EVALUATION OF *CRICOTOPUS MYRIOPHYLLI* OLIVER (DIPTERA: CHIRONOMIDAE) AS A POTENTIAL BIOCONTROL AGENT FOR EURASIAN WATER MILFOIL, *MYRIOPHYLLUM SPICATUM*.

by

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

in the department of

Biology

We accept this thesis as conforming to the required standard

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June 1988

ISBN 0-315-46536-0

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ABSTRACT

Myriophyllum spicatum (Eurasian Water Milfoil, or E.W.M.) is a rooted aquatic macrophyte which grows and spreads rapidly forming dense, mats of surfacing vegetation. These mats clog irrigation and navigation ditches, shallow lakes and beaches and has led to the plant being considered a nuisance weed in B.C. The phytophagous larvae of the chironomid, *Cricotopus myriophylli* Oliver, feed on the meristematic tissue located at the plant's apical tips, thereby suppressing growth. This insect has demonstrated the ability to locally control this aquatic weed through its feeding habits. Ecological studies and laboratory trials were preformed to assess this species as a potential biocontrol agent for E.W.M., and to determine any possible hazards associated with its introduction.

Life history investigations show that the insect has 4 larval instars, is univoltine, and the 2nd to 4th larval stadia are present throughout the entire year. Adult emergence of *C. myriophylli* is apparently affected by temperature, occurring only when water temperature is between 16 - 25 C. Trials indicate that: larval feeding significantly suppresses the growth of *M. spicatum*; the optimum number of larvae necessary to suppress growth of the plant is one per meristem ; and the larvae are specific to *Myriophyllum spp.*, preferring the introduced *M. spicatum* over all native plants tested. In addition, the larvae will relocate to fresh sources of *M. spicatum* growing tissue when original source has been depleted. Mechanical harvesting of *M. spicatum* was shown to decrease populations of *C. myriophylli*, although these populations subsequently recover, likely through immigration.

The insect has been reported only from B.C. and S.E. Ontario, and has not been found in any other E.W.M. infestations in North America. This limited distribution, coupled with its feeding preferences, strongly suggests that the species is introduced. Adults mate in swarms, and the conditions necessary to induce this behaviour are difficult to replicate. It is concluded that this species is safe to introduce anywhere in British Columbia and is considered a good candidate for a biocontrol agent of *Myriophyllum spicatum* in certain circumstances.

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Acknowledgements

This work was partly funded by a grant from the British Columbia Science Council. I would like to thank all the members of my committee, listed on the title page, for their input, criticism and patience. Although not a member of my committee, the advice and criticism of Neville Winchester is much appreciated. I also owe Neville thanks for help with my statistical procedures. I would also like to thank the B.C. Ministry of Environment and Parks for their logistical support, especially the Littoral Resources Branch for supplying lab space, boats and scuba gear over the past 3 years. The advice from the staff at the Water Quality Lab in Vernon was invaluable and I would like to thank Mel Maxnuk, Dennis Einarson, Mike Wallis, and Peter Wright for loaning their expertise on E.W.M. and other aquatic plants in the Okanagan Valley. Thanks are due to Greg Armour of the Okanagan Basin Water Board for his assistance, and to Gerry Lang and the staff at the Vernon campus of Okanagan College for the loan of their facilities. Thanks also to Ben Kangasniemi and to Don Oliver for listening to theories and relating their experiences with this species.

On a more personal level, I would like to thank all the friends who lent support to me during the process of this thesis. I was fortunate to have the support of my family, especially my parents, George and Miriam, and to them and my sisters, Heather and Shannon and my brother, Scott, I can only say 'thanks' does not seem enough, but thanks.

Last, but certainly not least, none of this would have been possible without the support of my wife, Colleen. Her help in preparing this thesis, listening to my ideas, and keeping me motivated were irreplaceable. To her, more than anyone, I say thank you.

INTRODUCTION

1. General Introduction

The introduction of an exotic species which has the ability to be more competitive than native species to a habitat devoid of natural enemies generally results in the exotic's population expanding and eventually displacing the native species. Such is the case with *Myriophyllum spicatum* L. where it has been introduced in to North America, and certainly in British Columbia (Newroth, 1980; Dearden, 1983).

Biological control procedures attempt to restore a habitat to a condition which existed prior to the introduction of an exotic species which has become a pest. The biological control of weeds is a relatively new technology and any attempt to implement such a program requires an extensive ecological study of both the target and control organisms. Although once argued to be an untenable proposal by some biologists (Huffaker, 1952; Williams, 1954), the use of insects as biological control agents of weeds was pioneered by entomologists as an alternative to herbicides (Wilson, 1953) and is now accepted as a viable control procedure (Wilson, 1960).

In the late 1970's and 1980, several well-established weed beds of *M. spicatum* in the Okanagan Valley lakes system and a weedbed in Magic Lake on Pender Island failed to surface and flower. Researchers from the Littoral Resources Unit of the British Columbia Ministry of Environment, found this failure to be the result of feeding damage by the larvae of several insect species (B.C. Ministry of Environment, 1979a; Kangasniemi, 1983). The majority of the damage in the Okanagan Valley, however, was attributed to the larvae of what was then, an undescribed chironomid (Kangasniemi and Oliver, 1983). This species has since been described by Oliver (1984) and named *Cricotopus* (*Isocladius*) *myriophylli* Oliver, after its association with the plant (Plates 1 & 2). Larvae of *C. myriophylli* become established on the apical portions of *M. spicatum* stems, construct cases and feed on the meristematic tissue which is located there. This behaviour is well documented (Kangasniemi, 1983; Kangasniemi and Oliver, 1983; B.C. Ministry Of Environment, 1981; Oliver, 1984). Although the Ministry of Environment initiated research on the possibility of using this insect as a biocontrol agent, funding and staff limitations led to the discontinuation of research in this field. The discovery of a species of the *Cricotopus* genus using *M. spicatum* as habitat is not surprising. Members of this genus have been recorded as utilizing the plant as substrate (Menzie, 1980, 1981) and *C. sylvestris* has even been reported as feeding on *M. spicatum* in Ohio (Boesel, 1983).

The purpose of this thesis is to investigate those aspects of the life history, feeding, reproduction, and ecology of *Cricotopus myriophylli* and to evaluate the abilities of this insect to function as a biocontrol agent. The need to control *Myriophyllum spicatum* and the rationale for using and the difficulty in establishing biocontrol agents is described.

Plate 1: Second instar larva of *Cricotopus myriophylli*. Note the setal brushes on abdominal segments 1 to 7 (*C. sylvestris* larvae have setal brushes on abdominal segments 1 to 6)

Plate 2: Adult *Cricotopus myriophylli*. The plumose antennae indicate it is a male.


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2. Background Information

2.1 Biological Controls - The control of pest species of plants have usually involved the blanket application of expensive and potentially hazardous herbicides. More recently, however, there has been a transition to integrated programs which incorporate several different control technologies to reduce the pest population to a level where the damage inflicted is economically tolerable (Sill, 1982). The different techniques are aimed at different points of attack on the plant with a constant monitoring of the population of the target organism to assess the effectiveness of each (Welch and Croft, 1979). The basic concept in these programs is to control rather than to eradicate the pest plant species. The use of biological control agents, such as insects, pathogens or parasites which selectively attack the target organism, is a major part of many of these integrated pest management programs (Sill,1982).

