretical probability (binomial distribution) of missing a dominant cytotype of 8.7%. In practise this error is lower as subsampling was done without replacement. The error was further reduced as cytotype frequency increased beyond the imposed minimum. A subsample size of 14 would require cytotyping 1680 larvae.

Data were examined using G-tests, cluster analysis and correlation analysis. G-tests were used to determine if the distribution of each cytotype was independent of stream type (Sokal and Rohlf 1981). To increase the rigor of this analysis, cytotypes occurring in low frequency due to seasonality were eliminated, thus G-tests were restricted to

cytotypes occurring in at least 20% of the spring, summer or total (spring + summer) collections.

A sequential, agglomerative, hierarchical cluster analysis was used to group stream sites by similarity of their cytotype fauna. The Jaccard coefficient was used to produce the resemblance matrix because it ignored cases where cytotypes were absent from both paired sites being compared when calculating similarity (Pielou 1984). Ties often occur when using nominal data; therefore single link clustering was chosen to produce the tree dendrogram as this algorithm is well behaved with ties, i.e., all alternative tree dendrograms are equivalent (Rohlf 1988). Cytotype associations (i.e., degree of co-occurrences) were examined using the Jaccard coefficient and clustering by the UPGMA algorithm, which is preferred when few or no ties occur (Romesburg 1984). The fit between a resemblance matrix and its dendrogram was judged using the cophenetic correlation coefficient (Romesburg 1984). A coefficient $\geq$ 0.8 was considered a good fit whereas coefficients below this were considered poor (Rohlf 1988). The statistical package NTSYS was used for cluster analysis (Rohlf 1988).

Correlation analysis was used to examine the relationship between cytotype occurrence and various stream variables. Since correlations were between a nominal (presence/absence of cytotypes) versus interval/ratio, ranked and other nominal variables, special cases of the Pearson product moment

correlation coefficient and tests of significance were used (Wherry 1984) (Table 5.1). For purposes of computation, cytotype presence was coded as 1 and absence as 0. As with contingency table analysis, correlations were restricted to those cytotypes identified from at least 20% of the spring or summer collections.

An understanding of these modified coefficients is important for a correct interpretation of results, hence a brief explanation is given. A correlation between the occurrence (presence/absence) of a cytotype and a continuous variable such as pH (Table 5.1), is equivalent to a t-test comparing stream pH at sites where a cytotype was present and sites where it was absent (Wherry 1984). Similarly, correlations between cytotype occurrence and ranked or dichotomous variables are equivalent to Mann-Whitney U or Chi square tests respectively. The advantage of these coefficients is that the significance, strength and direction of a relationship can be presented in one value. Significance tests for these coefficients are given in Table 5.1.

ANOVAS and Kruskal-Wallis procedures were used to determine if stream variables differed among stream types. ANOVAS were used on continuous variables when assumptions of normality and homogeneity of variance were satisfied, otherwise the Kruskal-Wallis procedure was used. The Kruskal-Wallis procedure was also used on ranked data. Multiple comparisons among groups for significant ANOVAS were performed

using the Tukey test and for significant Kruskal-Wallis tests using a Tukey type test ($q_{\alpha,\infty,k}$ or $Q_{\alpha,k}$). Differences in cytotype richness among stream types were detected with Kruskal-Wallis and Tukey type multiple comparison ($Q_{\alpha,k}$) procedures.

RESULTS

Cytotype fauna

Details of each collection site and cytological identifications are given in Appendix 1. All 60 sites were successfully sampled and larvae cytotyped in the spring collections. However, road construction destroyed one site (T) and failure to find larvae after repeated attempts at another (L), reduced summer collections to 58 sites. A total of 1652 Simulium venustum/verecundum complex larvae were cytotyped (Table 5.2). AA and CC2-3 (combined CC2 and CC3) were the most frequently encountered, comprising over 70% of larvae cytotyped. Based on 387 male identifications, CC2-3 consisted of 75.7% CC2 (CI = 71.1 - 79.9%) and 24.3% CC3 (CI = 20.1 - 28.9%).

From the 118 collections cytotyped, 51 sites contained a single cytotype, 53 sites had 2, 8 sites had 3, 5 sites had 4 and only 1 site contained 5 cytotypes (Fig 5.2). Kruskal-Wallis and Tukey-type multiple comparison analysis showed

cytotype richness was significantly lower in samples from large stream sites (Table 5.3).

In the spring collections, EFG/C was the cytotype most often identified at outlets, whereas CC2-3 and AC(gb) were the prevalent cytotypes identified from downstream sites (Fig. 5.3). In the summer collections, identifications from outlets were almost exclusively ACD and AA, with no CC2-3, while downstream sites were predominantly AA and CC2-3. The virtual absence of EFG/C in the summer collections (Fig. 5.3) suggests this cytotype was a univoltine spring simuliid. The single specimen of EFG/C likely represents an exceptional individual with prolonged or delayed development.

Analysis of stream variables

Analysis of stream variables among different stream types is given in Tables 5.4 and 5.5. Mean depth from L stream sites was significantly greater (spring and summer) than other sites. No significant differences in depth were found among O, T and S stream sites in the spring. However, T sites were shallower than O sites in the summer. Oxygen was significantly lower and canopy cover more extensive at T sites during the summer. The size of stream bed particles in T sites was also smaller than in other stream types, and the riparian vegetation reduced compared to other downstream sites (S and L). No other differences were found among site conditions. The range of continuous stream variables under which each cytotype

was found is given in Table 5.6. CC2-3 and AA were found under the broadest range of conditions and EFG/C and ACD the narrowest.

Frequency and correlation analyses

Frequency analysis showed cytotype occurrence was not independent of stream type (Table 5.7). In the spring collections EFG/C, CC2-3 and AC(gb) showed significant, nonrandom distributions among stream type, whereas AA did not. Frequency of ACD was too low for analysis. In the summer collections significant G-tests indicated the distributions of ACD, AA and CC2-3 were dependent on stream type. When data from the spring and summer collections were combined (i.e., temporal influences ignored) for ACD, AA, CC2-3 and AC(gb), all showed nonrandom distributions among stream types (Table 5.7). The frequency of EFG/C was too low for this part of the analysis.

Table 5.7 and Fig. 5.3 suggest that EFG/C, ACD and to a lesser extent AA were sublacustrine simuliids, whereas AC(gb) and CC2-3 were most frequently found at downstream locations. Correlation analysis supports this view (Table 5.8). In the spring collections cytotype occurrence showed either a significant positive (AA, EFG/C) or negative (AC(gb), CC2-3) correlation with outlets. Although AC(gb) and CC2-3 were found primarily in downstream sites, G-tests showed no significant differences ($df = 2$, $p < 0.05$) in frequency of occurrence

among the different downstream categories (T, S, L) for either AC(gb) (spring, $G_{adj.}$ = 5.44) or CC2-3 (spring, $G_{adj.}$ = 2.38; summer, $G_{adj.}$ = 1.23). However, based on male identifications, it was found that 52.4% (11/21) of sites positive for CC3 in the spring collections were L stream sites, as opposed to 27.0% (10/37) for CC2, suggesting CC3 adults preferentially oviposit at large streams.

In the summer collections, ACD showed a significant positive and CC2-3 a significant negative correlation with outlets. Although AA was not correlated with outlets in the summer (Table 5.8), occurrence of AA was significantly higher in O and T sites (pooled) ($G_{adj.}$ = 8.34, df = 1, $p < 0.01$) than S and L sites (pooled).

Other than outlets, most correlations between stream variables and cytotype occurrence were not significant (Table 5.8). In the spring, occurrence of AC(gb) was negatively correlated with temperature and stream bed particle size and positively correlated with mixed larval substrate and canopy cover. In the spring, CC2-3 was negatively correlated with dissolved oxygen. In the summer, ACD was positively correlated with conductivity; AA was negatively correlated with rock substrates and positively associated with vegetation substrates. No other significant correlations were found.

Cytotaxonomic identifications of CC2-3 males showed the spring collection consisted of 63.6% CC2 (CI = 57.2 - 69.7%) and 36.4% CC3 (CI = 30.3 - 42.8%). Correlation analysis must

be interpreted from the viewpoint of a mixed population. Nevertheless, an analysis (not shown) restricted to subsamples in which occurrence of CC2 (n = 53) and CC3 (n = 38) could be unequivocally determined (based on male identifications), yielded identical conclusions to those reached for the mixed population of CC2-3. The summer population consisted of 95.9% CC2 (CI = 91.2 - 98.5%) and 4.1% CC3 (CI = 1.5 - 8.8%). In this case CC3 was unequivocally identified from only 4 subsamples, all of which also contained CC2. Therefore, results of the summer analyses were considered to pertain to CC2.

Cluster analysis

Cluster analysis of stream type by cytotype fauna is given in Figs. 5.4 and 5.5. The cophenetic correlations for spring and summer data were 0.749 and 0.827 respectively, indicating a poor fit for spring data. For the most part clusters were not well separated, but it is apparent that outlet sites tended to cluster together as did downstream sites (T, S, L). Clustering by species association for the spring collections (Fig. 5.6) gave one distinct cluster consisting of EFG/C, ACD and AA and a second of CC2-3 and AC(gb). A cophenetic correlation of 0.884 and similar results (not shown) obtained using other measures of similarity (simple match, Phi, Dice) and clustering algorithms (single link, complete link) indicated that clusters in Fig. 5.6

strongly reflected the original data matrix. Cluster analysis for summer cytotype associations was not performed since EFG/C and AC(gb) were very scarce.

DISCUSSION

The occurrence of a species in a particular stream or stream section is largely the result of female ovipositional behavior (Zahar 1951; Colbo 1979; Colbo and Moorhouse 1979; Colbo and Wotton 1981), a point missed in many benthic studies. For example, the occurrence of Simulium truncatum (= EFG/C) and S. noelleri Fries larvae at outlets resulted from the upstream flight of females to a lentic outflow which provided the ovipositional cue (Carlsson et al. 1977; Wotton 1982). Species found further downstream apparently do not require this cue (Wotton 1979). This point is strengthened by the unpublished observation of EFG/C ovipositing at outlets (confirmed by cytotyping larvae reared from eggs obtained from ovipositing adults) (Colbo pers. comm. 1987). deMoor et al. (1986) showed the distribution of Simulium chutteri Lewis along the Vaal River, South Africa, was largely determined by the ovipositional requirements of the adult female. Lake and Burger (1983) suggested that future studies consider the role that gravid females play in the distribution of Simulium venustum/verecundum cytotypes.

Drift is likely to have its greatest influence on the spatial distribution within stream sections (Colbo and Moorhouse 1979). This is especially true in Newfoundland where streams are frequently interrupted by lentic bodies or large quiet reaches (Larson and Colbo 1983) that act as barriers to large scale movement via drift. Early instars may drift within a riffle from ovipositional sites to locations more suitable for larval development (Reisen 1977; Colbo and Moorhouse 1979; Colbo and Wotton 1981), and final instars may change location to pupate (Reisen 1977; Colbo and Moorhouse 1979). Drift in older larvae is often a response to biotic or abiotic disturbances (Colbo and Wotton 1981).

The spatial distribution pattern of each cytotype of the S. venustum/verecundum complex was nonrandom and to some extent predictable. Differences in the pattern of distribution among cytotypes were also apparent. Many factors have been considered by other authors to explain the distribution and/or abundance of preimaginal simuliids with varying amounts of success (see reviews by Colbo and Wotton 1981; Grunewald 1981; Ross and Merritt 1987). Reviewing the literature often reveals inconsistent results for individual morphospecies, as also noted by Lake and Burger (1983). This results, in part, from the fact that most morphospecies are complexes (Rothfels 1979, 1981b, 1987). For example, on the Avalon Peninsula, the S. venustum/verecundum complex was found in all streams able to support simuliid populations, indicating a broad ecological

distribution. However, individual cytotypes were largely restricted to specific habitats.

Interspecific differences in spatial (and temporal) distribution within species complexes frequently occur in the Simuliidae (Adler 1987) and such differences have often been claimed to represent habitat partitioning. For example, within the Simulium tuberosum complex in New Hampshire, sibling A was restricted to outlet sites, and FGH, FG, and CDE occurred primarily in nonoutlet streams (Pistrang and Burger 1988). In Alberta, FGH was confined to small cool streams and AB to wide warm rivers. FG occurred most frequently in streams of an intermediate nature, but did overlap with both AB and FGH (Adler 1986). Similar segregation among S. tuberosum cytotypes was reported in northern Saskatchewan by Ciborowski and Adler (1990). These same authors found that ACD dominated lake outlets, CC3 dominated midreaches and AA-A/C-CC (combined AA, A/C and CC verecundum cytotypes) reached its greatest relative abundance at sites even further downstream. Adler and Kim (1984) showed that the 2 sibling species of Simulium vittatum 'partitioned' the habitat on the basis of water temperature, velocity and oxygen content. As these factors changed along the water course a sibling gradient was produced. The diploid sibling of the Stegopterna mutata complex was restricted to headwaters and the triploid sibling to sites considerably removed from this habitat (Adler and Kim 1986).

Although interspecific differences in spatial

distribution may appear to represent habitat partitioning, there is very little evidence to support this idea. Hildrew and Townsend (1986) criticized the practice of invoking resource partitioning as a causal explanation for observed differences in the distribution of benthic organisms without appropriate experimental evidence. Hart (1986, 1987) has provided a mechanism by which competition might operate, albeit on a microdistributional scale. In the laboratory P. mixtum was displaced by Cnephia ornithophilia Davies, Peterson and Wood (Harding and Colbo 1981). In contrast, in situ observations over a rock showed only 2% of paired simuliid-simuliid interactions led to larval emigration (Wiley and Kohler 1981), which suggests that competitive displacement, at least in this instance, was not important. It can easily be envisioned how numerous larval interactions on a microdistributional scale could influence distribution on a much larger scale. Nevertheless, few studies have experimentally shown that interspecific competition plays any role in structuring the simuliid community (Hemphill and Cooper 1983; Hemphill 1988). The importance of competition and other biotic interactions in insect communities is not clear (Lawton and Hassell 1984; Price 1984; Lake et al. 1988).

To demonstrate if interspecific differences in spatial distribution within species complexes represent habitat partitioning in response to limited resources or simply reflect separate species following independent evolutionary

paths with different environmental requirements, will require laboratory and field experimentation with falsifiable hypotheses. Although Hart (1986, 1987) has provided an excellent basis upon which to design such studies, the ecological distribution of most cytotypes, and in some cases their species status, needs to be determined before the results of such investigations are fully interpretable.

The range of hydrological conditions under which each cytotype was found is given in Table 5.6. Ranges based on numerous collections over a period of time (e.g., one season) may characterize conditions under which cytotypes are found. Nevertheless, many of these factors probably have little or no effect on larval distribution (Grunewald 1976; Table 5.8). Therefore, interpreting these limits as determinants of distribution can be misleading. For example, EFG/C was found under the narrowest ranges of depth, dissolved oxygen, and conductivity, suggesting these factors may limit distribution. Correlation analysis, however, did not support this supposition. Similarly, ACD occurred over a very narrow range of pH, but again a significant correlation was not found. Adler (1983) noted that although the IS-7 Simulium vittatum sibling was absent from sites characterized by extremes in water chemistry, a lack of tolerance to these conditions should not be assumed.

Gordon and Cupp (1980) examined the distribution of ACD, A/C verecundum (cited as AA-AC) and CC larval cytotypes of the

S. venustum/verecundum complex in relationship to limnological factors. However, the analysis was based on data from 2 geographically separated areas (western and northern New York state) and only 2 sites from each area. Many differences between limnological profiles drawn for cytotypes reflected regional differences in geology. Under such conditions their conclusion that the complex divides into groups on the basis of pH, ionic content, and ratios of the water, is difficult to interpret.

In general the range of values given in Table 5.6 is similar to the few records available for the S. venustum/verecundum complex (Gordon and Cupp 1980; Lake and Burger 1983; Adler 1986; Adler and Kim 1986). The notable exceptions were: i) the higher pH range (7.2 - 9.9) and wider temperature range (4 - 25.5°C) reported for ACD in Alberta (Adler 1986); ii) the higher temperature range (16 - 24.5°C) for ACD in New York state (Gordon and Cupp 1980) and; iii) the wider temperature range (4 - 20.4°C) for AC(gb) in New Hampshire (Lake and Burger 1983). These differences reflect geographic variation in stream conditions, but whether local cytotypes have been selected for these conditions is at present unknown.

Although contingency table analysis showed cytotype occurrence was not random, many stream variables showed little variation among stream type. The single most important variable associated with cytotype distribution was the occurrence of outlets. Clearly, EFG/C, ACD and to a lesser

degree AA were sublacustrine in distribution, whereas CC2-3 and AC(gb) were found primarily downstream (Tables 5.7 and 5.8, Figs. 5.3 and 5.6). In a similar study, involving 20 cytotaxonomically defined simuliids from several complexes, Ciborowski and Adler (1990) found larval assemblage patterns were determined by proximity to outlets as well as stream size. The finding of these authors that ACD dominated lake outlets and CC3 dominated sites downstream in northern Saskatchewan concurs with the findings presented here.

In northern Saskatchewan, AA-A/C-CC verecundum (combined AA, A/C, CC verecundum) was most abundant at large river sites, removed from outlets, which is clearly contrary to the distribution of AA in Newfoundland. In Newfoundland the IIS arm of AA is almost without exception AA in both males and females (Table 3.5). On the other hand, males were preferentially A/C and females preferentially AA in Saskatchewan (Ciborowski and Adler 1990). Whether reported differences in habitat selection indicated 2 cytologically distinct populations, or a single variable cytospecies, warrants further investigation.

As discussed earlier (chapter 1), EFG/C and ACD are chromosomally equivalent to the European species S. truncatum and S. rostratum respectively (chapter 1). Both S. truncatum (Carlsson et al. 1977; Wotton 1979, 1982) and S. rostratum (Schröder 1983, 1988) are known as sublacustrine species in Europe, supporting the conspecificity of these European

species with North American cytotypes. In New Hampshire, EFG/C (cited as S. truncatum) occurs below small ponds and lakes (Burger 1987). The few remaining studies relevant to Newfoundland cytotypes do not specifically mention any affinities for outlet vs nonoutlet sites (Gordon and Cupp 1980; Cupp and Gordon 1983; Lake and Burger 1983; Adler 1986; Adler and Kim 1986; Currie and Adler 1986).

Black fly larvae occur in very high numbers at lake outlets and decline further downstream (Carlsson et al. 1977; Sheldon and Oswood 1977), presumably in response to changes in food quality, substrate stability, flow conditions and ovipositional cues (Carlsson et al. 1977; Wotton 1987). Although these factors may account for larval abundance, they do not, with the exception of ovipositional cues, explain why species would be restricted to such a habitat (Colbo 1979; Burger 1987). For example, Thompson (1987a b) concluded that simuliid occurrence was not explicable in terms of availability of specific foods, but food quality and quantity could influence density. Although Schröder (1988) found that interspecific food differences correlated with the distribution of 5 simuliids, gut contents also reflected local food availability. Accordingly, the relationship between food and distribution was unclear.

With the exception of proximity to outlets (i.e., outlet vs downstream locations) (Table 5.8), no other factor was consistently correlated with the occurrence of cytotypes.

In the case of EFG/C, which was almost exclusively found at outlets, no additional factors were associated with occurrence. One of the 3 nonoutlet sites harbouring EFG/C was located 500 m downstream from an outlet and probably represents the downstream limit of its distribution (Wotton 1979). The single specimen identified from a large stream site yielded poor chromosomes and its identification is in doubt. Alternatively, 100 m upstream from this site the stream was deep, slow and wide, similar to conditions found in a small pond.

The positive correlation of ACD with conductivity may indicate a preference for enriched sites. In a study of larval distribution over northwestern North America, Corkum and Currie (1987) found streams lacking black flies had higher conductivity (among other factors) than streams with these insects. These same authors also showed conductivity useful in predicting simuliid assemblages. On the basis of differences in conductivity and pH, Grunewald (1976) divided members of the Simulium damnosum complex into 3 groups. In Tanzania, Grunewald et al. (1979) found the distribution of Simulium nyasalandicum de Meillon shifted with seasonal changes in conductivity. In New York state, members of both the S. venustum/verecundum and Simulium jenningsi Malloch groups were divided on the basis of stream pH and ionic content (Gordon and Cupp 1980; Gordon 1984). However, as I have mentioned, I find the results presented by Gordon and Cupp (1980)

unconvincing.

AA was more frequently found on vegetation (positive correlation) than on rocks (negative correlation). Eggs of the summer generation(s) are deposited on emergent and trailing vegetation (Colbo pers. comm. 1987; chapter 4) which would account for the preponderance of larvae on vegetation. The high incidence of trailing and emergent vegetation at O and T sites may also explain why AA was often found at these sites (Table 5.7). Adler and Kim (1986) and Ciborowksi and Adler (1990) also found AA and AA-A/C-CC respectively, associated with trailing vegetation. It appears the larval distribution of the summer generation(s) of AA was influenced by the availability of vegetation for ovipositional sites or larval substrate. The availability of suitable substrate is known to limit simuliid distribution (Maitland and Penny 1967; Lewis and Bennett 1975). Larvae of the spring generation, which arise from eggs deposited over open water in the fall (Colbo pers. comm. 1987), were not correlated with substrate, but showed a significant positive relationship with outlets. In this case larval distribution could be the consequence of females from the fall population preferentially ovipositing at sublacustrine locations. Diptera are known to shift habitat requirements over time (Rae 1985).

Interestingly, ACD also oviposits on vegetation in the summer (chapter 4) but showed no substrate preference either here or in the study of Lake and Burger (1983). In further

contrast to AA, ACD was highly correlated with outlets in the summer collections. Though excluded from statistical analysis, 6 of the 7 positive identifications of ACD in the spring were also at outlets. Clearly, outlets exert considerable influence on the choice of breeding sites for ACD.

Correlation analysis (Table 5.8) showed AC(gb) was most frequently identified from sites with lower temperatures (present = 13.5°C ±2.8; absent = 15.9°C ±3.1), mixed larval substrates and a stream bed of small stones and/or rubble. The fact that AC(gb) was identified from all 3 locations with complete canopies accounts for the positive correlation with canopy cover. Canopy cover has been reported to increase the abundance of some simuliids (Towns 1981; Behmer and Hawkins 1986), but can reduce species richness (Bishop 1973; Colbo and Moorhouse 1979). Although AC(gb) was abundant at a canopied site in New Hampshire, boulders formed a significant part of the stream bed. (Lake and Burger 1983; Pistrang and Burger 1984).

Again differing from the results presented here, AC(gb) occurred in 2 of 5 outlets investigated in New Hampshire, and was very abundant at one of these locations (Lake and Burger 1983; Pistrang and Burger 1984). Adler (1986) noted that Simulium decorum and Cnephia dacotensis (Dyar and Shannon) were largely confined to outlets in eastern North America but showed no such restriction in Alberta. Thus, habitat selection in some cytotypes may vary with geographic location, a

possibility also raised by Pistrang and Burger (1988).

CC2-3 was negatively correlated with dissolved oxygen content in the spring. When the analysis was restricted to males it was found that both CC2 and CC3 showed a significant negative correlation with dissolved oxygen. The decrease in dissolved oxygen content of streams during the summer (spring = 10.0 ± 1.1 mg/L, summer = 9.2 ± 1.1 mg/L; $t = 3.95$, $df = 102$, $p < 0.001$) may account for the nonsignificant correlation between oxygen and CC2-3 at that time. Thus, the predictive value (or the biological importance) of a variable may be seasonal. Carlsson (1967) suggested the importance of dissolved oxygen has been overemphasised. Reisen (1977) did not believe changes in dissolved oxygen had any significant impact on simuliid density in an Oklahoma stream. On the other hand, Adler and Kim (1984) showed the distribution of IS-7 and IIIL-1 siblings of the S. vittatum complex was very much dependent on dissolved oxygen as well as other factors affecting respiratory stress (temperature, current).

The ability to reliably identify cytotype assemblages and predict their occurrence on the basis of stream type or other variables would greatly reduce the dependence on cytotaxonomic identification. However, the only information gained from cluster analysis (Figs. 5.4 and 5.5) was that outlet sites (O) tended to cluster together as did downstream sites (T, S, L). No other significant groupings were defined. Corkum and Currie (1987) were able to group simuliid morphospecies assemblages

throughout northwestern North America into 5 distinct clusters. They were also able to predict (discriminant analysis) a stream site's membership to one of these 5 groups with 71% accuracy, based on a few simply measured stream variables (latitude, distance from source, drainage basin, stream width, conductivity). Using 5 species of blackflies, including S. rostratum, Schröder (1988) was able to cluster 19 stream sites from 2 Irish drainage basins into headwater, downstream, and outlet sites. Schröder (1988) noted that S. rostratum was restricted to the outlet sites.

The majority of sites sampled in this study supported mixed cytotype populations (Table 5.3, Fig. 5.2). Future studies of the S. venustum/verecundum complex in Newfoundland and elsewhere must recognize that most sites support mixed populations (e.g., Lake and Burger 1983). In contrast, Adler and Kim (1984) found that 70% of 167 sites in the eastern United States appeared to support pure populations of either IS-7 or IIIL-1 S. vittatum siblings. In the present study, species richness was shown to be the lowest for large streams, a consequence of the rare identifications of EFG/C, ACD or AC(gb) (Table 5.7) from these sites. Species richness for simuliids has been linked to stream permanency (Merritt et al. 1978), stream size (Konurbayev 1978), canopy cover (Bishop 1973; Colbo and Moorhouse 1979), stream productivity (Lake and Burger 1983) and ecoregion (Colbo and Moorhouse 1979).

SUMMARY

1. EFG/C and ACD dominated outlet sites in the spring and summer respectively. EFG/C was rarely found at downstream sites.

2. The CC2-3 and AC(gb) cytotypes were most frequent at downstream sites. In these locations CC3 was most frequently identified from large streams, whereas AC(gb) was most frequently found in cool, canopied streams where the stream bed consisted mainly of small stones and/or rubble. CC2 was commonly encountered in all downstream locations.

3. In the spring AA was common to both outlet and nonoutlet sites but favored the former. In the summer AA was most frequently found in outlets and trickles. The summer generation(s) of AA was associated with the distribution of trailing and emergent vegetation.

4. It is argued that differences in the spatial distribution of cytotypes cannot be assumed to represent habitat partitioning without the appropriate experimental data.

TABLE 5.1. Correlation coefficients used in correlation analysis between cytotype occurrence and stream variables.

	Type of variable		
	Interval/ ratio	Ranked	Nominal (0 / 1)
	temperature pH dissolved oxygen conductivity depth	stream substrate canopy cover riparian vegetation	outlets larval substrate
Correlation coefficient (r)*	r_{pbs}	r_{dr}	$r\phi$
Significance test	$F = \frac{r^2}{1 - r^2} \cdot (N - 2)$	$z = r \cdot (N - 1)^{0.5}$	$z = r \cdot (N - 1)^{0.5}$

*Correlation coefficients and tests of significance taken from Wherry (1984). r_{pbs} and $r\phi$ are commonly referred to as the point-biserial and phi correlation coefficients respectively.

TABLE 5.2. Total number of individuals of each cytotype identified from 118 collections on the Avalon Peninsula.

Cytotype	Number identified	% of Total
EFG/C	125	7.6
ACD	181	11.0
AA	326	19.7
CC2-3[+]	874	52.9
AC(gb)	146	8.8
	1652	

[+]Based on 387 male identifications, CC2-3 consisted of 75.7% CC2 (CI = 71.1 - 79.9%) and 24.3% CC3 (CI = 20.1 - 28.9%).

TABLE 5.3. Kruskal-Wallis and Tukey-type multiple comparison analysis of cytotype richness among outlet, trickle, small and large stream habitats.

Stream type	Mean[+] rank	Median[++]	$H_{adj.}$
Outlet	63.8[a]	3	16.71***
Trickle	74.7[a]	2	
Small	57.2[a]	2	
Large	42.1[b]	1	

*** $p < 0.001$.

[+]Mean ranks with different letters were significantly different at $p < 0.05$.

[++]Median values given for comparative purposes only.

TABLE 5.4. ANOVA and Tukey multiple comparison analysis of depth and pH among outlet, trickle, small and large stream habitats.

	Mean[+] (±SD)	
	Depth	pH
Spring collections		
Outlet	0.16^{a} (±0.05)	6.2 (±0.4)
Trickle	0.12^{a} (±0.05)	5.9 (±0.5)
Small	0.15^{a} (±0.05)	6.1 (±0.5)
Large	0.26^{b} (±0.08)	6.2 (±0.4)
	$F_{3,56} = 15.57***$	$F_{3,46} = 0.74$
Summer collections		
Outlet	0.18^{a} (±0.07)	5.9 (±0.4)
Trickle	0.11^{b} (±0.04)	6.1 (±0.3)
Small	0.17^{ab} (±0.07)	6.0 (±0.5)
Large	0.26^{c} (±0.09)	6.3 (±0.6)
	$F_{3,54} = 11.22***$	$F_{3,52} = 1.57$

*** $p < 0.001$.

[+]Means (within season) for each collection with different letters were significantly different at $p < 0.05$.

TABLE 5.5. Kruskal-Wallis and Tukey-type multiple comparison analysis of stream variables among outlet, trickle, small and large stream habitats.

	Mean Rank* (Mean ±SD)			Mean rank		
	Temperature (°C)	Dissolved oxygen (mg/L)	Conductivity (µS/cm at 25°C)	Riparian vegetation	Stream bed	Canopy cover
Spring collections						
Outlet	33.6 (15.4 ±2.8)	37.3 (10.6 ±1.1)	24.5 '41.8 ±15.6)	28.1^{ab}	33.8^{a}	29.3
Trickle	25.5 (14.1 ±4.0)	21.4 (9.5 ±1.3)	33.7 (53.1 ±18.9)	17.9^{b}	13.5^{b}	35.7
Small	27.0 (14.4 ±2.9)	30.0 (10.1 ±1.0)	31.0 (48.5 ±17.4)	34.6^{a}	36.0^{a}	31.5
Large	35.8 (15.9 ±2.8)	26.6 (9.9 ±0.6)	24.3 (40.7 ±11.8)	40.3^{a}	38.7^{a}	25.5
$H_{adj.}$	3.71	6.48	3.45	15.40***	20.46***	6.30
Summer collections						
Outlet	34.7 (17.9 ±1.3)	26.2^{a} (9.3 ±0.7)	25.0 (42.9 ± 8.9)	28.1^{ab}	32.4^{a}	27.1^{a}
Trickle	24.2 (16.0 ±3.7)	14.1^{b} (8.2 ±1.1)	34.2 (55.8 ±21.1)	17.9^{b}	12.1^{b}	40.6^{b}
Small	25.6 (17.1 ±2.7)	28.6^{a} (9.4 ±1.0)	23.0 (46.1 ±18.0)	31.9^{a}	34.5^{a}	27.1^{a}
Large	33.4 (18.1 ±2.3)	32.0^{a} (9.6 ±1.0)	20.5 (40.2 ± 9.9)	40.1^{a}	38.5^{a}	23.5^{a}
$H_{adj.}$	4.34	10.60*	6.27	14.35***	21.64***	17.00***

* $p < 0.05$ *** $p < .001$.

*Mean ranks (within season) with different letters were significantly different at $p < 0.05$.

TABLE 5.6. Range of continuous stream variables under which each cytotype was found.

Stream variable	Observed range of variable	Observed range for cytotype				
		EFG/C	ACD	AA	CC2-3	AC(gb)
Depth (m)	0.04 - 0.48	0.09 - 0.23	0.06 - 0.33	0.06 - 0.40	0.04 - 0.48	0.04 - 0.41
Temperature (C°)	8.5 - 23.0	11.0 - 22.0	11.0 - 21.0	9.5 - 22.0	8.5 - 23.0	8.5 - 19.0
pH	4.8 - 7.4	5.3 - 7.2	5.4 - 6.5	5.3 - 7.4	4.8 - 7.2	4.8 - 7.2
Dissolved oxygen (mg/L)	6.3 - 12.4	7.8 - 12.4	6.3 - 12.4	6.3 - 12.4	6.3 - 12.2	7.1 - 12.2
Conductivity (µS/cm at 25°C)	13.0 - 99.0	26.0 - 73.0	34.0 - 99.0	16.0 - 99.0	13.0 - 99.0	18.0 - 49.0

TABLE 5.7. Frequency analysis (G-tests) of cytotype occurrence (+/-) among stream types.

	EFG/C*		ACD		AA		CC2-3		AC(gb)	
	+	-	+	-	+	-	+	-	+	-
Spring collections										
Outlet	14	1	6	9	8	7	4	11	1	14
Trickle	2	13	0	15	6	9	15	0	9	6
Small	0	15	1	14	3	12	13	2	10	5
Large	1	14	0	15	2	13	14	1	4	11
Total no. of streams	17	43	7	53	19	41	46	14	24	36
$G_{adj.}$**	42.7**		-		6.7		26.9**		16.0**	
Summer collections										
Outlet	1	14	13	2	10	5	0	15	0	15
Trickle	0	14	6	8	10	4	11	3	1	13
Small	0	15	2	13	5	10	14	1	3	12
Large	0	14	1	13	4	10	12	2	0	14
Total no. of streams	1	57	22	36	29	29	37	21	4	54
$G_{adj.}$	-		25.9**		8.4*		40.6**		-	
Total collections										
Outlet	15	15	19	11	18	12	4	26	1	29
Trickle	2	27	6	23	16	13	26	3	10	19
Small	0	30	3	27	8	22	27	3	13	17
Large	1	28	1	28	6	23	26	3	4	25
Total no. of streams	18	100	29	89	48	70	83	35	28	90
$G_{adj.}$	-		33.4***		14.5**		60.2***		18.3***	

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

*The + and - columns are number of streams each cytotype was, and was not identified from, respectively.

**$G_{adj.}$ is G value using William's continuity correction. Df = 3 for all tests.

TABLE 5.8. Correlation analysis between cytotype occurrence and stream site conditions.

	Correlation coefficients				
	EFG/C	ACD	AA	CC2-3	AC(gb)
Spring collections					
Depth (m)	-0.087	-	-0.093	0.136	-0.206
Temperature (°C)	0.071	-	-0.038	0.075	-0.396**
pH	0.058	-	0.161	-0.079	-0.008
Dissolved oxygen (mg/L)	0.161	-	-0.022	-0.460***	-0.020
Conductivity (μS/cm at 25°C)	-0.123	-	-0.064	0.078	0.091
Outlet (+/-)	0.833***	-	0.269*	-0.683***	-0.393**
Stream bed particle size	-0.069	-	0.058	-0.062	-0.356**
Riparian vegetation	0.034	-	-0.095	0.035	-0.052
Canopy cover	0.092	-	-0.212	0.046	0.287*
Larval substrate -rock (+/-)	0.189	-	-0.020	-0.051	-0.224
Larval substrate -vegetation (+/-)	-0.149	-	0.208	0.172	-0.136
Larval substrate -mixed (+/-)	-0.052	-	-0.177	-0.111	0.361**
Summer collections					
Depth (m)	-	-0.113	0.004	-0.005	-
Temperature (°C)	-	0.100	-0.064	-0.172	-
pH	-	-0.079	0.095	0.050	-
Dissolved oxygen (mg/L)	-	-0.204	-0.161	-0.025	-
Conductivity (μS/cm at 25°C)	-	0.405**	0.155	0.026	-
Outlet (+/-)	-	0.593***	0.197	-0.784***	-
Stream bed particle size	-	-0.079	-0.170	-0.009	-
Riparian vegetation	-	-0.200	-0.131	0.008	-
Canopy cover	-	0.027	0.254	0.118	-
Larval substrate -rock (+/-)	-	-0.137	-0.354**	0.117	-
Larval substrate -vegetation (+/-)	-	0.076	0.323*	-0.104	-
Larval substrate -mixed (+/-)	-	0.048	0.000	-0.000	-

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

FIG. 5.1. Location of collection sites, Avalon Peninsula, Newfoundland. The peninsula covers a 9000 km^2 area and lies between 46°.35' - 48°.11' N and 54°.13' - 52°.38' W.

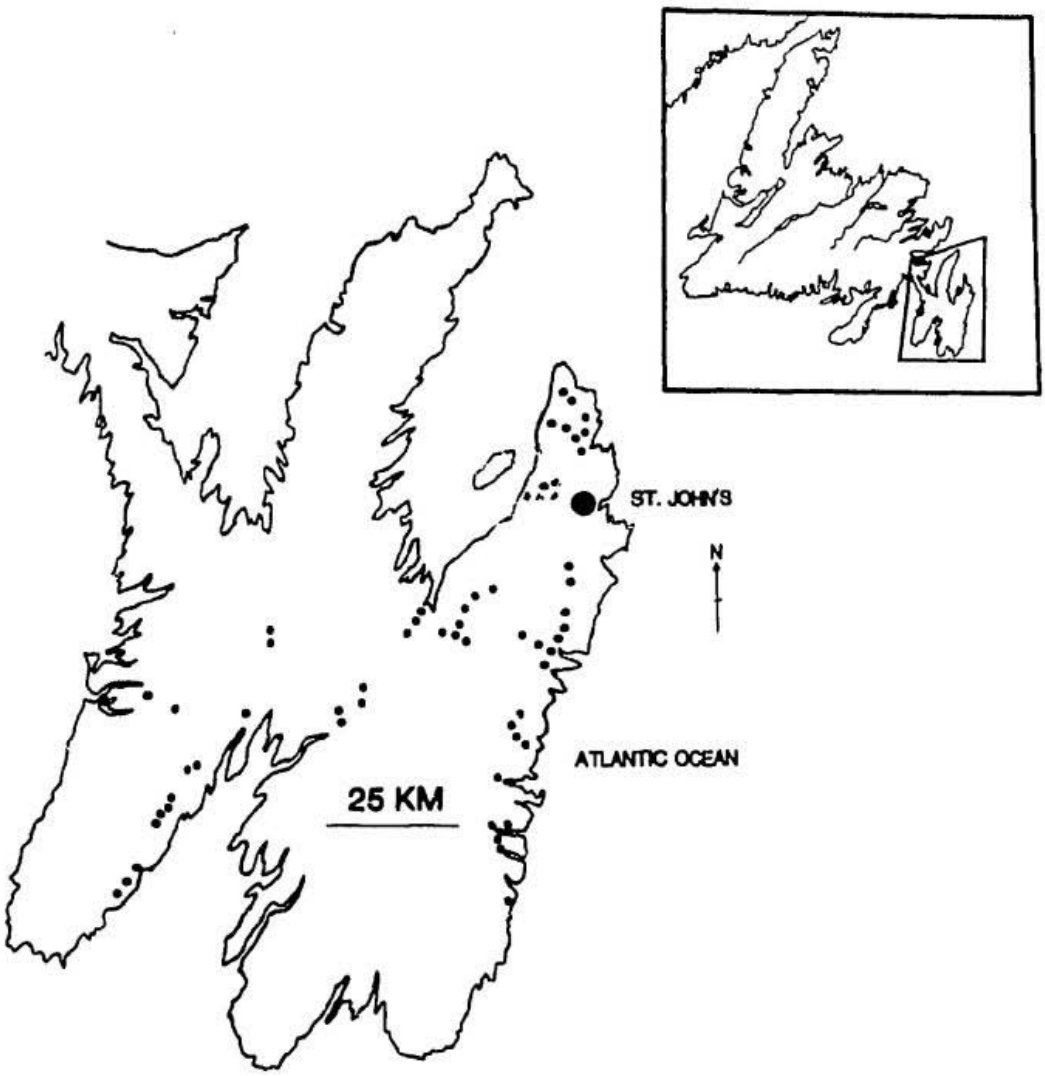

ST. JOHN'S
N
ATLANTIC OCEAN
25 KM

FIG. 5.2. Frequency histogram of cytotype richness among sites.