Although the results of biological controls are frequently cyclic and populations of control organisms tend to lag behind pest populations, they are much more specific than broad spectrum poisons, are non-toxic, and are therefore safer environmentally (O'Brien, 1978). In addition, many biocontrol programs have the advantage of becoming self-replicating, thereby eliminating the need for repeated applications and are thus more cost-effective in the long run (Andres, 1977). The development of biocontrol agents has historically involved exploratory trips to the pest's home range to search for any native species which may function as a natural enemy there. The release of exotic natural enemies is referred to as the "classical approach" to biocontrol (O'Brien, 1978). A second method involves the artificial augmentation of populations of native species of insects which already may be feeding on the target plant. This method is referred to as inundative control. This technique has been criticized (e.g. Frick, 1982) in that, while populations of native species may increase to the point where they inflict damage severe enough to control the target plant, the natural enemies of the control organism will still be present in the system. This results in a functional response in the population of the predators which in turn consume large numbers of the control species, leading to wide fluctuations in population levels. The continued reintroductions necessary to keep the population of control organisms at the level required for damage adds expense to the program. Therefore such techniques are often looked upon as possible but not economically effective. Regardless of the approach used, it is necessary to assess the insect to be used for both effectiveness and safety. The points most frequently considered in making this assessment involve aspects of the insect's life history, feeding habits, methods of reproduction, and any other factors affecting its ability to function as a control organism. The most effective way of gathering these data is to study the insect's general ecology. This information aids in the prediction of population responses to certain circumstances (Wilson, 1964). Aspects of climate, host-plant suitability, mode of attack, and risks involved in introductions all can be addressed by studies in this area.

2.2 Eurasian Water Milfoil - Eurasian Water Milfoil, also referred to as E.W.M., (*Myriophyllum spicatum* Linn.) is a rooted, submerged aquatic macrophyte (Figure 1). It is characterized by finely divided leaflets (usually 14-24 pairs) making up a leaf. Leaves are usually arranged in whorls of 4, each leaf being 1.5-4 cm. long, and feather-like in appearance. The inflorescence is a terminal spike 5-20 cm. long and is often pink (Amundsen and Brenkert, 1978; Aiken *et al.*, 1979). Morphologically, *Myriophyllum spicatum* so closely resembles a number of native species of milfoil that a number of taxonomic problems arise (Aiken, 1981). These difficulties are sometimes so great that the only way to differentiate the species is with phytochemical techniques (Ceska, 1977; Ceska and Ceska, 1985).

The plant is able to become established in a variety of substrates and tolerates a wide range of nutrient availability and water chemistry (Giesy and Tessier, 1979; Aiken and Picard, 1980; B.C. Ministry Of Environment, 1981). Growth is characteristically vertical in early spring with the plant forming dense mats of lateral growth when nearing the surface (Figure 2). This thick mat forms a canopy which effectively cuts off light to the plants below and in this way displaces the slower growing native species. This activity is well documented (Amundsen and Brenkert, 1978; Grace and Wetzel, 1978; Aiken *et al*., 1979; B.C. Ministry of Environment, 1981; Agami and Waisel, 1985). The ability of *M. spicatum* to displace native species has resulted in its becoming a noxious weed in many areas of North America .

Although some seed production and dispersal occurs, fragmentation appears to be a much more important method of reproduction and dispersal (Amundsen and Brenkert, 1978). *M. spicatum* can reproduce a new plant from a 1 cm-long fragment, provided it contains a node (B.C. Ministry of Environment, 1981). Human activity, such as boating and waterskiing, frequently contributes to this fragmentation and subsequent dispersal of the weed.

The floating mats formed by *M. spicatum* make boating and swimming difficult and sometimes dangerous, interfere with angling, can clog navigation, irrigation, and flood control channels, they can alter temperatures in the littoral zone by as much as 10 C, contribute to the overall eutrophication of small, shallow lakes, and can clog shallow inlets rapidly (Plate 3). In addition, fragments wash up on the beach, rot, and must be removed at public expense. The presence of dense weedbeds often lower the property values of lakeshore real estate, including Crown Land. A questionnaire circulated to tourists in the Okanagan Valley revealed that water quality and the presence of dense beds of aquatic plants was of concern and, therefore, the presence of E.W.M. may be damaging to the tourism industry (Phipps and James, 1980). Although the plant does provide habitat for invertebrates (Pardue and Webb, 1985), it is not thought to be of great food value to waterfowl (Elser, 1969) and is not as desirable a habitat for fish as native plants (Keast, 1983). Several attempts have been made to find some commercial use for *M. spicatum*, however, none has been successful (B.C. Ministry Of Environment, 1979b&c).

M. spicatum may have been introduced to North America as early as the turn of the century (Reed, 1977) although other authors maintain it was much later (Couch and Nelson, 1986) and was probably first introduced into B.C. in Vernon Arm of Lake Okanagan about 1970 (Aiken *et al.*, 1979). When first noted, it occupied approximately 20 ha, approximately 2000 ha; of littoral zone were infested by 1987 (Newroth, pers. comm.).

A variety of control techniques have been utilized in British Columbia, including an experimental chemical treatment program involving the application of 2,4-D (B.C. Ministry of Environment, 1979). This program was limited in application area and was halted due to lack of public support. Although this herbicide is still used in some areas of the United States, it is doubtful that a large scale 2,4-D treatment will ever be attempted again in B.C. waters

(Newroth, pers. comm.). Currently, some degree of control is achieved with mechanical treatments (harvesting, rototilling, cultivation, diver-operated dredges, and bottom barriers) (B.C. Ministry Of Environment 1978, 1986). All of these treatments are limited by expense, their relative effectiveness, and the time necessary for application. They are, therefore, confined to high priority areas such as public beaches and marinas. In addition, although rototilling, cultivation, and diver dredging do result in the removal of the plant roots, treated areas are invariably reinfested from neighbouring untreated areas, usually within 2 years. Harvesting is merely the removal of the plant stems to a maximum depth of 2 meters, but as the roots are left untouched, the plant recovers its full height within weeks during the growing season.

Figure 1: a) Apical stem of *M. spicatum*. b) Flower spike of the plant. c) Cross section of a node showing leaflets (from B.C. Ministry of Environment and Parks, 1981).

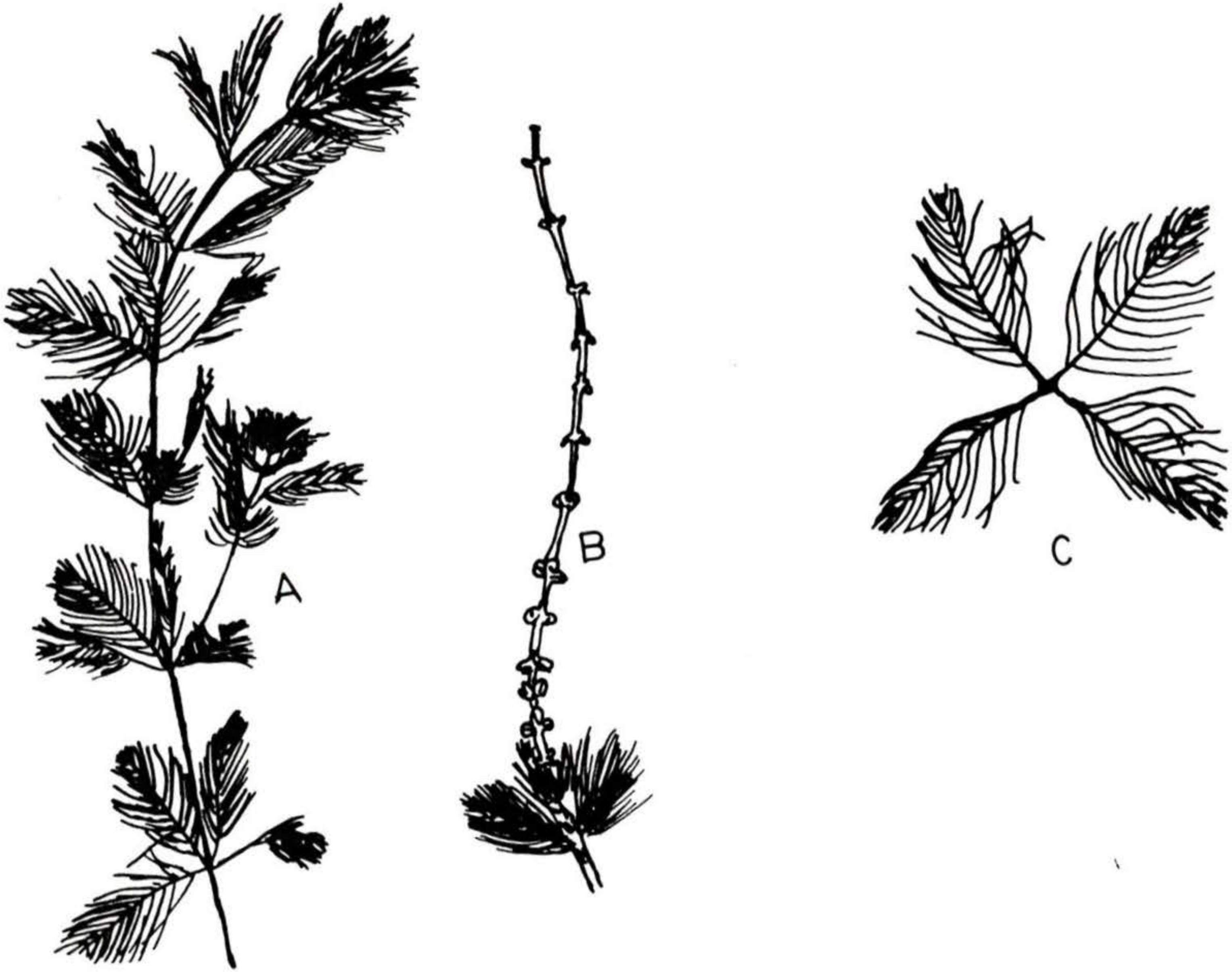
A
B
C

Figure 2: Cross section of *Myriophyllum spicatum* weed bed showing typical seasonal growth (from B.C. Ministry of Envorinment and Parks, 1981).

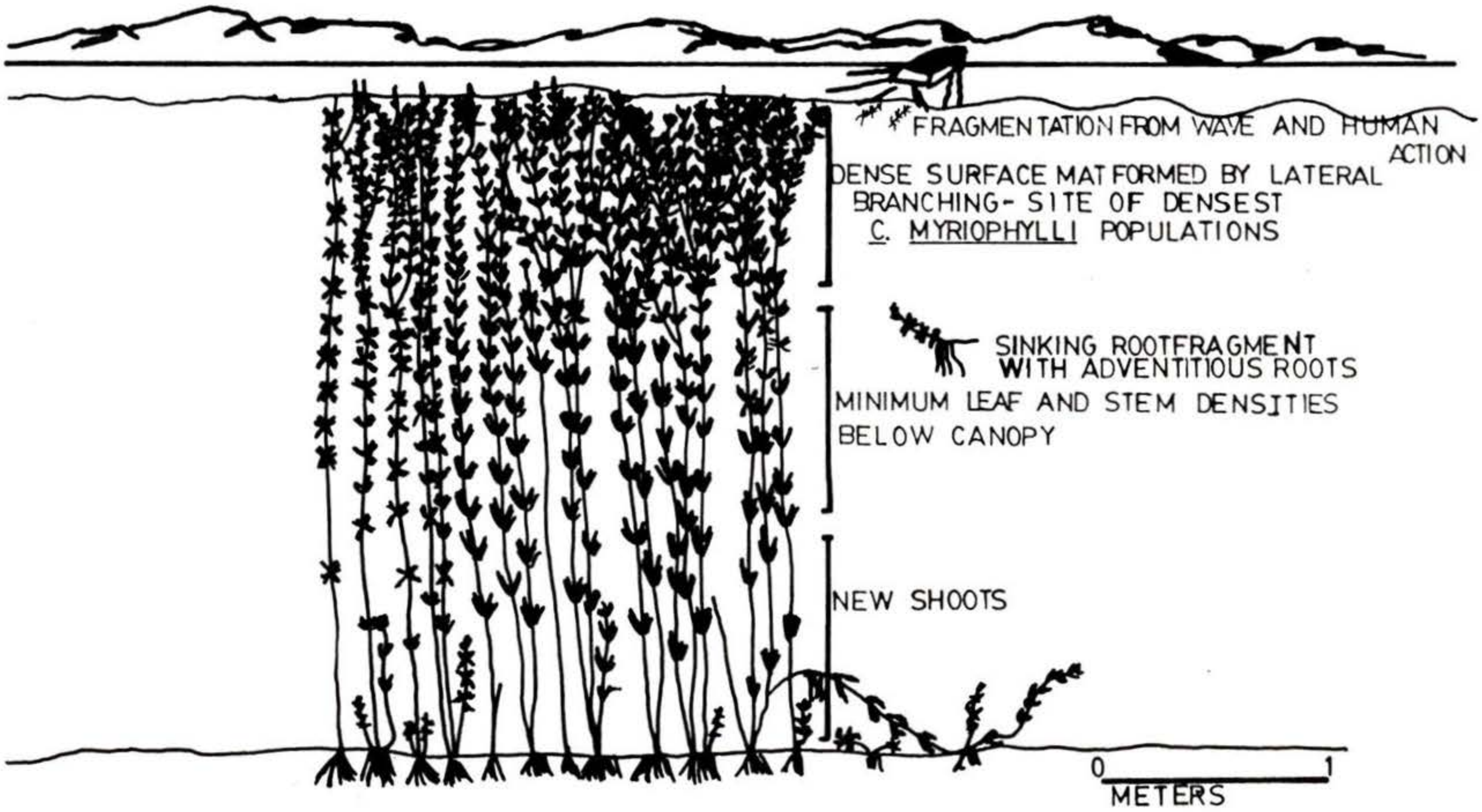
FRAGMENTATION FROM WAVE AND HUMAN ACTION
DENSE SURFACE MAT FORMED BY LATERAL BRANCHING- SITE OF DENSEST C. MYRIOPHYLLI POPULATIONS
SINKING ROOTFRAGMENT WITH ADVENTITIOUS ROOTS
MINIMUM LEAF AND STEM DENSITIES BELOW CANOPY
NEW SHOOTS
0
1
METERS

Plate 3: Clogged inlet, north end of Ellison Lake, Kelowna, B.C. Surfacing *M. spicatum* plants are visible as ripple-like marks on the surface of the water.