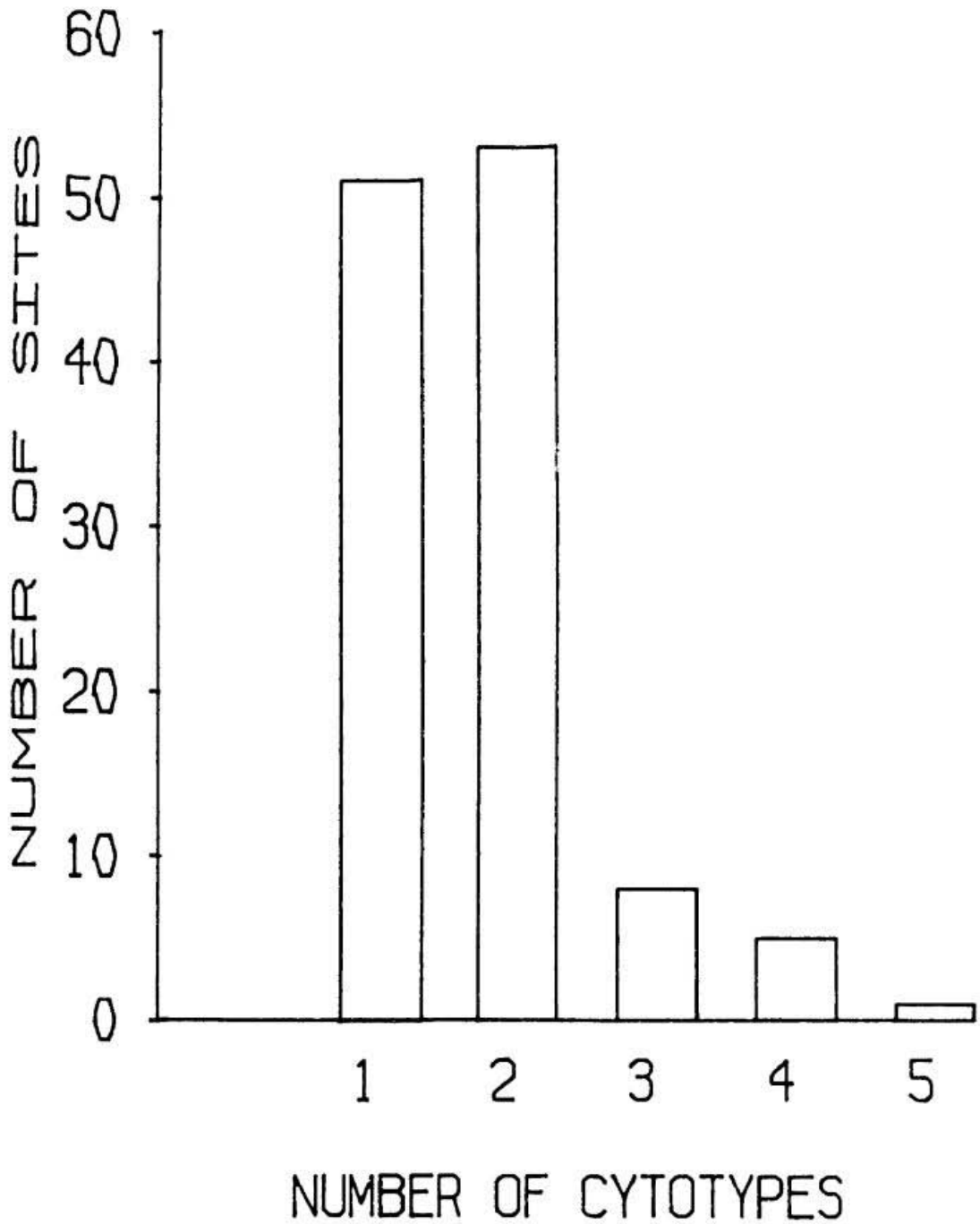

60
50
40
30
20
10
0
NUMBER OF SITES
1
2
3
4
5
NUMBER OF CYTOTYPES

FIG. 5.3. Combined percent cytotype composition for each stream type. For spring collections n = 210 scored larvae/stream type. For summer collections n = 196 for trickle and large stream sites and 210 for outlet and small stream sites. Total number of larvae scored = 1652.

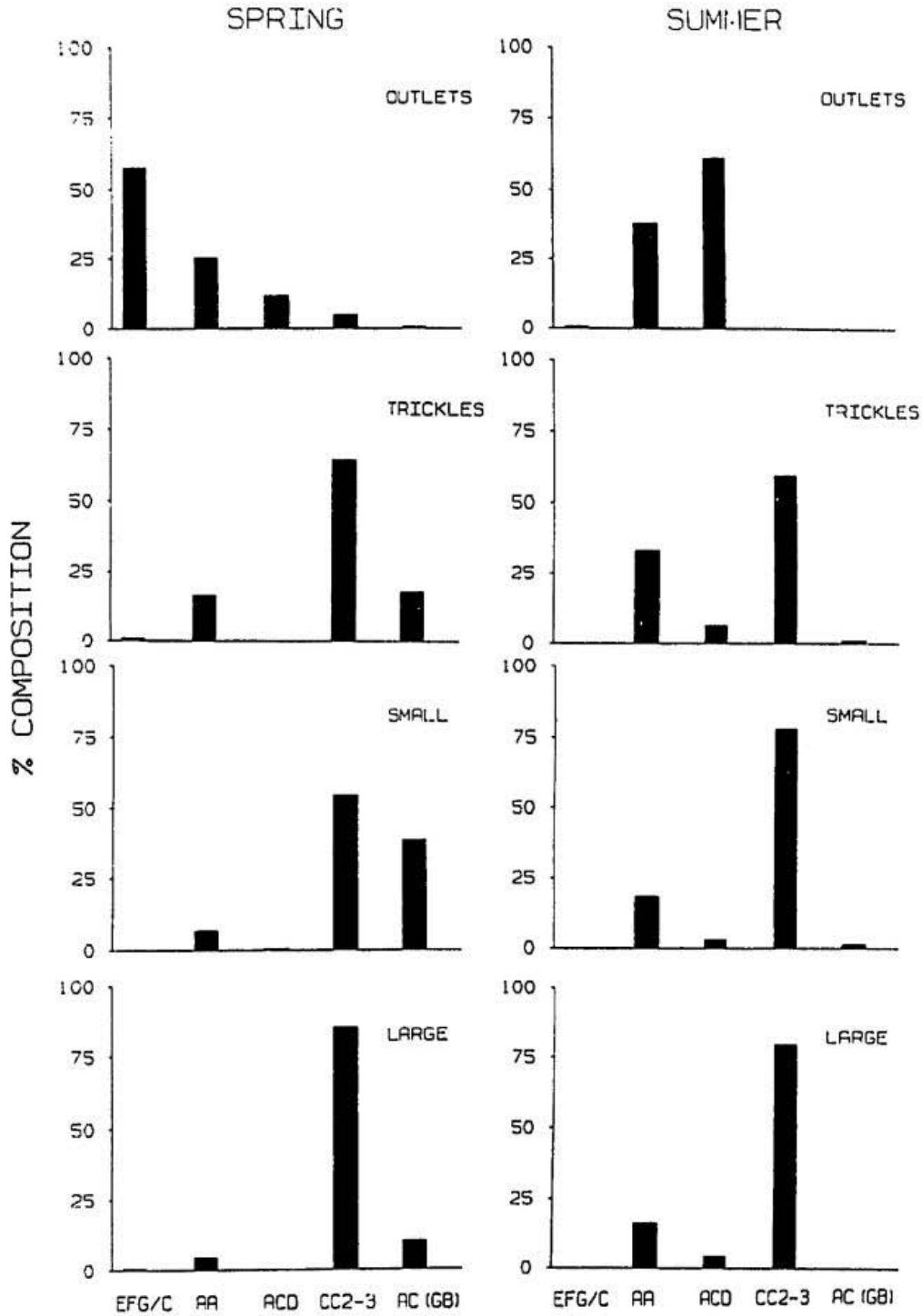

SPRING
SUMMER
OUTLETS
TRICKLES
SMALL
LARGE
% COMPOSITION
100
75
50
25
0
EFG/C
AA
ACD
CC2-3
AC (GB)

FIG. 5.4. Dendrogram of 60 stream sites based on cytotype fauna. Data are for spring collections. Dendrogram produced using the Jaccard similarity coefficient and single linkage clustering. O = outlet, T = trickle, S = small, and L = large stream site.

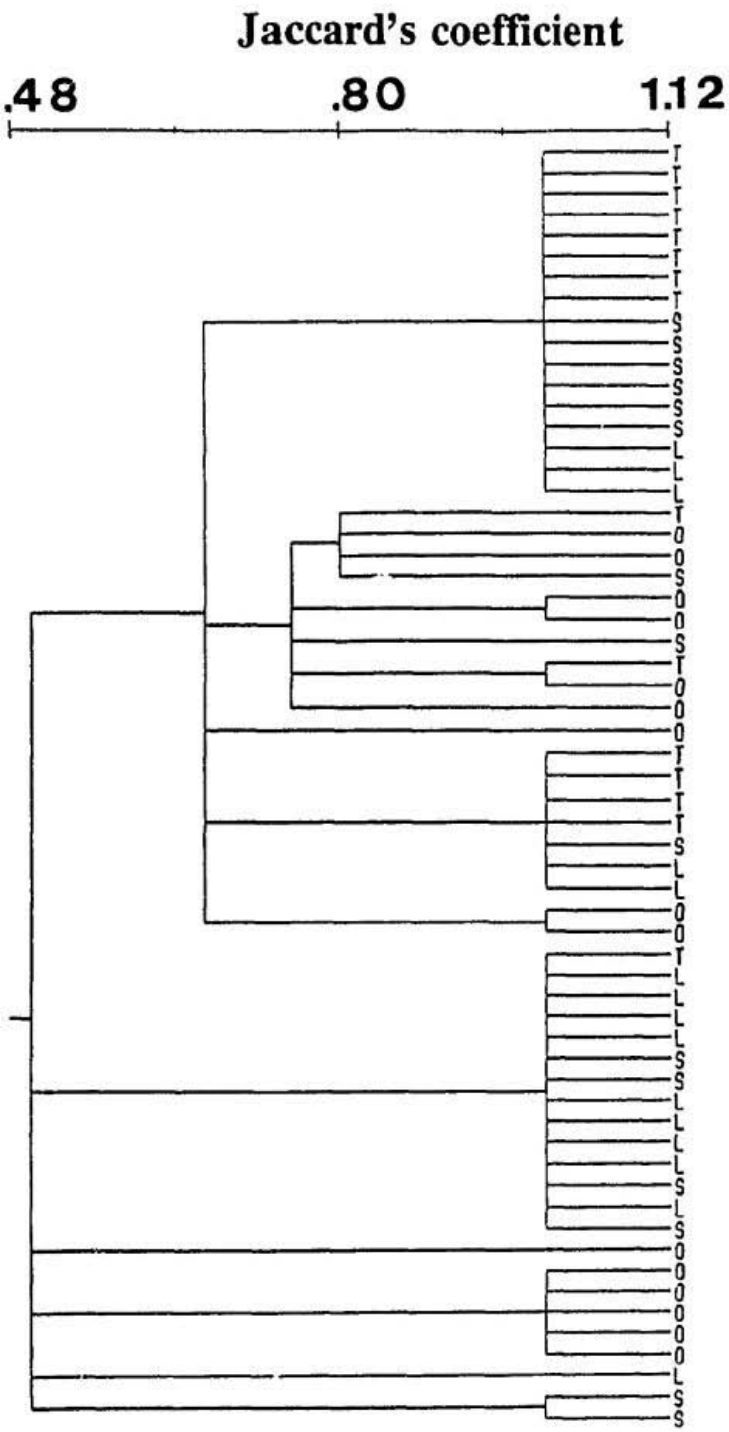

Jaccard's coefficient
.48
.80
1.12

FIG. 5.5. Dendrogram of 58 stream sites based on cytotype fauna. Data are for summer collections. Dendrogram was produced using the Jaccard similarity coefficient and single linkage clustering. O = outlet, T = trickle, S = small, and L = large stream site.

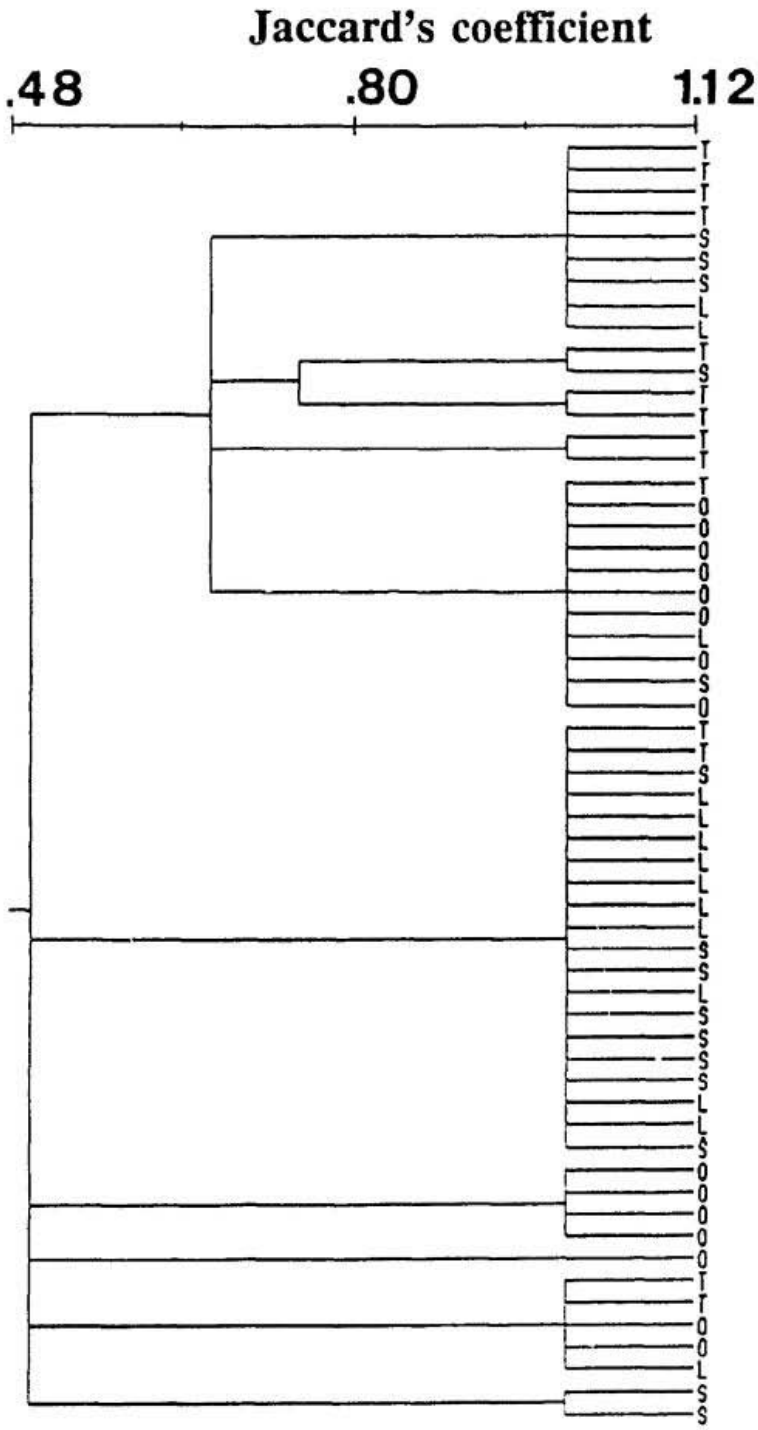
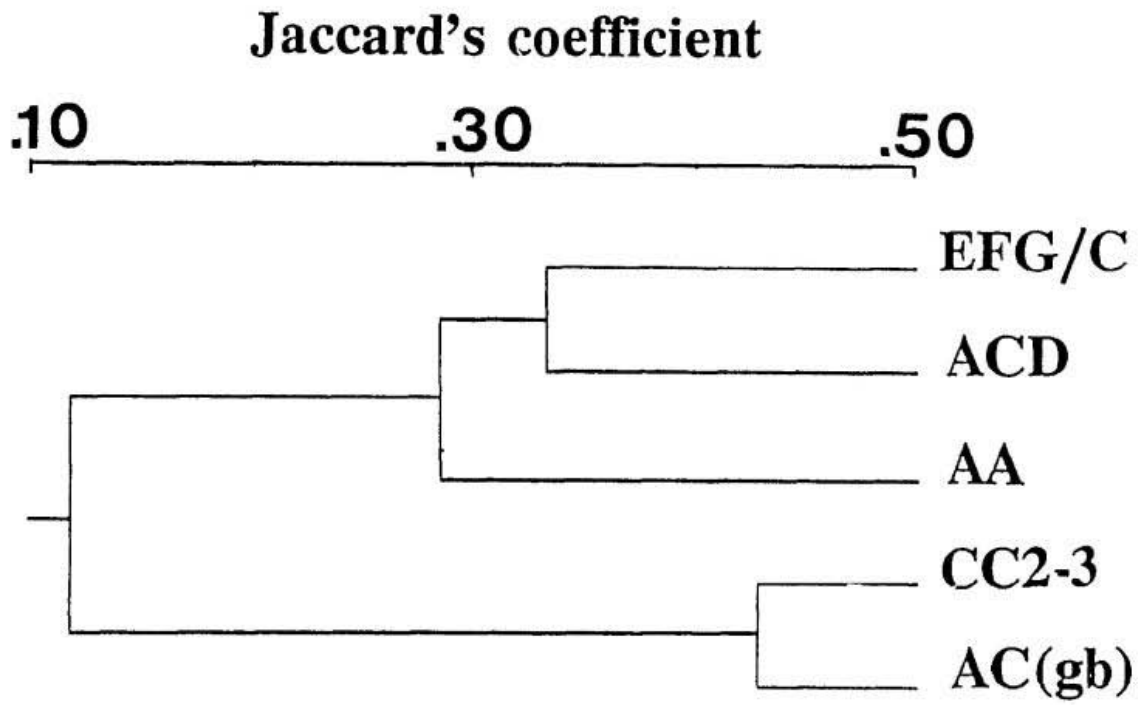

Jaccard's coefficient
.48
.80
1.12

FIG. 5.6. Dendrogram of cytotype association based on number of co-occurrences. Data are for spring collections. Dendrogram was produced using the Jaccard similarity coefficient and the UPGMA cluster algorithm.


Jaccard's coefficient
.10
.30
.50
EFG/C
ACD
AA
CC2-3
AC(gb)

CHAPTER 6

MACRODISTRIBUTION PATTERNS OF LARVAE. II. FACTORS ASSOCIATED WITH CYTOTYPE COMPOSITION AND ABUNDANCE

INTRODUCTION

In chapter 5 attention was focused on the pattern of cytotype occurrence (presence / absence). This chapter continues the analysis of macrodistribution by an examination of factors associated with changes in cytotype abundance and composition. Two data sets were examined; the first from a single drainage basin with the intent of developing empirical models relating cytotype abundance to stream site conditions. As results from chapter 5 suggested the existence of distinct outlet and downstream faunas, predictable changes in cytotype composition should ocurr with increased distance from lake outlets. A second data set allowed comparisons of field data to expectations generated from chapter 5. In addition, larval AA were taken in sufficient numbers from this second data set to examine adult dispersal based on the discontinuity of the IIL-4 polymorphism.

MATERIALS AND METHODS

Cytotype abundance within a single drainage basin

The Piccos drainage basin (Fig. 6.1), located 12 km N of St. John's, Newfoundland, Canada, (47°38' - 47°43' N and 52°50' - 52°42' W) was selected as the study site. The majority of streams were first and second order, with the largest, Piccos Brook, a fourth order stream draining into the Atlantic Ocean. The distribution and abundance of cytotypes was quantitatively investigated using single 10-cm lengths of stiff monofilament fishing line (diameter ≈ 2.0 mm), each individually anchored to a small piece of metal. Ten filament samplers were placed at each of 13 sampling stations (Fig. 6.1) using a stratified random sampling design (strata = fast and slow water velocity) and left for 1 week. Particulars of the filament samplers and the sampling design are deferred to chapter 8 (Phenology) where the performance of both sampler and sampling design are evaluated in detail. Each sampling station consisted of a 5-m or 10-m stretch of stream (depending on stream size). Sampling was conducted during the last week of May, 1987.

At retrieval, each filament was removed from the stream, covered with a culture tube, cut from the anchor, and the tube capped and placed on ice for transportation to the laboratory. Stream variables measured (for details see chapter 2) at each site during the time of filament retrieval included depth,

width, velocity, temperature, conductivity, pH, and dissolved oxygen. Depth (3 - 4 equidistant readings) and width were measured at the upstream, downstream, and midpoint boundaries of each sampling station and means determined. Measurements at the station midpoint of velocity (3 - 4 equidistant readings) 5 cm above the stream bed (i.e., centre of filament) and depth and width were used to calculate discharge. Three seston samples were collected 1 m upstream from the sampling station and results averaged (chapter 2). Stream order and distance to the nearest pond outlet were estimated for each station from a 1:25,000 topographical map.

Upon returning to the laboratory larvae were fixed and the number of last instar S. venustum/verecundum on each filament counted. An attempt was made to score all last instar larvae. It was assumed that unscorable cytotypes occurred in the same frequency as scorable cytotypes.

Influence of outlets on cytotype composition

Results of chapter 5 suggest predictable changes in cytotype composition should occur with increased distance from outlets, as downstream species replace those suited for sublacustrine conditions. If changes are assumed to be proportional to distance from outlet, then cytotype replacement could be described in a formal model as:

[1] % of Cytotype$_i$ = f(m)

where m equals the distance from a lake outlet in meters. If a second assumption is made that cytotype replacement is a linear or curvilinear function of distance then the model takes the form:

$$[2] \quad \% \text{ of Cytotype}_i = a_0 + a_1 m + a_2 m^2 + a_3 m^3$$

The purpose here was to compare field data with the expectations generated from the above model. Polynomials were restricted to quadratic and cubic terms as powers higher than these are seldom important in biological work (Sokal and Rohlf 1981).

Field data were from 2 systematic collections working in a downstream direction from pond outlets. The first collection (June 2, 1988) was from the second order stream draining Axes Pond (47°40'45'' N, 52°45'30'' W). This pond was located on the Piccos drainage basin (Fig. 6.1, upstream of station 7). Swath samples were taken at 10, 30, 40, 50, 60, 80, 120, 160, 360, 400, and 500 m from the outlet. When larvae were present on both rock and vegetation substrates separate collections were made. At each collection site depth (n = 3) and velocity (n = 3) were measured along the collection swath; conductivity and temperature were also noted. Due to technical difficulties pH (n = 7) and oxygen (n = 8) were not measured at all sites. The second collection (June 10, 1988) was from Broad Cove Brook (47°34'15'' N, 52°51'00'' W), a fourth order stream

below Healey's Pond, 11 km W of St. John's. Collections were restricted to vegetation as few larvae were found on rocks found further than 30 m from the outlet. An extra site at 1000 m was added to the Broad Cove collection. Depth (n = 3), seston (n = 3), dissolved oxygen, pH, conductivity and temperature were measured at 10, 50, 120, 320, and 500 m. All stream variables were measured according to methods presented in chapter 2.

Cytotaxonomic identification of all larvae in each swath sample would require a prohibitive amount of time. The percent of each cytotype in a sample was estimated from a random subsample of 22 with a mean confidence interval of 18.4% (Appendix 2). Substantially increasing subsample size does not greatly decrease error, but increases identification time considerably. For example, increasing subsample size from 22 to 50 decreases the mean confidence interval to 11.8%, but doubles identification time. In cases where larvae were collected from both rock and vegetation (Axes Pond), 22 larvae from each substrate were scored and the results combined.

Adult dispersal

Larval AA were taken in sufficient numbers at both the Axes Pond and Broad Cove sites for inference about adult dispersal. Larvae were scored for the IIL-4 autosomal polymorphism from the 2 populations (Axes Pond and Broad Cove) and separately tested (G-tests) for Hardy-Weinberg

equilibrium. Subsequently the frequency distribution for the IIL-4 inversion was compared between the 2 populations (G-test). A significant discontinuity between the 2 sites is interpreted as evidence of restricted gene flow resulting from low adult dispersal (Rothfels 1981b). Conversely, a non-significant result is evidence of unrestricted gene flow and high dispersal ability (Adler 1983). The 2 sites were 12 km apart.

Data analysis

Cytotype abundance. A detailed description of regression methodology is given in chapter 7 (where regression protocol was initially developed), therefore only a brief account is given here. Best-regression (exploratory) and stepwise (variable selection) routines were used to reduce the number of potential variables to significant predictor variables ($p < 0.05$) while producing an unbiased model. Variables which failed a tolerance of 0.01 were excluded from the analysis. Larval abundance counts were transformed to the log $(x + 1)$ (Elliott 1977) to satisfy the assumptions of regression analysis (Sokal and Rohlf 1981; Zar 1984). After initial variable selection models were scrutinized by an examination of residual plots, leverage points, Cook's distances and a lack of fit routine, which did not require replication.

A sequential, agglomerative, hierarchical cluster analysis was used to group stream sites by similarity of their

cytotype fauna. Since data were of a quantitative nature the coefficient distance (Sokal 1961; Kownacki 1985; Schröder 1988) was used to determine dissimilarity between each site. This coefficient was calculated as:

$$[3] \quad d_{jk} = \left(\sum_{i}^{n} (x_{ij} - x_{ik})^2\right)^{0.5}$$

where n = number of cytotypes at paired sites, x_{ij} = proportion of cytotype i at site j, and x_{ik} = proportion of cytotype i at site k. Sites were excluded if total larval abundance fell below 10 (see Schröder 1988). A tree dendrogram was produced using the UPGMA algorithm (Rohlf 1988).

Cytotype composition. The model presented in equation 2 was tested using a stepwise multiple regression routine. A significant regression was considered grounds for acceptance of the model. The significance of each distance term described the form of cytotype change (i.e., linear or curvilinear). The dependent variable (percent of cytotype$_i$) was subjected to an arcsine transformation as recommended by Sokal and Rohlf (1981). Distances were expressed as logs which improved both modeling and graphical representation of the data. After initial variable selection the resultant models were scrutinized as outlined above. At Axes Pond the influence of water depth, velocity, temperature and conductivity on cytotype composition was examined using stepwise regression.

RESULTS

Cytotype abundance within a single drainage basin

Cytotype abundance at each of the 13 sampling stations is shown in Table 6.1. CC2-3 and AC(gb) were the most abundant and widespread cytotypes, occurring at 10 and 7 sites respectively. Unequivocal male identifications (n = 52) showed the CC2-3 population consisted of 94.2% CC2 (CI = 84.1 - 98.8%) and 5.8% CC3 (CI = 1.2 - 15.9%). Hence, results for CC2-3 largely pertain to CC2.

The distinction between sublacustrine and downstream faunas is apparent in Table 6.1. EFG/C was collected in greatest numbers at station 7 (65 m from nearest outlet, Fig. 6.1) and ACD and AA were found only at this site. CC2-3 and AC(gb) were collected in greatest numbers at sites removed from outlets (Table 6.2, Fig. 6.1). The occurrence of CC2-3 was also significantly correlated with AC(gb) (r_{phi} = 0.592, df = 11, $p < 0.05$).

The abundance of S. venustum/verecundum larvae (i.e., total larvae) and CC2-3 larvae increased with increasing seston (total suspended particle) loads (range 0.43 - 4.00 mg/L) and discharge (range 0.01 - 0.75 m^3/s), and decreasing conductivity (range 39 - 67 μS/cm at 25°C) (Table 6.2). The abundance of AC(gb) increased with increasing stream width (range 0.7 - 7.4 m) (Table 6.2). An examination of residuals, leverage points, Cook's distances and the lack of fit routine

indicated regression models were an adequate fit to the data. Bivariate plots of log observed abundance and log predicted abundance (Fig. 6.2) showed modest and even scatter around the 1:1 diagonal line, a further indication of reasonable fit.

Seven stream sites had sufficient larvae for cluster analysis (Fig. 6.3). At the 90% level 2 distinct site clusters occurred. Group 1 included the 2 sites closest to outlets (5 and 7) and group 2 included remaining sites. At the 80% level 2 further clusters can be identified from group 2. The first cluster included sites where CC2-3 was the most abundant cytotype (4, 8, 9, 10), and the second cluster was the lone site (11) where AC(gb) was most abundant. The cophenetic correlation coefficient of 0.867 indicated that the dendrogram was a good fit to the resemblance matrix.

Influence of outlets on cytotype composition

At the Axes Pond site AA and CC2-3 together comprised 90.3% of the total collection (Table 6.3). Male identifications (n = 51) showed the CC2-3 population consisted of 92.2% CC2 (CI = 81.1 - 97.8%) and 7.8% CC3 (CI = 2.2 - 18.9%). At Broad Cove 75% of the total collection consisted of the EFG/C and AA cytotypes (Table 6.3). Consequently, attention was restricted to these cytotypes. Regression analysis (Table 6.4, Fig. 6.4) showed a continuous, directional, pattern of change in cytotype composition with distance from an outlet. Peak frequency of AA occurred much

further downstream at Broad Cove (330 m by model) than at Axes Pond (10 m by model) (Fig. 6.4). It is interesting to note that this difference for AA was concurrent with a high (Broad Cove) and low (Axes Pond) frequency of EFG/C (Table 6.3).

At Axes Pond no significant relationships (stepwise regression) were detected between cytotype composition and station depth (0.09 - 0.22 m), velocity (0.28 - 0.69 m/s), temperature (10.5 - 12.0°C) and conductivity (45 - 63 µS/cm at 25°C), or quadratic expressions of these variables. Since measurements of dissolved oxygen and pH were not available for each station, these variables were omitted from regression analysis. Both dissolved oxygen (10.8 - 11.4 mg/L, n = 8) and pH (5.9 - 6.2, n = 7) showed little variation among stations measured. At Broad Cove stream measurements were taken at 5 stations (10, 50, 120, 320, and 500 m). Dissolved oxygen (11.9 - 12.3 mg/L), pH (6.0), temperature (11.0 - 12.0°C) and conductivity (64 - 73 µS/cm at 25°C) showed little or no variation among stations measured. Seston varied between 0.37 - 1.73 mg/L and depth varied between 0.17 - 0.26 m.

Adult dispersal

AA populations from both Axes Pond and Broad Cove sites were in Hardy-Weinberg equilibrium for the IIL-4 autosomal polymorphism (Table 6.5). In contrast, frequency distributions for the IIL-4 inversion between sites were significantly different ($G_{adj.} = 10.75$, df = 2, $p < 0.01$). This difference is

evidence of restricted gene flow between populations, suggesting adults from these 2 sites do not freely interbreed.

DISCUSSION

In chapter 5 it was argued that the occurrence of a species in a particular stream, or section of a stream, was largely the result of female ovipositional behavior. Abundance on the other hand, is a function of the number of females ovipositing at a site, as well as larval survival, immigration and emigration. Although selection would favor adults that discriminate and select ovipositional sites where larval survival is maximized, future stochastic events will nevertheless influence larval abundance. It follows that occurrence and abundance can be influenced by different abiotic or biotic factors acting on different stages of the life cycle, as the results presented in Table 5.8 and Table 6.2 would suggest.

The analysis presented in Table 6.2 is the first attempt at modelling the macrodistribution of individual members of any North American simuliid complex. Conductivity, discharge, and seston explained a significant amount of the variation in both total larval (R^2 = 73.3%) and CC2-3 larval (R^2 = 68.8%) abundance. As 48.7% of all larvae cytotyped were CC2-3, selection of identical independent variables for total larvae

and CC2-3 is not surprising.

The response of preimaginal black flies to discharge has shown considerable variation. Shipp and Procunier (1986) found positive correlations between discharge and the seasonal abundance of Simulium defoliarti (IIS-14.15) and Prosimulium onychodactylum (2a, 2b) Dyar and Shannon, which they suggested was probably related to food availability. Reisen (1977) suggested that the negative correlation between the abundance of Simulium virgatum Coquillett and discharge was either a response to increased periphyton following increased rainfall or scouring during spates. Spates were also found to greatly reduce larval abundance in both Scotland (Maitland and Penny 1967) and Newfoundland (Lewis and Bennett 1975), however, in the latter case larval density returned to pre-spate levels within 3 - 4 days. The low population level of preimaginal Austrosimulium bancrofti (Taylor) during the summer months in Australia was partly the result of reduced substrate suitability resulting from a fluctuating discharge (Colbo and Moorhouse 1979). The high abundance of Simulium chutteri Lewis at a site on the Vaal River, South Africa, was due to the lack of competitors and predators which was presumably the result of marked changes in discharge (Chutter 1968). In Michigan, Ross and Merritt (1978) reported a negative correlation between the number of winter larvae colonizing artificial substrates and discharge. During February, high discharge further influenced population dynamics by stimulating a second

hatch of Prosimulium mixtum/fuscum and Stegopterna mutata Malloch. It is evident that the relationship between discharge and simuliid density may be of a local nature and have many underlying causes.

In the present study, as stream discharge increased so did width, depth, and stream order, but not water velocity. Therefore, increased abundance of CC2-3 with discharge may be related to an increase in stream size (width) or order, as was the case for AC(gb). Both species occurrence and abundance have been shown to vary with stream size and/or order (Konurbayev 1978; Colbo 1982; Burger 1987; Corkum and Currie 1987; Schröder 1988). Stream size and order may in turn be related to food supply, as algae, an important food source (Thompson 1987c; Morin and Peter 1988), can be expected to increase from headwaters to mouth (Anderson and Sedell 1979; Kurtak 1979).

Total larval and CC2-3 larval abundance increased with elevated seston load. Although larvae may be expected to graze to some extent, most species obtain the bulk of their food supply from the seston (Chance 1970; Currie and Craig 1987; Ross and Merritt 1987). Both field and laboratory studies have shown the influence of food (quantity and/or quality) on abundance and survival (Carlsson et al. 1977; Colbo and Porter 1981; Gilasson 1985; Gilasson and Gordsarsson 1988; Morin and Peters 1988). As the quality and quantity of the organic content of seston varies between sites (Kurtak 1979; Morin and

Peters 1988), increasing seston load is not necessarily indicative of an increasing food supply. Furthermore, a measure of total organic matter in the seston is of limited value as only a portion of this material (particles ≤ 350 μm in diameter) is ingested by simuliids (Chance 1970; Kurtak 1979). Nonetheless, Maitland and Penny (1967) suggested the increased abundance of 3 species of simuliids in the lower stretches of the River Endrick was a result of increased seston (suspended solids) which they believed was directly or indirectly related to food availability. Ladle et al. (1977) also thought suspended solids may play some role in the distribution of simuliids in the River Frome and its tributaries. The importance of seston in the present study may be that it represents bulk (Colbo and Wotton 1981). This in turn decreases passage time through the gut (Thompson 1987b), which may result in processing high quality, easily digested foods (e.g., some algae), while rapidly voiding inferior food (e.g., refractory detritus).

Total larvae and CC2-3 decreased as conductivity increased. As discussed in the previous chapter, other studies have shown that conductivity may influence preimaginal distribution (Grunewald 1976; Grunewald et al. 1979; Corkum and Currie 1987; Table 5.8). In contrast to these studies Mohsen and Mulla (1982) found no correlation between fluctuations in conductivity and the seasonal abundance of Simulium argus Williston, S. canadense Hearle or S. piperi

Dyar and Shannon.

Cytotype composition changed in a continuous, directional and predictable (R^2 = 52.4 - 92.2%) manner with increased distance from outlets (Table 6.4, Fig. 6.4). Olejnicek (1986) found species composition changed over a 1 km section of stream starting at an artificial lake and passing through a forest, forest-meadow transition zone, and pasture. Carlsson et al. (1977), showed a striking difference in the simuliid composition between an outlet site and 2 sites further downstream. Although percentage data are adequate for detection of changes in stream fauna, such data do not explain the underlying dynamics of change, a point often ignored. For example, the observed changes in the frequency of AA and CC2-3 with increased distance from the outlet at Axes Pond (Figs. 6.4a and b) could have resulted from any of the following scenarios: i) abundance of AA decreases and CC2-3 remains constant; ii) abundance of CC2-3 increases and AA remains constant or; iii) abundance of AA decreases and CC2-3 increases. Similar scenarios can be forwarded for EFG/C and AA at Broad Cove. Percentage data alone could not determine which of the above possibilities explained the observed patterns at Axes Pond. Quantitative data (Table 6.1, Figs. 8.8 - 8.11) shows EFG/C and AA most abundant at outlets, and CC2-3 and AC(gb) most abundant at downstream sites, which supports the last alternative as responsible for Figs. 6.4a and b. As well, these data suggest the decline in percent EFG/C at Broad Cove

(Fig. 6.4d) resulted from decreasing abundance with increasing distance from the outlet.

The peak frequency of AA occurred much further downstream at Broad Cove (330 m, Fig. 6.4c), where EFG/C dominated the outlet (= 71.6% of larvae identified in the first 50 m of the stream), than at Axes Pond (10 m, Fig. 6.4a), where EFG/C was scarce (= 8.3% of larvae identified in first 50 m). Although further investigation is required, results do suggest that a high density of EFG/C larvae may displace AA downstream, and AA may occupy areas close to outlets when the EFG/C population has declined. It has been shown that one species of black fly can displace another (Harding and Colbo 1981; Wotton 1982). Displacement would be expected when resources (food, space) are limiting, although evidence of such competition is limited (Hart 1987). Intersibling competition is an area in need of attention.

The analysis shown in Table 6.4 and Fig. 6.4 has a significant implication for future sampling procedures. Since cytotype composition changes significantly over very short distances from outlets (≤ 80 m), studies which replicate samples over such a longitudinal gradient may in fact change the study population, as opposed to replicating results. In future, samples near outlets should include distance from the outflow as part of site description. Clearly, it is not valid to assume that S. venustum/verecundum larvae collected as little as 80 m from an outlet represent the same population as

larvae collected within the first 30 m. In addition, the pattern of cytotype replacement can be expected to vary (compare Figs. 6.4a and c).

Discontinuity between frequency of the IIL-4 polymorphism in 2 AA populations 12 km apart suggested restricted gene flow between these sites. Rothfels (1981b) noted a conspicuous difference in the frequency of inversion polymorphism between populations of Prosimulium fuscum, which he interpreted as inbred and isolated populations. On the other hand, Prosimulium mixtum showed no such discontinuities. Rothfels (1981b) suggested differences may be due to P. fuscum being autogenous, with females tending to oviposit at emergence sites, whereas P. mixtum is anautogenous. Siblings of the autogenous S. vittatum complex readily disperse (Adler 1983) while anautogenous AA appears to have restricted movement, suggesting dispersal is not simply a function of autogeny. Pistrang and Burger (1988) have raised the intriguing possibility that many simuliids might return to oviposit in the same streams from which they emerged.

SUMMARY

1. Both the abundance of S. venustum/verecundum complex (total larvae) and CC2-3 increase with increasing seston loads (total suspended particles) and discharge, and decreasing conductivity. The abundance of AC(gb) increased with increasing stream width.

2. Two of the 3 factors accounting for the abundance of CC2-3 (discharge, seston) and the single factor for AC(gb) (width) were probably related to food supply.

3. Cytotype composition changed in a continuous, directional and predictable manner with increased distance from outlets. Such changes must be considered in future sampling protocols.

4. Discontinuity in the frequency of the IIL-4 polymorphism suggested restricted gene flow due to limited adult AA disperal over distances greater than 12 km.

TABLE 6.1. Estimated abundance (no./site) of each cytotype collected at each sampling station on the Piccos drainage basin.

Sampling* station	Cytotypes**					Total larvae
	EFG/C	ACD	AA	CC2-3	AC(gb)	
1	0	0	0	0	0	0
2	0	0	0	2	1	3
3	0	0	0	6	0	6
4	0	0	0	14	3	17
5	10	0	0	3	0	13
6	0	0	0	6	1	7
7	26	22	43	17	8	116
8	2	0	0	38	20	60
9	5	0	0	46	31	82
10	0	0	0	58	0	58
11	0	0	0	9	38	47
12	0	0	0	0	0	0
13	0	0	0	0	0	0
Total	43	22	43	199	102	409
% of total	10.5	5.4	10.5	48.7	24.9	

*Of the 409 last instar larvae collected, 248 (60.1%) were cytotyped. Estimated abundance of a cytotype$_i$ at each station was calculated as total larvae at station$_i$ x %cytotype$_i$.

**Based on male identifications (n = 52) CC2-3 population consisted of 94.2% CC2 (CI = 84.1 - 98.8%) and 5.8% CC3 (CI = 1.2 - 15.9%).

TABLE 6.2. Regression analysis between larval abundance and stream site conditions for S. venustum/verecundum larvae and the CC2-3 and AC(gb) cytotypes. Listed are regression coefficients for significant predictor variables. Blanks (-) indicate insignificant coefficients.

Regression[+] variables	S. venustum/ verecundum complex	CC2-3	AC(gb)
Constant	1.95*	1.70*	3.08*
Conductivity (μS/cm @25°C)	-0.05**	-0.42**	-
Seston (mg/L)	0.37*	0.27*	-
Log discharge (m^3/s)	0.98**	0.79**	-
Log width (m)	-	-	1.54**
Velocity (m/s)	-	-	-
$(Velocity)^2$ (m/s)	-	-	-
Depth (m)	-	-	-
Dissolved oxygen (mg/L)	-	-	-
Temperature (°C)	-	-	-
pH	-	-	-
Log distance from outlet (m)	-	-	-
$R^2_{(adj.)}$	73.3%	68.8%	45.8%

* $p < 0.05$, ** $p < 0.01$.

[+]Velocity and discharge were for station midpoint. Width and depth were measured at the upstream, downstream and midpoint boundaries of stations and then averaged.

TABLE 6.3. Total number of individuals of each cytotype identified at Axes Pond and Broad Cove Brooks.

Cytotype	Axes Pond		Broad Cove	
	Number cytotyped	% of Total	Number cytotyped	% of Total
EFG/C	13	3.7	92	34.8
ACD	20	5.7	33	12.5
AA	191	54.2	106	40.2
CC2-CC3[+]	127	36.1	29	11.0
AC(gb)	1	0.3	4	1.5
Total	352		264	

[+]Male identifications (n = 51) showed the CC2-3 population at Axes Pond was 92.2% CC2 (CI = 81.1 - 97.8%) and 7.8% CC3 (CI = 2.2 - 18.9%).