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3. Biocontrol of Aquatic Weeds

Several vertebrate species have been utilized or suggested as biocontrol agents for aquatic weeds including various fish, waterfowl, and even manatees (Blackburn, Sutton, and Taylor, 1971; B.C. Ministry of Environment, 1979; Dutante and Duboise, 1986). Recently, much research has also been conducted on pathogens and this area seems to be a bright hope for biocontrol of aquatic weeds in general (Zettler and Freeman, 1972; Freeman *et al.*, 1974; Freeman *et al.*, 1976; Addor, 1977; Andrews, 1980; Charudattan *et al.*, 1984; Hoffman *et al.*, 1984). The fertilization of lakes has been advocated in an attempt to encourage the growth of phytoplankton to reduce the amount of light reaching the plants below (Bernatowicz, 1966) but the associated increase in the amount of epiphytes and filamentous algae is undesirable (Philips *et al.*, 1978). The use of insects, however, in the field of weed control is a relatively new and successful method (Bennett, 1973, 1974).

The technique of using phytophagous insects to control aquatic weeds was first attempted with the release in 1964 of a chrysomelid beetle, *Agasicles hygrophila* Selman and Vogt, into the southwestern United States to control Alligatorweed, *Alternanthera philoxeroides* (Hawkes *et al.*, 1967; Andres and Bennett, 1975; Spenser and Coulson, 1976). This project has expanded to include a species of thrips, *Amynothrips andersoni* (Thysanoptera: Phlaeothripidae), and a stem boring moth larva, *Vogtia malloi* Pastrana (Lepidoptera: Pyralidae) (Maddox *et al.*, 1971). Recent publications on the program describe it as a success in those areas where the insects have become established (Cofrancesco, 1984). The success with Alligatorweed has been followed by advances in the control of several other aquatic weed species. Waterhyacinth, *Eichornia crassipes*

(Mart.) Solms, a floating plant, is controlled in many areas of the world with programs that include the use of 2 weevil species, *Neochetina eichorniae* and *N. bruchi*, and a pyralid moth, *Sameoides albigutallis* (Perkins and Maddox, 1976; Center and Durden, 1981; Center, 1979, 1981, 1982; Irving and Beshir, 1982; Center *et al.*, 1984; Sanders and Theriot, 1985). In addition, another pyralid moth, *Acigona infusella*, has been suggested as a possible biocontrol agent (DeLoach *et al.*, 1980; Sands and Kassulke, 1983), and research has also been conducted on a pathogenic control for this plant (Pennington and Theriot, 1983).

Salvinia molesta, a floating aquatic fern, has also become the target of insect biocontrol agents. A weevil, *Crytobagous singularis*, a leaf-eating grasshopper, *Paulinia acuminata*, and a pyralid moth, *Samea multiplicalis*, were released in the mid 1960's in areas where *Salvinia* is a problem (Andres and Bennett, 1975). Of these three, the weevil is considered the most successful and is now used in a number of *Salvinia* control programs (Room *et al.*, 1981; Sands *et al.*, 1983). Another insect that has been suggested for *Salvinia* control is the waterlily aphid, *Rhopalosiphum nymphaeae* (John and Nair, 1983). This insect, however, has a broad host range (Sarup *et al.*, 1973) is not an obligate aquatic and has been reported as vectoring the watermelon mosaic virus from aquatic to terrestrial plants, and so may vector other commercially harmful mosaic viruses in the same manner (Wyman *et al.*, 1979).

Possibly the most worrisome aquatic weed at present in the southern United States is *Hydrilla verticillata* (Newroth, pers. comm.). Although there is little in the literature regarding advances in finding suitable biocontrols for this species, some initial research has been conducted into biocontrol programs using insects (Baloch, 1974) and pathogens (Charudattan *et al.*, 1984). In 1987 *Bagous* *affinis*, a weevil which feeds on *H. verticillata* tubers during low water conditions, and *Hydrellia pakistanae*, a fly which feeds on *H. verticillata* stems and leaves, were both introduced to Florida (Bartnik, pers. comm.). In addition, two species of chironomid native to Africa are being considered as possible biocontrol agents for this plant (Buckingham, pers. comm.).

4. Biocontrol of Eurasian Water Milfoil

Many potential agents have been examined as possible biocontrols for Eurasian Water Milfoil, the majority of the studies having been conducted in the past 15 years. Probably the most controversial is the white amur or grass carp, *Ctenopharyngodon idella*, which feeds extensively on a wide range of aquatic vegetation. However, in the past some species of true carp, which are quite distinct from *C. idella*, have become problems when introduced into other areas, displacing sports fish (Beach, 1973). For this reason, the species has been banned from introduction in Canada and many areas of the United States (Sutton, 1977). There is, at present, a group at the University of Washington assessing a sterile genotype of the fish, and investigating the possibility of introductions into milfoil-infested lakes. Recent reports of this study indicate that the white amur may have applications in small, enclosed, reservoir-like habitats, but it is doubtful that it will be as effective in large open systems, such as the location of most of the *M. spicatum* infestations in B.C. (Bowers *et al.*, 1985; Gilbert *et al.*, 1987).

Two plant species have been suggested as possible controls. Dwarf spikerush forms dense mats on the substrate restricting rooting sites for *M. spicatum*, and American Lotus produces a floating barrier which can restrict fragment dispersal (Frank, 1975). However, both plants are considered pests themselves in certain areas of the United States, and their ability to outcompete E.W.M. makes their use as biocontrol agents limited and their introduction inadvisable (B.C. Ministry of Environment, 1979a).

In the early to mid 1970's, a number of exploratory surveys were conducted in the potential home ranges of *M. spicatum*. Twenty-five different species of insects were found feeding on milfoil in Pakistan (Habib-UR-Rehman *et al.*, 1969; Baloch *et al.*, 1972) and 16 in Yugoslavia (Lekic and Mihajlavic, 1970; Lekic, 1972). Of these, only three species were considered to have potential as biocontrol agents. These were two species of pyralid moth, *Parapoynx stratiotata* L. and *Acentropus niveus* (= *Acentria nivea* Olivier), and a stem boring weevil, *Litodactylus leucogaster* Marsham (Spenser and Lekic, 1974; Urbane, 1975). *Parapoynx stratiotata* feeds on a number of aquatic macrophytes (Habek, 1974, 1983) but seemed to have a high preference for *M. spicatum* over other plant species (Lekic, 1970). Unfortunately, this species is tropical and cannot survive North American winters north of Florida. It is closely related to two native species of the same genus; *P. allionealis* Walker has been collected from Nova Scotia and Florida, but its entire range is not fully known; and *P. badiusalis* has been reported as being associated with the natural decline of E.W.M. in North Carolina (Apperson and Axtell, 1981). Both species are polyphagous, although their host preferences and tolerances have not been fully tested.