TABLE 6.4. Regression analysis between percent cytotype composition (arcsine) of AA, CC, and EFG/C and distance (log) from an outlet. Listed are regression coefficients for significant predictor variables. Blanks (-) indicate insignificant coefficients.

	Distance (log)			F	$R^2_{adj.}$
	m	m^2	m^3		
Broad Cove					
AA	-	42.0***	-11.1***	33.2***	85.4%
EFG/C	40.4***	-	-	131.1***	92.2%
Axes Pond					
AA	-	-	-2.05**	12.00**	52.4%
CC2-3	-	9.91***	-	21.82**	67.5%

** $p < 0.01$, *** $p < 0.001$.

TABLE 6.5. Tests of Hardy-Weinberg equilibrium for the IIL-4 autosomal polymorphism in 2 AA populations. The Axes Pond and Broad Cove sites were 12 km apart.

Site	IIL-4			n	$G_{adj.}$	df
	s/s	s/i	i/i			
Axes Pond (June 2, 1988)	107	68	12	187	0.03	2
Broad Cove (June 10, 1988)	62	29	0	91	3.27	2

FIG. 6.1. The Piccos drainage basin showing the location of each sampling station. This drainage basin was located 12 km N of St. John's, Newfoundland (47°38' - 47°43' N and 52°50' - 52°42' W).

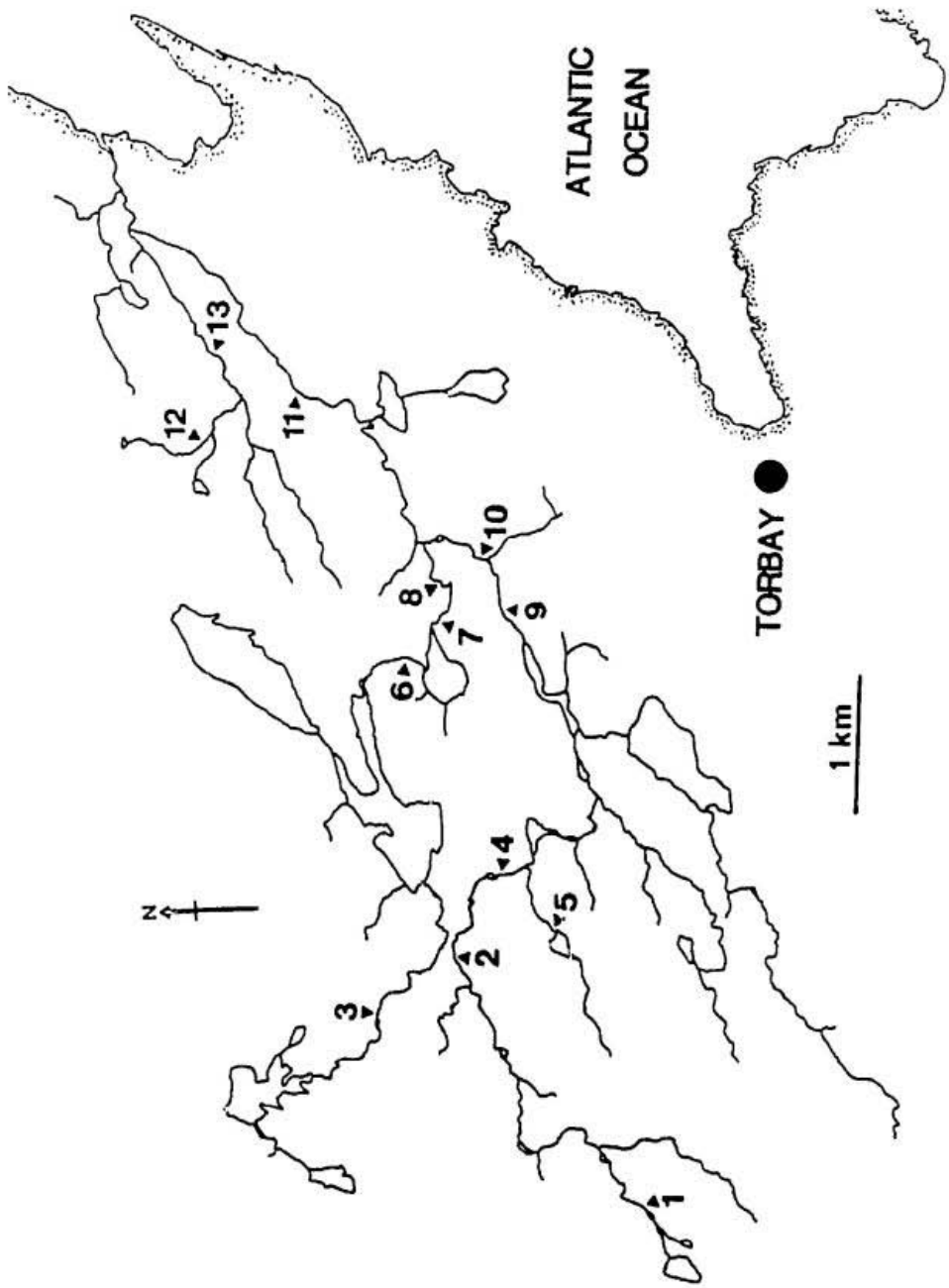

ATLANTIC
OCEAN
TORBAY
1 km
N
1
2
3
4
5
6
7
8
9
10
11
12
13

FIG. 6.2. Observed abundance (log x + 1) in the Piccos drainage basis vs predicted abundance (log x + 1) by regression analysis. A) total larval abundance, B) CC2-3 and C) AC(gb).

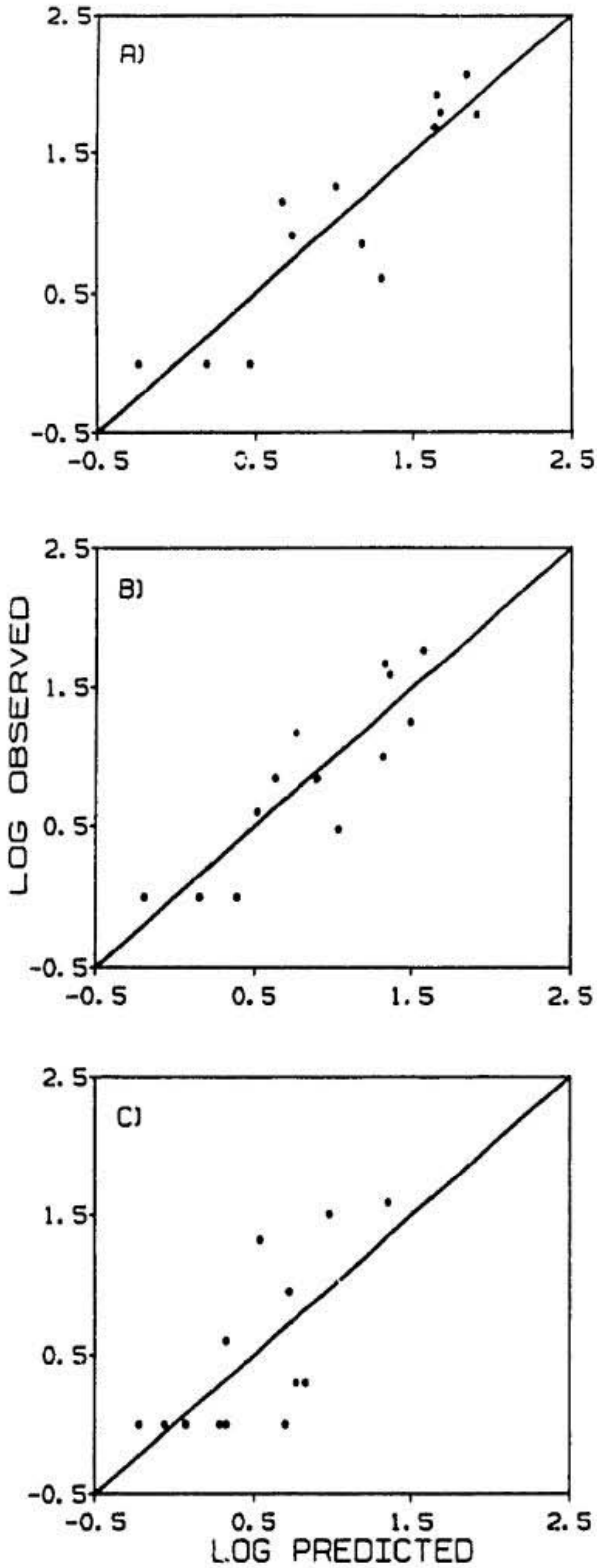

A)
B)
C)
2.5
1.5
0.5
-0.5
LOG OBSERVED
LOG PREDICTED

FIG. 6.3. Dendrogram of 7 stream sites based on cytotype fauna. Data is for the Piccos drainage basin. Dendrogram was produced using the coefficient distance and the UPGMA cluster algorithm. Location of each site is given in Fig. 6.1.

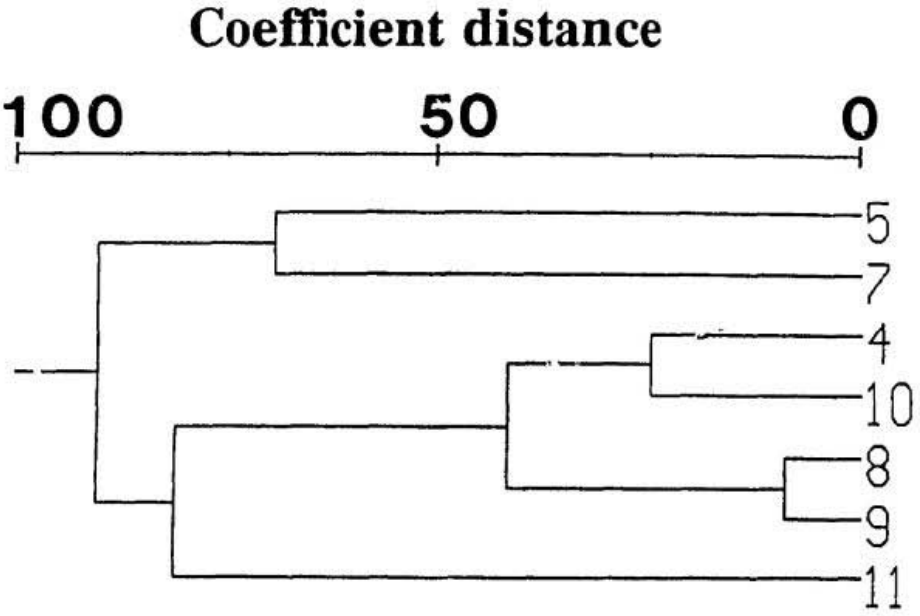
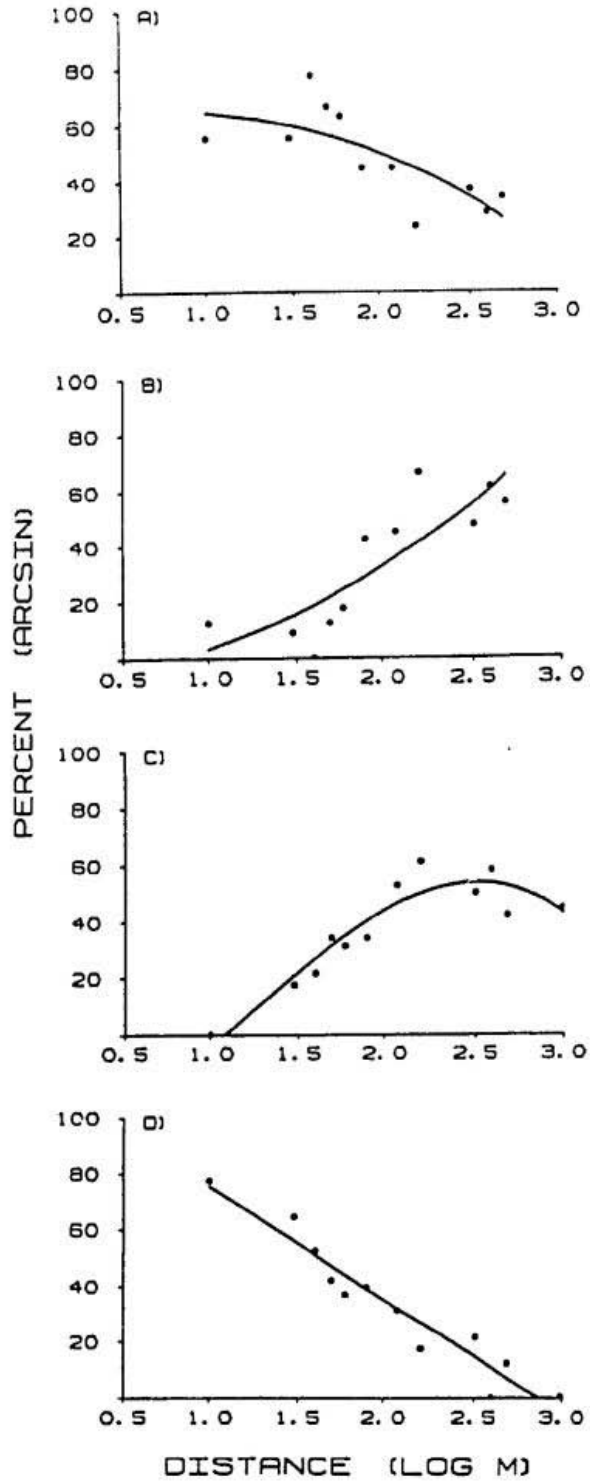

Coefficient distance
100
50
0
5
7
4
10
8
9
11

FIG. 6.4. Regression analysis between cytotype composition and distance from a pond outlet. A) AA, Axes Pond site, B) CC2-3, Axes Pond site, C) AA, Broad Cove site and D) EFG/C, Broad Cove site.


A)
B)
C)
D)
100
80
60
40
20
0.5
1.0
1.5
2.0
2.5
3.0
PERCENT (ARCSIN)
DISTANCE (LOG M)

CHAPTER 7

MICRODISTRIBUTION PATTERNS OF SUBLACUSTRINE CYTOTYPES

INTRODUCTION

The literature (Table 1.2) clearly identified water velocity, water depth and substrate type as primary correlates of preimaginal microdistribution. Consequently, microhabitat was quantified on the basis of velocity and depth preference curves, while controlling for substrate by sampling with ceramic tiles. Because Morin et al. (1986) showed that distance from an outlet correlates with larval distribution over a stretch of as little as 50 m, a distance term was also included in the description of microhabitat. Dissimilarities in preference curves among cytotypes were considered to indicate differences in microhabitat selection. The present study investigates microhabitat selection between different sublacustrine cytotypes.

MATERIALS AND METHODS

Study sites

The multiple regression technique used to fit preference

curves is not suitable for low numbers (Morin et al. 1986) hence, sites with high larval abundance would be required. Cytotaxonomic identification of larvae from these sites could be greatly reduced if pure populations were studied. Accordingly, sites draining lake outlets were selected as they support large populations (Carlsson et al. 1977; Sheldon and Oswood 1977; Burger 198'; Wotton 1987) and in Newfoundland produce temporally separated EFG/C and verecundum (ACD and AA) populations (chapters 5 and 8). On occasion, the verecundum line existed as pure ACD or AA, permitting comparisons of curves among individual cytotypes.

Three sites, each draining an oligotrophic pond, were selected. Site 1 was the first 20 m length of a second order stream draining Middle Three Island Pond (47°41'00'' N, 52°45'45'' W), 250 m upstream of sampling station 6, shown in Fig. 6.1. Stream width varied between 5 - 10 m. The stream bed was primarily rubble and the riparian vegetation brush (Table 2.1) with no significant canopy. The site can only be reached by foot or boat and is relatively undisturbed by humans.

Site 2 (Fig. 6.1, station 7) was a 10-m stream section located 50 - 60 m downstream from the outlet of Axes Pond (47°40'45'' N, 52°45'30'' W). This second order stream varied between 3 - 4 m in width. The bed was primarily rubble and the riparian vegetation was brush which produced a partial canopy. Axes Pond can only be reached by foot, and except for the occasional angler, is relatively undisturbed.

Site 3 was the first 20 m length of a 40 m run (47°35'30'' N, 52°50'45'' W) connecting Mitchells Pond North, and Hughs Pond. Stream width varied between 1 - 4 m, with a stream bed largely of rubble. Riparian vegetation was brush with no canopy. A small wooden bridge crossed this second order stream 23 m from the outlet. Numerous summer homes exist on both ponds but the connecting stream showed only moderate signs of human disturbance (i.e., few signs of human debris).

Sampling procedures

Red-brown, unglazed, square ceramic tiles (100 cm^2) were used to collect larvae (Fig. 7.1). Data obtained from artificial substrates, particularly tiles, provide reasonable indications of velocity, depth and distance preferences (Morin et al. 1986; Colbo 1987). Artificial substrates minimize habitat destruction as well (Rosenberg and Resh 1982), allowing a site to be repeatedly sampled.

Each site was divided into a 40 x 6 grid system (0.5 m units) and a random number generator used to select the placement of tiles at each site; 35 - 45 tiles were used at each site. A new set of grid numbers was generated for each stream collection. Peak colonization on tiles usually occurs within 5 - 7 days and fouling can be significant after 2 weeks (Gersabeck and Merritt 1979). Consequently, tiles were left in place for 1 - 2 weeks, depending on population levels. A period of this length also allowed sufficient time to repeat

collections for univoltine EFG/C in the event of catastrophic disturbance, and to sample pupae. Eleven collections were successfully completed (Site 1 = 2, Site 2 = 2, Site 3 = 7) from May to July, 1988 and during July, 1989.

It was thought that measurements of velocity and depth (chapter 2) would disturb larvae on the tiles, which could affect results if many larvae entered the water column. Instead, the position of a tile on the stream bed was first marked with orange metal markers prior to tile retrieval. Larvae were removed from the top surface of the tile with curved forceps and randomly assigned to a small plastic container of either 70% ethanol or water. Larvae placed in water were kept live on ice for later cytotaxonomic identification. Using the metal markers as guides, the tile was returned to the stream in its original position and water velocity 2.5 cm above, and water depth over the middle of the tile recorded. Distance from the outlet was also noted. This procedure was repeated for each tile and sampling proceeded in an upstream direction to minimize disturbance. Since the diameter of the propeller was 2.5 cm, velocity readings were integrated values from 1 - 4 cm above the tiles. Although water velocity experienced by larvae is less than that measured above the tile by an indeterminate amount (Ciborowski and Craig 1989), velocity in the boundary layer is related to velocity at various distances above the substrate (Chow 1959; Statzner et al. 1988).

Nets were not used to capture larvae that may have been released during tile removal since the exact origins of these larvae (i.e., from sides, bottom or top of tile, or from the benthos) could not be ascertained. However, direct observation (together with results from the artificial stream, see below) indicated that few, if any, last instar larvae entered the drift from the top surface of tiles during retrieval.

In the laboratory, for each stream collection, the number of last instar S. venustum/verecundum larvae and pupae colonizing individual tiles was counted. A subsample of larvae preserved in acetic ethanol from each collection was identified cytologically.

Significant differences in stream depth and stream velocity between the 3 study sites were detected with ANOVAS and Kruskal-Wallis procedures respectively. A log (100 x d) transformation was required to stabilize the variance of depth. Multiple comparisons among groups for depth were performed with the Tukey test and for velocity with the Tukey-type test ($Q_{\alpha,k}$).

Calculation of preference curves

Three techniques are available to fit preference curves for stream macroinvertebrates: the incremental method (Gore and Judy 1981), polynomial regression on a single factor (Orth and Maughan 1983) and multiple regression on several habitat variables (Gore and Judy 1981; Morin et al. 1986). The

multiple regression approach was used as it produces the most accurate estimates of abundance and unbiased preference curves (Morin et al. 1986).

Using multiple regression, larval or pupal abundance was related to the independent (predictor) variables of water velocity, water depth, and distance from the outlet. Because variance exceeded the mean and data were skewed to the right for each collection, a log (y + 1) transformation was required (Elliott 1977; Allan 1984; Montgomery 1984) to satisfy the assumptions of regression analysis (Draper and Smith 1981; Sokal and Rohlf 1981; Zar 1984). To reduce the problem of multicollinearity (correlations among predictors) associated with polynomial terms, independent variables were expressed as deviations around their means before being raised to second and third order powers (Sokal and Rohlf 1981; Minitab 1988; Neter et al. 1990). Therefore, the form of the saturated model relating larval abundance to stream conditions becomes:

$$[1]\quad \log (y + 1) = a_0 + a_1 v + a_2 (v - \bar{v})^2 + a_3 (v - \bar{v})^3 + a_4 d + a_5 (d - \bar{d})^2 + a_6 (d - \bar{d})^3 + a_7 m + a_8 (m - \bar{m})^2 + a_9 (m - \bar{m})^3 + a_{10} md + a_{11} vm + a_{12} vd$$

where y = number of larvae/tile, m = distance from lake outlet (m), v = velocity (m/s) and d = depth (m). The success of the above transformation on independent variables was evaluated by a tolerance test of 0.01 (i.e., a measure of the correlation

between variables) (Norusis 1985). Occasionally some variables failed a tolerance of 0.01 even after transformation and were subsequently excluded from the analysis. Polynomials were restricted to the square and cube as initial analysis showed higher powers were not useful. Powers higher than cubes are rarely important in biological work (Sokal and Rohlf 1981). Interaction terms (md, vm, vd) were also included in the model.

The intent of regression procedures was to reduce the number of terms in equation 1 to only significant ($p < 0.05$) predictor variables, while attempting to maximize R^2 (coefficient of determination) and reduce bias (C_p statistic). Different variable selection procedures may select different predictors (Draper and Smith 1981). The best-regression routine (based on R^2 criterion), used as an exploratory method, and the stepwise routine (based on the partial F-test) used as the variable selection routine were recommended by Draper and Smith (1981) and therefore adopted here. The best-regression routine is useful to determine bias regressions (C_p statistic) and to approximate the maximum number of predictor variables as indicated by stabilizing mean square error (MSE) and R^2 values. The forward selection procedure, using both the partial F-test (Draper and Smith 1981) and sequential sum of squares (Sokal and Rohlf 1981; Morin pers. comm. 1987) was used as a comparative procedure.

After initial variable selection, resultant models were

scrutinized by an examination of studentized residuals, leverage points, Cook's distances (Draper and Smith 1981; Sokal and Rohlf 1981; Neter et al. 1990) and a lack of fit routine which did not require replication (Burn and Ryan 1983). As a visual examination of residuals was subjective, plots suggesting heteroscedasticity were formally tested using the procedure of Goldfield and Quandt (1965). Outlying cases with respect to the dependent and independent variables were detected by an examination of studentized residual and leverage points respectively. Cook's distances were used to determine if outlying cases were unduly influential, i.e., exclusion of suspect cases changes the significance and/or sign of estimated regression coefficients. Once each model was considered acceptable, bracketed higher order terms in equation 1 were expanded out and then reduced to produce the final form of the model.

A regression model was converted to preference curves by first back-transforming the log (y + 1) transformation to arithmetic units. The larval or pupal response to an independent variable (e.g., velocity) was isolated by holding remaining variables in the equation (e.g., depth, distance) constant at their mean values, while permitting the variable of interest to vary over the range of observed values. The resulting curve was standardized by dividing the regression equation by the maximum abundance as derived from this equation. Such standardization produced a preference factor

which measured larval response to observed changes in a stream variable on a scale of 0 (conditions least suited to larvae) to 1 (optimal conditions). Factors were presented graphically as preference curves (Morin et al. 1986). Preference factors for 2 variables were considered simultaneously by plotting a surface.

Size-velocity and size-depth relationships

Head capsule measurements were used to determine the influence of velocity and depth on larval size from pure EFG/C (Table 7.1, collection 3) and ACD (Table 7.1, collection 6) populations. From each collection, 4 last instar larvae were randomly selected from each tile with ≥ 5 final instar larvae. Head capsules were prepared following methods in chapter 2. Viewing each head capsule from above CAW, HCW, HCL and PAW lengths (Fig. 2.1) were measured. Stepwise regression (with replication) was used to determine any size-velocity and size-depth relationships.

Laboratory velocity studies

Artificial streams. Artificial streams (Fig. 7.2) constructed from plexiglass troughs (l x w x d = 152 x 7.7 x 10 cm) were used to: i) observe the response of final instar larvae to changes in water velocity and; ii) determine if laboratory based results have predictive value in the field. A fibreglass container (182 x 90 x 60 cm) located below the

troughs filled with 0.74 m^3 of dechlorinated water (i.e., tap water allowed to stand for 48 h) served as the main water reservoir (Fig. 7.2a). Water (20°C) was pumped by a recirculating pump (Flotec Inc., Norwalk Calif., model R2P1-1106V) from the main reservoir to a small reservoir (33.5 x 28.5 x 13.5 cm) 155 cm above the floor. Outlets from the small reservoir (one per trough) consisted of plastic elbows (length = 11.0 cm; diameter = 1.0 cm) attached to pieces of 1.2 cm diameter Tygon® tubing, which drained water to a reservoir (7.5 x 7.7 x 3.7 cm) at the head of each trough (Fig. 7.2b). Water then entered each trough over a 3.7 cm high weir. Water leaving each trough drained into the main reservoir and was recycled. Sieves (mesh size = 250 μm) located at the mouth of each trough captured larvae that had entered the drift.

Different water velocities were achieved by raising or lowering the head of each trough through 6 different levels (Fig. 7.2b), therefore slope was altered simultaneously with velocity. Water velocity was measured following Wotton (1985). All experiments were run at room temperature and no food was provided.

Larvae used for the first experiment were collected within 20 m of the outlet at Round Pond (47°24'30'' N, 53°01'30'' W) on June 17, 1987 and for experiments 2 and 3 from the outlet at Mitchells Pond North on May 18, 1988. Larvae, left attached to substrates, were placed in plastic containers with a small amount of water and held on ice for

transportation to the laboratory. In the laboratory larvae were gently washed off substrate with distilled water into a plexiglass container (36.5 x 36.5 x 12.0 cm) filled with a mixture of stream and distilled water. Plexiglass baffles (36 x 10.5 cm) placed in the container provided substrate for attachment. Aeration was supplied by compressed air pumped through 0.7 cm diameter Tygon® tubing and released through 0.6 cm diameter air-wands placed on the bottom of each baffle. Larvae were given 25 mL of blended TETRA (4 g/L solution), held overnight at 15°C and used the following morning. For experiments 1, 2 and 3 respectively, a mean of 349 ±86, 662 ±239 and 494 ±117 larvae per trough were used.

Experiment 1. Before the start of the experiment, each trough was held level, sealed at the mouth and a small amount of water added. Larvae were transferred from baffles to troughs using a small paint brush and allowed to attach and acclimate to trough conditions for 30 min. Seals were then removed, each trough moved to a randomly selected level (velocity) and after 2 min the experiment started. Each trial lasted 2 h at which time sieves were changed and troughs positioned at the next randomly selected level. The procedure was repeated until all 6 levels had been tested. Each level was replicated 3 times (i.e., 3 troughs). Larvae that entered the drift (i.e., captured in sieves) were assumed to have done so in response to unfavourable flow conditions (e.g., Gersabeck and Merritt 1979). For each trial, the number of

last larval instars entering the drift was expressed as a percent of total larvae (drift + benthos) present for that trial. Fifty last instar larvae were randomly selected and cytotyped.

Experiment 2. The same protocol as in experiment 1 was followed except that each trial lasted only 1 h.

Experiment 3. This experiment was designed to detect both migration (i.e., looping in an upstream or downstream direction) and drift. After the completion of experiment 2, larvae were removed from the first 30.0 cm and the last 54.5 cm of each trough, leaving a 60.0 cm band of larvae. Troughs were positioned at levels 1, 3 and 6 respectively and left for 3 h. The number of larvae migrating outside the 60 cm band and those entering the drift were noted for each trough. Fifty last instar larvae were randomly selected and cytotyped.

Analysis of data. Data from experiments 1 and 2, expressed as percentages, were arcsine transformed (Sokal and Rohlf 1981) and ANOVAS used to detect significant differences in drift rate among different velocities. Data from experiment 3 were subjected to frequency analysis (G-tests) to determine if movement (migration, drift) was independent of velocity.

RESULTS

Preference curves

Data from 7 of the 11 field collections were considered appropriate for analysis (Table 7.1). In order to facilitate reference to these collections, they are numbered 1 - 7 (Table 7.1). The remaining 4 collections either produced too few larvae or pupae for analysis, or were from mixed populations. Examinations of studentized residuals plots showed no systematic trends or patterns, indicating that the assumptions of regression analysis were at least approximated. The routine of Burn and Ryan (1983) provided no evidence of lack of fit for any of the regression models. Cook's distances indicated no outliers of significant influence, thus all data points were kept for each model. One or 2 interaction terms had to be removed due to multicollinearity from collections 1 (md), 4 (md, vm), and 5 (md, vm). In all cases these variables did not influence the selection of predictor variables. Stepwise and forward routines selected identical or similar predictor variables. The only exception to this was collection 2, where the forward selection routine using the sequential sum of squares F-test selected variables different from those of other procedures. In view of the above, final regression models (Table 7.2) were considered reasonable descriptions of the relationship between preimaginal abundance and stream conditions.

Velocity, distance and depth preference curves are shown in Figs. 7.3, 7.4, and 7.5 respectively. Comparisons of velocity and depth conditions among larval collections are given in Tables 7.3 and 7.4. Since collections 4 and 5 were taken from the same site (site 2), involved the same cytotypes (mixed ACD-AA) and sampled in tandem (Table 7.1), collection 5 can be considered a replication or validation set of collection 4. Similarly, collection 7 (site 3) can be considered a replication of collection 6. Note the similarity between the preference curves produced from collections 4 and 5 (Figs. 7.3b and c) and the similarity between curves produced from collections 6 and 7 (Figs. 7.3d and e).

Larval EFG/C responded differently to velocity, depth and distance than ACD or ACD-AA (Table 7.2; Figs. 7.3 - 7.5). Velocity was found to have a significant effect on the distribution of ACD-AA and ACD in all cases examined. In contrast, larval density of EFG/C was shown to vary with velocity at site 1 only (Table 7.2; Fig. 7.3). The optimal velocity for EFG/C, as derived from the regression model, was 0.36 m/s (range 0.03 - 0.52 m/s), whereas the density of ACD-AA increased exponentially with increasing velocity in both collections 4 (range 0.10 - 0.62 m/s) and 5 (range 0.03 - 0.68 m/s). At site 3, the optimal current velocity for ACD, estimated from regression, was 0.73 m/s in collection 6 and 0.69 m/s in collection 7.

The microdistribution of larval EFG/C was clearly related

to outlets (Figs. 7.4a and b). Larvae showed a parabolic response to distance, with maximum density estimated to occur at 10.5 m and 16.0 m from the outlets of sites 1 and 3, respectively. No significant distance terms were found for ACD-AA (site 2), but this is not surprising as collections were confined to a 10-m stream section removed (50 - 60 m) from the outlet. Interestingly, the distance-preference curve for ACD at site 3 (Fig. 7.4c) was opposite to that of EFG/C.

The number of larval EFG/C decreased exponentially with depth (Fig. 7.5). The microdistribution of ACD-AA and ACD was not influenced by water depth (Table 7.2). Water depth was usually lower during times when ACD and AA were present in streams, than when EFG/C was present (Table 7.4).

Depth and distance EFG/C pupal preference curves were similar to larval curves. However, the significant md term (Table 7.2) showed pupal response to distance was modified by extreme depth, and vice versa (Fig. 7.6). Pupae were most abundant at sites removed from the outlet (15.5 - 20.0 m) over most of the observed depth range (0.09 - 0.27 m), but high abundance would be expected at sites immediately below the outlet in shallow water (0.03 - 0.06 m). At a depth of 0.08 m, calculations from the partial regression produced a bimodal preference curve with peak preferences at both the outlet and 16 m downstream. Similarly, pupae preferred shallower water except at sites most removed (15.5 - 20.0 m) from the outflow where deeper water was sought. As expected, empirical

calculations showed no depth preference at 16.0 m.

Pupal ACD showed a distinct preference for positions immediately below the outlet. Abundance increased exponentially with water velocity up to the maximum recorded velocity of 0.85 m/s (Fig. 7.7). The microdistribution patterns of larval and pupal ACD for collection 6 were highly correlated ($r = .808$, $df = 36$, $p < 0.001$).

Larval preference surface plots for EFG/C and ACD are given in Fig. 7.8. In all plots maximum preference was restricted to a small percent of each surface and sharply decreased outside a narrow range of conditions. The depth-distance and depth-velocity plots for EFG/C (Fig. 7.8) were flat over the bulk of their surface. Results of surface plots were interpreted to indicate that optimal conditions exist over a very narrow range of stream conditions, which was reflected in their contiguous distributions (Table 7.1, compare mean to SD).

Size-velocity and size-depth relationships

Stepwise regression failed to find any significant relationships between larval size and velocity, or larval size and depth, for either EFG/C (collection 3) or ACD (collection 6). Velocity-size and depth-size scatter plots are given in Figs. 7.9 and 7.10 for EFG/C and ACD, respectively. Both populations showed relatively little variation in size as indicated by low coefficients of variation, which ranged from

3.6 - 5.5%, depending on the character. An analysis of variance (not shown) for each of the 4 head capsule characters found no significant differences in the mean size of larval EFG/C among the 19 different velocities examined.

Laboratory velocity studies

Results of the laboratory velocity trials are shown in Tables 7.5 and 7.6. Cytotaxonomic identifications showed that larvae used in experiment 1 were 84% EFG/C and 16% ACD. In experiments 2 and 3, all 50 larvae identified were EFG/C. Although 6 different levels were used in experiments 1 and 2, adjacent levels often produced very similar velocities. Consequently, levels were combined to give 3 experimental velocities which included 0.06 ±0.02 m/s (n = 3), 0.22 ±0.02 m/s (n = 6) and 0.35 ±0.01 m/s (n = 6). These same values were used in experiment 3.

In experiments 1 and 2 (Table 7.5) no significant differences were found in the mean percent releasing (i.e., entering drift) among the 3 velocities examined. For all trials combined, the average percent of larvae entering the drift per hour was 0.64% ±0.68 (n = 15) and 0.13% ±0.24 (n = 15) for experiments 1 and 2, respectively. In experiment 3, G-tests showed no significant difference in the frequency of migrating larvae or total larval movement (migration + drift) between different velocities. Hourly drift was significantly higher at 0.35 m/s than at 0.22 or 0.06 m/s (Table 7.6).

Larval movement in experiment 3 was also low with a combined (all 3 velocities) hourly total movement (migration + drift) of 0.64% ±0.11 (n = 3). Under the conditions of these experiments, few [illegible] instar larvae relocated in response to changes in velocity.

DISCUSSION

Micropreference curves have been published for several unrelated simuliids which included Simulium aureum Fries, Prosimulium mixtum/fuscum, Stegopterna mutata, Simulium vittatum, and Simulium spp. (Orth and Maughan 1983; Morin et al. 1986; Morin and Peters 1988). Results presented here were the first attempt at modelling the microhabitat preferences of S. venustum/verecundum cytotypes and results showed differences in habitat selection within this complex. The preferred microhabitat (as defined by velocity, depth and distance) of EFG/C was distinctly different from that of ACD-AA and ACD. Within a stream stretch, optimal conditions for EFG/C as predicted from preference curves was shallow water and a location removed (≈ 10.5 - 16.0 m) from the outlet. At site 1, the optimal velocity was estimated at 0.36 m/s (Fig. 7.3a). In contrast to this, depth did not appear to influence the microdistribution of ACD and ACD-AA (Table 7.2) and their optimal velocity was estimated to range from 0.62 - 0.73 m/s

(Figs. 7.3b - e). Larval ACD (site 3) also showed a maximum preference for the location immediately below the outlet (Fig. 7.4c).

Numerous studies have demonstrated an association between larval microdistribution and stream velocity (Phillipson 1956, 1957; Maitland and Penny 1967; Ulfstrand 1967; Chutter 1969; Dècamps et al. 1975; Lewis and Bennett 1975; Minshall and Minshall 1977; Colbo 1979; Colbo and Moorhouse 1979; Gersabeck and Merritt 1979; Boobar and Granett 1980; Orth and Maughan 1983; Osborne et al. 1985; Wotton 1985; Yamagata and Kanayama 1985; deMoor et al. 1986; Morin et al. 1986; Rühm and Pegel 1986; Morin and Peters 1988; Ciborowski and Craig 1989; present study).

It has been generally assumed that simuliid larval behaviour and choice of microhabitat is directly related to filter feeding (Chance and Craig 1986; Craig and Galloway 1987; Morin and Peters 1988). Ingestion rate or ingestion efficiency varies in a linear or curvilinear fashion with water velocity (Elouard and Elsen 1977; Moore 1977a; Kurtak 1978; Lacey and Mulla 1979; Schröder 1980; Lacey and Lacey 1983; Braimah 1987; Thompson 1987b). Consequently, preference for a particular velocity may reflect larvae positioning themselves in currents that provide maximum ingestion rate or ingestion efficiency (Craig and Galloway 1987). Decreased larval abundance at velocities exceeding the optimum may result from decreased ingestion rate (e.g., Lacey and Mulla

1979), decreased ingestion efficiency (e.g., Thompson 1987b), or increased drag (e.g., Morin and Peters 1988). Different optimal velocities shown for ACD and EFG/C could be interpreted as species specific differences in the ingestion-velocity relationship (Kurtak 1978).

It follows that larval size should vary with stream velocity and that maximum size would occur at the optimal velocity given that: i) microdistribution is influenced by velocity and reflects feeding requirements; ii) water velocity influences ingestion of food and; iii) size is dependent on the availability of food (Colbo and Porter 1979; Edman and Simmons 1985). The selective advantage of larger size is increased fecundity (Chutter 1970; Colbo and Porter 1981; Simmons and Edman 1981; Post 1983) and superior mating ability (Edman and Simmons 1985). That neither EFG/C nor ACD demonstrated a relationship between size and velocity was unexpected. In addition, Ciborowski and Craig (1989) found in the laboratory that abundance data and feeding data produced contradictory indications of optimal flow conditions for S. vittatum (cytotype IIIL-1). Two hypotheses are offered as possible explanations for results presented here.

First, lake outlets produce a superior food supply in terms of quality and/or quantity (Carlsson et al. 1977; Morin and Peters 1988) which is believed to be partially responsible for the high larval abundance occurring in these habitats (Wotton 1987). Although velocity determines the rate at which

food particles pass the labral fans (e.g., Chance 1977), nutrient availability at outlets may be sufficiently high to realize maximum growth under almost any flow regime. The highly uniform size of both EFG/C and ACD supports this idea. It would be of interest to examine the size-velocity relationship at sites removed from outlets, where the food supply is usually of inferior quality (Carlsson et al. 1977; Colbo 1982; Morin and Peters 1988). In this regard, AA would be an ideal study animal because it occurs at both sublacustrine and downstream locations (chapters 5 and 6).

Second, it is hypothesized that the lack of correlation with size may reflect a trade-off between the amount of material ingested and the energetic expenditure of maintaining the feeding stance over the range of velocities examined. Craig and Galloway (1987) suggested larvae would attempt to maximize the difference between the benefit and cost at a particular site by maximizing filtration efficiency while reducing energetic costs, such as drag, to a minimum. If the net difference between increased filtration rate and energy expended to overcome drag shows little variation over a large range of velocities, then the insignificant size-velocity relationship may reflect a constant energetic gain. The advantage to larvae in this instance would be that reduced food acquisition under poor flow conditions could be offset by a reduction in the energetic cost of the feeding stance, at least over the ascending limb of the velocity curve. The

increase in drag forces between 0.1 - 0.9 m/s has been measured in the laboratory for S. vittatum (Eymann 1988), but the energetic costs associated with changing drag have not. Phillipson (1956) found that larval Simulium ornatum Meigen in moving water consumed more O_2/g (wet weight) than larvae in standing water, suggesting an energetic cost associated with moving water. Statzner et al. (1988) suggest the cost of drag would be a significant amount of a benthic insect's energy budget.

If one accepts one or both of the above hypotheses (as they are not mutually exclusive), it follows that different velocities, with the possible exception of extreme values, impart no great advantage to larvae under field conditions. This leads to the question: "Why do larvae distribute themselves in relation to velocity?" It has been generally assumed that the choice of microhabitat, and in particular the selection of velocity, is directly related to filter feeding (Maitland and Penny 1967; Chance and Craig 1986; Craig and Galloway 1987; Morin and Peters 1988). Nevertheless, velocity can also influence many factors including substrate suitability (Ulfstrand 1967; deMarch 1976; Rabeni and Minshall 1977; Reice 1980; Minshall 1984), substrate stability (Newbury 1984) and the distribution of potential predators and competitors (Ulfstrand 1967; Minshall and Minshall 1977; Orth and Maughan 1983).

Biotic interactions between simuliids and other insects

can lead to local displacement of simuliids (Wiley and Kohler 1981; Hemphill and Cooper 1983; Malmqvist and Ott 1987). Since potential competitors and predators also exhibit rheopreferunda, simuliid distribution in response to velocity may in part reflect predator or competitor free refugia. Under laboratory conditions significantly more Simulium larvae sought predator free refuges in the presence of predators than in their absence (Fuller and deStaffan 1988). The preference of benthic macroinvertebrates (including simuliids) for simple hydraulic variables such as velocity may be altered by other species or by intraspecific interactions of different age groups (Statzner et al. 1988).

It could be argued that failure to find a size-velocity relationship (Figs. 7.9 and 7.10) reflected inadequate sampling procedures because velocity data were available only at the time of collection. If stream conditions were highly variable while tiles were in place, any relationship between size and velocity might be obscured. The highly uniform size of larvae, especially EFG/C, is not consistent with this explanation. As well, mean flow conditions between tile collections taken in sequence (collections 6 vs 7) suggest velocity conditions did not change to any great extent over the period larval ACD was under investigation at site 3. Finally, while stratifying (strata = fast and slow water velocity) phenology sites (chapter 8) it became apparent that relative velocities within a stream section usually remained

stable for a period of 2 - 3 weeks.

An inverse relationship between depth and larval microdistribution has been shown by many studies (Ulfstrand 1967; Lewis and Bennett 1975; Reisen 1977; Gersabeck and Merritt 1979; Yamagata and Kanayama 1985; Morin et al. 1986; Morin and Peters 1988; present study). Preference for deeper water has been shown by some species (Granett 1979; Morin and Peters 1988). The underlying causes for depth-abundance relationships are often unclear (e.g., Morin and Peters 1988). Wotton (1982) showed experimentally the outlet specialist Simulium noelleri Friedrichs can ingest material from the lipid rich surface film of outflowing lake water. The lack of correlation between size and depth for EFG/C and ACD suggests this was of little consequence to these simuliids.

Ulfstrand (1967) suggested changes in flow conditions in deeper water may be responsible for the shallow water preference exhibited by blackflies and other benthic insects. Shear stress, a force acting directly on the stream bottom, will increase with depth (Statzner et al. 1988). Consequently, a preference for shallow water may reflect an avoidance of high shear stress. Some authors have shown that larvae prefer, at least on a gross scale, turbulent water (Décamps et al. 1975; Colbo 1979; Orth and Maughan 1983; Wetmore et al. 1990). Turbulence can be expected to decrease with increased depth which may explain why larvae may avoid deeper water (Orth and Maughan 1983; Morin and Peters 1988). Froude number is an

index of turbulence (Statzner 1981b; Orth and Maughan 1983) and is calculated as:

$$[2] \quad F = v / (g \cdot d)^{0.5}$$

where v = water velocity (m/s), d = depth (m) and g = acceleration of gravity (9.8 m/s^2). When data for EFG/C were fitted to Froude number (Appendix 3), significant relationships were found, suggesting larval distribution could be influenced in part by turbulence, which in turn varied inversely with depth.

Although the abundance of larval ACD-AA and ACD was also shown to vary with Froude number (Appendix 3), no significant depth relationships were found. For the ACD collections, the range of depths encountered (0.03 - 0.19 m) may have been too narrow to detect significant variation in abundance with changes in depth. A non-significant relationship between larval abundance and a narrow depth range (0.10 - 0.23 m) was reported by Chutter (1969). Finally, as was suggested for velocity, depth selection may represent the cumulative effect of many factors such as flow conditions, feeding, predator and competitor free refugia. For example, at some critical depth large predators such as fish would be excluded from the larval habitat.

The orientation of EFG/C to the lake outlets was very similar to the distance-preference curve described for S.

aureum over a 50 m stream reach (Morin et al. 1986), which for this latter species was attributed to a competitive interaction with hydropsychids (Trichoptera). In the present study no such competitive interactions were apparent for either EFG/C or ACD. When Froude number was plotted against distance for ACD, a bimodal pattern, similar in appearance to the distance-preference curve, was found. As ACD showed a preference for higher Froude numbers its orientation to the lake outlet may have resulted from selecting areas with higher Froude numbers. Craig and Galloway (1987) have suggested that diminishing larval abundance with increasing distance from outlets may be related to changing Froude number.

In Labrador (as part of a separate project), I have made aerial observations (25 - 50 m above ground) at lake outlets which revealed very distinct bands of S. venustum/verecundum complex larvae. Running at right angles to the bank, each band was approximately 1 - 2 m wide, and separated from adjacent bands by several meters. Larval density within bands appeared to decrease with increasing distance downstream. Distance-preference curves shown in Fig. 7.4 suggest also EFG/C and ACD aggregate in bands. The advantage of such aggregations may be a decrease in shear stress (Ciborowski and Craig 1989) or possibly enhanced nutrition by coprophagy (Wotton 1980b).

Morin et al. (1986) have suggested that data be examined from several streams to determine the general applicability of preference curves. Dissimilarity in preference curves among

cytotypes could be ascribed to differences in stream conditions at the time of sampling, since data were collected at different sites and times. However, the analyses shown in Tables 7.3 and 7.4 indicate preference curves reflect differences between siblings, not stream conditions. For example, mean depth was not significantly different between site 1, where EFG/C was negatively correlated to depth, and site 2, where the abundance of ACD-AA showed no relation to depth.

Pupal preference curves (Figs. 7.6 and 7.7) and the high correlation between larval and pupal ACD in collection 6 ($r = 0.808$) showed pupal distribution was similar to that of the final instars. Previous studies have shown late last instar larvae may move to more sheltered microhabitats to pupate (Maitland and Penny 1967; Colbo and Moorhouse 1979). As the majority of examined EFG/C (72/100, collection 1) and ACD (74/100, collection 6) had light histoblasts (newly moulted last instar larvae), it is clear that last instar larvae did not seek out a different microhabitat in which to pupate.

Although depth and distance preference curves for pupal EFG/C were similar to larval EFG/C, a significant interaction term (md) showed pupal response to depth (d) was modified by extreme values of distance (m) and vice versa (Fig. 7.6). Statistically, the significant interaction term means depth and distance did not act independently of each other (Gore and Judy 1981). Whether the interaction term has any biological

significance or simply represents the best empirical fit to the observed data is not readily apparent. Because the md term was influential only at extreme values, was not selected by the forward regression routine, and other interaction terms were not found, no biological significance can be given to the md term on the basis of the present work.

Under experimental conditions few larvae responded, either by drift or migration, to changes in velocity and most responses were not statistically significant among treatments. Results from these experiments have little value for predicting the occurrence of larvae under stream conditions. Significant movement in laboratory trials may have been detected if replications were run longer; extrapolation of data suggests daily movement could be as high as 18.0%. At comparable laboratory velocities of 0.05 and 0.25 m/s, Gersabeck and Merritt (1979) found similar hourly rates of relocation (interpolated from their Fig. 1) for 5th - last instar Prosimulium mixtum/fuscum (0.14 and 0.75%) and Cnephia dacotensis Dyar and Shannon (0.30 and 0.50%). In contrast, Wotton (1985) found considerable upstream movement of larval Simulium noelleri of all ages in an artificial stream using velocities of 0.05 - 0.49 m/s. At lower velocities (0.05 - 0.15 m/s) up to 42% of the larvae in the experimental stream were observed to migrate over 0.50 m upstream within a 90 min period. He also noted that the larger a larva was, the more likely it was to move in lower water velocities. Both Wotton

(1985) and Ciborowski and Craig (1989) found larval movement decreased with increased velocity.

The generally low rate of larval movement during experimental trials (Tables 7.5 and 7.6) agreed with the *in situ* observation of Mokry (1975). This author noted mass movements of a population of 4th to final instar *S. venustum/verecundum* (referred to as *S. venustum*) were minimal during normal flow conditions and only occurred during spates, drying conditions or mechanical perturbation of the substrate. Larvae would, however, move their relative positions slightly. *In situ* observation also suggested that larvae remained stationary as long as stream conditions remained suitable (Colbo 1979). Lack of movement should not be surprising as movement is energetically costly (Wotton 1985; Ciborowski and Craig 1989) and can increase the risk of predation (Mokry 1975). Hence, large scale changes in location should be restricted to: (i) dispersal stages such as early instars (e.g., Reisen 1977; Colbo and Moorhouse 1979: Colbo and Wotton 1981; deMoor *et al.* 1986); (ii) extreme changes in local conditions (Mokry 1975; Ciborowski and Clifford 1984) and; (iii) immediate threat to survival such as predation (Wiley and Kohler 1981). Considerable movement by late instars occurred under stream conditions as tiles were readily colonized. As Mokry (1975) noted, individual larvae often made small scale movements, thus tiles were most likely colonized by larvae occurring on natural substrates within the immediate

vicinity of tiles. The fact that last instars of EFG/C and ACD were very abundant immediately below the outlet at site 3 shows a significant part of the population remains relatively stationary.

SUMMARY

1. Larval EFG/C and ACD selected different microhabitats as shown by their response to velocity, depth and distance. Prepupae did not select a different microhabitat than larvae in which to pupate.

2. Optimal velocity for larval EFG/C was 0.36 m/s. Larvae showed a parabolic orientation to the lake outlets with maximum abundance estimated at 10.5 - 16.0 m from the outflow. The abundance of larval EFG/C decreased exponentially with depth.

3. Optimal current velocity for larval ACD was estimated to be between 0.69 - 0.73 m/s. The distance-preference curve for ACD at site 3 was opposite to that of EFG/C, with maximum larval abundance just below the outlet. The microdistribution of ACD-AA and ACD was not influenced by water depth.

4. Although both EFG/C and ACD were influenced by both

velocity and depth it was not possible to explain patterns of distribution in relation to larval size.

5. Experimental troughs were of little value in determining velocity preferences under field conditions.

TABLE 7.1. Tile collections used for fitting preference curves.

Collection No.	Date of retrieval	Site	Identification		No. of larvae (l) or pupae (p)	Mean No./ tile ±SD (n)
			n	Cytotype(s)		
1	16/05/88	3	50	EFG/C	6549 (l)	192.6 ±369.1 (35)
2	29/05/88	3	-	EFG/C[+]	7711 (p)	220.3 ±408.2 (35)
3	17/05/88	1	50	EFG/C	6247 (l)	152.4 ±273.8 (41)
4	08/07/88	2	40	ACD, AA	821 (l)	19.1 ± 32.4 (43)
5	16/07/88	2	7	ACD, AA[++]	215 (l)	5.4 ± 8.8 (40)
6	13/07/89	3	50	ACD	2386 (l)	62.8 ± 90.8 (38)
6	13/07/89	3	-	ACD[§]	662 (p)	17.4 ± 38.7 (38)
7	22/07/89	3	30	ACD	144 (l)	3.5 ± 10.0 (41)

[+]Pupae were assumed to be EFG/C as all last instar larvae identified (n = 50) the previous week at this site (collection 1) were EFG/C.

[++]Very poor chromosome preparation quality preventing cytotaxonomic identification of any more larvae.

[§]As all last instar larvae identified (n = 50) from this site at the time of collection were ACD, pupae were also assumed to be ACD.

TABLE 7.2. Microdistribution models for larval and pupal abundance (log y + 1). Note that v = velocity, d = depth and m = distance. Models were used to construct preference curves for each stream variable.

Collection No.	Cytotype(s)*	Regression models	$R^2_{adj.}$
1	EFG/C (l)**	$2.978 - 0.870m - 4.036d + 0.112m^2 - 0.004m^3$	73.6%
2	EFG/C (p)	$4.300 - 0.483m - 27.993d + 0.048m^2 - 0.002m^3 + 1.750md$	67.0%
3	EFG/C (l)	$0.690 + 0.240m - 10.970d + 11.513v - 0.011m^2 - 16.000v^2$	62.2%
4	ACD-AA (l)	$0.211 + 3.178v$	31.3%
5	ACD-AA (l)	$0.051 + 2.039v$	41.3%
6	ACD (l)	$0.779 - 0.212m + 5.237v + 0.010m^2 - 3.611v^2$	79.2%
6	ACD (p)	$1.265 - 0.204m + 1.395v + 0.007m^2$	66.9%
7	ACD (l)	$0.063 - 0.132v + 7.203v^2 - 6.870v^3$	62.4%

*Cytotaxonomic identifications given in Table 7.1.

**l = larvae, p = pupae.

TABLE 7.3. Kruskal-Wallis and Tukey-type multiple comparison analysis of stream velocities among larval collections. Analysis based on mean rank. Mean and range given for comparative purposes.

Collection No.	Site	Cytotype(s)[+]	Mean[++] rank	Mean velocity ±SD (m/s)	Velocity range (m/s)	$H_{adj.}$
1	3	EFG/C	101.2[a]	0.23 ±0.21	0.03 - 1.03	16.71***
3	1	EFG/C	86.5[b]	0.18 ±0.14	0.03 - 0.52	
4	2	ACD-AA	144.3[a]	0.31 ±0.13	0.10 - 0.62	
5	2	ACD-AA	113.0[a]	0.24 ±0.15	0.03 - 0.68	
6	3	ACD	127.6[a]	0.32 ±0.24	0.03 - 0.85	
7	3	ACD	137.6[a]	0.35 ±0.25	0.03 - 0.92	

*** $p < 0.001$.

[+]Cytotaxonomic identifications given in Table 7.1.

[++]Mean ranks with different letters were significantly different at $p < 0.05$.

TABLE 7.4. ANOVA and Tukey multiple comparison analysis of stream depth among larval collections. Analysis based on log mean (d x 100). Mean and range given for comparative purposes.

Collection No.	Site	Cytotype(s)*	Mean ** log	Mean depth ±SD (m)	Depth range (m)	$F_{5,231}$
1	3	EFG/C	1.31^{a}	0.21 ±0.05	0.07 - 0.34	27.97***
3	1	EFG/C	1.17^{b}	0.16 ±0.05	0.03 - 0.28	
4	2	ACD-AA	1.15^{b}	0.15 ±0.04	0.05 - 0.23	
5	2	ACD-AA	1.08^{bc}	0.13 ±0.05	0.04 - 0.23	
6	3	ACD	1.02^{cd}	0.11 ±0.03	0.04 - 0.19	
7	3	ACD	0.93^{d}	0.09 ±0.03	0.03 - 0.16	

*** $p < 0.001$.

*Cytotaxonomic identifications given in Table 7.1.

**Mean logs with different letters were significantly different at $p < 0.05$. Depth values (m) were first multiplied by 100 before log transformation to avoid negative log terms.

TABLE 7.5. ANOVA of the mean hourly release rate of last instar larvae among 3 trough velocities.

	Mean % releasing / h (±SD)			$F_{2,14}$**
	0.06 m/s	0.22 m/s	0.35 m/s	
Experiment 1*	0.75 ±0.40	0.74 ±1.02	0.49 ±0.36	0.24
Experiment 2	0.08 ±0.13	0.18 ±0.27	0.11 ±0.28	0.32

*Based on 50 cytotaxonomic identifications larvae in experiment 1 were 84% EFG/C (CI = 70.9 - 92.8%) and 16% ACD (CI = 7.2 - 29.1%). In experiment 2, all 50 larvae identified were EFG/C.

**Analysis of variance based on arcsine transformation of percentages.

TABLE 7.6. Frequency analysis (G-tests) of the hourly movement of last instar larval EFG/C at 3 velocities*.

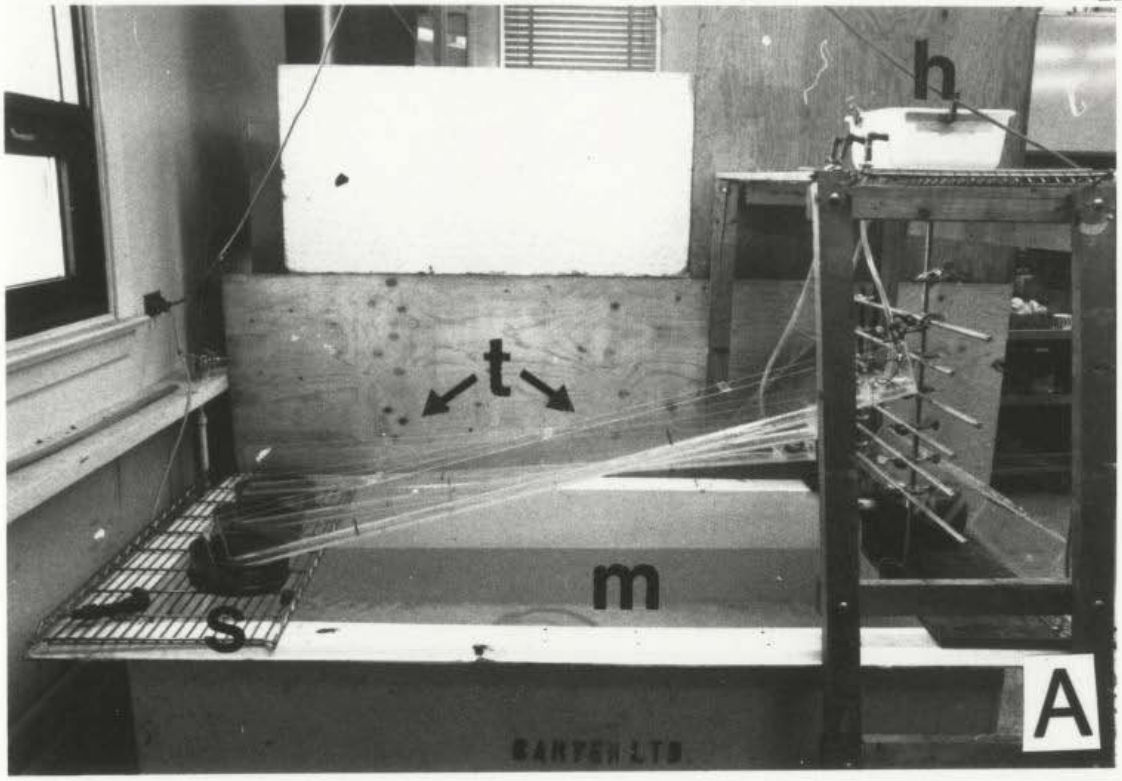
	Trough velocity (m/s)			$G_{adj.}$
	0.06 (m/s)	0.22 (m/s)	0.35 (m/s)	
% migrating / h	0.41	0.37	0.06	4.99
% in drift / h	0.12	0.37	0.61	6.05*
% total movement / h	0.53	0.74	0.67	0.58

*All 50 larvae cytotyped in this experiment were EFG/C.

FIG. 7.1. Ceramic tiles used as artificial substrate to collect larvae and pupae. (Top) Placement of tiles in stream. (Bottom) Close up view of a single tile showing attached larvae and pupae.

FIG. 7.2. Artificial stream system used to test the response of final larval instars to changes in water velocity. (A) View of complete apparatus. (B) Close up view of plexiglass trough channel and trough reservoir.

b = bar used to position trough at required level
c = trough channel
h = small reservoir
m = main reservoir
p = pump used to move water from main to small reservoir
r = trough reservoir
s = sieve
t = plexiglass trough
tt = Tygon tubing


h
t
m
s
A

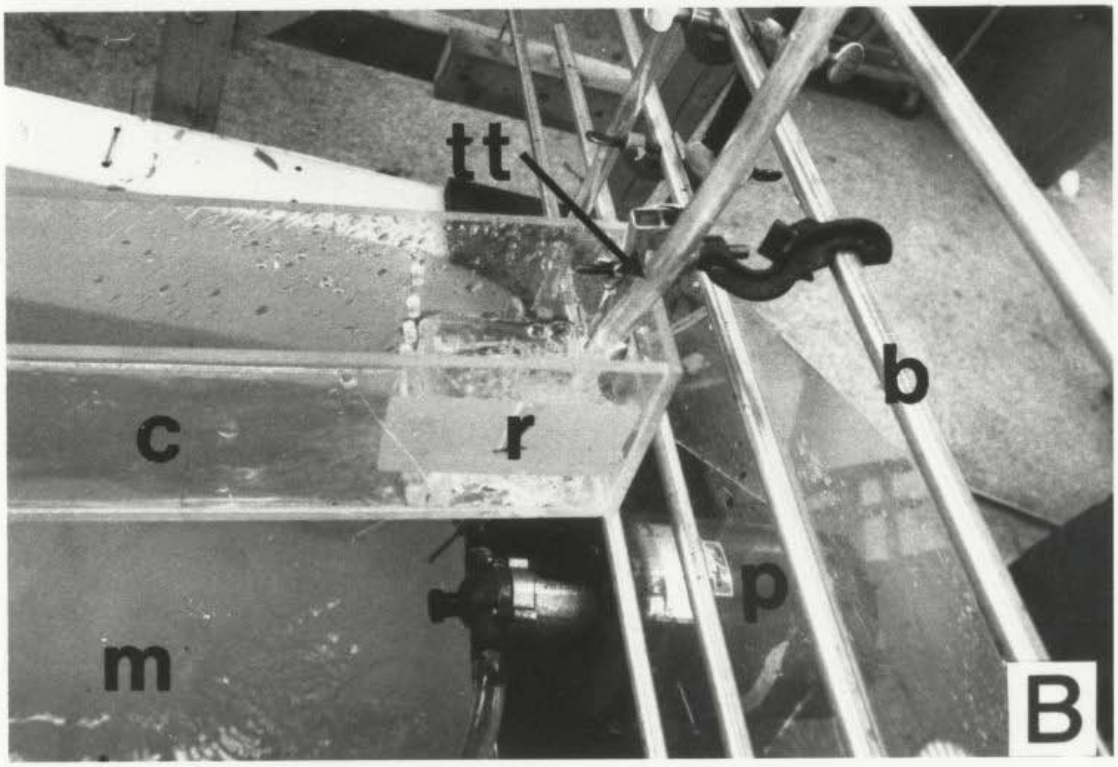

tt
b
c
r
p
m
B

FIG. 7.3. Velocity-preference curves for larval EFG/C, ACD-AA and ACD. Preference factor measures last larval instar response to observed changes in velocity on a scale of 0 (conditions least suited for larvae) to 1 (optimal conditions).

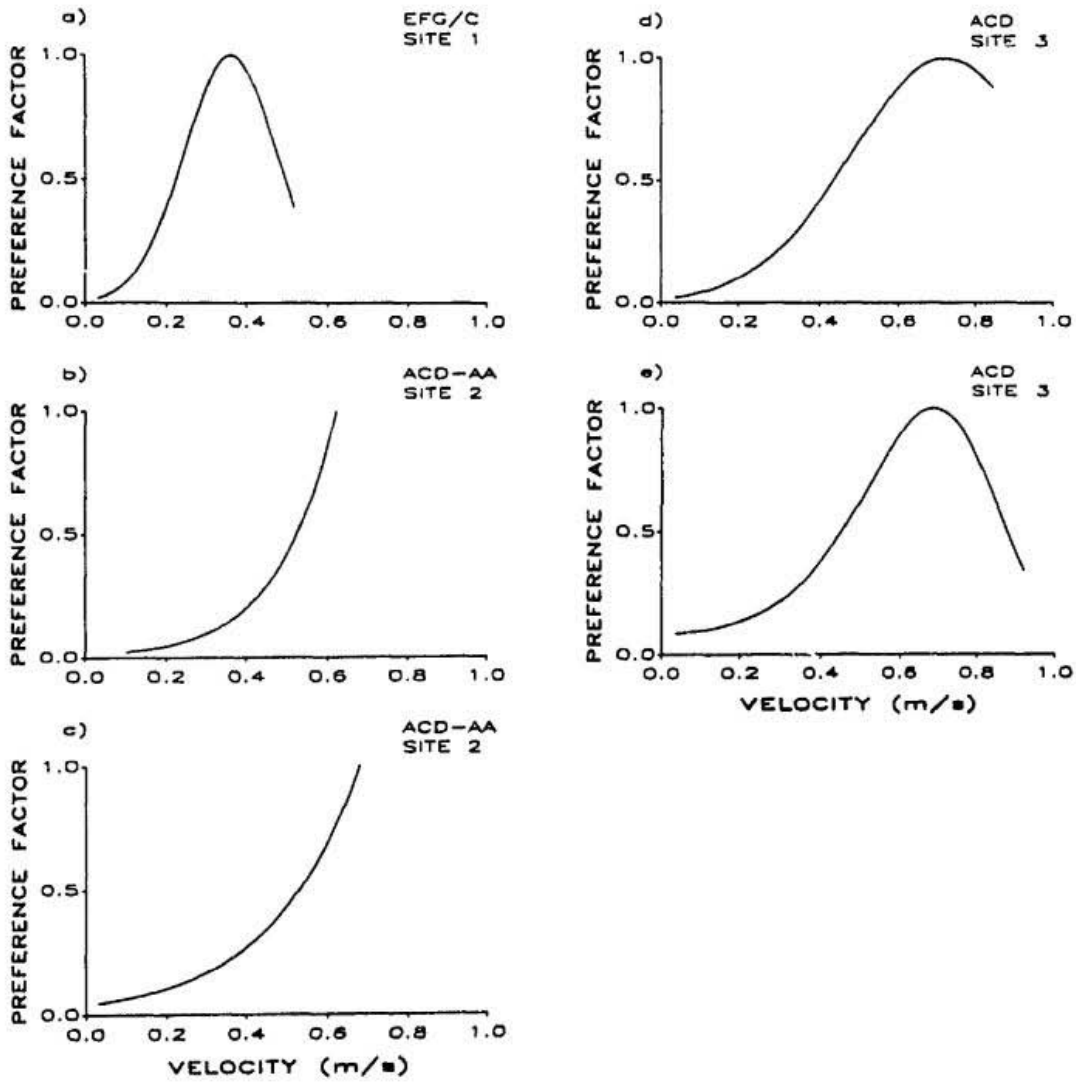

a)
EFG/C
SITE 1
b)
ACD-AA
SITE 2
c)
ACD-AA
SITE 2
d)
ACD
SITE 3
e)
ACD
SITE 3
PREFERENCE FACTOR
0.0
0.5
1.0
0.0
0.2
0.4
0.6
0.8
1.0
VELOCITY (m/s)

FIG. 7.4. Distance-preference curves for larval EFG/C and ACD. Preference factor measures last larval instar response to observed changes in distance from an outlet on a scale of 0 (conditions least suited for larvae) to 1 (optimal conditions).

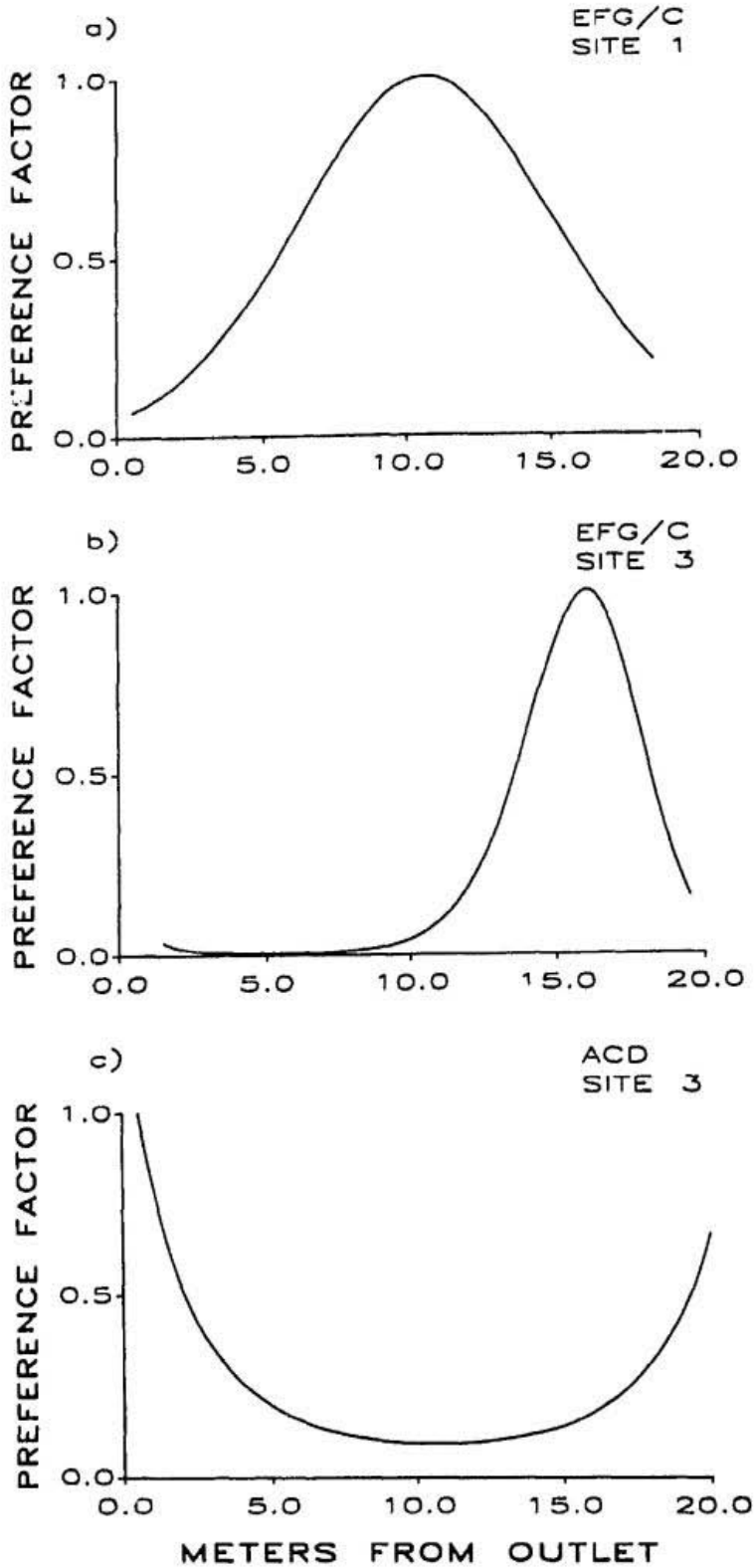

a)
EFG/C
SITE 1
PREFERENCE FACTOR
1.0
0.5
0.0
0.0
5.0
10.0
15.0
20.0
b)
EFG/C
SITE 3
PREFERENCE FACTOR
1.0
0.5
0.0
0.0
5.0
10.0
15.0
20.0
c)
ACD
SITE 3
PREFERENCE FACTOR
1.0
0.5
0.0
0.0
5.0
10.0
15.0
20.0
METERS FROM OUTLET

FIG. 7.5. Depth-preference curves for larval EFG/C. Preference factor measures last larval instar response to observed changes in depth on a scale of 0 (conditions least suited for larvae) to 1 (optimal conditions).

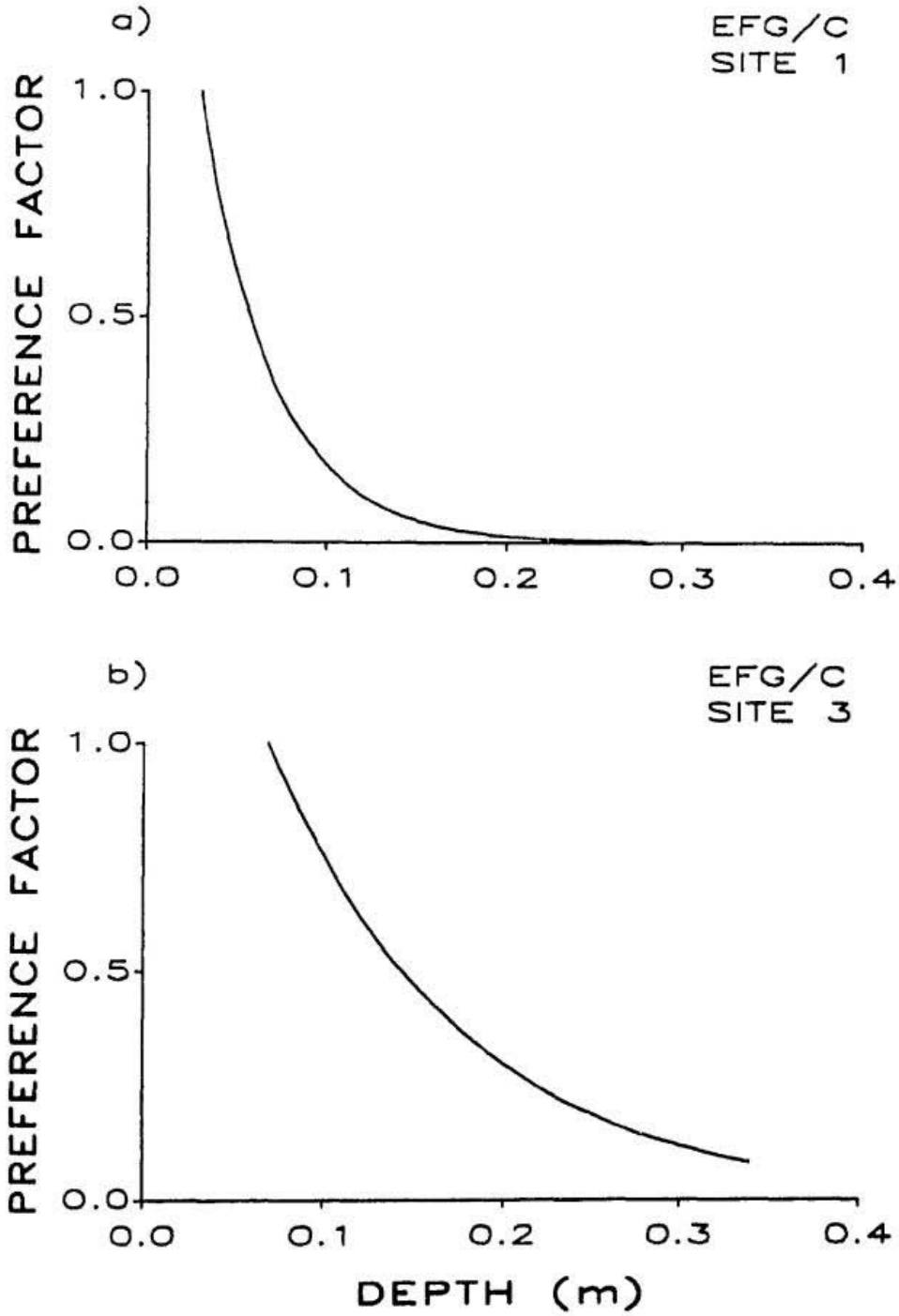
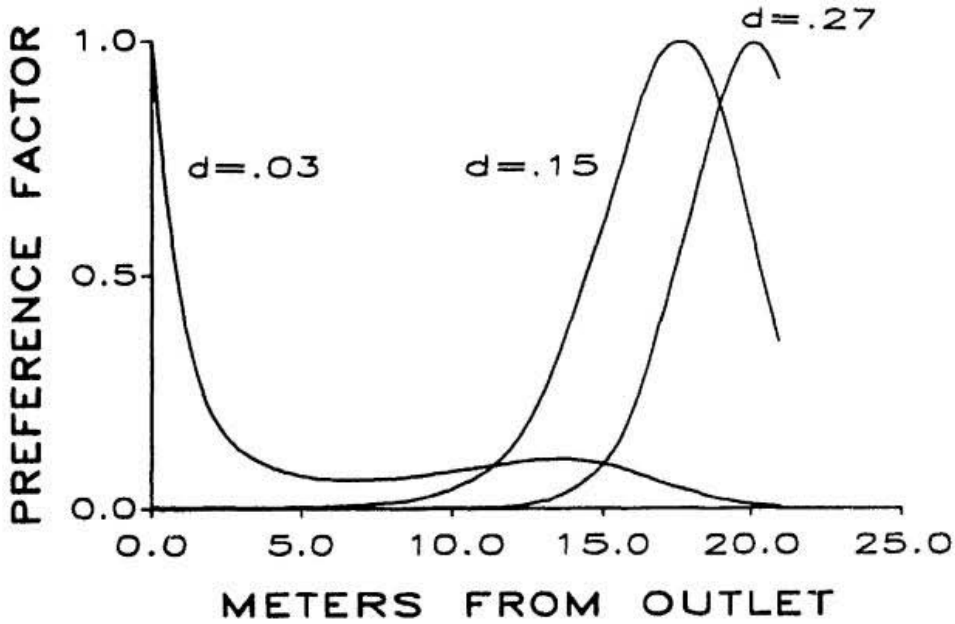

a)
EFG/C
SITE 1
PREFERENCE FACTOR
1.0
0.5
0.0
0.0
0.1
0.2
0.3
0.4
b)
EFG/C
SITE 3
PREFERENCE FACTOR
1.0
0.5
0.0
0.0
0.1
0.2
0.3
0.4
DEPTH (m)

FIG. 7.6. Distance-preference and depth-preference curves for pupal EFG/C (collection 2). Preference factor measures pupal response to observed changes in a stream variable on a scale of 0 (conditions least suited for pupae) to 1 (optimal conditions). Note the effect the interaction term (md) has on curves, i.e., pupal response to depth (d) was modified by extreme values of distance (m) and vice versa.


PREFERENCE FACTOR
1.0
0.5
0.0
d=.03
d=.15
d=.27
0.0
5.0
10.0
15.0
20.0
25.0
METERS FROM OUTLET

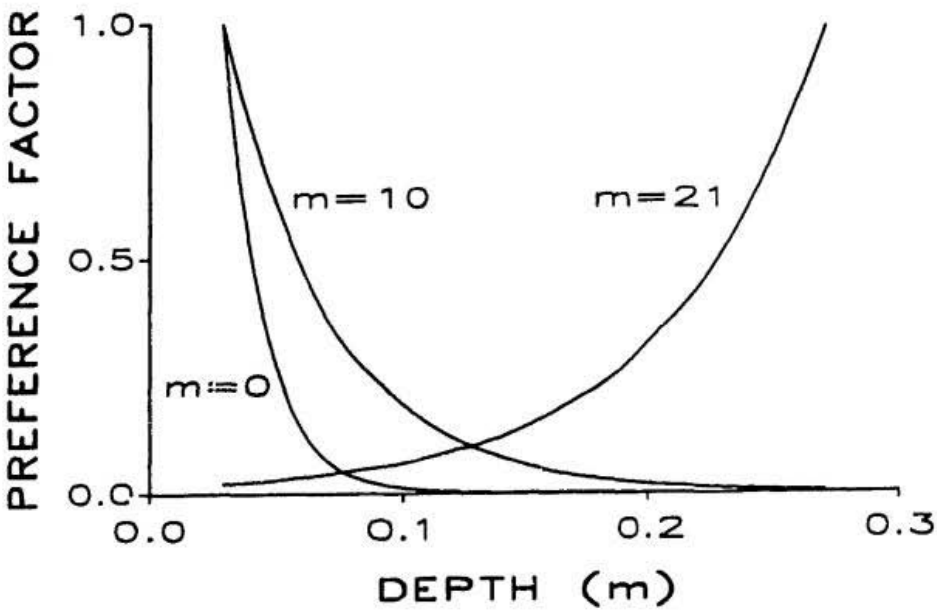
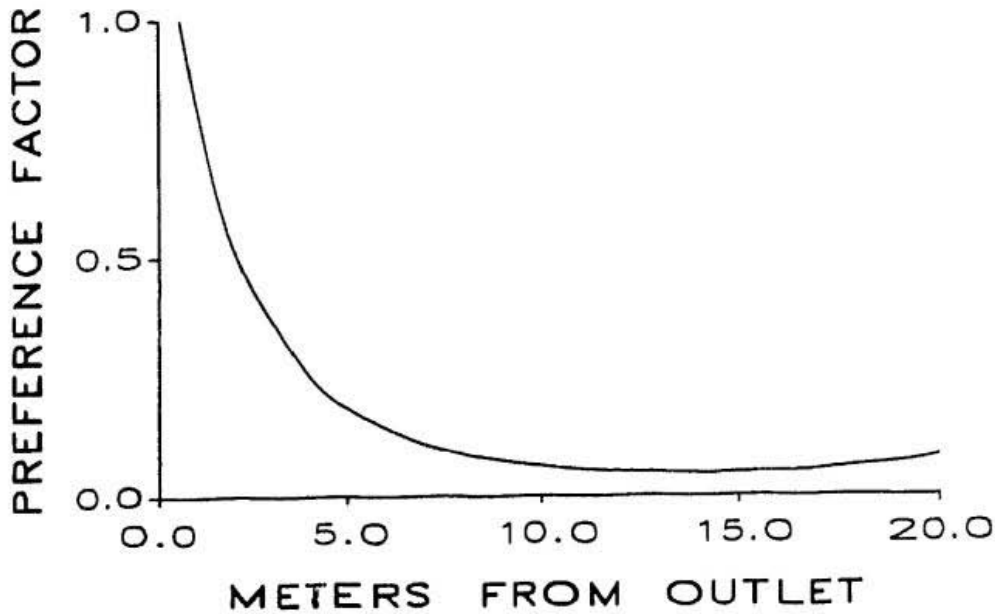

PREFERENCE FACTOR
1.0
0.5
0.0
m=10
m=21
m=0
0.0
0.1
0.2
0.3
DEPTH (m)

FIG. 7.7. Distance-preference and velocity-preference curves for pupal ACD (collection 6). Preference factor measures pupal response to observed changes in a stream variable on a scale of 0 (conditions least suited for pupae) to 1 (optimal conditions).