Acentria nivea, the other pyralid moth, was originally thought to be absent from N. America, but was subsequently found to have been introduced to Montreal, Que. in 1927 (Sheppard, 1945). This species overwinters as a larva and appears to inflict extensive feeding damage early in the spring before it pupates and emerges as an adult (Batra, 1977). Although a voracious feeder and credited with decimating milfoil populations in several Ontario lakes (Painter, pers. comm., and Painter and McCabe, 1988), researchers in the United States found its host range to be quite wide and milfoils were not high in feeding preference (Buckingham, 1980; Spenser, 1974). In addition, the species was very difficult to rear since the females often do not emerge from the water, but merely lift the tip of their abdomens above the surface, enabling the males, skimming the surface, to mate with them (Buckingham *et al.*, 1981; Buckingham and Ross, 1981; Spenser and Lekic, 1974). For this reason, most authors recommended that, while this species may eventually be accidentally introduced or naturally disperse throughout North America, intentional introduction of *A. nivea* should be decided by local governmental concerns. *Litodactylus leucogaster* Marsham, was found residing in North America, but it is not clear if it is native or introduced (Buckingham, pers. comm.). This species was present in North America and had been described as *Phytobius griseomicans* Schwarz and so biological investigations were conducted in North America rather than in Yugoslavia (Buckingham *et al.*, 1979). Larvae of this species mine the stems of the plants and feed on the plant material and the adults feed on the leaves in a similar manner to *Neohydronomus pulchellis*, a weevil used in preliminary control programs for water lettuce in South America (DeLoach *et al.*, 1976). Another weevil found feeding on milfoil in Yugoslavia, but not pursued by the researchers, was *Eubrichiopsis velatus* (Beck). A considerable amount of damage in the Okanagan Valley lakes has been attributed to this species (Kangasniemi, 1983) and further study may be warranted.

L. leucogaster exhibits a specialized biology which indicates it is highly adapted to milfoils. Both larvae and adults are very specific to milfoils in their feeding, but adults have been collected from other species of aquatic plants as well. In addition, the species is restricted to milfoils for oviposition. Researchers have found this species to be the most suitable for introduction with regard to safety (Buckingham *et al.*, 1981; Buckingham and Bennett, 1981). It is present in the Okanagan, and while it does inflict some damage to the plants there, this occurs after the milfoil has formed surface mats when it is too late in the year to effect any measure of control.

Stem boring might, however, be a perfect inoculation pathway for plant pathogens, several species of which have been investigated as possible controls (Andrews and Hecht, 1980, 1981; Andrews *et al.*, 1982; Gunner, 1983; Zattau, 1985). Many of these have been native species of pathogens isolated from naturally declining populations of *M. spicatum* (Bayley *et al.*, 1968; Bean *et al.*, 1973). Most of the pathogens studied have been secondary infections and require some damage to the plant or a break in its integument for entry.

From the perspective of inundative control, searches have also been conducted in N. America for native species which may feed and inflict damage to milfoil (Balciunas, 1982b). These surveys did not reveal any species of interest other than those already mentioned.

5. Assessment Methods

The first step in a classical biocontrol program is to assess the problem the weed creates. Then, after determining the center of origin of the problem plant, an exploratory survey is conducted to search for natural enemies

(Huffaker, 1957, 1959; Wilson, 1960, 1964). Any potential candidates for biocontrol must be studied to assess the amount of damage they inflict on the target species (Bennett, 1973, 1977; Balciunas, 1982b). This whole endeavour is time consuming and involves considerable logistical and monetary support. The discovery of *Cricotopus myriophylli* feeding on, and locally controlling *M. spicatum* was, therefore, an important event since it obviated the necessity for the entire exploratory process.

There are some criticisms of the exploratory process; it has been said that relationships in home ranges, areas of origin, may be too highly evolved into interspecific balances to allow control (Hokanen and Pimentel, 1984). However, the arguments the authors made for this point of view were all specific to cactaceous plants and do not stand up to generalizations (Goeden and Lok, 1986). In addition, control programs take place out of home ranges in the absence of environmental conditions which may contribute to interspecific balances.

The next step in a classical control program, that which is addressed by this thesis, is the investigation of the candidate's potential as a biocontrol. The last two steps of a biocontrol program are introductions, through field releases, and assessment of success. These latter points, however, are beyond the scope of this study.

Much of the information necessary to evaluate an insect's suitability as a potential biocontrol organism can be extrapolated from life history studies to give indications of its suitability. Other factors such as phenology of attack and host preferences can be tested directly. Of primary importance is determination of the safety of the insect for introduction and i/n ensuring it will not become a pest species itself if introduced (Wilson, 1964). Traditionally, this involved starvation trials; feeding isolated insects only those plants of concern (Huffaker, 1959). However, crossing over to alternate hosts does occur and testing every plant species which is important would be too time consuming. In addition, insects may feed and oviposit on plants in isolation, while in nature these behaviours may not occur (Dunn, 1976). For these reasons, the widely-used starvation trials are now considered inadequate and have been modified. Testing now includes not only lab isolation trials of certain commercially important crop species, but also examining the feeding behaviour of the insect on other plant species in the same family as the target plant, and the feeding behaviour of closely related insect species on important crop plants (Harris and Zwolfer, 1969; Wapshere, 1971; and Zwolfer and Harris, 1971). Aspects of the insects' biology which may restrict the host range are also considered.

Although determining the effect of any exotic phytophagous insect on important crop plants is necessary before an introduction is attempted, Harris (1973) considers this has pre-empted attention from the selection of effective agents. Early selection of biocontrol organisms was often by intuition. To avoid this, Harris (1973) has suggested a scoring system whereby the effectiveness of an insect as a biocontrol can be rated. This rating system incorporates aspects of host-specificity, type of damage inflicted, phenology of attack, number of generations, number of progeny, mortality factors, feeding behaviour, compatibility with other control organisms, distribution, and the size of the insect. Most if not all of these points can be addressed with life history studies and field observations.

Some of the criteria listed above may contradict those used to determine the safety of an insect for introduction. A good rating of effectiveness does not preclude host-range trials; rather, it merely indicates if field trials and releases can be expected to be successful. While not following Harris' scoring method precisely, the trials and observations incorporated in this study do provide a basis for the assessment of *C. myriophylli* as a biocontrol agent to be made on conscious analysis rather than intuition.