PREFERENCE FACTOR
1.0
0.5
0.0
0.0
5.0
10.0
15.0
20.0
METERS FROM OUTLET

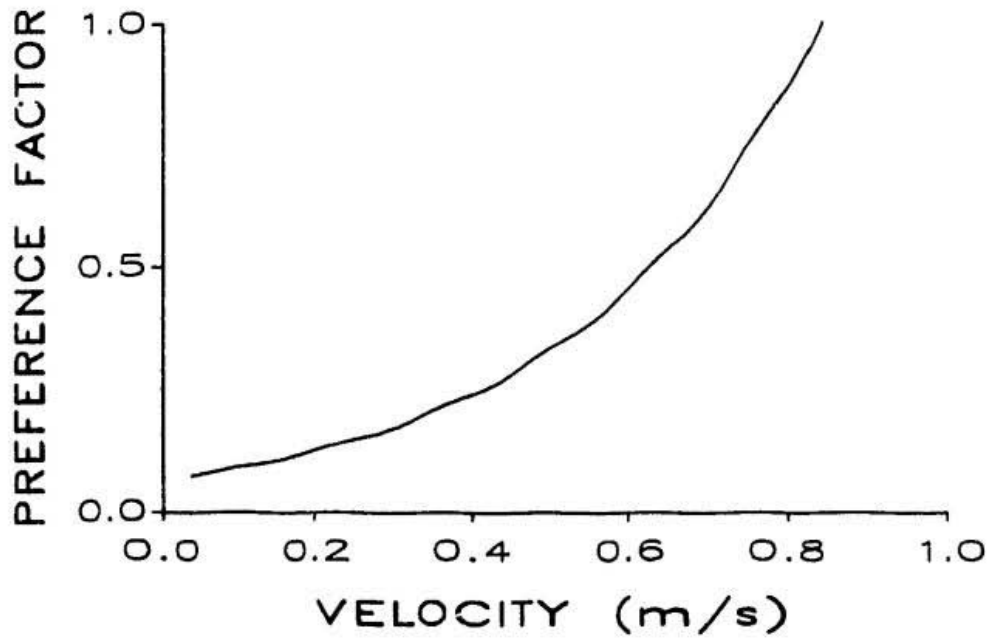
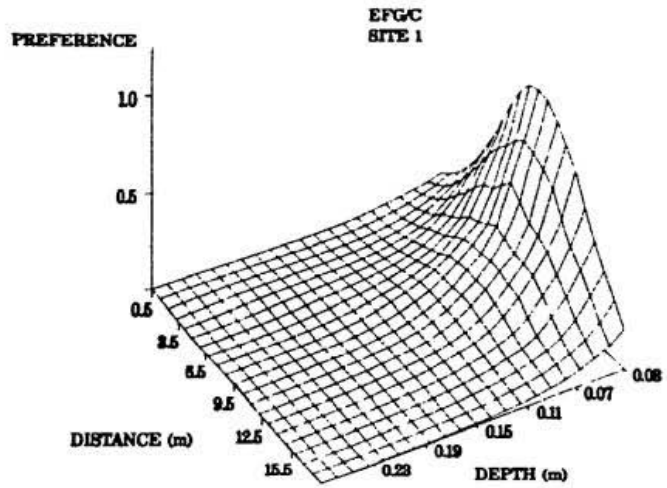
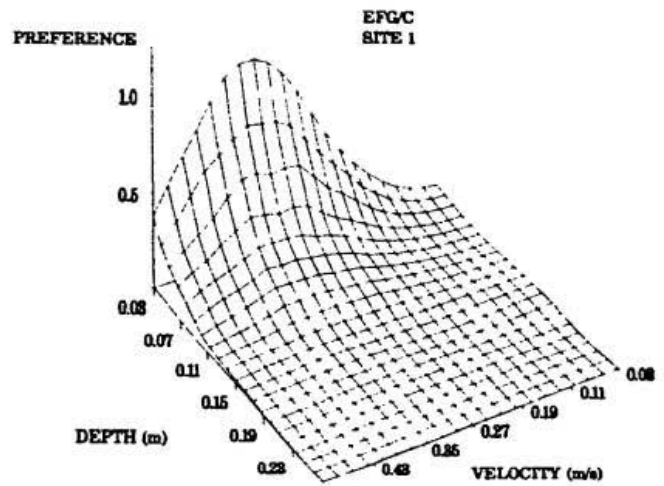
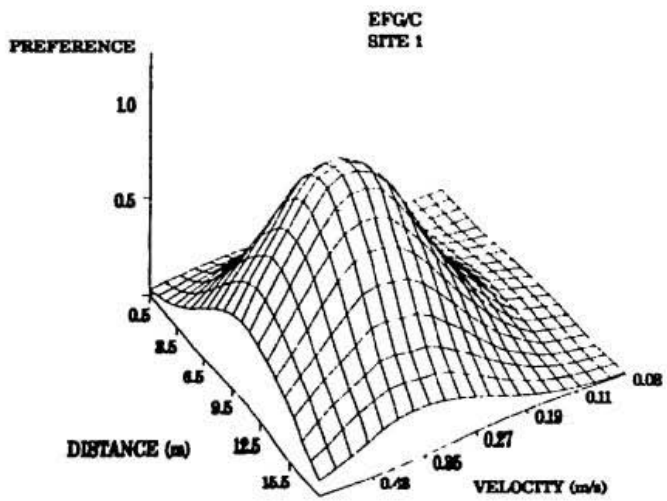

PREFERENCE FACTOR
1.0
0.5
0.0
0.0
0.2
0.4
0.6
0.8
1.0
VELOCITY (m/s)

FIG. 7.8. Distance-depth, depth-velocity and distance-velocity preference surface plots for larval EFG/C (collection 3) and ACD (collection 6). Preference factor measures last instar larval response to observed changes in 2 stream variables on a scale of 0 (conditions least suited for larvae) to 1 (optimal conditions).


EFG/C
SITE 1
PREFERENCE
1.0
0.5
0.5
2.5
6.5
9.5
12.5
15.5
DISTANCE (m)
0.08
0.07
0.11
0.15
0.19
0.23
DEPTH (m)


EFG/C
SITE 1
PREFERENCE
1.0
0.5
0.08
0.07
0.11
0.15
0.19
0.23
DEPTH (m)
0.08
0.11
0.19
0.27
0.35
0.43
VELOCITY (m/s)


EFG/C
SITE 1
PREFERENCE
1.0
0.5
0.5
2.5
6.5
9.5
12.5
15.5
DISTANCE (m)
0.08
0.11
0.19
0.27
0.35
0.43
VELOCITY (m/s)

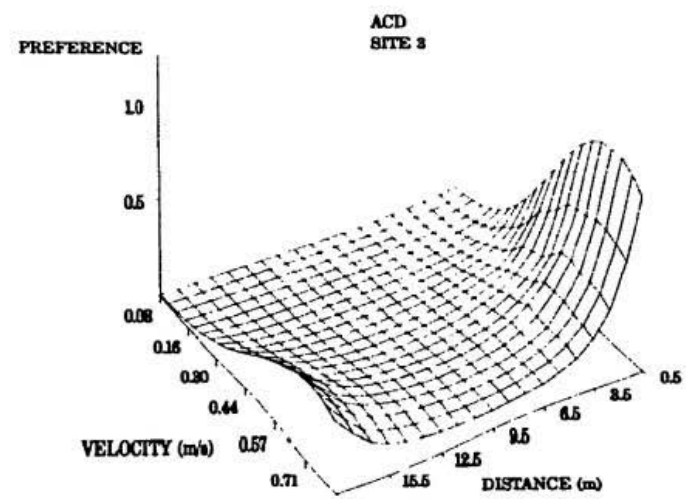

ACD
SITE 3
PREFERENCE
1.0
0.5
0.08
0.16
0.30
0.44
0.57
0.71
VELOCITY (m/s)
0.5
3.5
6.5
9.5
12.5
15.5
DISTANCE (m)

FIG. 7.9. Velocity-size and depth-size scatter plots for larval EFG/C (collection 3). For each head capsule character, each point represents a mean (n = 4). Error for sample means was low (CV = 3.6 - 4.2%) and omitted for clarity of presentation. CAW = cephalic apotome width, HCW = head capsule width, HCL = length from lateral-dorsal aspect of the postantennal buttress to the lateral-dorsal aspect of the postocciput, and PAW = distance between the dorsal aspects of the postantennal buttresses.

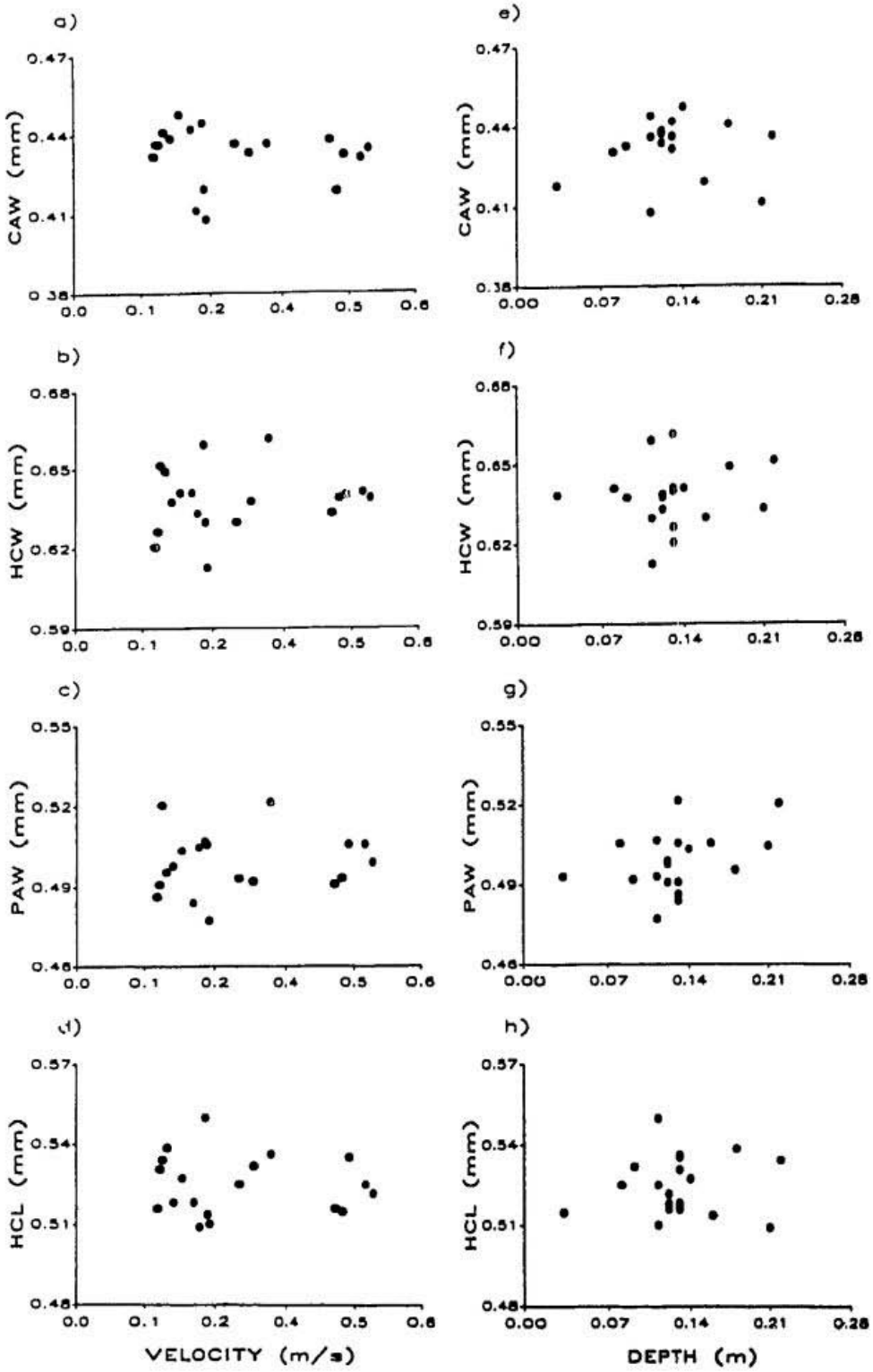
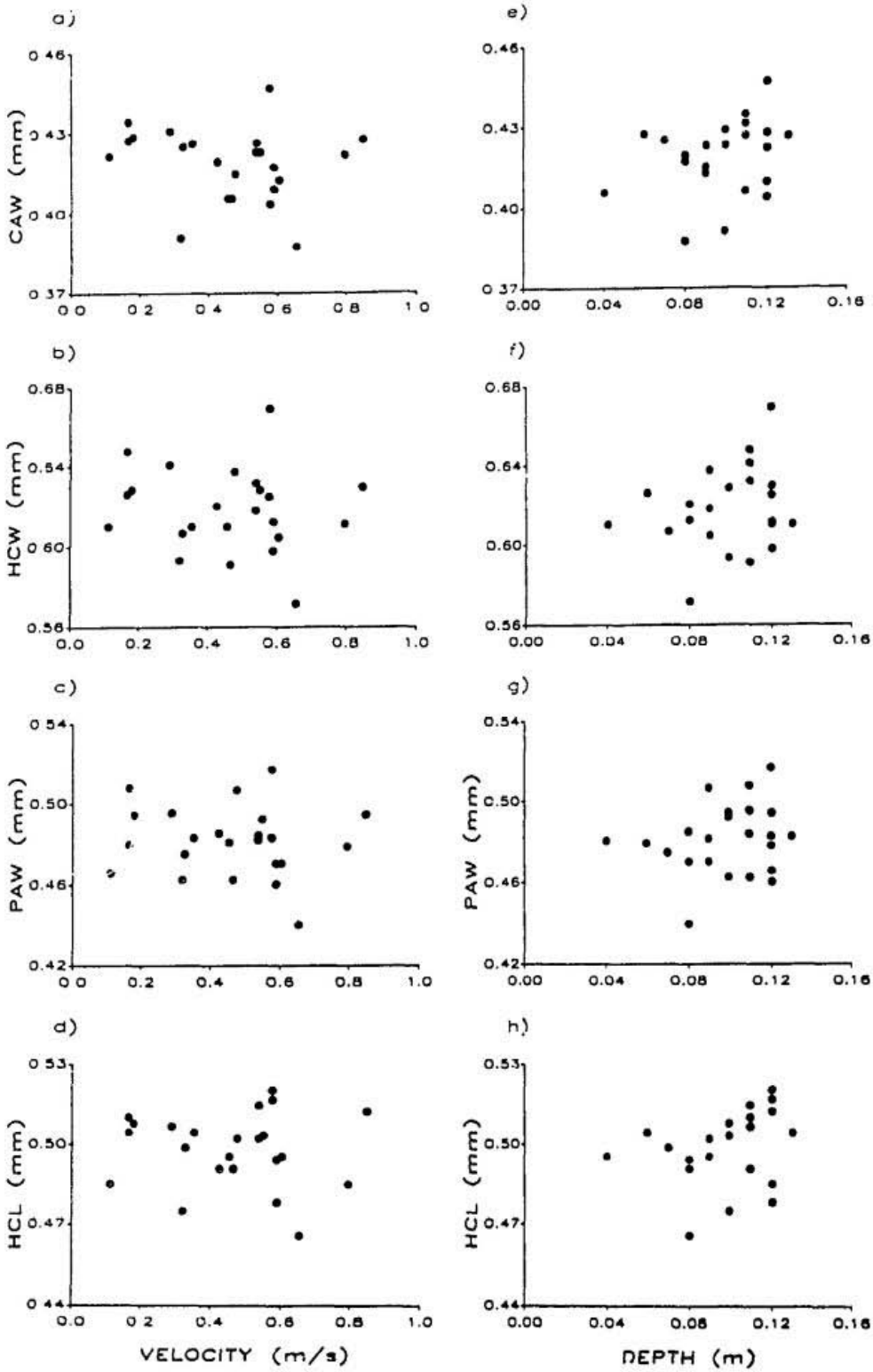

a)
CAW (mm)
0.47
0.44
0.41
0.38
0.0 0.1 0.2 0.4 0.5 0.6
e)
CAW (mm)
0.47
0.44
0.41
0.38
0.00 0.07 0.14 0.21 0.28
b)
HCW (mm)
0.68
0.65
0.62
0.59
0.0 0.1 0.2 0.4 0.5 0.6
f)
HCW (mm)
0.68
0.65
0.62
0.59
0.00 0.07 0.14 0.21 0.28
c)
PAW (mm)
0.55
0.52
0.49
0.46
0.0 0.1 0.2 0.4 0.5 0.6
g)
PAW (mm)
0.55
0.52
0.49
0.46
0.00 0.07 0.14 0.21 0.28
d)
HCL (mm)
0.57
0.54
0.51
0.48
0.0 0.1 0.2 0.4 0.5 0.6
VELOCITY (m/s)
h)
HCL (mm)
0.57
0.54
0.51
0.48
0.00 0.07 0.14 0.21 0.28
DEPTH (m)

FIG. 7.10. Velocity-size and depth-size scatter plots for larval ACD (collection 6). For each head capsule character, each point represents a mean (n = 4). Error for sample means was low (CV = 4.6 - 5.5%) and omitted for clarity of presentation. CAW = cephalic apotome width, HCW = head capsule width, HCL = length from lateral-dorsal aspect of the postantennal buttress to the lateral-dorsal aspect of the postocciput, and PAW = distance between the dorsal aspects of the postantennal buttresses.


a)
CAW (mm)
0.46
0.43
0.40
0.37
0.0 0.2 0.4 0.6 0.8 1.0
e)
0.46
0.43
0.40
0.37
0.00 0.04 0.08 0.12 0.16
b)
HCW (mm)
0.68
0.64
0.60
0.56
0.0 0.2 0.4 0.6 0.8 1.0
f)
0.68
0.64
0.60
0.56
0.00 0.04 0.08 0.12 0.16
c)
PAW (mm)
0.54
0.50
0.46
0.42
0.0 0.2 0.4 0.6 0.8 1.0
g)
PAW (mm)
0.54
0.50
0.46
0.42
0.00 0.04 0.08 0.12 0.16
d)
HCL (mm)
0.53
0.50
0.47
0.44
0.0 0.2 0.4 0.6 0.8 1.0
VELOCITY (m/s)
h)
HCL (mm)
0.53
0.50
0.47
0.44
0.00 0.04 0.08 0.12 0.16
DEPTH (m)

CHAPTER 8

PHENOLOGY

INTRODUCTION

Phenology is an important aspect of any aquatic insect's life history strategy (Butler 1984). The phenology of S. venustum/verecundum can be broadly divided into 2 cycles: TYPE 1 - Univoltine species (most venustum ?) with eggs deposited in early summer and hatching the following spring. Pupation starts by April or May; TYPE 2 - Bivoltine and multivoltine species (verecundum, some venustum) with eggs often hatching later than TYPE 1. Two or more generations per year occur and larvae, pupae and adults can be present all summer. Eggs laid by the last summer or fall generation overwinter until the following spring (Rothfels et al. 1978; Cupp and Gordon 1983; Lake and Burger 1983; Adler 1986; Adler and Kim 1986).

Much is known about the seasonal distribution of all stages of the S. venustum/verecundum complex in Newfoundland (Lewis and Bennett 1973, 1974; Ezenwa 1974; Mokry 1976; McCreadie et al. 1985), however, other than a few collection records (Rothfels et al. 1978; Colbo 1985), little is known about the phenology of individual cytotypes. The present study examines the seasonal succession of S. venustum/verecundum cytotypes in both sublacustrine and downstream habitats.

MATERIALS AND METHODS

Study sites

Four sampling stations were selected for study. Three of these stations, designated as Axes Pond outlet (APO), Big Piccos (BP) and Little Piccos (LP), were located on the Piccos drainage basin (Chapter 6). The locations of these sites are given in Fig. 6.1 (station 2 = LP, station 7 = APO, station 11 = BP). The fourth site, designated Beachy Cove outlet (BCO), was located on the Hogan Pond drainage basin (47°34' - 47°37' N, 52°50' - 52°53' W), 11 km W of St. John's (Fig. 8.1). Each station consisted of a 10-m stretch of stream. APO and BCO were located close to outlets (< 80 m) whereas Little and Big Piccos were located further downstream (> 1 km).

The APO sampling station (47°40'45'' N, 52°45'30'' W) was located on Piccos Brook, 65 - 75 m downstream from the outlet of Axes Pond (pond area = 14.1 ha). Over the course of the field season this second order stream varied between 3 - 4 m in width. The bed was primarily rubble and the riparian vegetation brush (Table 2.2) which produced a partial canopy. The site can only be reached on foot, and is relatively undisturbed by man; there are no buildings on Axes Pond.

The BP station (47°12'15'' N, 52°44'00'' W) was located on a third order section of Piccos Brook, approximately 2.5 km downstream of APO. The closest outlet to BP was Upper Gallows Cove (pond area = 10.0 ha) approximately 1 km upstream. Stream

width varied between 7 - 10 m. The stream bed was primarily a rubble-boulder mix and the riparian vegetation forest which did not produce a significant canopy over the stream. Although this site was located less than 100 m from a secondary highway, it was well concealed from view and showed little disturbance by man (i.e., no signs of human debris).

The LP station (47°40'30'' N, 52°47'15'' W) was located on a second order stream. Stream width varied between 1 - 5 m. The stream bed was primarily rubble with significant amounts of moss (Fontinalis spp.). This site was located in a small spruce forest which produced a partial canopy over the stream. Although several houses were located 100 - 200 m upstream, the sampling station was free of debris and appeared relatively undisturbed by man. This site was approximately 3.5 km downstream from the nearest pond outlet.

The BCO station (47°35'45'' N, 52°51'00'' W) was established on Beachy Cove Brook, 55 - 65 m downstream from Hughs Pond (pond area = 7.5 ha). Width of this second order stream varied between 1 - 5 m. The stream bed was primarily rubble and the banks were lined by trees producing a partial canopy. Several houses and summer homes were located on Hughs Pond and Beachy Cove Brook which is a more disturbed and enriched (by human activity) system than sites on the Piccos drainage basin. The sampling station was located on private land and free from public tampering.

Sampling procedures

Sites were quantitatively sampled each week in 1987 and 1988 from the beginning of May to the end of August, and 1 - 2 times in September. LP was sampled in 1987 only. Each station was sampled with an artificial substrate in 1987. Natural and artificial substrates were used in 1988. The artificial sampler consisted of a single 10-cm length (diameter ≈ 2.0 mm) of stiff monofilament fishing line (Triple Fish Commercial Line, Slonek KG., Saal/Donau, West Germany) anchored to a small metal weight such that the filament projected upward into the water column (Fig. 8.2). Filaments were selected because they: i) collected large numbers of larvae (Colbo 1987); ii) were inexpensive (1.5 cents/filament) and; iii) were easily retrieved. Ten filaments were placed at each station and replaced weekly. In 1988, rocks (n = 10) at APO, BP, and BCO, and vegetation (Sparganium spp.) at BCO (n = 5) were also sampled. A sample size of 10 was selected because it yields modest precision (Allan 1984), with reasonable sorting time, and in the case of rocks, minimizes stream bed destruction. Sampling individual rocks as opposed to a set area of stream bed further reduces sorting time and stream bed destruction. The scarcity of vegetation at BCO in the spring lead to a reduced sample size (n = 5). The lack of vegetation precluded its sampling at BP and APO.

A random stratified sampling design (strata = fast and slow water velocity) was used because samples selected in this

manner have reduced error and are more representative than samples of equal size from a simple design (Cummins 1975; Bhattacharyya and Johnson 1977; Elliott 1977). Both these points were important considerations with the limited sample size used here. As velocity is a primary correlate of simuliid distribution (Tables 1.1 and 1.2), strata were based on this variable. Strata were determined weekly. At a station, 3 or 4 equidistant velocity readings, measured 5 cm above the stream bed (i.e., filament midpoint) and perpendicular to the stream bank, were taken. This procedure was repeated at every 2.5 m length of stream, giving a total of 15 to 20 readings for a station. Any velocity measurement exceeding one half the maximum recorded velocity was considered fast; all other values were considered slow. Velocity measurements were then used to determine the area of fast and slow water and the 10 artificial samplers proportionally allocated to each stratum. Placement of filaments within each stratum was achieved by dividing the site into a 20 x 20 grid and using a random number generator to select sampler position. Samplers were retrieved the following week. Rock and vegetation were simultaneously collected with filaments using this stratified protocol.

At retrieval, each filament was removed from the stream, covered with a culture tube, cut from the anchor, and the tube capped. Individual rocks were collected by hand and placed in plastic bags. Vegetation was collected using a 15 cm long

(diameter = 6 cm) piece of PVC tubing. One end was closed with 50 μm mesh plankton netting held in place by an 'O' ring. Sampling was accomplished by sliding vegetation into the open end of the tubing and cutting off the required amount. Each tube with its contents was then placed into a plastic bag. All collections proceeded in an upstream direction to minimize disturbance. All samples were placed on ice for transportation to the laboratory.

Stream variables measured at each collection (see chapter 2 for details) included stream width (upstream, midpoint and downstream boundaries), temperature (present, weekly minimum and maximum), conductivity, pH, dissolved oxygen, seston, and mean depth. At the station midpoint, 3 - 4 equidistant readings of velocity (at 0.6 the depth) and depth, along with midpoint width, were used to calculate discharge.

In the laboratory larvae were fixed live and the number of last instar S. venustum/verecundum from each filament, rock and vegetation sample was counted. The surface area of each rock was estimated following McCreadie and Colbo (1991). The surface area of each vegetation sample was estimated by drying several 1 x 1 cm sections for 24 h at 60°C and weighing them to the nearest mg. Thus the weight of a specified area of vegetation was known and this weight-area relationship used to estimate the surface area of remaining vegetation in each sample. Quantitative density estimates from natural substrates were expressed as number/m^2 and for the artificial samplers as

number/filament.

Each substrate was analyzed separately. An attempt was made to score all individuals in weekly samples with ≤30 larvae. Above this number the percent cytotype composition for each week and substrate was estimated from a random stratified subsample of 22 (see Appendix 2). Since chromosome preparation quality continually diminished throughout the summer months it was not always possible to cytotype 22 larvae; nonetheless, cytotype composition was usually estimated within an error of ±20%.

Data analysis and evaluation of the sampling program

As sample variance generally exceeded the mean in most instances, a log (y + 1) transformation was used when calculating confidence intervals for mean number of S. venustum/verecundum (Elliott 1977). In cases where all larvae in a sample were successfully cytotyped, mean number of each cytotype was calculated directly. More often only a subsample of larvae was identified; accordingly, mean number of each cytotype was estimated as:

$$[1]\quad \text{Mean number of cytotype}_i = \frac{\text{total larvae in sample} \times \%\text{cytotype}_i \text{ in subsample}}{\text{sample size}}$$

Since means calculated in this manner provide no estimate of error, confidence intervals cannot be calculated. The

inability to calculate confidence limits for individual cytotypes was not considered essential to quantify trends in seasonal succession over time. Confidence intervals were essential only for evaluating the performance of stratification and for this mean number of S. venustum/ verecundum was used (see below). Means for each cytotype were plotted as logs since seasonal trends in abundance over 4 orders of magnitude were to be compared.

The stratified random sampling design used here required considerable time, hence an evaluation of this protocol was deemed appropriate. From select data sets percent confidence intervals (%CI = CI/mean x 100) and coefficients of variation (CV) were calculated using procedures for both stratified and simple designs (Elliott 1977). Mean differences in %CI and CV between these calculations were considered an indication of the precision gained from stratifying collections. Data sets for filaments included the 3 weeks of peak abundance from each site and year (for APO in 1988, n = 2). Rock and vegetation collections with abundant larvae from BCO were also used.

An evaluation of the effectiveness of filament samplers to monitor population changes was also undertaken. Correlation analysis between the seasonal log mean abundance of individual cytotypes on rocks, vegetation and filaments was used to evaluate the facility of filaments to monitor population changes on natural substrate. Data from APO and BCO for 1988 were employed in the analysis. The low number of larvae

collected from rocks at BP (n = 23) precluded this site from the analysis.

Loss of larvae from filaments at retrieval was estimated as follows: on May 14, 1988, 3 rows, each with 3 filament samplers, were placed in a 3 meter wide stream draining Gull Pond (47°34'30'' N, 52°50'30'' W). At time of retrieval (May 24, 1988) a collecting net was positioned immediately downstream of each filament to capture releasing last instar larvae. Release rate was calculated as number of larvae in the net divided by the total number (= net + filaments).

RESULTS

Evaluation of the sampling program

Mean percent confidence intervals (%CI) and mean coefficients of variation (CV) for selected data sets of S. venustum/verecundum larvae are given in Table 8.1. For each substrate and year, results shown for simple and stratified designs were for the same data set; however, calculations of %CI and CV followed procedures for simple and stratified designs (Elliott 1977). The difference in error between these calculations was minimal, with error, based on either of these calculations, moderate to high. Mean log density, confidence intervals and coefficient of variation, for each weekly collection and substrate for S. venustum/verecundum larvae, is

provided in Appendix 4. As well, estimates of log mean density for each cytotype are given.

Only 3.6% (= 41/1152) of last instar S. venustum/ verecundum larvae released from filaments during retrieval. Correlation analysis between seasonal trends in cytotype abundance observed from different substrates is presented in Table 8.2. With the exception of EFG/C from APO, each cytotype showed a similar pattern of seasonal population change among the different substrates.

Cytotype composition among stations and substrates

A total of 1428 S. venustum/verecundum complex larvae were cytotyped (Table 8.3). Based on 134 male identifications (pooled from all sites) CC2-3 consisted of 78.4% CC2 (CI = 70.4 - 85.0%) and 21.6% CC3 (CI = 15.0 - 29.6%). Cytotype composition for each site, year, and substrate is shown in Fig. 8.3. Three points should be noted. First, the cytotype fauna near outlets (BCO, APO) was markedly different from sites further downstream (BP, LP). EFG/C and ACD were found only at BCO and APO. Although AA was found at all sites, it was most frequently collected from APO. CC2-3, AC(gb), AA were the only cytotypes found at BP and LP, with CC2-3 comprising most of the larvae collected. AC(gb) was rarely found at APO and never identified from BCO. Second, cytotype assemblage at a particular site was usually consistent from year to year, although percentages varied.

Finally, data from BCO during 1988 showed cytotype composition varied with substrate. Chi square analysis (Table 8.4) showed highly significant differences in composition among substrates at BCO. Proportionally more EFG/C larvae were collected from rocks than filaments or vegetation. In contrast, the proportion of AA and ACD was considerably higher on vegetation than on rocks. The incidence of CC2-3 was similar among the different substrates. Overall, cytotype composition was most similar between filaments and vegetation.

Because means for individual cytotypes were not suited to statistical analysis (i.e., no error terms, see equation [1]), rigorous comparisons of cytotype density among different substrates were not possible. However, at BCO all larvae cytotyped (n = 291) from July to September 1988 were either ACD or AA and therefore t-tests were calculated between the density of mixed ACD-AA on rocks and vegetation (Table 8.5). In 4 out of 5 cases, density of ACD-AA larvae was significantly ($p < 0.05$) greater on vegetation than rocks.

Seasonal succession of larval cytotypes

Seasonal variations in stream variables are given in Figs. 8.4 - 8.7, and Table 8.6. From the beginning of June to September 1988, water levels, water velocities and seston loads were usually higher than in 1987. During May, this trend was reversed. In fact, low water levels and a heavy build up of algae during 1987 forced sampling to be abandoned at APO by

the second week of July. Stream pH was similar for each site and year, varying between 5.5 - 6.8 over the course of the field season. Conductivity was similar at APO, BP, and LP, but slightly higher at BCO, suggesting some pollution at this latter site. Dissolved oxygen was usually close to saturation, but varied from 70.2 - 119.2%.

The patterns of larval seasonal succession are given in Figs. 8.8 - 8.10. EFG/C larvae were consistently the first to appear, occurring 1 - 5 weeks earlier than other cytotypes (Figs. 8.8 - 8.10). First instars collected from BCO on April 5, 1988 (stream temperature = 4°C), reared to last instar and cytotyped (n = 27), proved to be EFG/C. Peak larval density occurred in mid May but EFG/C was found as late as June 18. Only a single generation was found at either APO or BCO.

Last instar larval ACD and AA consistently appeared 2 - 4 weeks later than EFG/C (Figs. 8.8 and 8.9). During 1987, the summer generation(s) of ACD at BCO were greatly reduced and AA was not found. Only a single peak of high density was seen at APO for ACD and AA before stream conditions forced an end to sampling at this station. In 1988, ACD and AA were most plentiful after June at APO and BCO. Two - three peaks of high larval density were seen for both ACD and AA (Figs. 8.8 and 8.9). Interestingly, AA invariably appeared, though in low numbers, at both LP and BP, after CC2-3 and AC(gb) had emerged (Fig. 8.10). Last instar larvae of ACD and AA were collected from May 27 to September 16. When ACD and AA were sympatric,

patterns of abundance were similar with seasonal peaks often coinciding.

The pattern of seasonal succession for AC(gb) and CC2-3 at the LP and BP sampling stations is shown in Fig. 8.10. Although seasonal occurrence of AC(gb) and CC2-3 overlapped, AC(gb) was present in streams for a much shorter period of time. AC(gb) was found as early as May 20 and as late as June 18. CC2-3 was found from May 20 to July 9. Based on 113 male identifications from BP and LP, CC3 was present from May 28 to June 26, whereas CC2 was found May 20 to July 9. Only single generations of AC(gb) and CC2-3 were observed.

DISCUSSION

Evaluation of the sampling program

Mean sample errors, as indicated by %CI and CV were comparable to previous studies using a simple random design of similar sample size (e.g., Allan 1984). The additional time (1 - 2 h/site) required to implement a stratified design was not justified on the basis of reduced error.

Few last instars were lost from filaments during retrieval. Larvae found in the collecting net could have originated from the metal weights or surrounding benthos, as well as from filaments. Hence, the release rate of 3.6% is undoubtedly an overestimation, but lower than previous reports

(≈ 10%) for other artificial substrates (Rosenberg and Resh 1982).

In a recent review of the literature, Colbo (1987) concluded that artificial samplers were effective for monitoring the seasonal distribution of larval and pupal simuliids. With the exception of EFG/C at APO, the results of Table 8.2 support this conclusion. At present I have no explanation for the complete absence of EFG/C from rocks at APO during the spring of 1988. Disney (1972) suggested the extent to which a particular species exploits an artificial substrate may vary with stream conditions.

Filaments also collected large numbers of larvae which was not always true for natural substrates. For example, adequate numbers of larvae were taken from filaments at BP (1987, n = 238; 1988, n = 254) to establish the seasonality of CC2-3 and AC(gb), whereas few larvae (1988, n = 23) were found on stones. In addition, it has been my experience that fixation of live larvae produces the best chromosome preparations. At retrieval, filaments placed in culture tubes permitted easy storage and transportation of live larvae from field locations to the laboratory where fixative could be applied. In conclusion, filaments are recommended for sampling larval cytotypes of the S. venustum/verecundum complex because: i) filaments reflected population trends on natural substrates; ii) filaments were cheap and easy to produce; iii) few larvae released from filaments on retrieval; iv) few other

insects colonized filaments and; v) live larvae collected on filaments were easily stored and transported under field conditions. Evaluation of their effectiveness for other simuliids is encouraged.

Cytotype composition among stations and substrates

The results in Fig. 8.3 corroborate the conclusions of chapters 5 and 6, i.e., the cytotype fauna at sublacustrine locations is distinct from the fauna at sites further downstream. EFG/C, ACD, and AA were found to dominate the 2 sites near outlets (APO and BCO), whereas almost all larvae at the 2 sites further downstream (BP and LP) were CC2-3 and AC(gb).

Cytotype composition differed among substrates. Proportionally more ACD and AA were found on vegetation than on rocks, whereas the reverse was true for EFG/C. It was also noted that the density of a mixed ACD-AA population was usually higher on vegetation than on rocks. Microhabitat selection of EFG/C differed from ACD and AA not only in terms of water velocity, depth and orientation to outlets (chapter 7), but also in the choice of substrate. The preponderance of AA on vegetation is in agreement with both the findings in Chapter 5 (Table 5.8) and the studies of Adler and Kim (1986) and Ciborowksi and Adler (1990). In northern Quebec Wolfe and Peterson (1959) found that during July and August S. venustum/verecundum larvae (reported as S. venustum) were more

abundant on aquatic grasses than on stones. Judging by the time of year of this report it is likely that larvae referred to were largely S. verecundum complex (Adler 1986; Adler and Kim 1986; present study). In the S. vittatum complex, Adler and Kim (1984) showed substrate preferences between siblings at mixed sibling sites. These authors found larvae of IIIL-1 attached more commonly to rocks and IS-7 to grasses.

Seasonal succession of larval cytotypes

Species complexes investigated to date have shown that the seasonal occurrence of sympatric members often overlaps, although development is typically staggered and population peaks asynchronous (Adler 1987). In the present study a similar trend was observed. When EFG/C, ACD and AA were sympatric, the latter 2 simuliids invariably appeared after the peak abundance of EFG/C. In 1988, CC2-3 was present at APO in significant numbers and though synchronic with EFG/C, ACD and AA, development of cytotypes was staggered and population peaks asynchronous. Although AC(gb) and CC2-3 showed a great deal of seasonal overlap, AC(gb) occurred over a much shorter period of time.

Gordon and Cupp (1980) suggested that the seasonal sequence of cytotypes in New York state (CC, ACD, AA-AC) was driven largely by temperature. This does not appear to be the situation in Newfoundland as stream temperature varied considerably from year to year, yet cytotype occurrence

remained relatively consistent. For example, ACD and AA invariably appeared in the last week of May or the first week of June, regardless of the fact that mean weekly temperatures varied from 11.0 (range 7.5 - 15.0°C) - 19.0°C (15.5 - 22.5°C). Similarly CC2-3 or AC(gb) always appeared near the end of May despite the fact mean stream temperature varied from 10.0°C (5.5 - 16.0°C) - 18.0°C (11.5 - 23.0°C). The broad range of temperatures survived by EFG/C, ACD and AA (chapter 4) indicates differences in the seasonal distribution of these cytotypes were not due to specific temperature requirements of the larvae. Although temperature strongly influences the rate of development (chapter 4), it was apparently less influential in determining when on the temporal axis development begins.

Eggs of EFG/C probably commenced hatching by late March as first instars were abundant by early April. EFG/C was found to be a univoltine spring species in Newfoundland, which agrees with previous North American reports (Rothfels et al. 1978; Cupp and Gordon 1983). In contrast to the North American populations, overwintering S. truncatum (= EFG/C) does not hatch until mid June in Sweden (Carlsson et al. 1977), and in Finland a second summer generation develops (Wotton 1982).

In New Hampshire AC(gb) was reported to be bivoltine, with a late summer generation present in streams in August and September (Lake and Burger 1983). No evidence of a second generation was found at BCO, APO, BP, or LP. However, the

occurrence of AC(gb) in the same trickle on May 25 and July 21, 1988, (Appendix 1, site 8), suggested a second generation did occur at some sites. Similarly, no evidence was found for a second generation of CC2-3, whereas its presence in 37 out of 58 summer macrodistribution collections (Table 5.7; Fig. 5.3) clearly indicated this simuliid was bivoltine and possibly multivoltine. Classic CC2 males (IIS = CC, IIL-1 = s/i, IIIL-5 = ii, Rothfels et al. 1978) were collected as late as August 2 in the macrodistribution collections, confirming the suspicion of Rothfels et al. (1978) that this cytotype was bivoltine in Newfoundland. The occurrence of a few classic CC3 males (IIS = CC, IIL-1 = ss, si or ii, IIIL-1 = s/i, Rothfels 1981a) in mid July suggested this cytotype might have more than 1 generation. Alternatively it is quite possible these few CC3 males might have been univoltine individuals with delayed development.

Two possibilities would account for the discrepancy between the data presented here and Table 5.7: either a summer generation(s) of AC(gb) and CC2-3 was present at one or more of the phenology sampling stations but was not detected, or, only a single generation of AC(gb) and CC2-3 occurred at these sites. It seems very unlikely that with over 1400 cytological identifications of larvae from natural and artificial substrates, collected at 4 sites and over 2 field seasons, a second generation would not be detected. It seems much more likely that only a single generation of AC(gb) and CC2-3

occurred at the phenology sampling stations. This implies that within a limited geographic area, the number of generations can vary between streams, a situation previously noted for CC in Pennsylvania (Adler and Kim 1986).

ACD and AA appeared later in the season and remained longer than EFG/C, CC2-3 or AC(gb). Other authors have reported members of the verecundum line tend to occur later than most members of the venustum line (e.g., Cupp and Gordon 1983). The extended time ACD and AA were present in the field clearly showed these simuliids were multivoltine in Newfoundland. ACD and AA egg masses collected from vegetation in late June (chapter 4) further corroborates a multivoltine life history. AA and ACD have been reported as multivoltine in other areas of North America (Rothfels et al. 1978; Gordon and Cupp 1980; Cupp and Gordon 1983; Lake and Burger 1983; Adler 1986; Adler and Kim 1986). Reduced flow during 1987 may have contributed to the greatly diminished summer generation(s) of ACD at BCO (Jamnback 1976). In fact flow was sufficiently reduced during the summer of 1987 to preclude sampling at APO by the middle of July.

The life history of the venustum line of S. venustum/ verecundum complex has often been described as univoltine and that of the verecundum line as multivoltine (e.g., Adler 1987). Since 6 of the known 10 cytotypes of the venustum line have been reported to be bivoltine or multivoltine, at least under certain circumstances, (Rothfels et al. 1978; Wotton

1982; Lake and Burger 1983; Adler 1986; Adler and Kim 1986; present study) such generalizations are oversimplified.

SUMMARY

1. EFG/C appeared 1 - 5 weeks earlier than all other cytotypes. ACD and AA usually appeared later and remained in streams longer than EFG/C, AC(gb) or CC2-3. Although the seasonal occurrence of sympatric cytotypes often overlapped, development was typically staggered and population peaks asynchronous.

2. EFG/C is univoltine. AC(gb) may have a second generation at a few sites. CC2-3 has at least 2 generations per year in Newfoundland. ACD and AA are multivoltine.

3. Proportionally more ACD and AA were found on vegetation than rocks, whereas the reverse was true for EFG/C. The density of a mixed ACD-AA population was usually higher on vegetation than rocks.

4. The stratified random sampling design did not substantially reduce sampling error but probably yields more representative samples than a simple random sampling design.

5. Filaments are recommended as an artificial sampler.

TABLE 8.1. Comparisons of calculations of mean percent confidence intervals (%CI) and coefficients of variation (CV) for simple and stratified random sampling designs. Number of sample sets used for each comparison is given in parentheses.

	Filaments[+]		Rocks	Vegetation
	1987 (12)	1988 (8)	1988 (3)	1988 (5)
Mean %CI[++]				
Simple design	55.9%	31.5%	51.7%	92.0%
Stratified design	48.1%	28.2%	50.4%	87.7%
Difference	7.8%	3.3%	1.3%	4.3%
Mean CV				
Simple design	74.6%	42.1%	69.9%	74.1%
Stratified design	64.0%	37.9%	67.9%	70.6%
Difference	10.6%	4.2%	2.0%	3.5%

[+]For each substrate and year, results for simple and stratified designs were for the same data set. Calculations %CI and CV, however, followed procedures for simple and stratified designs.

[++]CI for each sample was expressed as a percent of the mean, i.e., $\%CI_i = (CI_i/mean_i) \times 100$.

TABLE 8.2. Correlation analysis between the seasonal log mean abundance of individual cytotypes on rocks, vegetation and filaments. Data were from the 1988 field season.

	Filaments* vs rocks	Filaments vs vegetation	Vegetation vs rocks
BCO (1988)**			
Total	0.894***	0.821***	0.708**
EFG/C	0.868***	0.958***	0.870***
ACD	0.661**	0.712***	0.643**
AA	0.476*	0.580*	0.512*
APO (1988)**			
Total	0.629**	-	-
EFG/C	0.000	-	-
ACD	0.637**	-	-
AA	0.846***	-	-
CC2-3	0.967***	-	-

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

*Too few larvae (n = 23) were collected from rocks at BP for a meaningful analysis, thus its omission here.

**n = 18, df = n - 2.

TABLE 8.3. Total number of individuals of each cytotype identified at the 4 phenology sites.

Cytotype*	Number identified	% of Total
EFG/C	359	25.1
ACD	308	21.6
AA	379	26.6
CC2-3	293	20.5
AC(gb)	89	6.2
	1428	

*Based on 134 male identifications (pooled from all sites) CC2-3 consisted of 78.4% CC2 (CI = 70.4 - 85.0%) and 21.6% CC3 (CI = 15.0 - 29.6%).

TABLE 8.4. Chi square analysis of cytotype composition among different substrates for the BCO collections, 1988.

	% Composition[+]				n[++]	X^2
	EFG/C	ACD	AM	CC -3		
Filaments	77.6%	12.1%	6.3%	4.1%	762	571.5***
Vegetation	65.8%	11.0%	15.4%	7.8%	1743	
Rocks	95.3%	0.8%	0.7%	3.3%	1339	

*** $p < 0.001$.

[+]% Composition based on 479 cytotyped larvae.

[++]Total number of last instar S. venustum/verecundum larvae collected.

TABLE 8.5. Comparisons of ACD-AA larval density on rock and vegetation substrates from BCO, 1988.

Week[+]	Mean No. larvae/m^2		t^{++} (df = 4)
	Rocks	Vegetation	
10 (July)	33.7 ± 83.9	1128.0 ±1083.0	-2.88*
11 (July)	35.0 ± 73.9	1468.0 ±2337.0	-2.62*
13 (July)	25.0 ± 66.1	569.0 ± 497.0	-5.40***
17 (Aug.)	115.0 ±356.0	586.0 ± 542.0	-2.99*
18 (Aug.)	23.3 ± 73.7	228.0 ± 405.0	-1.80

* $p < 0.05$, *** $p < 0.001$.

[+]Weeks were numbered in sequence with week 1 the last week in April and week 22 the last week in September (see Appendix 4 for further details).

[++]t-tests were performed on log (y + 1) tranformed data. Raw data have been presented to allow comparisons with previously published work.

TABLE 8.6. Mean (±SD) stream pH, conductivity and dissolved oxygen content at each sampling station for the 1987 and 1988 field seasons (early May to mid September). Readings were taken weekly at time of larval collection. Range of values are given in parentheses.

Site*		pH	Conductivity (μS/cm at 25°C)	Dissolved oxygen (%saturation)
BCO	1987	6.2 ±0.2 (5.7 - 6.6)	91.0 ±11.1 (65 - 105)	84.4 ±10.0 (72.0 - 111.1)
	1988	6.4 ±0.2 (6.0 - 6.6)	85.7 ±15.8 (46 - 105)	89.8 ±10.9 (74.0 - 114.4)
APO	1987	6.2 ±0.4 (6.0 - 6.6)	47.7 ±3.4 (46 - 55)	96.0 ±8.4 (74.0 - 113.0)
	1988	6.2 ±0.3 (5.7 - 6.7)	63.2 ±5.5 (52 - 72)	92.7 ±5.5 (79.8 - 100.8)
BP	1987	6.4 ±0.3 (5.9 - 6.8)	62.2 ±10.0 (41 - 75)	97.6 ±8.1 (81.9 - 119.2)
	1988	6.2 ±0.4 (5.[illegible] - 6.6)	68.0 ±5.8 (56 - 78)	94.6 ±5.8 (81.1 - 106.5)
LP	1987	6.1 ±0.3 (5.6 - 6.6)	59.8 ±10.4 (39 - 77)	95.4 ±7.6 (84.8 - 113.5)

*Sampling at APO was discontinued after the first of July during 1987.

FIG. 8.1. The Hogans Pond drainage basin showing location of the BCO sampling stations. This drainage basin was located 11 km N of St. John's, Newfoundland (47°34' - 47°37' N, 52°50' - 52°53' W).

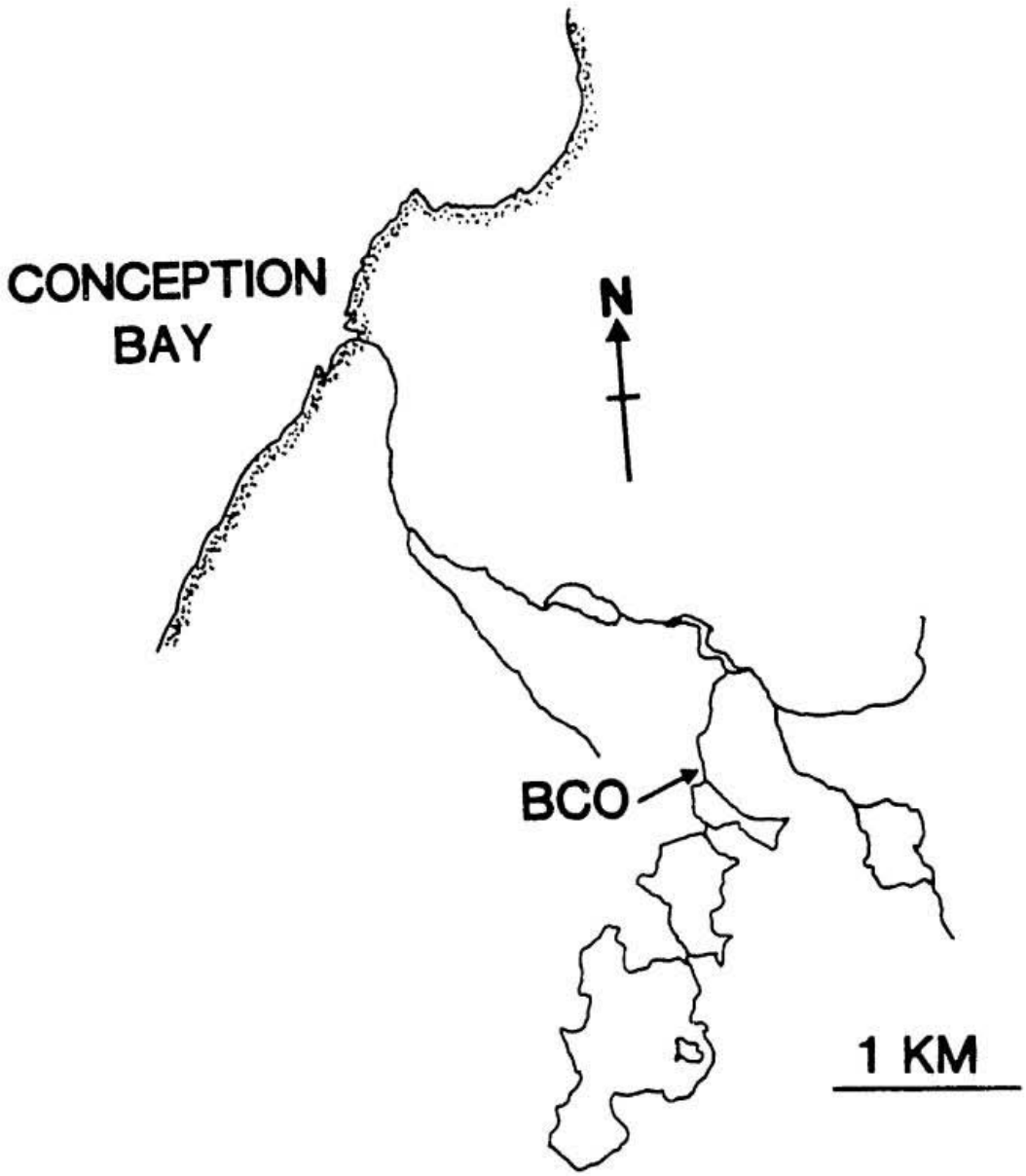

CONCEPTION
BAY
N
BCO
1 KM

FIG. 8.2. Filament artificial sampler (filament plus orange metal anchor) *in situ*. Note larvae attached to filament. Ceramic tile in background was used to provide contrast for photographic purposes.

FIG. 8.3. Percent cytotype composition at each of the 4 sampling stations for the 1987 and 1988 collections (early May to mid September). Composition was calculated separately for each site, year, and substrate.

LP = Little Piccos
BP = Big Piccos
AP = Axes Pond outlet
BC = Beachy Cove outlet
F = filament substrate
R = rock substrate
V = vegetation substrate

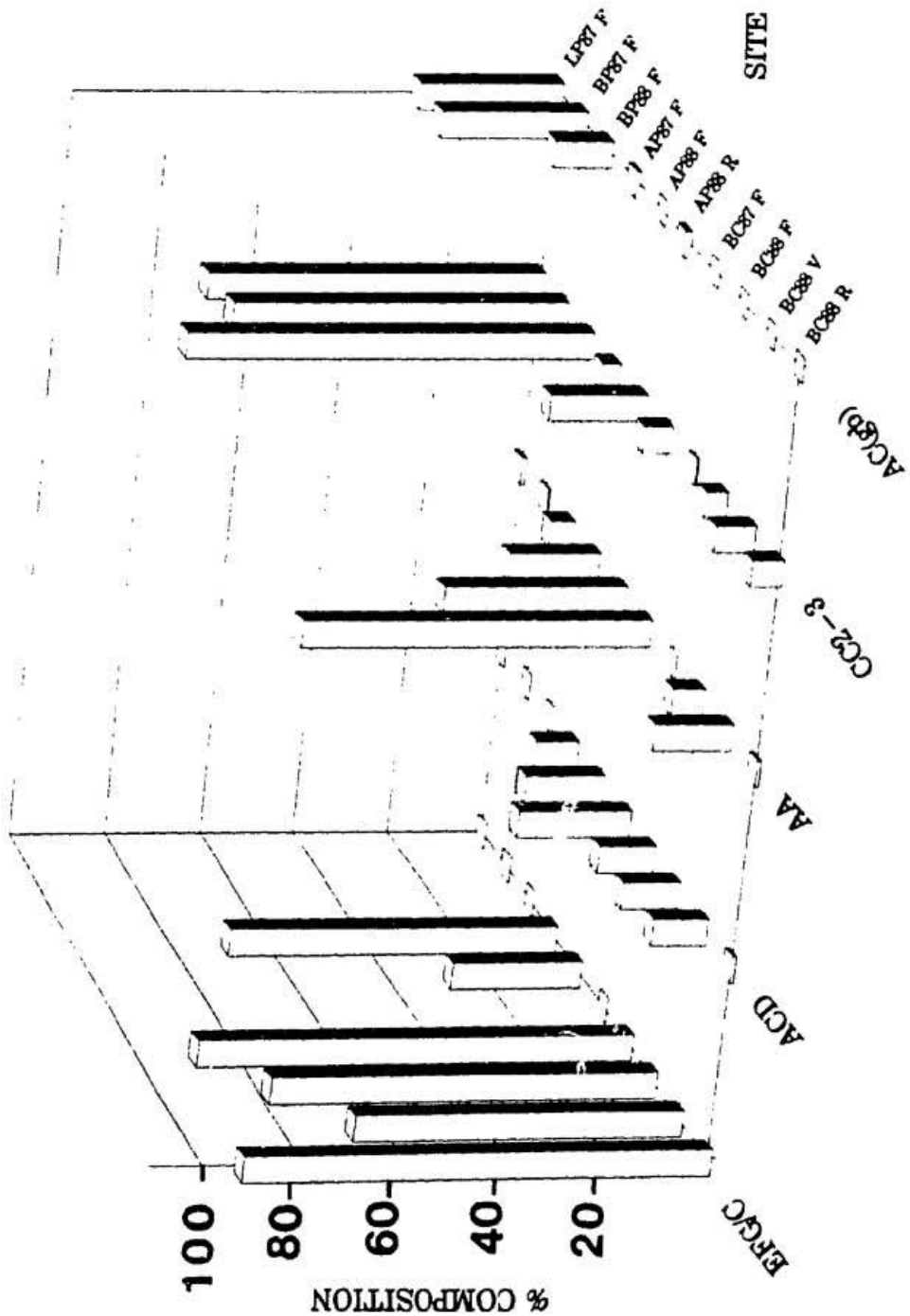

% COMPOSITION
100
80
60
40
20
ERGC
ACD
AA
CC2-3
AC(gb)
SITE
LP87 F
BP87 F
BP88 F
AP87 F
AP88 F
AP88 R
BC87 F
BC88 F
BC88 V
BC88 R

FIG. 8.4. Weekly variation in stream conditions at the Beachy Cove outlet (BCO) sampling station for 1987 and 1988. Readings were taken at time of larval collection. Velocity was measured 5 cm above the stream bed at the station midpoint. Seston was suspended material between 0.45 - 1000 μm. Temperatures are for the entire week up to the time of larval collection. Percent fast water was determined at time of stratification.

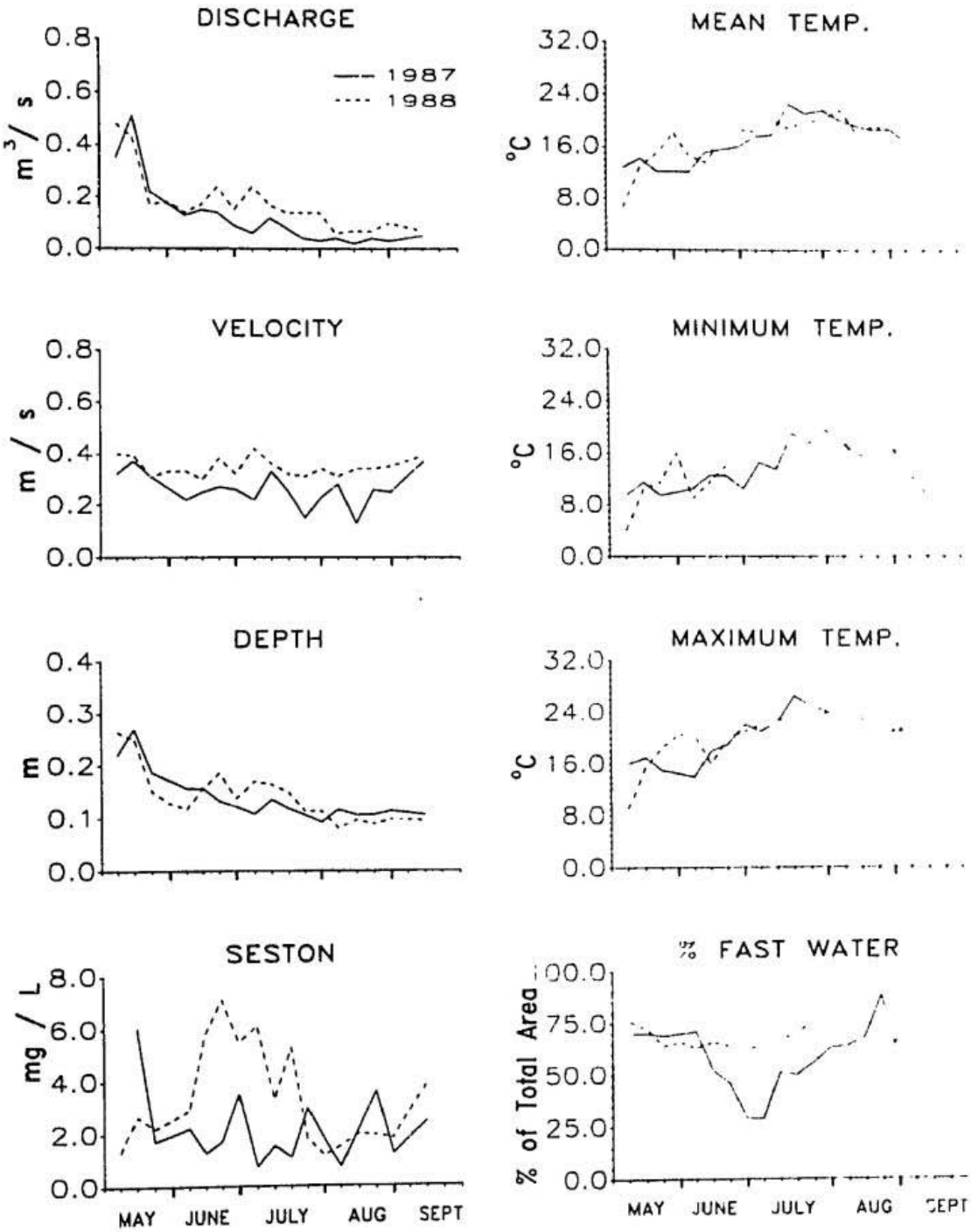

DISCHARGE
1987
1988
m³/s
0.8
0.6
0.4
0.2
0.0
MEAN TEMP.
°C
32.0
24.0
16.0
8.0
0.0
VELOCITY
m/s
MINIMUM TEMP.
DEPTH
m
0.4
0.3
0.2
0.1
MAXIMUM TEMP.
SESTON
mg/L
8.0
6.0
4.0
2.0
MAY
JUNE
JULY
AUG
SEPT
% FAST WATER
% of Total Area
100.0
75.0
50.0
25.0

FIG. 8.5. Weekly variation in stream conditions at the Axes Pond outlet (APO) sampling station for 1987 and 1988. Readings were taken at time of larval collection. Velocity was measured 5 cm above the stream bed at the station midpoint. Seston was suspended material between 0.45 - 1000 μm. Temperatures are for the entire week up to the time of larval collection. Percent fast water was determined at time of stratification.

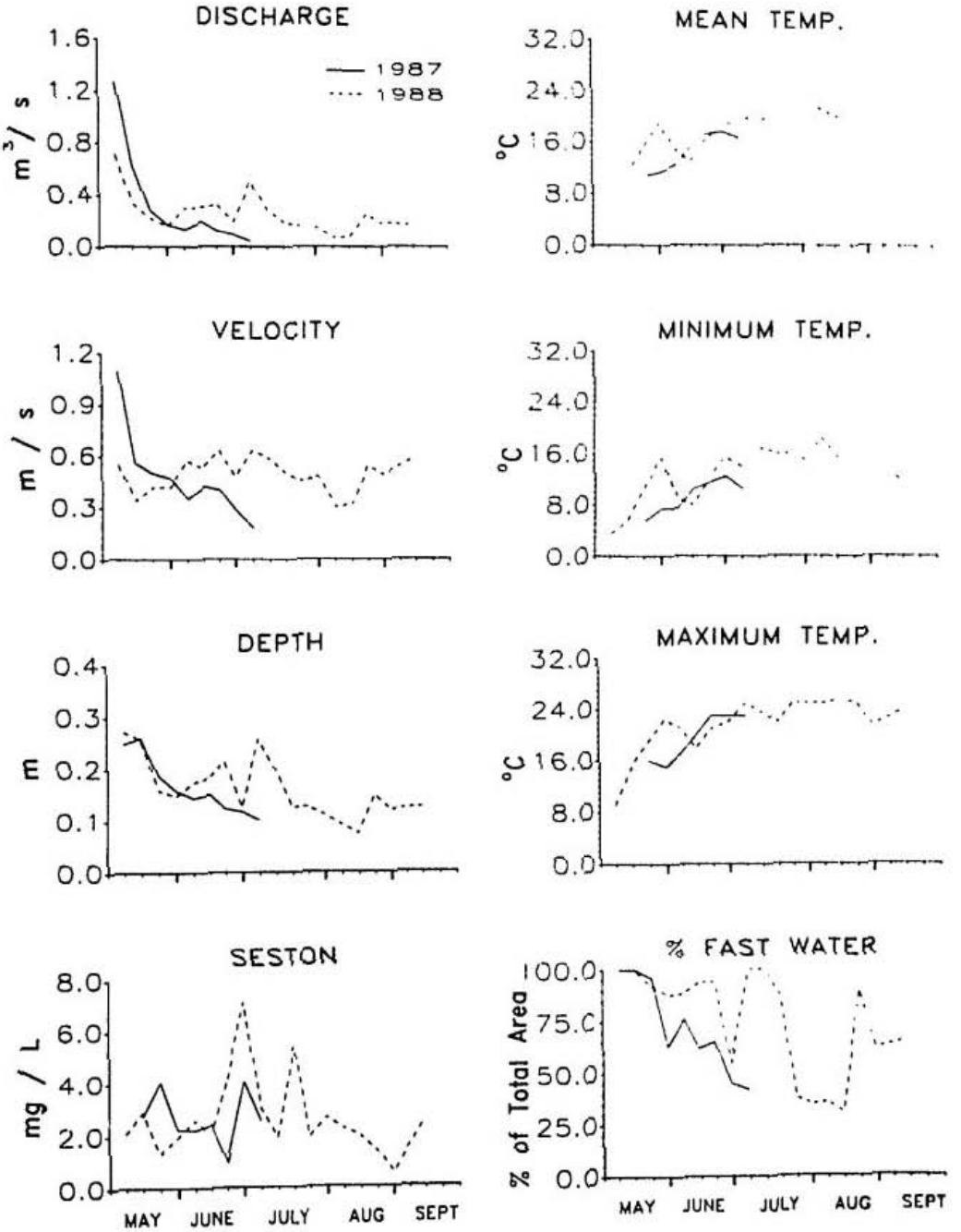

DISCHARGE
1987
1988
m³/s
1.6
1.2
0.8
0.4
0.0
MEAN TEMP.
°C
32.0
24.0
16.0
8.0
0.0
VELOCITY
m/s
1.2
0.9
0.6
0.3
0.0
MINIMUM TEMP.
°C
32.0
24.0
16.0
8.0
0.0
DEPTH
m
0.4
0.3
0.2
0.1
0.0
MAXIMUM TEMP.
°C
32.0
24.0
16.0
8.0
0.0
SESTON
mg/L
8.0
6.0
4.0
2.0
0.0
MAY
JUNE
JULY
AUG
SEPT
% FAST WATER
% of Total Area
100.0
75.0
50.0
25.0
0.0
MAY
JUNE
JULY
AUG
SEPT

FIG. 8.6. Weekly variation in stream conditions at the Big Piccos (BP) sampling station for 1987 and 1988. Readings were taken at time of larval collection. Velocity was measured 5 cm above the stream bed at the station midpoint. Seston was suspended material between 0.45 - 1000 μm. Temperatures are for the entire week up to the time of larval collection. Percent fast water was determined at time of stratification.

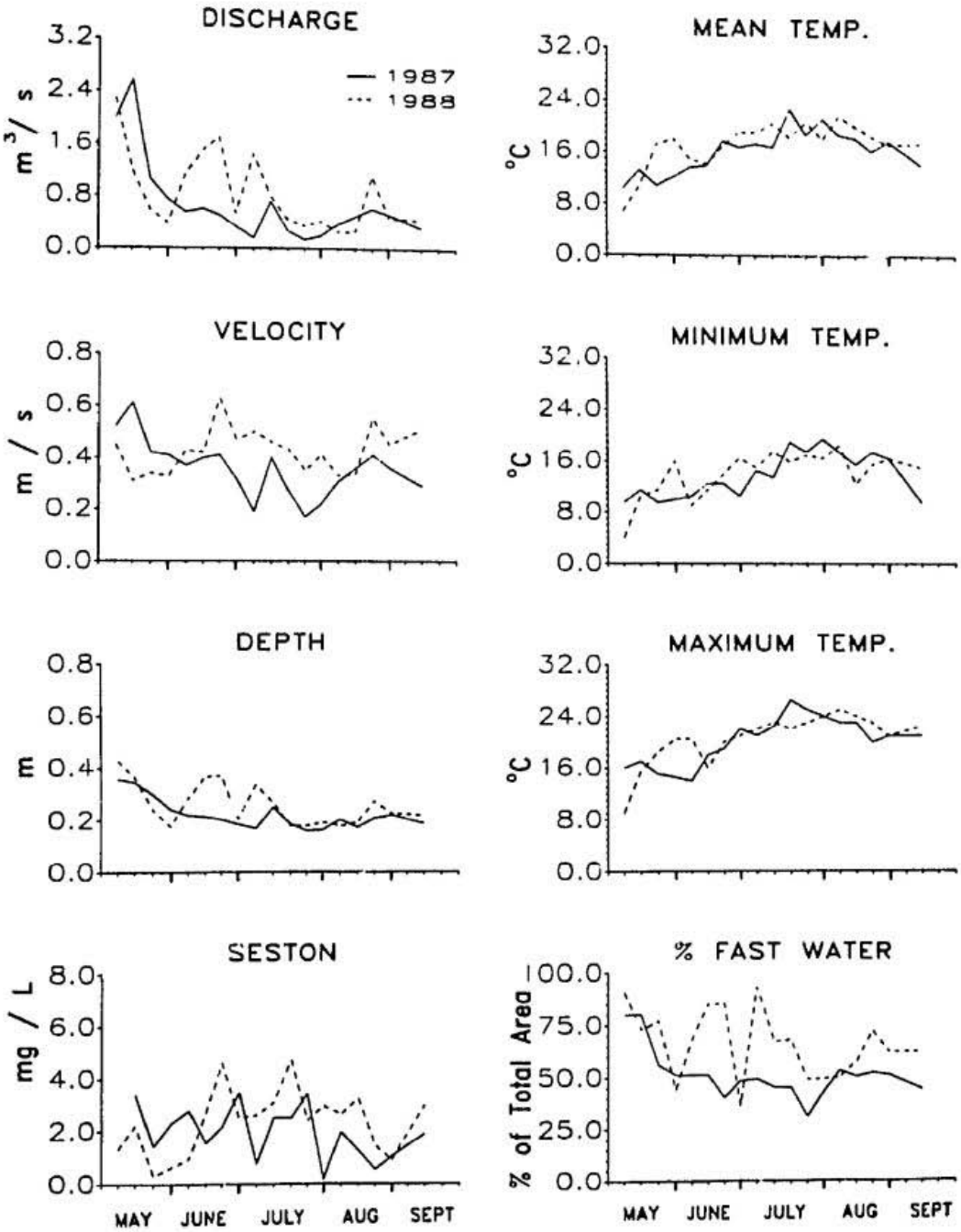

DISCHARGE
1987
1988
m³/s
3.2
2.4
1.6
0.8
0.0
MEAN TEMP.
°C
32.0
24.0
16.0
8.0
0.0
VELOCITY
m/s
0.8
0.6
0.4
0.2
0.0
MINIMUM TEMP.
°C
32.0
24.0
16.0
8.0
0.0
DEPTH
m
0.8
0.6
0.4
0.2
0.0
MAXIMUM TEMP.
°C
32.0
24.0
16.0
8.0
0.0
SESTON
mg/L
8.0
6.0
4.0
2.0
0.0
% FAST WATER
% of Total Area
100.0
75.0
50.0
25.0
0.0
MAY
JUNE
JULY
AUG
SEPT

FIG. 8.7. Weekly variation in stream conditions at the Little Piccos (LP) sampling station for 1987. Readings were taken at time of larval collection. Velocity was measured 5 cm above the stream bed at the station midpoint. Seston was suspended material between 0.45 - 1000 μm. Temperatures are for the entire week up to the time of larval collection. Percent fast water was determined at time of stratification.

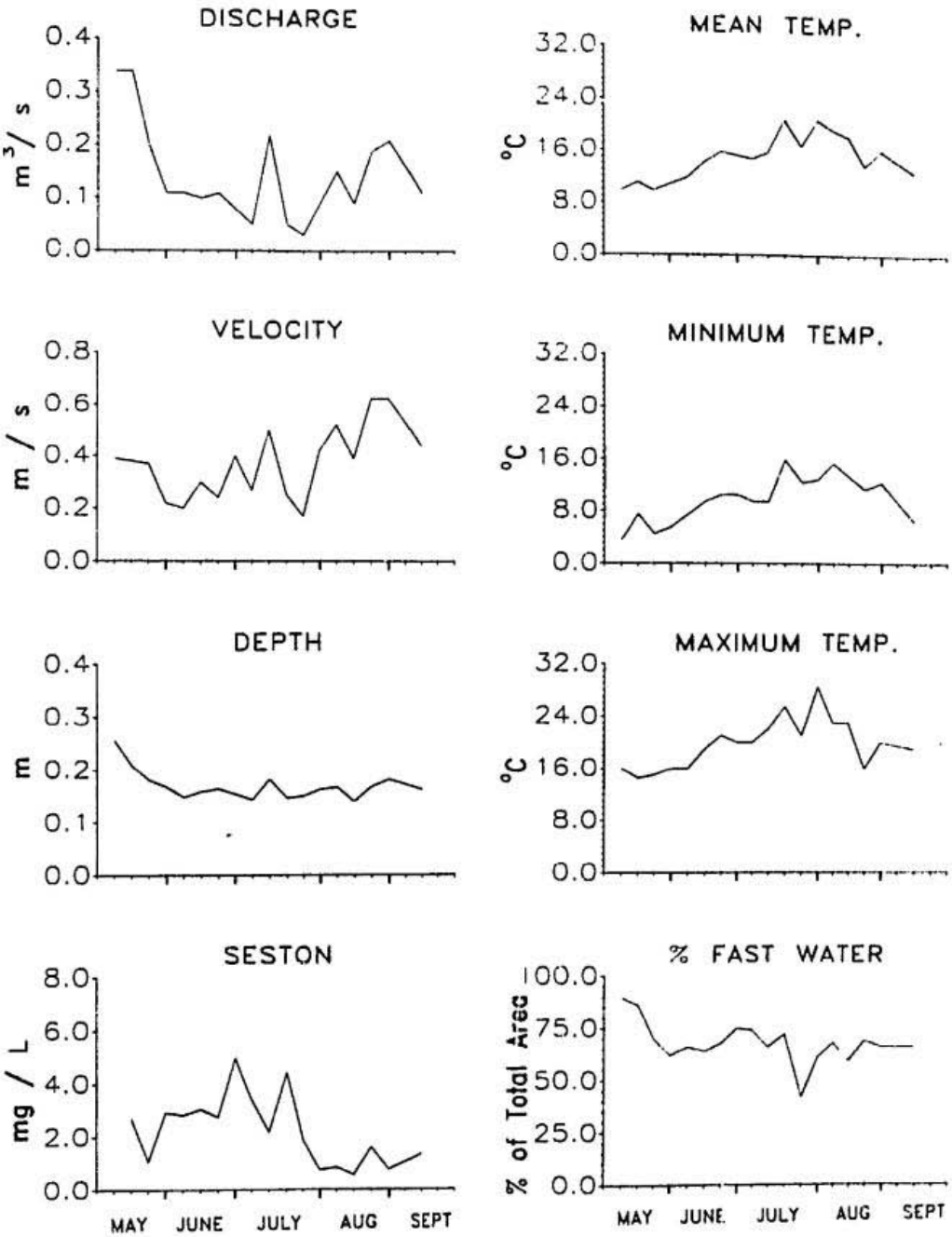

DISCHARGE
m³/s
0.4
0.3
0.2
0.1
0.0
MEAN TEMP.
°C
32.0
24.0
16.0
8.0
0.0
VELOCITY
m / s
0.8
0.6
0.4
0.2
0.0
MINIMUM TEMP.
°C
32.0
24.0
16.0
8.0
0.0
DEPTH
m
0.4
0.3
0.2
0.1
0.0
MAXIMUM TEMP.
°C
32.0
24.0
16.0
8.0
0.0
SESTON
mg / L
8.0
6.0
4.0
2.0
0.0
% FAST WATER
% of Total Area
100.0
75.0
50.0
25.0
0.0
MAY
JUNE
JULY
AUG
SEPT
MAY
JUNE
JULY
AUG
SEPT

FIG. 8.8 (continued on next leaf). Seasonal succession of last larval instar cytotypes at the Beachy Cove outlet (BCO) sampling station during the 1987 and 1988 field seasons. Open squares with a smaller black square shown on the date axis mark weeks samples were not taken.

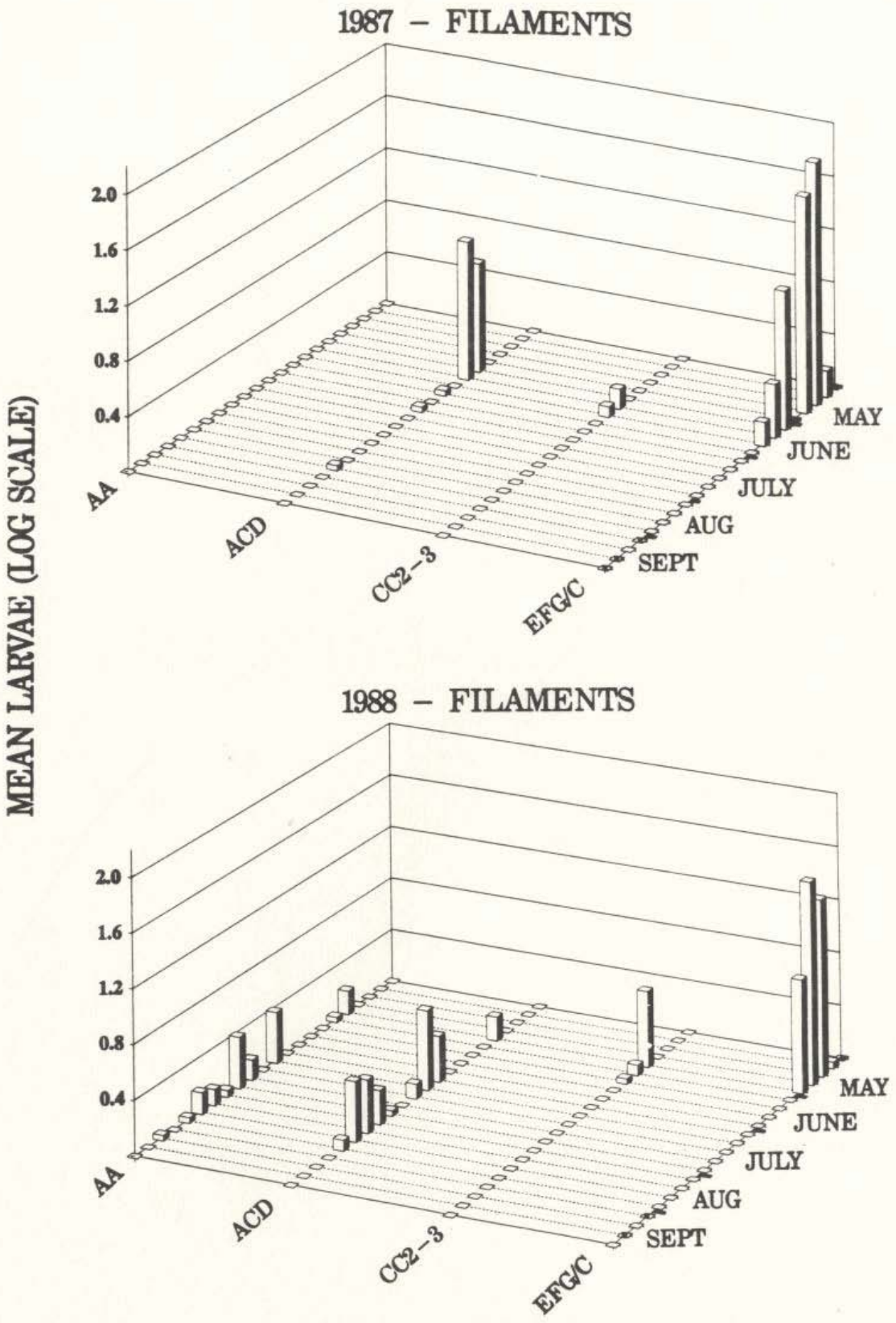

1987 – FILAMENTS
MEAN LARVAE (LOG SCALE)
2.0
1.6
1.2
0.8
0.4
AA
ACD
CC2–3
EFG/C
MAY
JUNE
JULY
AUG
SEPT
1988 – FILAMENTS
2.0
1.6
1.2
0.8
0.4
AA
ACD
CC2–3
EFG/C
MAY
JUNE
JULY
AUG
SEPT

FIG. 8.8 (continued). Open squares with a smaller black square shown on the date axis mark weeks samples were not taken.