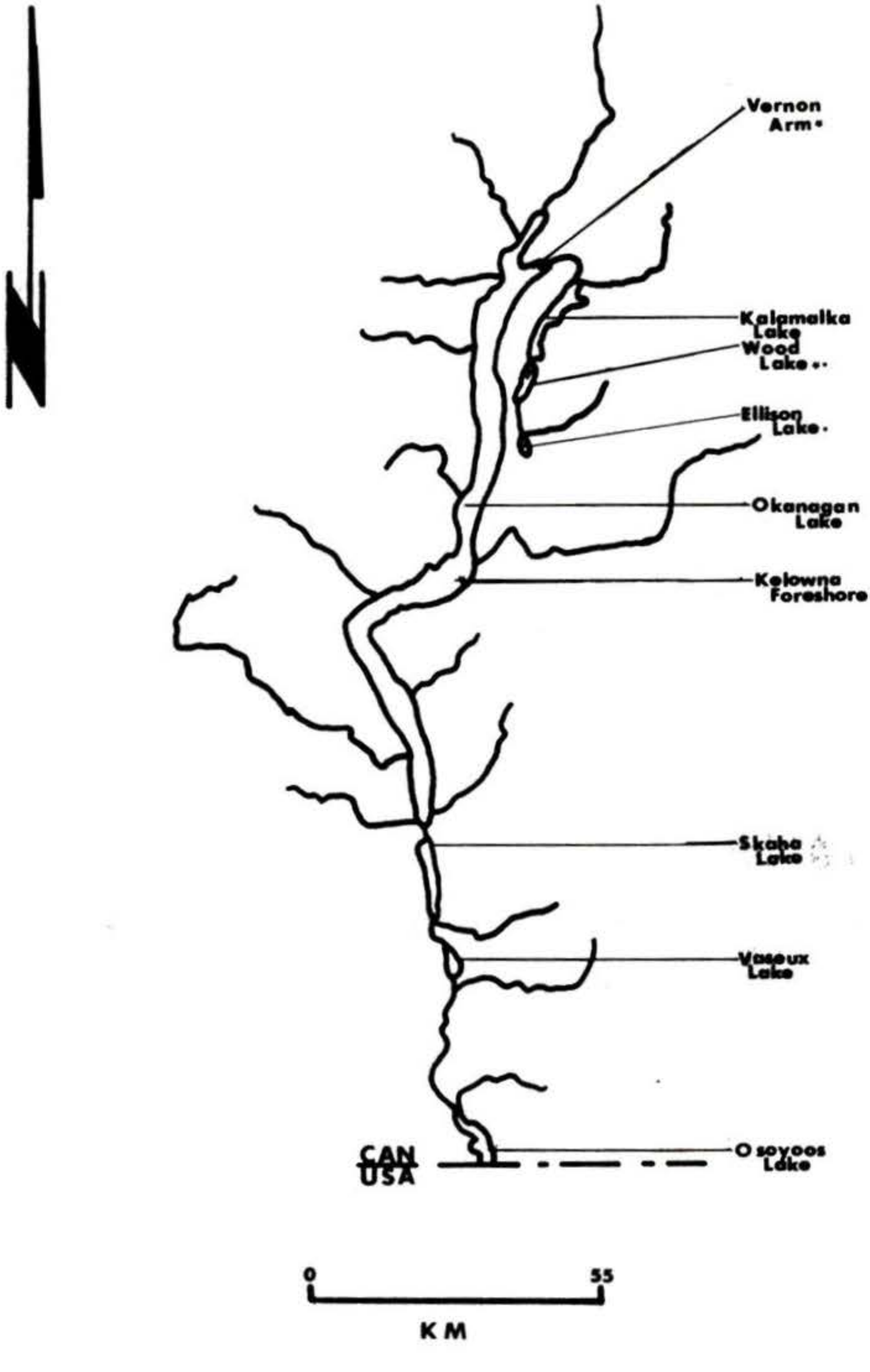
METHODS AND MATERIALS

1. Description of Study Site

All field work was conducted in the Okanagan Valley lakes system, which lies in the south central interior of British Columbia (Figure 3). The Okanagan Valley is a wide, deep basin formed by stream and glacial action, with a length of 286 km (176 km in Canada), and with a maximum width of 96 km. The Canadian area of the valley is approximately 8000 km^2, or 1% of the land area of B.C. Climatically, the area has very warm, dry summers and relatively mild winters. The valley is drained by a series of lakes, most of which are deep and generally mesotrophic. This system starts in the north Okanagan near Kelowna, B.C. and, connected by the Okanagan River, flows south to join the Columbia River near Brewster, Wash. The chain of lakes supports an extensive series of irrigation and flood control systems as part of the agricultural industry of the area including ranching and fruit production, which involves modern fertilization practices. Runoff from areas where these agricultural activities occur, almost the entire area of both banks of the lakes in the chain, in addition to the runoff associated with logging activities and septic tanks, contributes to the accumulation of nutrients in the lakes and has resulted in a general increase in their biological productivity over the past 100 years (Marr *et al.*, 1974). This has enhanced the ability of *M. spicatum* to expand its population and range. This expansion has had an adverse effect on the tourism industry of the area (Phipps and James, 1980). This is of great importance because the economy of the area has been shifting since the early 1960's from resource-based activities to secondary and tertiary activities, of which tourism is the leader (Marr *et al.*, 1974).

Species composition of native aquatic plant communities in this area consists of a number of species grouped together in mixed beds. In those areas infested with *M. spicatum*, communities usually have become restricted to only one or two species in addition to the E.W.M. This is a result of *M. spicatum* outcompeting the other, native species (Aiken *et al.*, 1979).

Figure 3: The Okanagan Valley Basin, South Central B.C. Metamorphotype sample areas are indicated by ·, post-harvest survey sites are indicated by *. Larvae of *C. myriophylli* are found throughout the entire system from Ellison Lake to Osoyoos Lake.


N
Vernon Arm•
Kalamalka Lake
Wood Lake••
Ellison Lake•
Okanagan Lake
Kelowna Foreshore•
Skaha Lake
Vaseux Lake
CAN
USA
Osoyoos Lake
0
55
KM

2. Life History

2.1 Emergence - Emergence data were collected from sites in two lakes during the summers of 1986 and 1987: a) Ellison Lake, a shallow, eutrophic lake north of the Kelowna airport, and b) Wood Lake, a deeper, mesotrophic lake, more typical of the Okanagan lakes system (Figure 3). Sample sites in Ellison Lake were located in the northern inlet, which, during the summer months, supports an extensive, dense bed of E.W.M. Sites in Wood Lake were located in the northwestern corner, to the west of Oyama beach which also supports extensive weedbeds.

Traps used to monitor the emergence of *C. myriophylli* were 1/2 m^2 clear polyethylene sheets (referred to hereafter as "windows") with grommets fixed to each corner and at the middle of each edge (Figure 4; Plate 4). The undersides of the windows were coated with stickum (3/4 Tree Tanglefoot : 1/4 naphtha). Windows were attached with twist ties to square metal frames which had a styrofoam float at each corner. This arrangement suspended the window 18-24 cm. above the surface of the water. In addition, each trap had dark polyethylene skirts around the edges, extending from the bottom of the window to 4 cm. below water level to prevent the loss of emerging insects through the sides of the trap. As the skirts were opaque and the top window clear, insects were attracted upwards onto the window rather than remaining on the sides.

Three traps were placed at each of the two sites and monitored at 2-3 day intervals, the windows cleaned of adult *C. myriophylli* and the water temperature and the dissolved oxygen at each site recorded at 1/2 m depth. DO_2 and water temperature measurements were taken using a "Y.S.I." aquatic DO_2

meter/thermometer, and the weekly means of both were calculated. Insects were removed from the trap windows using a no. 5 camel hair brush soaked in naphtha and preserved initially in Khale's Fluid (Martin, 1977) and later transferred to 70% EtOH. Windows were periodically cleaned of all insect material by soaking them in naphtha.

Since *C. myriophylli* mates in swarms, flight time could also be considered a good indication of mating period. Therefore, a Malaise trap was set just onshore at the Ellison Lake site in an effort to correlate emergence with flight time. Trap design was similar to that recommended by Townes (1972) with the main body of the trap being black and the top white. All specimens were collected and preserved in 70% EtOH.

2.2 Instar Differentiation - Weekly collections of 10 *C. myriophylli* larvae were taken from each sample site from Apr. 28 - Sept. 14, 1986 with monthly samples of at least 10 larvae/sample taken also in Mar. 1986 and Dec., Jan., and Feb of 1987. Weekly summer samples were hand-picked while swimming on SCUBA. Plants were examined below branching and followed up to the apical tips, the first ten larvae encountered were taken. Winter sample sizes were not equal as they were hand-picked through the ice. Larvae were initially preserved in Khale's Fluid and later transferred to 70% EtOH. Larvae were identified according to Oliver's description (1984).

Head capsule measurements were taken using a "Hipad" digitizing board and "Bioquant" program run on an Apple IIE computer. Larval head capsules were removed with microscissors, positioned on a bed of plasticine and examined under a Wild M5 dissecting scope to reconfirm identification using the shape of the sub-mentum (Oliver,1984). A 'camera lucida' was used to simultaneously view the head capsule and the digitizing pad and cursor. Head capsule lengths were then plotted against widths and the groupings used to indicate larval instars. As the collection date was known for each metamorphotype sample, temporal distribution of larval instars was charted to indicate the overwintering stage and predict the kind of feeding pattern that can be expected.

Plate 4: Floating emergence traps, used to monitor emergence of *C. myriophylli*, at the Ellison Lake sample site.

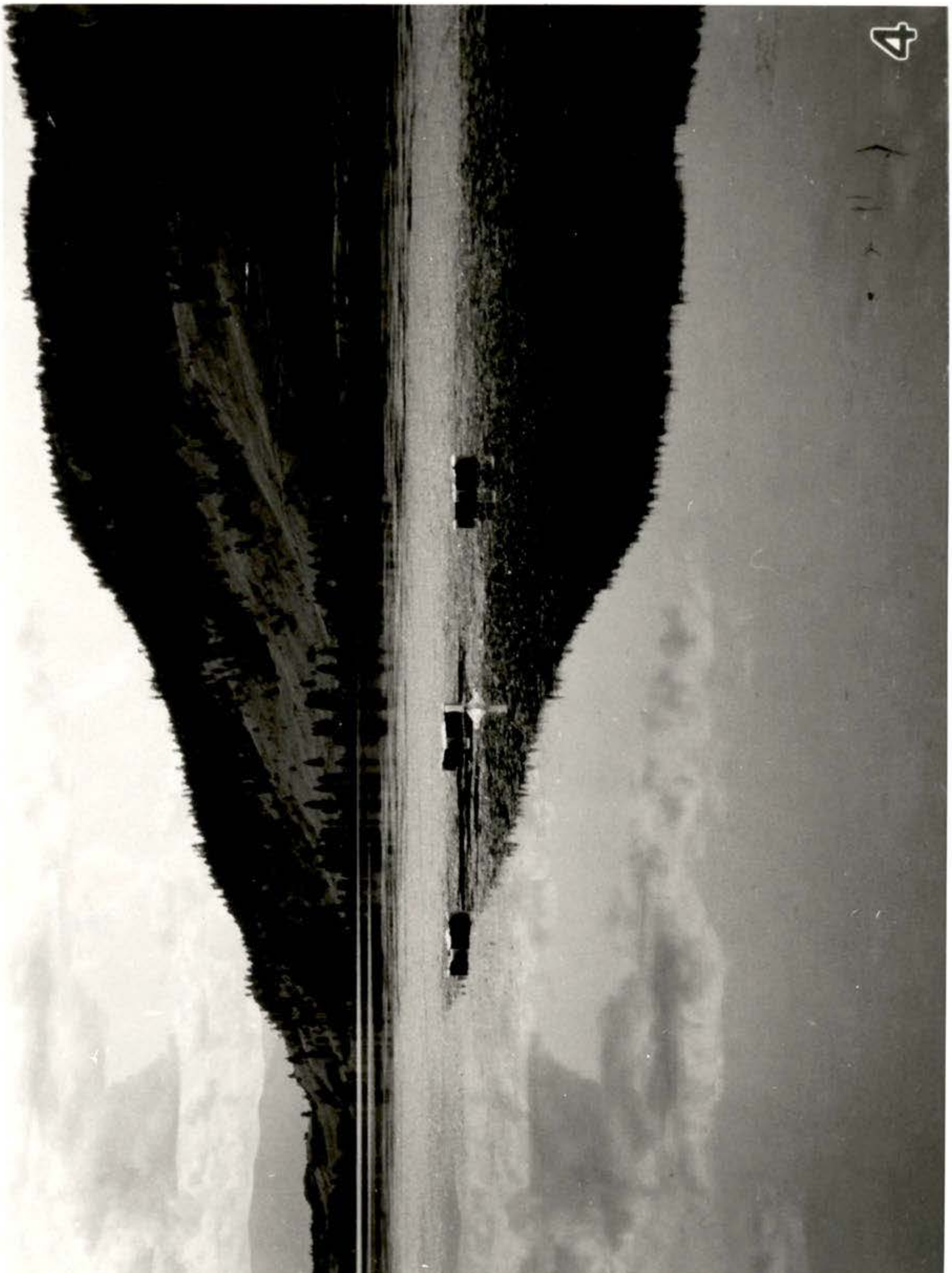
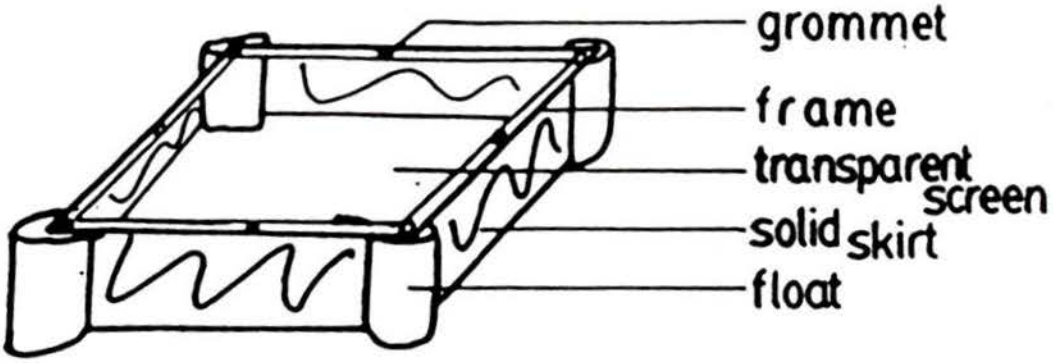
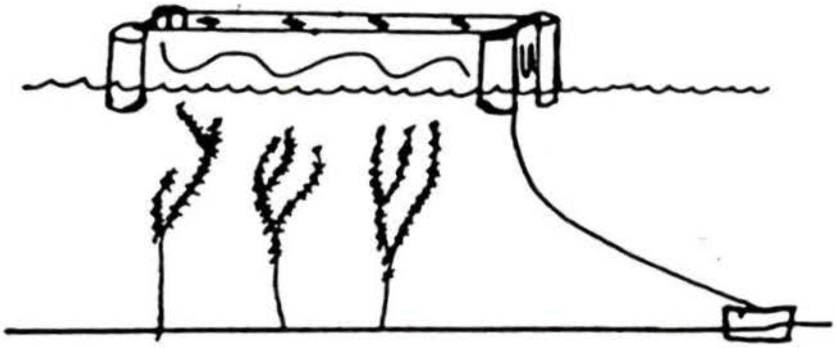