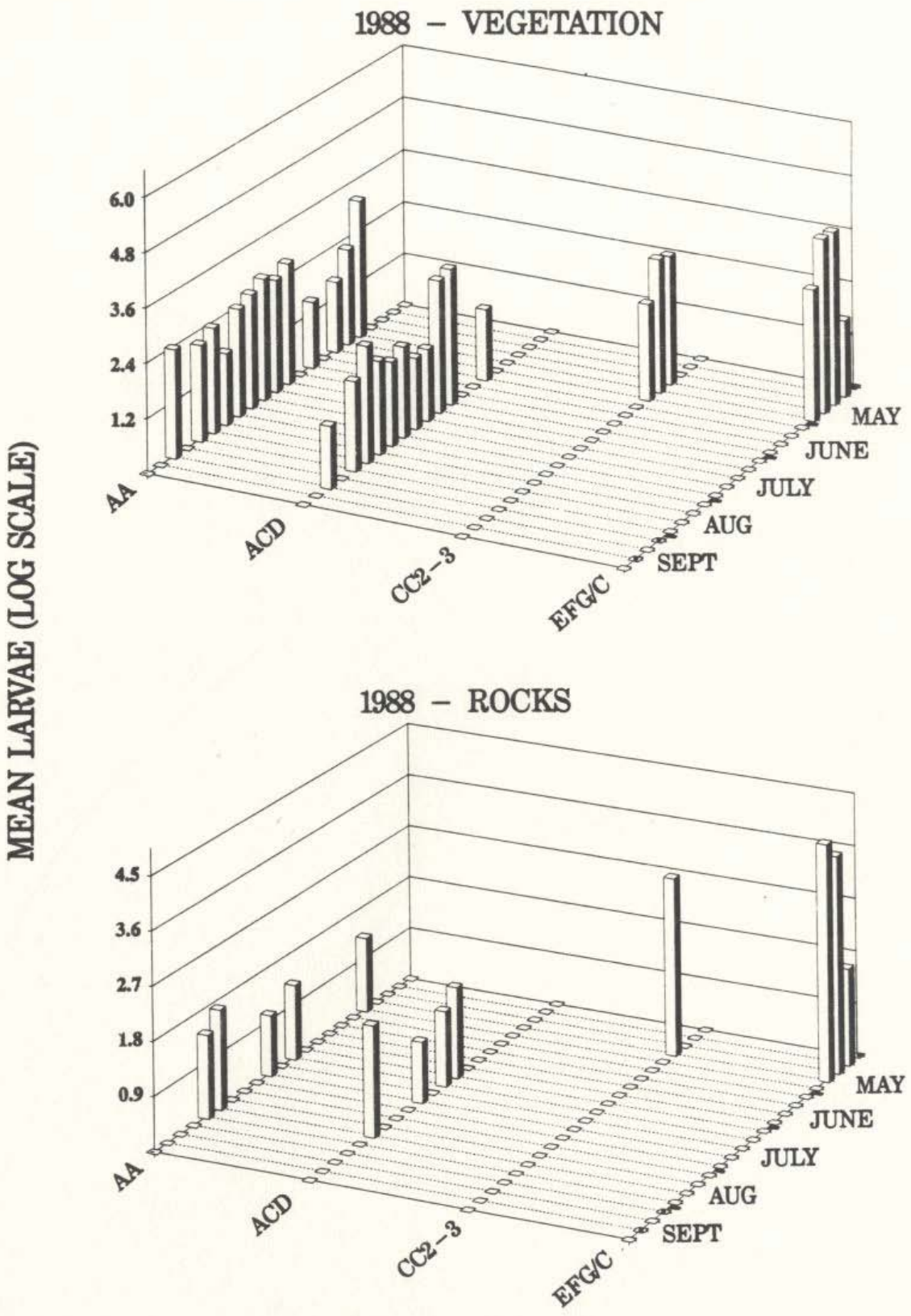

1988 – VEGETATION
MEAN LARVAE (LOG SCALE)
6.0
4.8
3.6
2.4
1.2
AA
ACD
CC2–3
EFG/C
MAY
JUNE
JULY
AUG
SEPT
1988 – ROCKS
4.5
3.6
2.7
1.8
0.9
AA
ACD
CC2–3
EFG/C
MAY
JUNE
JULY
AUG
SEPT

FIG. 8.9. Seasonal succession of last larval instar cytotypes at the Axes Pond outlet (APO) sampling station during the 1987 and 1988 field seasons. Open squares with a smaller black square shown on the date axis mark weeks samples were not taken.

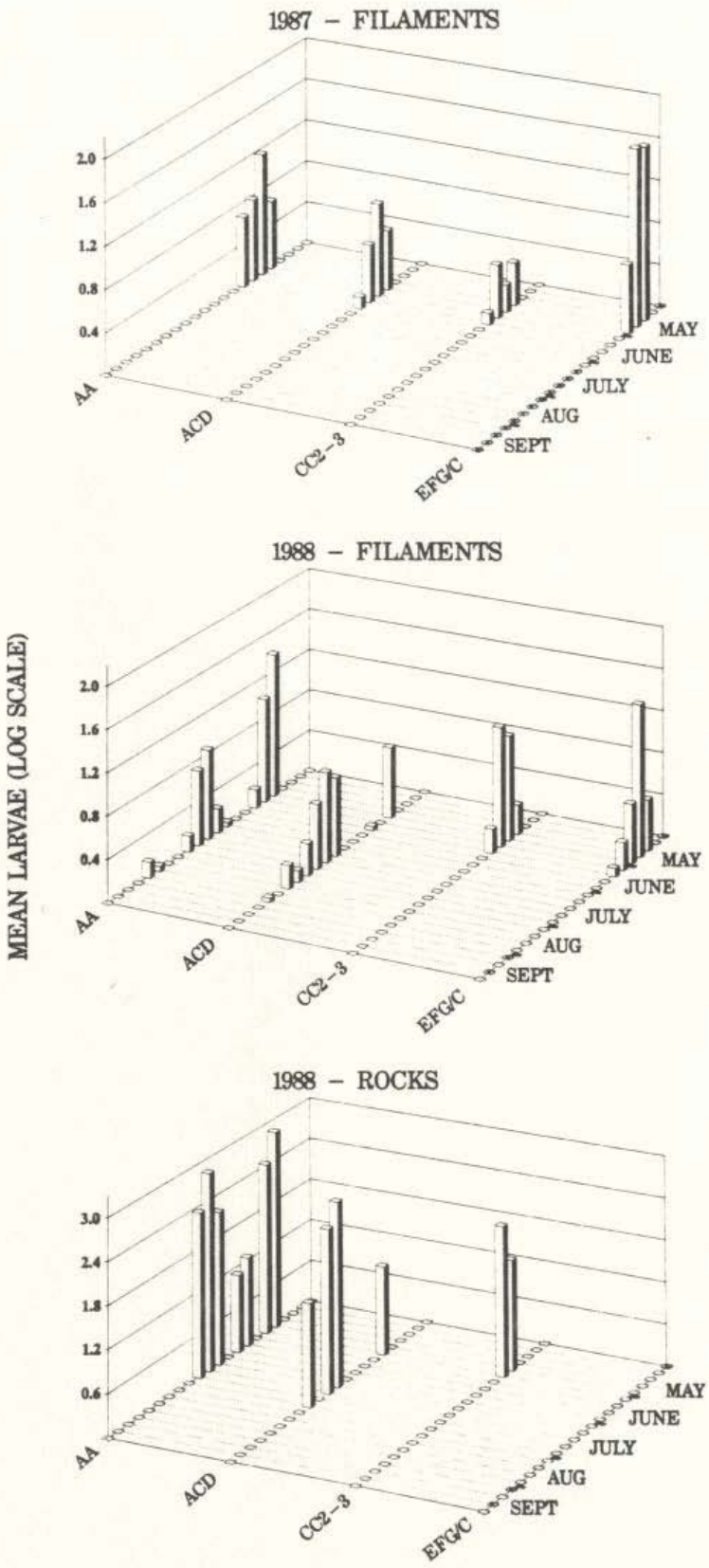
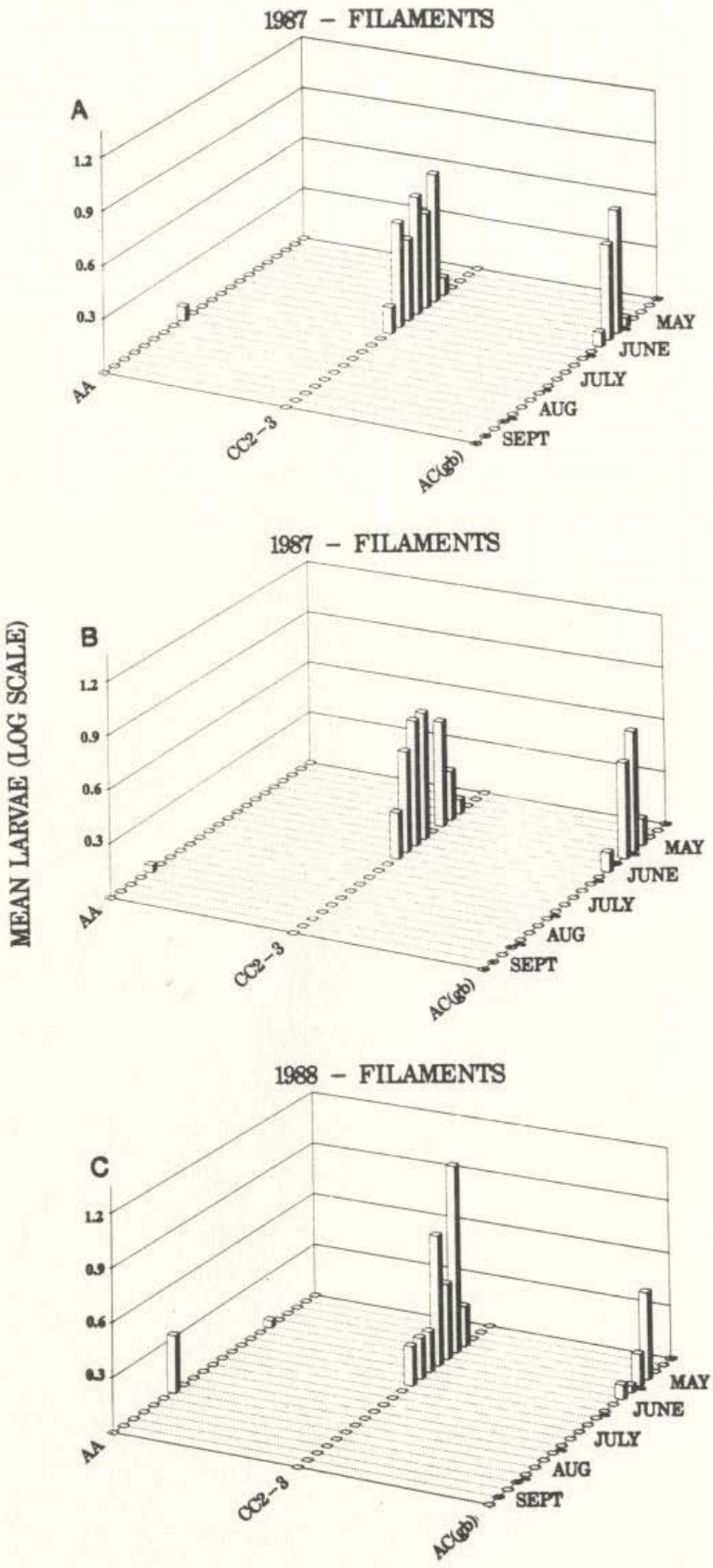

1987 – FILAMENTS
1988 – FILAMENTS
1988 – ROCKS
MEAN LARVAE (LOG SCALE)
2.0
1.6
1.2
0.8
0.4
3.0
2.4
1.8
0.6
AA
ACD
CC2–3
EFG/C
MAY
JUNE
JULY
AUG
SEPT

FIG. 8.10. Seasonal succession of last larval instar cytotypes at the Little Piccos (A) and Big Piccos (B and C) sampling stations during the 1987 and 1988 field seasons. Open squares with a smaller black square shown on the date axis mark weeks samples were not taken. Due to the low number of larvae (n = 23) collected from rocks at BP in 1988, results for these collections have been omitted.


1987 – FILAMENTS
A
1.2
0.9
0.6
0.3
AA
CC2–3
AC(gb)
MAY
JUNE
JULY
AUG
SEPT
1987 – FILAMENTS
MEAN LARVAE (LOG SCALE)
B
1.2
0.9
0.6
0.3
AA
CC2–3
AC(gb)
MAY
JUNE
JULY
AUG
SEPT
1988 – FILAMENTS
C
1.2
0.9
0.6
0.3
AA
CC2–3
AC(gb)
MAY
JUNE
JULY
AUG
SEPT

CHAPTER 9

ALLOMETRY IN LAST LARVAL INSTARS OF EFG/C AND ACD

INTRODUCTION

Biological studies have lagged far behind the rapid designation of simuliid cytotypes largely due to the difficulty in separating cytotypes using conventional morphological characters (e.g., Rothfels et al. 1978). As few simuliid ecologists are presently capable of cytotaxonomic identification the need for practical morphological characters is critical if cytotype ecology is to progress beyond the descriptive phase (Adler pers. comm. 1987; Feraday pers. comm. 1987). Most morphological characters of practical use have already been examined and Crosskey (1987) concluded 'the character-future appears to lie more in the morphometric study of old ones (characters) rather than in the discovery of new ones'. This same author pointed out that morphometrics has been the most neglected aspect of simuliid morphotaxonomy.

Recently, morphometric studies have attempted to use linear measurements and, of concern here, ratios of these measurements as variables in discriminant analysis for sibling separation (Adler 1983; Snyder and Linton 1983). Ratios have also been used as characters in traditional dichotomous keys of morphospecies (e.g., Stone 1964). However, in both

instances simple allometry of size was never considered.

It has been generally thought that ratios act as a means of scaling morphometric variables, thereby removing the effect of variation in general body size, which for blackfly larvae is largely a function of rearing conditions (e.g., Merritt et al. 1982). Where isometry exists between 2 measurements, proportionality of size is maintained despite different absolute body sizes, whereas allometry implies the ratio changes over the range of body sizes (Daly 1985). If ratios are not 'environmentally stable', i.e., if isometry is not the case, their use as taxonomic characters is questionable and can lead to erroneous conclusions (Janzon 1986).

This study tests the hypothesis that although linear measurements may vary with rearing conditions (Table 4.5), ratios of these characters remain stable and thus have taxonomic potential. Morphometric data from laboratory reared EFG/C and ACD (chapter 4) and wild caught EFG/C (chapter 7) were used to test this hypothesis.

MATERIALS AND METHODS

Head capsule measurements from the temperature study (chapter 4) for last instar EFG/C (10 - 30°C) and ACD (10 - 25°C) and wild EFG/C from the microdistribution study (chapter 7) were used for analysis. The 6 ratios that can be calculated

from the 4 linear head capsule measurements (Fig. 2.1) were subjected to morphometric analysis to determine if allometry existed. The allometric equation:

[1] log y = alogx + logb

is a straight line where y and x are linear measurements, a = the slope and logb = intercept (Gould 1966). If isometry is present, a = 1, otherwise allometry is indicated. Regression equations were computed (all temperatures combined) for each sibling, using both principal axis regression (PA) and reduced major axis regression (RMA). Isometry was not rejected if the value of 1 fell within the 95% confidence limits for slope. Correlations between head capsule measurements were also calculated.

RESULTS

Results of correlation and regression analyses are shown in Table 9.1. A high degree of correlation existed between the size of head capsule characters. In only 2 cases did PA and RMA reach different conclusions. In detail, RMA showed all 6 ratios of ACD were isometric over the range of experimental temperatures, however, the CAW/HCL ratio is suspect due to a low correlation between these two characters and rejection of

isometry by PA. Only 2 ratios showed isometry for laboratory reared EFG/C (PAW/HCL and HCL/HCW). Results for laboratory reared larvae did not change appreciably when the analysis (not shown) was restricted to temperatures (10 - 20°C) more typical of boreal streams. Isometry could not be rejected for 5 of the 6 ratios examined for wild caught EFG/C larvae.

DISCUSSION

A high degree of correlation was found between the size of head capsule characters in contrast to other species (Colbo and Okaeme 1988; Colbo 1989). Although linear head capsule measurements were sensitive to temperature (Table 4.5), model II regression analysis suggested that in many cases the ratios between these measurements were temperature stable as indicated by the acceptance of isometry (Table 9.1).

Most notably, RMA showed all 6 ratios of ACD were isometric over the range of experimental temperatures. However, only 2 ratios showed isometry for laboratory reared EFG/C (PAW/HCL and HCL/HCW). This suggested that head capsule shape of EFG/C is much more variable in response to temperature than ACD, with the implication that shape is a poor taxonomic character.

Isometry could not be rejected for 5 of the 6 ratios examined for wild caught EFG/C larvae which is in contrast to

the laboratory reared larvae. Allometry of size may indicate that a population is a composite of 2 or more distinct populations (Simpson et al. 1960), which could account for the above results. Laboratory reared EFG/C were treated as a single group, but could be considered a collection of populations, each one defined by its rearing temperature. Wild EFG/C came from a single collection with individual larvae exposed to the same environmental conditions, thus forming a homogeneous population. Accordingly, analysis of a population based on a single collection may not be sufficient to fully evaluate disproportionate changes in size.

Colbo (1989) showed that certain head capsule measurements in Simulium vittatum Zetterstedt did not remain stable, thus vitiating their taxonomic value. As shown by the above analysis, a ratio between varying linear measurements may not always constitute a stable third character. Jedlicka (1978) showed that both head capsule width and cephalic apotome width (referred to as the frons-clypeus width) in Odagmia ornata (Meigen) and O. spinulosa (Doby and Deblock) show a high degree of variability between sites and generations and he suggested that temperature was a primary cause of this variation. He concluded that the application of a character for taxonomic purposes without an examination of spatial and temporal variability was questionable. Similar problems have been noted with other insects. For example, Janzon (1986) found that about 60% of the ratios used to

separate species of adult Pteromalus (Chalcidoidea) produced erroneous results due to allometry. However, once detected such ratios could be corrected to produce useful characters in many cases. To my knowledge, no one has critically examined how allometry affects the usefulness of ratios as taxonomic characters for simuliid sibling separation.

Although the detection of simple allometry of size is relatively easy, the most widely accepted Model II regression procedures for morphometric analysis (PA and RMA) are not found in some of the more commonly used statistical packages. Therefore, a Minitab macro was written to perform the essentials of model II regression required for allometric detection (Appendix 5).

SUMMARY

1. Data from larval head capsule measurements were used to determine allometry of size for final instar EFG/C and ACD. Measurements were evaluated using principal axis and reduced major axis regression.

2. Isometry of size was accepted for only some ratios and it is argued that a character's 'environmental stability' should be assessed before it is considered as a taxonomic character.

TABLE 9.1. Slope and 95% confidence limits for principal axis (PA) and reduced major axis (RMA) regression lines of head capsule measurements for laboratory reared EFG/C (10 - 30°C) and ACD (10 - 25°C) and field collected EFG/C.

	Variables* regressed	PA slope	Confidence Limits Lower	Upper	RMA slope	Confidence Limits Lower	Upper	r**
EFG/C (reared) (n = 120)	CAW/HCW	2.104	1.638	2.834	1.560	1.324	1.794	0.563
	CAW/PAW	1.844	1.364	2.646	1.373	1.156	1.590	0.495
	CAW/HCL	1.819	1.411	2.441	1.419	1.205	1.633	0.563
	PAW/HCW	1.156	1.046	1.279	1.136	1.036	1.235	0.877
	PAW/HCL	1.047	0.880	1.247	1.034	0.904	1.163	0.726
	HCL/HCW	1.130	0.971	1.318	1.099	0.971	1.227	0.769
EFG/C (wild) (n = 87)	CAW/HCW	1.243	0.946	1.661	1.144	0.950	1.338	0.618
	CAW/PAW	0.894	0.434	1.740	0.963	0.767	1.158	0.338
	CAW/HCL	1.332	0.948	1.939	1.166	0.953	1.380	0.532
	PAW/HCW	1.261	1.040	1.542	1.188	1.017	1.360	0.743
	PAW/HCL	1.407	1.025	2.000	1.212	0.994	1.429	0.555
	HCL/HCW	0.971	0.750	1.255	0.981	0.820	1.141	0.651
ACD (n = 64)	CAW/HCW	1.250	0.890	1.806	1.143	0.911	1.377	0.598
	CAW/PAW	1.150	0.739	1.844	1.074	0.840	1.310	0.510
	CAW/HCL	2.279	1.057	14.462	1.293	0.978	1.607	0.282
	PAW/HCW	1.074	0.934	1.236	1.065	0.935	1.194	0.877
	PAW/HCL	1.361	0.970	1.984	1.203	0.958	1.449	0.695
	HCL/HCW	0.817	0.569	1.144	0.885	0.706	1.064	0.604

*CAW = cephalic apotome width, HCW = head capsule width, PAW = postantennal buttress width, HCL = head capsule length.

**All r values were significant at $p < 0.001$ with $df = n - 2$, except $r = 0.282$ and $r = 0.338$ which were significant at $p < 0.01$.

CHAPTER 10

CONCLUDING DISCUSSION AND SUMMARY

The present study has emphasised the influence of abiotic factors on preimaginal development and distribution, although some evidence was presented for competitive interactions (chapter 6). The emphasis on abiotic factors is justified because numerous studies have shown that the lotic community is largely structured by locally operating abiotic factors (e.g., Lake et al. 1988). In addition, many biotic interactions between species in lotic systems, such as competition, may be mediated by abiotic factors (e.g., Hemphill and Cooper 1983). Nevertheless, future attention should be paid to biotic interactions among siblings and between siblings and other macroinvertebrates. Data on biotic interactions will be crucial in determining if interspecific differences in spatial and temporal distribution indeed represent habitat partitioning in response to limited resources.

Rothfels provided strong evidence that many S. venustum/ verecundum cytotypes were reproductively isolated and biologically distinct sibling species (Rothfels et al. 1978; Rothfels 1981a). The results presented here provided the complementary ecological evidence to support the distinct species status of the 6 cytotypes (EFG/C, CC2, CC3, AC(gb),

ACD, AA) found on the Avalon Peninsula, i.e., each cytotype examined was in fact a cytospecies. Spatial-temporal data showed each cytospecies had a unique ecological profile, therefore the null hypothesis of chapter 1 is rejected.

Differences among the ecological profiles of each cytospecies are well illustrated by simultaneously comparing differences along gross spatial (stream type) and gross temporal (months) axes. Spatial-temporal profiles were drawn (Fig. 10.1) assuming that: i) cytospecies distribution in the macrodistribution collections (chapter 5) was indicative of the mean percent occurrence among stream types and; ii) filament collections at the phenology sampling stations (chapter 8) reflected cytospecies seasonality.

Results of the present study are significant for several reasons. First, in North America previous studies on simuliid sibling ecology have either been: i) restricted to 1 or 2 sibling species (e.g., Adler and Kim 1984); ii) focused on a particular aspect of ecology such as phenology (e.g., Lake and Burger 1983) or macrodistribution (Gordon and Cupp 1980) or; iii) faunistic surveys consisting primarily of collection records with limited ecological information (e.g., Cupp and Gordon 1983; Adler and Kim 1986).

The present study is the first broad-based ecological investigation of 6 sympatric cytospecies occurring within a limited geographic area. Differences among cytospecies were found in: i) physiology (i.e., response to temperature); ii)

habitat selection on both the macrohabitat (stream type, along a stream continuum) and microhabitat (velocity, depth and substrate preferences) scale; iii) temporal segregation (seasonality, voltinism) and; iv) morphology (allometry of size). Future studies must take into account the unique ecologies of individual cytospecies. Clearly we are approaching the limit of useful ecological insight that can be gained from research centred on the species complex rather than the cytospecies. Although the logistics of cytotaxonomy greatly reduce the number of individuals that can be identified, it is my opinion that each investigator should at least be aware of which cytospecies constitute their study populations. This is of particular importance for the S. venustum/verecundum complex in Newfoundland where most sites supported mixed sibling populations.

Another significant finding of the current study was that the cytospecies fauna can change substantially over relatively small distances. Although cytotype composition is known to change along a stream continuum (e.g., S. tuberosum complex), these changes usually occur over a scale measured in kilometers (Adler 1987). However, in the present study it was found that the cytospecies assemblage of the S. venustum/ verecundum complex at lake outlets may be very different from the cytospecies assemblage as little as 100 m downstream. Even within a specific habitat (i.e., a 10 - 20 m section of stream) it was concluded that cytospecies composition varied

between different substrates, depths and velocities.

The findings presented here also support Rothfel's supposition that most cytologically defined members of the S. venustum/verecundum complex are valid species (Rothfels et al. 1978; Rothfels 1981a). Each cytospecies on the Avalon was a distinct species with a unique spatial-temporal distribution (Fig. 10.1), and it seems reasonable to assume other members of the complex will likewise prove to be distinct species with characteristic ecological profiles. Although some synonymy will likely occur (e.g., AA verecundum = A/C verecundum = CC verecundum) the current trend to lump members of the venustum line for convenience (e.g., Currie pers. comm. 1990) is not justified in light of my findings.

Finally, conclusions reached here may have significant ramifications for the Nematocera (lower flies) in general. Cytological studies of mosquitoes (Culicidae) and midges (Chironomidae), sister families to simuliids, have also revealed the existence of sibling species. If sibling complexes are widespread throughout the Nematocera, and, as suggested here, each sibling has a characteristic spatial-temporal distribution, ecological data based on the morphospecies, with no consideration of siblings, would have limited value. As is the case for simuliids, re-evaluation of taxonomic, behavioural, physiological and ecological data for other Nematocera may also be necessary. The species is the basic unit of ecology (Krebs 1985) and without a clear

perception of what this means, serious questions also arise about our understanding of community structure.

CYTOSPECIES LIFE HISTORY - A SYNOPSIS

EFG/C

EFG/C is a sublacustrine species which was rarely found more than 500 m from an outlet. Its decreasing contribution (percent) to the cytospecies fauna with increased distance from an outlet (10 - 1000 m) was highly predictable. At the level of the microhabitat, this cytospecies was most abundant in shallow (optimal depth $\leq$ 0.05 m), slow (optimal velocity = 0.36 m/s) water and at a position slightly removed (ca. 10 -15 m) from the outlet. Pupae did not select a microhabitat different from that of last larval instars.

Eggs of EFG/C commenced hatching by late March with last instar larvae consistently appearing 1 - 5 weeks earlier than other cytospecies. EFG/C, a univoltine spring species, was most abundant in May. Larvae were found from May 5 to June 20, with a single specimen collected on July 13. This latter larva is not indicative of a second generation but was most likely a delayed or diseased individual.

Laboratory experiments showed that larval EFG/C had a high tolerance to warm water (25 - 30°C) which could be an adaptation to the unpredictable and wide temperature

fluctuations characteristic of the spring climate on the Avalon Peninsula. It was also noted that as temperature changed so did aspects of the reproductive life history, i.e., size (and presumably fecundity) was maximized at 10 - 15°C, survival was highest at 15 - 20°C, and development was fastest at 25 - 30°C. Threshold temperature of development was 0°C. Males required significantly less time to reach the last instar than females; however, development time from the prepupal stage to adult emergence did not differ between the sexes.

AC(gb)

AC(gb) was a downstream cytospecies. This species showed no significant differences in frequency of occurrence among trickle, small stream, or large stream sites. Nevertheless, AC(gb) was most frequently found in cool, canopied streams, where the stream bed consisted mainly of small stones and/or rubble and a wide variety of larval substrates were available. A comparison of sites between 0.9 and 7.4 m wide showed larval abundance tended to increase with increasing stream width. This in turn may have been related to the food supply. At most locations only a single generation of AC(gb) occurred per year, with larvae most abundant in late May and early June. A second generation sporadically occurred at a few sites.

CC2 and CC3

CC2-3 was the most commonly encountered blackfly at downstream locations. Its increasing contribution (percent) to the cytospecies fauna with increased distance from an outlet (10 - 1000 m) was predictable. Larvae showed no preference for different downstream (T, S, L) sites. Larval abundance at downstream sites was highest at those sites with a high food (quality/quantity) supply and low conductivity. CC2-3 larvae were found in streams from May 20 to Aug. 2.

Based on male identifications, CC2 showed no preference for T, S or L sites and can be considered a habitat generalist within downstream locations. In Newfoundland, CC2 has one generation per year at some sites and at least a second generation at others. What determines the number of generations at a site is at present unknown. Larvae were found from May 20 to Aug. 2 and it is likely that individuals persist in streams throughout August.

Based on male identifications, CC3 was most frequently found in large streams. CC3 is most likely a univoltine cytospecies, or (similar to AC(gb)) a species which has a very limited second generation in a small number of streams. Larvae were found from May 21 to July 15.

AA

AA was most often found at lake outlets and trickles. Eggs of the summer generation(s) were deposited on emergent

and trailing vegetation which would account for the larval preponderance on this substrate. Adults of the last summer generation oviposit eggs directly into the stream where they sink to the stream bottom. Larval distribution of the summer generation(s) was influenced by the availability of vegetation for ovipositional sites and/or larval substrate. It was concluded that the distribution of the spring generation was, in part, the consequence of females from the fall population preferentially ovipositing at sublacustrine locations.

AA has at least 2 and probably 3+ generations per year in Newfoundland. This species was most abundant in streams after May, but was found from May 21 to Sept. 16. Cytological evidence suggested that the normal dispersal range of adult females was less than 12 km.

Laboratory experiments showed that larvae could tolerate temperatures of 10 - 25°C. Sex did not influence the rate of development.

ACD

Larval ACD is a sublacustrine species although it can be found at other locations. At the level of the microhabitat, this cytospecies was most abundant in fast water (optimal velocity = 0.69 - 0.73 m/s). Depth did not influence microhabitat selection. Pupae did not appear to move to a different microhabitat in order to pupate.

ACD has at least 2 and probably 3+ generations per year

in Newfoundland. Larvae were found from May 21 to Sept. 16 but were most abundant in streams after May. Adult females of the summer generation(s) oviposited eggs in masses on trailing and emergent vegetation. Adults of the last summer generation oviposit eggs directly into the stream where they sink to the stream bottom.

Laboratory experiments showed that larvae could tolerate a wide range of temperatures (5 - 25°C). Sex did not influence the rate of development.

FIG. 10.1 (continued on next leaf). Ecological profiles based on gross spatial (stream type) and gross temporal (months) differences. Due to the uncertainty regarding the seasonality of CC2 and CC3, these cytospecies have been omitted.

O = outlet site
T = trickle site
S = small stream site
L = large stream site

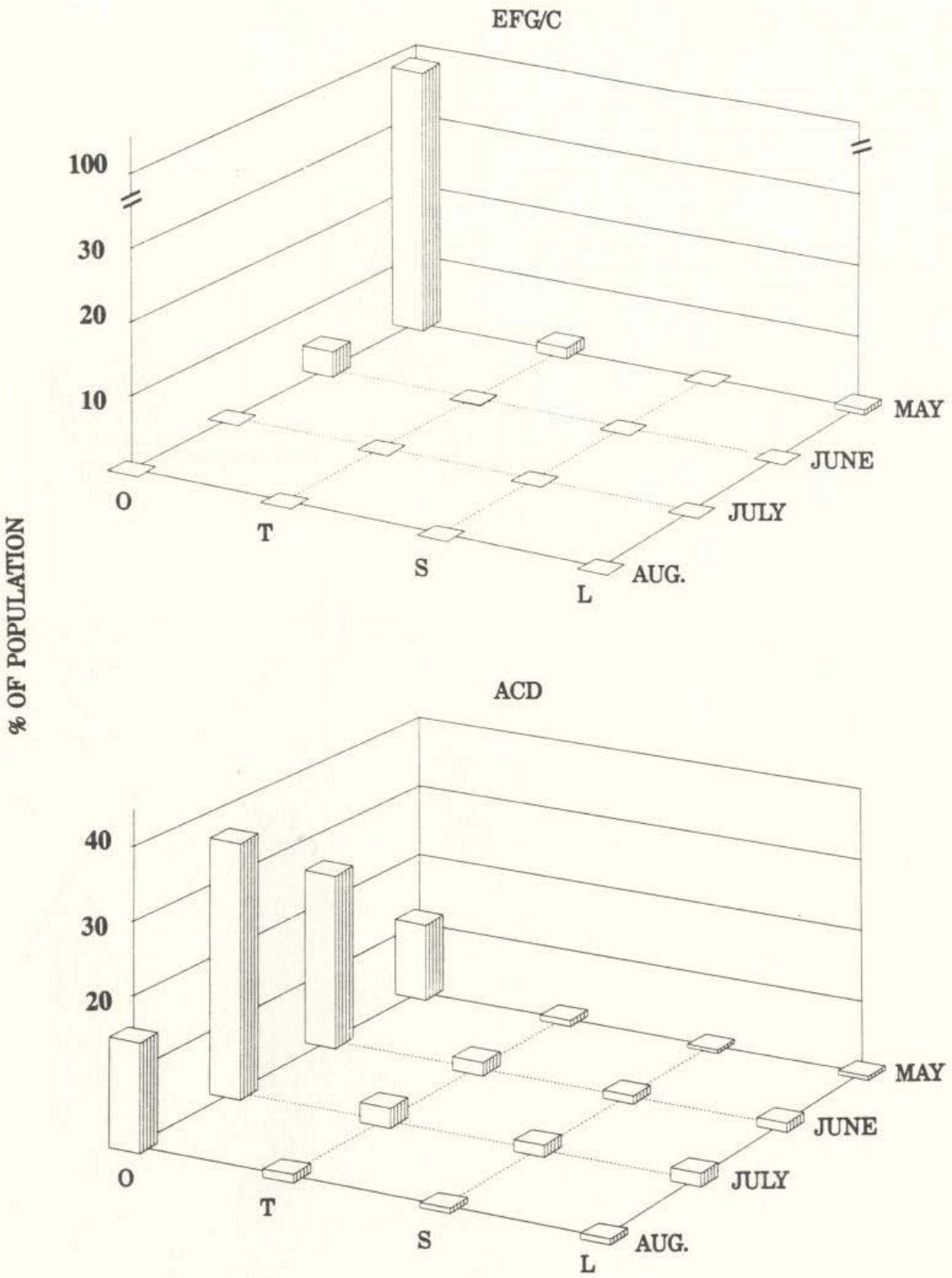
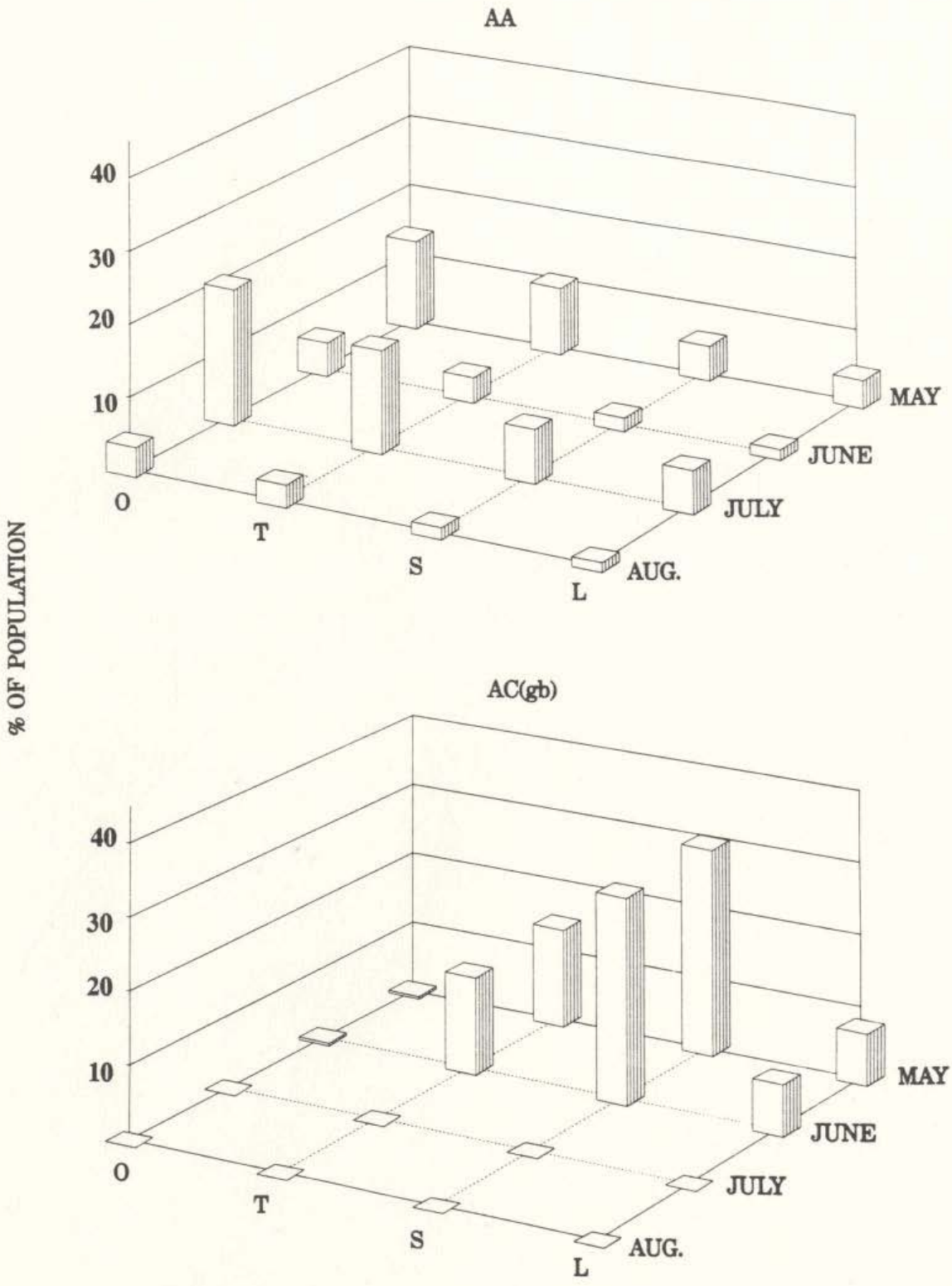

EFG/C
100
30
20
10
O
T
S
L
MAY
JUNE
JULY
AUG.
% OF POPULATION
ACD
40
30
20
O
T
S
L
MAY
JUNE
JULY
AUG.

FIG. 10.1 (continued). Note that a second generation of AC(gb) may occur in a very limited number of sites.

O = outlet site
T = trickle site
S = small stream site
L = large stream site


AA
40
30
20
10
O
T
S
L
AUG.
JULY
JUNE
MAY
% OF POPULATION
AC(gb)
40
30
20
10
O
T
S
L
AUG.
JULY
JUNE
MAY

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APPENDIX 1

This appendix (Table A.1) provides the location, stream conditions at the time of sampling and the results of cytological identifications (present/absent) from each site sampled for the macrodistribution analysis presented in chapter 5. Location names (B. = Brook; R. = River; P. = Pond) and latitudinal-longitudinal coordinates were taken from 1: 50,000 scale topographical maps produced by the Department of Energy, Mines and Resources, Surveys and Mapping Branch, Ottawa, Canada. Names of outlet sites were of the ponds immediately upstream from the collection site. In many cases (especially trickles) location names were not available.

TABLE A.1. *Site location, stream conditions* at time of collection and results of cytological identifications for macrodistribution collections (chapter 5).*

Si	Dt	Lat-long	Name	Co	St	Sb	Vg	Cp	Bd	D	T	pH	Ox	Cd	EF	AC	AA	CC	GB
1	150688	472115 N	-	1	2	2	1	2	1.0	0.11	11.0	5.2	10.1	58	0	0	0	1	1
1	290786	530345 W		2	2	2	1	2	1.0	0.07	13.0	-	-	-	0	0	1	1	0
2	*90687*	*471400 N*	-	1	2	3	1	1	3.0	0.05	8.5	6.2	12.2	46	0	0	0	1	1
2	120789	531900 W		2	2	3	1	1	3.0	0.06	18.0	6.0	10.0	43	0	0	1	1	0
3	180688	474145 N	-	1	2	3	3	3	3.5	0.16	10.0	5.7	9.5	70	0	0	0	1	1
3	220787	524415 W		2	2	3	3	3	3.5	0.09	9.0	5.8	7.3	45	0	0	0	1	0
4	140689	473930 N	-	1	2	2	1	1	3.5	0.10	9.5	6.1	10.8	86	0	0	1	1	0
4	180789	524415 W		2	2	2	1	1	3.5	0.13	15.0	6.3	9.4	97	0	1	0	1	0
5	150688	472015 N	-	1	2	2	1	1	5.0	0.19	14.0	6.6	10.0	40	0	0	1	1	0
5	*190789*	*530345 W*		2	2	3	1	1	4.5	0.14	21.0	6.1	8.8	36	0	1	0	1	0
6	210686	474045 N	Rocky B.	1	2	3	1	1	3.0	0.18	17.0	-	-	-	0	0	1	1	0
6	280786	524745 W		2	2	2	1	1	3.0	0.09	21.0	-	-	-	0	0	1	0	0
7	150688	472100 N	-	1	2	3	1	2	3.5	0.11	11.0	5.7	10.1	42	0	0	0	1	1
7	210788	524830 W		2	2	2	1	2	3.5	0.13	17.0	6.2	7.6	50	0	0	1	1	0
8	250588	471815 N	Maggotty	1	2	2	1	1	1.0	0.24	12.0	5.9	11.2	66	0	0	0	1	1
8	210788	524915 W	Cove B.	2	2	2	1	3	1.0	0.21	14.0	6.0	7.1	66	0	1	1	1	1
9	250588	472015 N	-	1	2	2	2	1	3.5	0.06	20.0	5.7	8.7	41	0	0	0	1	0
9	210788	525345 W		2	2	2	2	1	3.5	0.09	19.0	5.8	8.1	40	0	1	1	0	0

TABLE A.1. continued.

Si	Dt	Lat-long	Name	Co	St	Sb	Vg	Cp	Bd	D	T	pH	Ox	Cd	EF	AC	AA	CC	GB
10	150688	471200 N	-	1	2	2	3	1	1.0	0.11	13.0	6.4	8.8	42	1	0	1	1	1
10	200788	525400 W		2	2	2	3	3	1.0	0.16	10.0	6.4	7.3	53	0	0	1	0	0
11	310588	470145 N	-	1	2	3	1	1	3.0	0.08	19.0	6.8	9.4	35	0	0	0	1	1
11	240788	534915 W		2	2	2	1	1	3.0	0.08	19.0	6.5	9.0	47	0	0	0	1	0
12	160688	471230 N	-	1	2	2	1	1	3.5	0.08	15.0	5.5	8.1	44	0	0	1	1	0
12	-	533630 W		2	2	-	1	-	-	-	-	-	-	-	-	-	-	-	-
13	160688	471800 N	-	1	2	3	3	3	3.0	0.06	14.0	5.8	8.3	42	0	0	0	1	1
13	70789	533230 W		2	2	3	3	3	3.0	0.06	14.5	5.5	9.2	49	0	0	1	1	0
14	210688	474415 N	-	1	2	3	2	2	3.0	0.04	16.0	6.2	7.8	94	0	0	0	1	1
14	130788	524800 W		2	2	3	2	2	3.0	0.06	15.0	6.4	6.3	99	0	1	1	1	0
15	200688	472445 N	-	1	2	2	1	1	1.0	0.17	22.0	5.3	7.8	37	1	0	1	1	0
15	20889	525830 W		2	2	2	1	2	1.0	0.10	18.5	5.9	8.2	44	0	1	1	1	0
16	100687	474245 N	Bauline B.	1	3	1	3	1	5.0	0.14	14.5	6.4	11.7	80	0	0	0	1	0
16	10786	524915 W		2	3	1	3	1	5.0	0.18	16.0	-	-	-	0	0	0	1	0
17	150688	472745 N	Doyles R.	1	3	3	1	1	4.0	0.10	11.0	6.5	11.3	54	0	0	0	0	1
17	150787	524700 W		2	3	3	1	1	4.0	0.08	22.0	6.7	8.4	63	0	0	1	1	0
18	200688	472330 N	Seal Cove	1	3	2	3	1	5.0	0.26	21.0	6.2	8.5	46	0	0	0	1	0
18	200787	530330 W	R.	2	3	3	3	1	5.0	0.17	23.0	6.6	8.0	40	0	0	0	1	0
19	20688	474045 N	-	1	3	2	3	1	4.0	0.15	10.5	6.2	10.8	63	0	0	1	1	0
19	180789	524500 W		2	3	2	3	1	4.0	0.22	18.5	6.2	8.8	64	0	1	1	0	0

TABLE A.1. continued.

Si	Dt	Lat-long	Name	Co	St	Sb	Vg	Cp	Bd	D	T	pH	Ox	Cd	EF	AC	AA	CC	GB
20	100688	473415 N	Broad Cove	1	3	2	3	1	5.0	0.22	11.0	6.0	11.9	68	0	1	1	1	1
20	170789	525130 W	R.	2	3	2	3	1	5.0	0.19	18.5	6.5	9.6	89	0	1	1	1	1
21	150688	422215 N	-	1	3	1	2	2	4.0	0.14	13.0	5.3	10.3	40	0	0	0	1	1
21	190789	524800 W		2	3	1	2	2	4.0	0.17	16.0	5.8	10.4	27	0	0	0	1	0
22	150688	471645 N	Witless	1	3	1	2	1	4.5	0.24	14.0	5.6	10.1	43	0	0	0	1	0
22	190789	525030 W	Bay B.	2	3	1	2	1	4.5	0.19	19.0	5.8	9.2	32	0	0	0	1	0
23	240589	471245 N	Solomons R.	1	3	1	2	1	3.5	0.08	16.0	7.2	9.8	45	0	0	0	1	1
23	70789	534745 W		2	3	1	2	1	3.5	0.08	16.0	5.9	9.0	38	0	0	0	1	0
24	140688	470545 N	Cape	1	3	3	2	1	4.0	0.13	16.0	5.4	9.6	40	0	0	0	1	1
24	70788	525730 W	Broyle R.	2	3	3	2	1	4.0	0.14	14.0	6.1	9.7	44	0	0	0	1	0
25	60689	471345 N	-	1	3	3	2	1	4.0	0.18	18.5	6.4	8.4	32	0	0	1	1	1
25	120789	532000 W		2	3	2	2	1	4.0	0.36	17.5	6.1	9.4	34	0	0	1	1	0
26	140688	465830 N	-	1	3	1	3	3	3.5	0.16	12.0	5.7	9.6	40	0	0	0	1	1
26	150789	525[illegible]15 W		2	3	1	2	1	3.5	0.23	14.0	4.8	11.6	33	0	0	0	1	1
27	140688	465900 N	-	1	3	1	2	1	3.5	0.17	14.0	6.6	9.8	47	0	0	0	1	1
27	70788	525745 W		2	3	3	2	1	3.5	0.21	14.0	6.1	9.8	43	0	0	0	1	0
28	310588	470230 N	-	1	3	1	3	1	4.0	0.11	15.0	6.3	10.4	18	0	0	0	1	1
28	70789	534900 W		2	3	1	3	1	4.0	0.09	15.0	6.1	10.8	30	0	0	1	1	0
29	310588	470315 N	-	1	3	3	2	1	3.5	0.09	13.0	6.3	10.0	33	0	0	0	0	1
29	240788	534835 W		2	3	1	2	1	3.5	0.09	16.5	5.7	9.1	34	0	0	0	1	1

TABLE A.1. continued.

Si	Dt	Lat-long	Name	Co	St	Sb	Vg	Cp	Bd	D	T	pH	Ox	Cd	EF	AC	AA	CC	GB
30	210688	474345 N	Pouch Cove	1	3	2	3	2	4.5	0.14	17.0	5.8	9.4	79	0	0	0	1	0
30	130788	524800 W	B. West	2	3	2	3	2	4.5	0.14	16.5	5.9	8.3	74	0	0	0	1	0
31	60689	471145 N	Salmonier R.	1	4	3	3	1	4.0	0.29	17.0	6.4	9.6	58	0	0	0	1	1
31	300686	532230 W		2	4	2	3	1	4.0	0.48	16.0	-	-	-	0	0	0	1	0
32	0687	471215 N	Back R.	1	4	3	3	1	4.0	0.19	11.0	6.5	11.5	33	1	0	0	0	1
32	120789	532215 W		2	4	3	3	1	4.0	0.22	16.0	6.2	11.4	32	0	0	0	1	0
33	140688	470045 N	-	1	4	1	3	1	4.5	0.28	16.0	5.5	10.2	38	0	0	0	1	0
33	150789	525600 W		2	4	3	3	1	4.5	0.40	16.5	5.4	10.0	31	0	0	1	1	0
34	160688	472030 N	Hodge R.	1	4	2	3	1	4.0	0.32	16.0	6.0	10.0	55	0	0	0	1	1
34	200787	533300 W		2	4	3	3	1	4.0	0.26	22.0	7.4	9.2	54	0	0	1	0	0
35	310588	470145 N	Little	1	4	1	3	1	4.5	0.24	17.0	6.4	10.0	29	0	0	1	1	0
35	120787	534915 W	Barachois R.	2	4	3	3	1	4.5	0.18	22.0	6.6	8.0	35	0	0	0	1	0
36	160688	465645 N	Red Head R.	1	4	1	3	1	4.5	0.41	16.0	5.5	9.8	44	0	0	0	1	1
36	120787	535130 W		2	4	1	3	1	4.5	0.22	21.0	6.5	8.3	42	0	0	0	1	0
37	140688	465045 N	-	1	4	3	2	1	3.5	0.29	13.0	6.8	9.8	46	0	0	1	1	0
37	70788	525815 W		2	4	2	2	1	3.5	0.29	14.5	6.4	9.3	43	0	0	1	1	0
38	140688	470045 N	Aquaforte R.	1	4	1	3	1	5.0	0.26	17.0	5.9	10.0	37	0	0	0	1	0
38	150789	525900 W		2	4	1	3	1	5.0	0.26	17.0	5.3	8.4	30	0	0	0	1	0
39	140688	471115 N	La Manche R.	1	4	2	2	1	3.5	0.14	16.0	6.4	9.0	39	0	0	0	1	0
39	-	525500 W		2	4	-	2	1	3.5	-	-	-	-	-	-	-	-	-	-

TABLE A.1. continued.

Si	Dt	Lat-long	Name	Co	St	Sb	Vg	Cp	Bd	D	T	pH	Ox	Cd	EF	AC	AA	CC	GB
40	150688	472615 N	Raymonds B.	1	4	1	2	1	4.5	0.36	14.0	6.1	10.4	41	0	0	0	1	0
40	310789	524615 W		2	4	3	2	1	4.5	0.21	16.5	6.1	10.4	46	0	1	1	0	0
41	170689	472215 N	Daniels B.	1	4	3	2	1	4.0	0.17	22.0	6.3	9.4	45	0	0	0	1	0
41	120789	531030 W		2	4	3	2	1	4.0	0.24	19.0	6.9	10.2	51	0	0	0	1	0
42	310588	470645 N	Big	1	4	3	3	1	4.5	0.13	12.0	6.1	10.2	29	0	0	0	1	0
42	70789	534530 W	Barachois R.	2	4	3	3	1	4.5	0.13	18.5	5.8	10.2	32	0	0	0	1	0
43	310588	470715 N	Little	1	4	1	3	1	4.5	0.30	16.0	6.0	9.6	13	0	0	0	1	0
43	70789	534400 W	Salmonier R.	2	4	1	3	1	4.5	0.21	18.5	5.9	10.4	28	0	0	0	1	0
44	160688	465400 N	Beckford R.	1	4	1	3	1	4.0	0.22	16.0	5.5	10.0	51	0	0	0	1	0
44	240788	535430 W		2	4	1	3	1	4.0	0.25	17.0	6.6	9.0	41	0	0	0	1	0
45	200688	472245 N	North Arm R.	1	4	2	2	1	4.0	0.35	20.0	6.9	9.0	53	0	0	0	1	0
45	120789	531015 W		2	4	2	2	1	4.0	0.28	19.0	6.3	10.6	58	0	0	0	1	0
46	100688	473415 N	Healeys P.	1	1	2	2	1	4.5	0.22	12.0	6.0	12.2	73	1	0	0	0	0
46	210786	525100 W		2	1	3	2	1	4.5	0.17	18.0	-	-	-	0	1	0	0	0
47	40689	473430 N	Gull P.	1	1	1	3	2	4.5	0.10	14.0	6.0	12.4	62	1	1	1	0	0
47	210786	525030 W		2	1	1	3	2	4.5	0.13	19.0	-	-	-	0	1	1	0	0
48	20688	474045 N	Axes P.	1	1	1	2	1	4.0	0.12	12.0	6.0	11.3	52	1	1	1	0	0
48	180789	524530 W		2	1	2	2	1	4.0	0.17	18.0	6.3	10.4	59	0	1	0	0	0
49	210586	472015 N	Louis P.	1	1	3	1	1	4.0	0.14	11.0	-	-	-	1	1	1	1	1
49	120789	531115 W		2	1	2	1	1	4.0	0.20	18.5	6.5	10.0	48	0	1	1	0	0

TABLE A.1. continued.

Si	Dt	Lat-long	Name	Co	St	Sb	Vg	Cp	Bd	D	T	pH	Ox	Cd	EF	AC	AA	CC	GB
50	180686	472430 N	Round P.	1	1	1	2	1	3.0	0.20	13.0	-	-	-	1	0	0	0	0
50	130788	530130 W		2	1	1	2	1	3.0	0.17	19.0	5.4	9.0	41	1	1	0	0	0
51	70689	472045 N	Four mile P.	1	1	1	2	1	4.5	0.23	17.0	6.2	10.6	34	1	1	0	1	0
51	130788	530645 W		2	1	3	2	1	4.5	0.33	17.0	5.4	9.0	43	0	1	1	0	0
52	240588	473530 N	Mitchells P.	1	1	3	2	1	4.0	0.21	19.0	6.2	9.9	44	1	0	0	0	0
52	210786	525045 W	North	2	1	1	2	1	4.0	0.15	18.0	-	-	-	0	1	1	0	0
53	180588	472045 N	Five mile P.	1	1	3	1	1	3.0	0.18	15.0	5.9	11.1	33	1	0	0	0	0
53	130788	530430 W	West	2	1	3	1	1	3.0	0.26	17.0	5.9	8.9	39	0	1	1	0	0
54	250588	472015 N	Long P.	1	1	1	2	1	3.0	0.11	17.0	5.8	8.3	37	1	1	1	1	0
54	200788	524900 W		2	1	3	2	1	3.0	0.09	19.0	5.8	8.9	49	0	1	0	0	0
55	250588	471900 N	Long P.	1	1	3	2	1	3.5	0.09	17.0	5.8	10.3	47	1	1	0	0	0
55	210788	525115 W		2	1	1	2	1	3.5	0.07	19.5	5.6	8.1	50	0	1	0	0	0
56	250588	471015 N	-	1	1	1	3	1	4.0	0.21	20.5	6.2	9.8	31	1	0	1	0	0
56	150789	525500 W		2	1	2	3	1	4.0	0.26	17.0	6.2	9.8	35	0	1	1	0	0
57	140688	470900 N	-	1	1	1	3	1	4.5	0.17	13.0	6.4	10.6	46	1	0	1	0	0
57	200788	525300 W		2	1	3	3	1	4.5	0.20	17.5	5.9	8.7	44	0	1	1	0	0
58	310588	465930 N	-	1	1	2	1	1	5.0	0.15	16.0	6.1	11.0	16	0	0	1	0	0
58	240788	535000 W		2	1	2	1	1	5.0	0.24	19.0	5.6	9.1	39	0	0	1	0	0
59	240589	471415 N	Beaver P.	1	1	1	3	1	4.5	0.19	17.0	7.2	9.6	26	1	0	1	1	0
59	70789	534915 W		2	1	2	3	1	4.5	0.19	18.0	6.0	10.2	25	0	0	1	0	0

TABLE A.1. continued.

Si	Dt	Lat-long	Name	Co	St	Sb	Vg	Cp	Bd	D	T	pH	Ox	Cd	EF	AC	AA	CC	GB
60	150586	473545 N	Hughs P.	1	1	1	3	2	4.0	0.13	17.0	-	-	-	1	0	0	0	0
60	70786	525100 W		2	1	3	3	2	4.0	0.11	14.5	-	-	-	0	1	1	0	0

*Si = Site number; Dt = date (d/m/y); Lat-long = latitudinal and longitudinal coordinates for collection site given in degrees, minutes and seconds (e.g., 472115 N = 47°21'15''); Co = collection, 1 = spring, 2 = summer; St = stream type, 1 = outlet, 2 = trickle, 3 = small, 4 = large; Sb = larval substrate, 1 = rock, 2 = vegetation, 3 = mixed; Vg = riparian vegetation, 1 = open, 2 = brush, 3 = forest (see Table 2.1); Cp = canopy cover, 1 = none, 2 = partial, 3 = complete; Bd = size of dominant stream bed particles, 1 = mud, 2 = sand, 3 = small stones, 4 = rubble, 5 = boulders (see Table 2.1); D = water depth (m); T = water temperature (°C); Ox = dissolved oxygen (mg/L); Cd = water conductivity (μS/cm at 25°C); EF = cytotype EFG/C *venustum*, 0 = absent in subsample cytotyped, 1 = present in subsample; AC = ACD *verecundum*; AA = AA *verecundum*; CC = combined CC2 and CC3 *venustum*; GB = AC(gb) *venustum*.

APPENDIX 2

Tabled below are the 95% confidence limits associated with estimating the percent of a cytotype in a sample, from a subsample of 22. Calculations followed Zar (1984). For ease of computation, sample size was considered infinite, as subsampling was without replacement. Note that confidence limits were computed using the relationship between the F distribution and the binomial distribution which produces asymmetrical limits. The advantages of this method over the normal approximation to the binomial distribution are: i) calculated limits are accurate even when percents (p) are at extremes (i.e., 20.0% < p > 80.0%) and; ii) nonsense limits (i.e., 0.0% < CL > 100.0%) typical of the normal approximation are avoided.

TABLE A.2. 95% confidence limits for the estimated percent (p) of cytotype$_i$ in a sample based on a subsample size of 22.

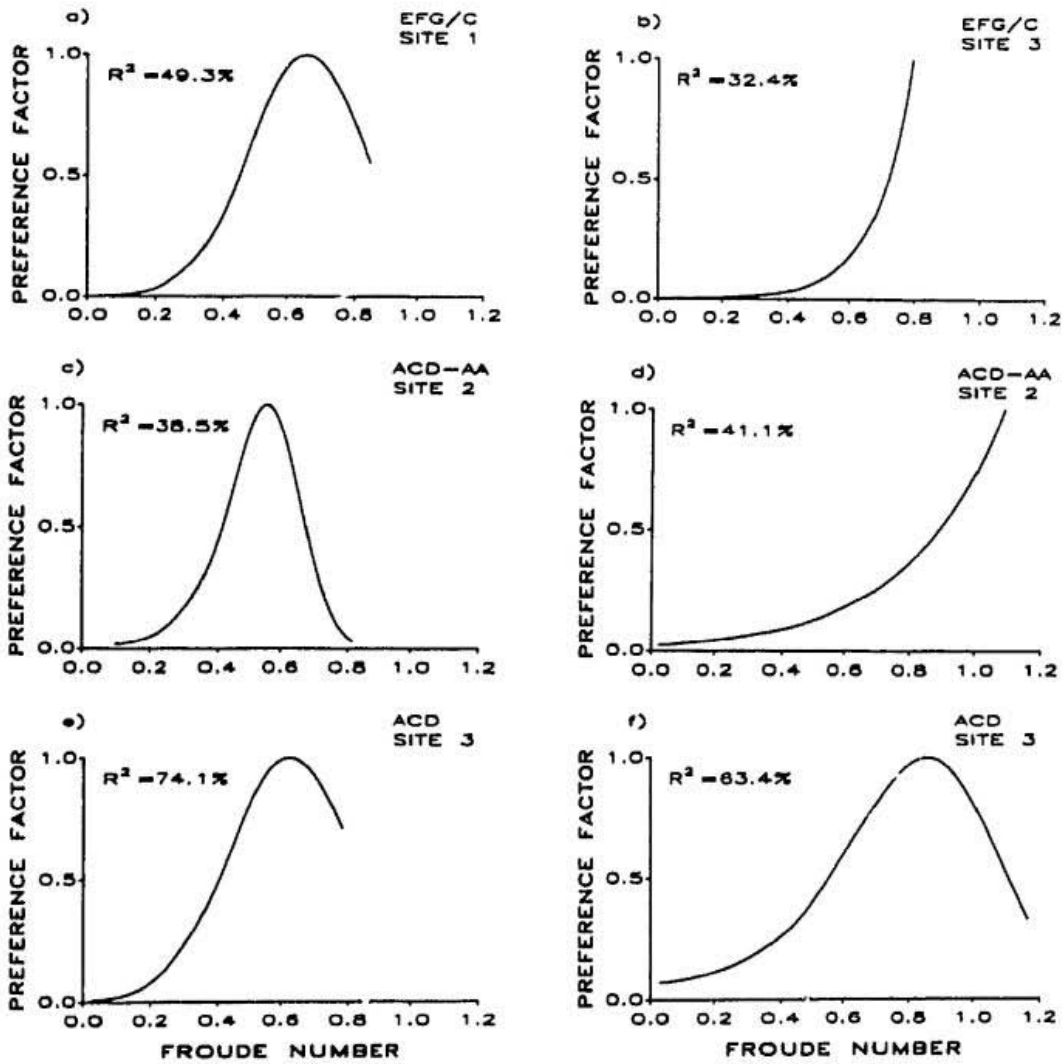
No. of cytotype$_i$ in subsample	Estimated p of cytotype$_i$ in sample	Lower confidence limit	Upper confidence limit	p - Lower limit	Upper - p limit
1	4.6	0.1	22.8	4.5	18.3
2	9.1	1.1	29.2	8.0	20.1
3	13.6	2.9	34.9	10.7	21.3
4	18.2	5.2	40.3	13.0	22.1
5	22.7	7.8	45.4	14.9	22.6
6	27.3	10.7	50.2	16.5	23.0
7	31.8	13.9	54.9	18.0	23.1
8	36.4	17.2	59.3	19.2	23.0
9	40.9	20.7	63.7	20.2	22.7
10	45.5	24.4	67.8	21.1	22.3
11	50.0	28.2	71.8	21.8	21.8
12	54.6	32.2	75.6	22.3	21.1
13	59.1	36.4	79.3	22.7	20.2
14	63.6	40.7	82.8	23.0	19.2
15	68.2	45.1	86.1	23.1	18.0
16	72.7	49.8	89.3	23.0	16.5
17	77.3	54.6	92.2	22.6	14.9
18	81.8	59.7	94.8	22.1	13.0
19	86.4	65.1	97.1	21.3	10.7
20	90.9	70.8	98.9	20.1	8.0
21	95.5	77.2	99.9	18.3	4.5
Mean confidence interval				18.4	18.4
Minimum confidence interval				4.5	4.5
Maximum confidence interval				23.1	23.1

APPENDIX 3

This appendix provides Froude number preference curves for EFG/C, mixed ACD-AA and ACD cytotypes. Details of sampling procedures, statistical analysis and calculation of Froude number can be found in Chapter 7. Mean stream velocity (= velocity at 0.6 the depth) is required to calculate Froude number (Statzner et al. 1988). However, in the present study velocity was measured at 2.5 cm above the substrate. Nevertheless, it is a reasonable assumption that the calculations presented here are at least a relative index of Froude number (Craig pers. comm. 1990). Therefore curves should accurately represent the relationship between larval abundance and Froude number, although optimal values would be higher than indicated here (see equation 2, chapter 7).

The response of larval ACD to depth and velocity was similar for collections 6 and 7 (Table 7.2), but curves for Froude number differed (Fig. A.1). A similar comparison can be made for ACD-AA (Table 7.2, Fig. A.1). Wetmore et al. (1990) on the other hand found that the larval habitat of the filtering caddisfly Brachycentrus occidentalis Banks at 2 sites differed in terms of the velocity and depth but was consistent in terms of Froude number. A comparison of R^2 values in Table 7.2 and Fig A.1 showed that Froude number alone did not improve the ability to predict larval abundance compared to velocity, depth and distance as predictors.

FIG. A.1. Froude number-preference curves for larval EFG/C, ACD-AA and ACD. Preference factor measures last larval instar response to observed changes in Froude number on a scale of 0 (conditions least suited for larvae) to 1 (optimal conditions). Details of preference curve calculations can be found in chapter 7.


a)
EFG/C
SITE 1
R^2 =49.3%
b)
EFG/C
SITE 3
R^2 =32.4%
c)
ACD-AA
SITE 2
R^2 =38.5%
d)
ACD-AA
SITE 2
R^2 =41.1%
e)
ACD
SITE 3
R^2 =74.1%
f)
ACD
SITE 3
R^2 =63.4%
PREFERENCE FACTOR
0.0
0.5
1.0
0.0 0.2 0.4 0.6 0.8 1.0 1.2
FROUDE NUMBER

APPENDIX 4

This appendix gives the weekly variation in abundance of last larval instar S. venustum/verecundum (total larvae) and individual cytotypes for the 1987 and 1988 field seasons. All values are expressed as logs. Sampling stations were located at Little Piccos (47°40'30'' N, 52°47'15'' W), Big Piccos (47°12'15'' N, 52°44'00'' W), Axes Pond outlet (47°40'45'' N, 52°45'30'' W) and Beachy Cove outlet (47°35'45'' N, 52°51'00'' W). Graphic representations of these data are presented in chapter 8. Week 1 was the last week in April and Week 22 the last week in September: May = weeks 2 - 5; June = weeks 6 - 9; July = 10 - 14; August = 15 - 18; September = 19 - 22. Note that SD = standard deviation (stratified random design); L-CI = lower confidence limit; U-CI = upper confidence limit; EF = EFG/C; AC = ACD; AA = AA; CC = combined CC2 and CC3; GB = AC(gb).

TABLE A.3. Phenology filament collections, Little Piccos, 1987.

Wk	Total larvae (log)				Cytotypes (log mean)		
	Mean	SD	L-CI	U-CI	CC	GB	AA
1	-	-	-	-	-	-	-
2	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	0.08	0.21	-0.10	0.25	0.10	0.05	0.00
6	0.68	0.42	0.32	1.03	0.73	0.70	0.00
7	0.58	0.45	0.26	0.90	0.54	0.54	0.00
8	0.54	0.21	0.39	0.68	0.67	0.08	0.00
9	0.22	0.38	-0.06	0.49	0.46	0.00	0.00
10	0.39	0.39	0.11	0.67	0.59	0.00	0.00
11	0.09	0.20	-0.05	0.23	0.15	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14	0.06	0.11	-0.02	0.14	0.00	0.00	0.08
15	0.03	0.10	-0.04	0.10	0.00	0.00	0.00
16	0.03	0.10	-0.04	0.10	0.00	0.00	0.00
17	0.03	0.10	-0.04	0.10	0.00	0.00	0.00
18	0.00	0.00	0.00	0.00	0.00	0.00	0.00
19	-	-	-	-	-	-	-
20	0.00	0.00	0.00	0.00	0.00	0.00	0.00

TABLE A.4. Phenology filament collections, Big Piccos.

Wk	Total larvae (log)				Cytotypes (log mean)		
	Mean	SD	L-CI	U-CI	CC	GB	AA
1987							
1	-	-	-	-	-	-	-
2	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0.17	0.13	0.07	0.26	0.08	0.15	0.00
5	0.55	0.45	0.23	0.87	0.28	0.68	0.00
6	0.76	0.20	0.61	0.91	0.60	0.54	0.00
7	-	-	-	-	-	-	-
8	0.48	0.50	0.12	0.83	0.72	0.11	0.00
9	0.46	0.45	0.14	0.78	0.71	0.00	0.00
10	0.41	0.37	0.15	0.67	0.57	0.00	0.00
11	0.19	0.17	0.07	0.30	0.26	0.00	0.00
12	0.07	0.22	-0.09	0.23	0.00	0.00	0.00
13	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14	0.03	0.10	-0.04	0.11	0.00	0.00	0.00
15	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16	0.03	0.10	-0.04	0.10	0.00	0.00	0.00
17	0.03	0.10	-0.04	0.11	0.00	0.00	0.00
18	0.03	0.10	-0.04	0.10	0.00	0.00	0.04
19	-	-	-	-	-	-	-
20	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1988							
1	-	-	-	-	-	-	-
2	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0.48	0.31	0.26	0.70	0.23	0.49	0.00
5	0.88	0.52	0.51	1.26	1.07	0.18	0.00
6	0.38	0.30	0.16	0.60	0.43	0.04	0.04
7	0.73	0.19	0.59	0.86	0.74	0.08	0.00
8	0.19	0.21	0.04	0.33	0.23	0.00	0.00
9	0.18	0.22	0.02	0.34	0.23	0.00	0.00
10	0.15	0.24	-0.03	0.34	0.22	0.00	0.00
11	0.12	0.17	0.00	0.24	0.00	0.00	0.00
12	0.05	0.15	-0.06	0.16	0.00	0.00	0.00
13	0.00	0.00	0.00	0.00	0.00	0.00	0.00
14	0.03	0.10	-0.04	0.10	0.00	0.00	0.00
15	0.26	0.19	0.13	0.40	0.00	0.00	0.00
16	0.24	0.26	0.05	0.44	0.00	0.00	0.32
17	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18	0.00	0.00	0.00	0.00	0.00	0.00	0.00
19	-	-	-	-	-	-	-
20	0.00	0.00	0.00	0.00	0.00	0.00	0.00

TABLE A.5. Phenology filament collections, Axes Pond outlet.

Wk	Total larvae (log)				Cytotypes (log mean)				
	Mean	SD	L-CI	U-CI	EF	AC	AA	CC	GB
1987									
1	-	-	-	-	-	-	-	-	-
2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	1.51	0.39	1.24	1.79	1.64	0.00	0.00	0.00	0.00
4	1.61	0.26	1.42	1.80	1.68	0.00	0.00	0.41	0.00
5	1.03	0.28	0.84	1.23	0.65	0.57	0.65	0.26	0.34
6	0.98	0.79	0.41	1.55	0.00	0.88	1.16	0.51	0.00
7	0.78	0.44	0.37	1.19	0.00	0.55	0.78	0.11	0.00
8	0.48	0.45	0.16	0.81	0.00	0.11	0.67	0.00	0.00
9	0.03	0.10	-0.04	0.10	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-
1988									
1	-	-	-	-	-	-	-	-	-
2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.42	0.26	0.22	0.62	0.48	0.00	0.00	0.00	0.00
4	1.25	0.53	0.87	1.63	1.44	0.00	0.00	0.28	0.00
5	1.32	0.59	0.90	1.75	0.56	0.68	1.38	1.00	0.00
6	1.34	0.20	1.19	1.48	0.26	0.00	1.00	1.14	0.00
7	0.31	0.30	0.09	0.52	0.08	0.04	0.18	0.23	0.00
8	0.09	0.20	-0.06	0.24	0.00	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	0.03	0.10	-0.04	0.10	0.00	0.00	0.04	0.00	0.00
11	0.60	0.44	0.28	0.91	0.00	0.74	0.23	0.00	0.00
12	1.04	0.22	0.88	1.20	0.00	0.85	0.85	0.00	0.00
13	0.86	0.23	0.69	1.02	0.00	0.61	0.71	0.00	0.00
14	0.32	0.21	0.17	0.47	0.00	0.30	0.15	0.00	0.00
15	0.08	0.15	-0.03	0.18	0.00	0.11	0.00	0.00	0.00
16	0.15	0.24	-0.03	0.33	0.00	0.22	0.00	0.00	0.00
17	0.03	0.10	-0.04	0.11	0.00	0.00	0.05	0.00	0.00
18	0.12	0.22	-0.04	0.28	0.00	0.04	0.15	0.00	0.00
19	-	-	-	-	-	-	-	-	-
20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

TABLE A.6. Phenology rock collections, Axes Pond outlet, 1988.

Wk	Total larvae (log)				Cytotypes (log mean)				
	Mean	SD	L-CI	U-CI	EG	AC	AA	CC	GB
1	-	-	-	-	-	-	-	-	-
2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	0.87	1.45	-0.17	1.91	0.00	0.00	2.83	1.57	1.57
6	0.64	1.37	-0.33	1.62	0.00	1.26	2.44	2.14	0.00
7	0.23	0.74	-0.29	0.76	0.00	0.00	0.00	0.00	0.00
8	0.22	0.71	-0.28	0.73	0.00	0.00	1.26	0.00	0.00
9	0.21	0.65	-0.26	0.67	0.00	0.00	1.10	0.00	0.00
10	0.22	0.70	-0.28	0.72	0.00	0.00	0.00	0.00	0.00
11	0.59	1.30	-0.34	1.53	0.00	2.61	2.17	0.00	0.00
12	1.71	1.78	0.35	3.08	0.00	2.31	2.81	0.00	0.00
13	1.44	0.95	0.76	2.12	0.00	0.00	2.33	0.00	0.00
14	0.24	0.77	-0.31	0.79	0.00	1.45	0.00	0.00	0.00
15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
19	-	-	-	-	-	-	-	-	-
20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

TABLE A.7. Phenology filament collections, Beachy Cove outlet.

Wk	Total larvae (log)				Cytotypes (log mean)				
	Mean	SD	L-CI	U-CI	EF	AC	AA	CC	GB
1987									
1	-	-	-	-	-	-	-	-	-
2	0.15	0.18	-0.01	0.32	0.20	0.00	0.00	0.00	0.00
3	1.71	0.39	1.39	2.03	1.83	0.00	0.00	0.00	0.00
4	1.19	0.41	0.90	1.48	1.63	0.00	0.00	0.00	0.00
5	-	-	-	-	-	-	-	-	-
6	0.68	0.61	0.21	1.15	1.04	0.82	0.00	0.00	0.00
7	0.66	0.52	0.29	1.03	0.40	1.04	0.00	0.15	0.00
8	0.14	0.23	-0.02	0.30	0.18	0.00	0.00	0.08	0.00
9	0.03	0.10	-0.04	0.10	0.00	0.04	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11	0.03	0.10	-0.04	0.10	0.00	0.04	0.00	0.00	0.00
12	0.03	0.10	-0.04	0.10	0.00	0.00	0.00	0.00	0.00
13	0.06	0.19	-0.08	0.20	0.00	0.00	0.00	0.00	0.00
14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18	0.03	0.10	-0.04	0.10	0.00	0.04	0.00	0.00	0.00
19	-	-	-	-	-	-	-	-	-
20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1988									
1	-	-	-	-	-	-	-	-	-
2	0.03	0.10	-0.04	0.11	0.05	0.00	0.00	0.00	0.00
3	1.23	0.40	0.92	1.54	1.33	0.00	0.00	0.00	0.00
4	1.33	0.38	1.03	1.62	1.52	0.00	0.00	0.00	0.00
5	0.85	0.37	0.54	1.16	0.85	0.18	0.18	0.57	0.00
6	0.08	0.16	-0.04	0.20	0.00	0.00	0.04	0.08	0.00
7	0.03	0.10	-0.04	0.10	0.00	0.00	0.00	0.04	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9	0.03	0.10	-0.04	0.10	0.00	0.00	0.00	0.00	0.00
10	0.23	0.33	-0.01	0.46	0.00	0.34	0.00	0.00	0.00
11	0.41	0.48	0.07	0.75	0.00	0.59	0.38	0.00	0.00
12	0.08	0.16	-0.04	0.20	0.00	0.11	0.00	0.00	0.00
13	0.11	0.20	-0.07	0.29	0.00	0.00	0.15	0.00	0.00
14	0.22	0.36	-0.04	0.47	0.00	0.04	0.38	0.00	0.00
15	0.17	0.30	-0.06	0.40	0.00	0.25	0.05	0.00	0.00
16	0.20	0.38	-0.09	0.49	0.00	0.39	0.12	0.00	0.00
17	0.29	0.40	-0.02	0.59	0.00	0.44	0.16	0.00	0.00
18	0.06	0.19	-0.08	0.20	0.00	0.08	0.04	0.00	0.00
19	-	-	-	-	-	-	-	-	-
20	0.03	0.10	-0.04	0.10	0.00	0.00	0.04	0.00	0.00
21	-	-	-	-	-	-	-	-	-
22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

TABLE A.8. Phenology collections, natural substrate, Beachy Cove outlet, 1988.

Wk	Total larvae (log)				Cytotypes (log mean)				
	Mean	SD	L-CI	U-CI	EF	AC	AA	CC	GB
ROCK									
1	-	-	-	-	-	-	-	-	-
2	0.26	0.84	-0.33	0.86	1.66	0.00	0.00	0.00	0.00
3	2.59	1.54	1.49	3.70	3.71	0.00	0.00	0.00	0.00
4	3.12	1.27	2.22	4.03	4.04	0.00	0.00	3.04	0.00
5	0.23	0.72	-0.29	0.74	0.00	0.00	1.28	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10	0.43	0.70	-0.07	0.93	0.00	1.54	0.00	0.00	0.00
11	0.45	0.92	-0.21	1.10	0.00	1.27	1.27	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13	0.32	0.85	-0.46	1.11	0.00	1.03	1.03	0.00	0.00
14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
17	0.31	0.97	-0.39	1.00	0.00	1.85	1.67	0.00	0.00
18	0.24	0.75	-0.30	0.77	0.00	0.00	1.39	0.00	0.00
19	-	-	-	-	-	-	-	-	-
20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
21	-	-	-	-	-	-	-	-	-
22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
VEGETATION									
1	-	-	-	-	-	-	-	-	-
2	0.48	1.08	-0.86	1.83	1.73	0.00	0.00	0.00	0.00
3	3.75	0.47	3.16	4.33	3.91	0.00	0.00	0.00	0.00
4	3.95	0.16	3.75	4.15	3.93	0.00	0.00	2.93	0.00
5	2.90	1.62	0.89	4.91	2.96	0.00	3.14	3.03	0.00
6	0.64	1.43	-1.13	2.41	0.00	0.00	2.19	2.19	0.00
7	0.92	1.45	-0.89	2.72	0.00	1.62	1.62	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9	0.73	1.17	-0.72	2.18	0.00	0.00	1.51	0.00	0.00
10	2.42	1.11	1.04	3.79	0.00	3.05	0.00	0.00	0.00
11	2.30	1.36	0.62	3.98	0.00	2.97	2.73	0.00	0.00
12	2.02	0.81	1.02	3.02	0.00	1.61	2.53	0.00	0.00
13	2.57	0.53	1.72	3.42	0.00	1.59	2.73	0.00	0.00
14	2.62	0.24	2.33	2.91	0.00	2.02	2.55	0.00	0.00
15	1.98	1.17	0.52	3.43	0.00	1.86	2.40	0.00	0.00
16	1.35	1.39	-0.37	3.07	0.00	2.06	1.59	0.00	0.00
17	2.24	1.27	0.66	3.82	0.00	2.57	2.33	0.00	0.00
18	1.39	1.47	-0.43	3.21	0.00	1.98	2.13	0.00	0.00
19	-	-	-	-	-	-	-	-	-
20	1.49	0.39	1.01	1.97	0.00	1.38	2.39	0.00	0.00
21	-	-	-	-	-	-	-	-	-
22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

APPENDIX 5

Given below is the Minitab macro used to calculate the slope and 95% confidence limits for simple (i.e., 2 variables) principal axis (PA) and reduced major axis (RMA) regression. These regressions were used to detect simple allometry of size in last larval instar EFG/C and ACD (chapter 9). This program also produces a scatter plot and computes the Pearson's product moment correlation coefficient between variables. This program will run on Minitab Versions 6.0 and 7.0 and should also work on any newer versions. Computational algorithms were taken from Sokal and Rohlf (1981).

Minitab Macro

```
NOECHO
# PROGRAM CALCULATES SLOPE & 95% CONFIDENCE LIMITS FOR     #
# SIMPLE PRINCIPAL AXIS AND REDUCED MAJOR AXIS REGRESSIONS.#
# COMPUTATIONAL ALGORITHMS FROM SOKAL AND ROHLF (1981).    #
# USES C1-C6 & K1-K13; C3-C6 & K1-K13 ERASED AT END OF     #
# PROGRAM. TO RUN ENTER LOG Y IN C1 AND LOG X IN C2 AND    #
# USE EXECUTE 'FILENAME' COMMAND.                          #
# WRITTEN BY J. W. McCREADIE, (31/03/90) DEPT. BIOL.,      #
# M.U.N. ST. JOHN'S, NF, CANADA  A1B 3X9                   #
NOTE
NOTE                ============================
NOTE                   VARIABLE DESCRIPTION
NOTE                ============================
NAME C1 'Y' C2 'X'
CORR C1 C2
HEIG=15
PLOT C1 C2
LET C3=C1/C2
NAME C3 'RATIO'
DESC C1-C3
#-----------------------------------------------------------#
# PRINCIPAL AXIS REGRESSION (PAR)                           #
# ONLY SLOPE FOR PRINCIPAL AXIS GIVEN AS THIS IS SLOPE      #
# USED TO DETERMINE ISOMETRY                                #
#-----------------------------------------------------------#
LET K1=(STDEV(C1))**2
LET K2=(STDEV(C2))**2
LET K3=SUM((C1-MEAN(C1))*(C2-MEAN(C2)))/((N(C1)-1))
LET K4=((K1+K2)**2-4*(K1*K2-K3*K3))**.5
LET K5=(K1+K2+K4)/2
LET K6=K1+K2-K5
LET K7=K3/(K5-K1)
LET C4=K7+0
LET C3=MEAN(C1)-K7*MEAN(C2)
let k10=N(C1)-2
invcdf .95 K11;
F  1 K10.
let K9=K11/(((K5/K6)+(K6/K5)-2)*K10)
LET K12=TAN((ATAN(K7)-.5*ASIN((2*(K9**.5)))))
LET K13=TAN((ATAN(K7)+.5*ASIN((2*(K9**.5)))))
LET C5=K12+0
LET C6=K13+0
NOTE
NOTE
NOTE=============================================================
NOTE
NOTE                  PRINCIPAL AXIS REGRESSION
NOTE=============================================================
```

```
NOTE
NOTE
NAME C3 'Y-INTER' C4 'SLOPE' C5 '95%LOWER' C6 '95%UPPER'
PRINT C3-C6
#-----------------------------------------------------------#
# REDUCED MAJOR AXIS REGRESSION  (RMA)                      #
# PROGRAM USES STANDARD ERROR OF ORDINARY LEAST SQUARES TO  #
# ESTIMATE ERROR OF RMA                                     #
#-----------------------------------------------------------#
LET K1=SUM((C1-MEAN(C1))**2)
LET K2=SUM((C2-MEAN(C2))**2)
LET K3=(K1-((SUM(C1*C2)-(SUM(C1)*SUM(C2)/N(C1)))**2)/K2)
LET K3=((K3/(N(C1)-2))/K2)**.5
LET K7=SSQ((C2-MEAN(C2)))
LET K8=SSQ((C1-MEAN(C1)))
LET K9=(K8/K7)**.5
LET K10=MEAN(C1)-K9*(MEAN(C2))
LET C4=K9+0
LET C3=K10+0
LET K5=N(C1)-2
INVCDF .975 K4;
T K5.
let C5=C4-K3*K4
let C6=C4+K3*K4
NOTE
NOTE
NOTE=======================================================
NOTE                  REDUCED MAJOR AXIS REGRESSION
NOTE=======================================================
NOTE
PRINT C3-C6
ERASE C3-C6 K1-K13
NOTE
NOTE
NOTE
NOTE
NOTE--------------------END OF PROGRAM---------------------
END
```