4

Figure 4: Diagram of the floating emergence traps used to monitor the emergence of adult *C. myriophylli*. Traps were anchored with a cinder block.


grommet
frame
transparent screen
solid skirt
float



3. Reproduction and Rearing

Observations were made twice weekly in the field on mating swarms of *C. myriophylli* from mid-May to early July in both 1985 and 1986. Swarms were sampled by aerial net to ensure that *C. myriophylli* were being observed and to establish its gender composition. Notes were made on swarming activity. Observations on the orientation, size, timing, and movement of swarms were made in a rearing tent during the summer of 1987 both from outside the tent and from the floor inside the tent (standing caused swarms to elevate). In addition, observations were made by B.C. Ministry of Environment personnel when rearing was first attempted in 1983 at Okanagan College (Wallis, pers. comm.).

In order to simulate the natural conditions which would allow mating to occur a rearing tent (6 m. tall, 3.5 m. long, 2 m. wide) was constructed of small gauge nylon netting. The tent included a full floor, ceiling, and a door sealed with velcro. This was suspended from a tree by a 3.75 cm. P.V.C. piping frame on the south shore of Vernon Arm, Okanagan Lake (Figure 5; Plate 5).

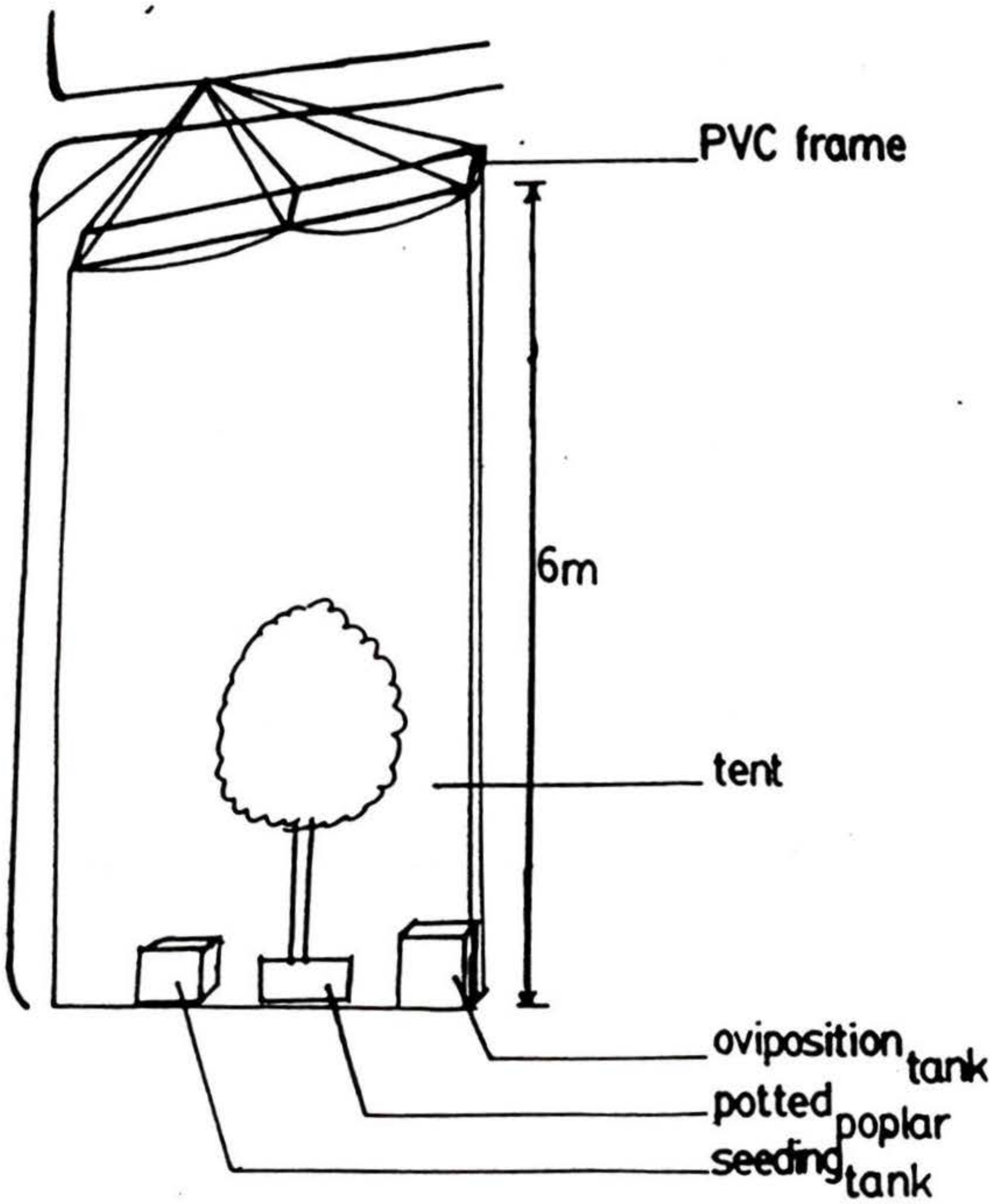
Inside the tent was placed a potted poplar, a seeding tank (to 'seed' the tent with insects), and an oviposition tank. The potted poplar served to provide a vertical object on which the swarm could "mark", shade in which the adults could shelter in the day (preventing desiccation), and a certain amount of food for the adults in the form of sugar water. The seeding tank was an aquarium filled with meristems collected from the field, sorted, and found to be occupied by *C. myriophylli* prepupae or pupae only (283 in the first trial, 237 in the second). This method of seeding was chosen over inoculation with adults from naturally-occurring swarms gathered by aerial net sweeps as all swarms sampled in the field contained only males.

The oviposition tank in the first trial, lasting from June 21 - July 30,1987 inclusive, a period of 40 days, was a 10 cm. deep plastic wading pool, 1.5 m. in diameter, containing lake substrate planted with milfoil. These plants were gathered from Shuswap Lake, an area where *C. myriophylli* was not present, and were cleaned of insect material by sealing them in mason jars for 24-36 hours (longer sealing periods killed the plants) to lower the DO_2, which killed any insects present. Any feeding on these plants or the presence of *C. myriophylli* larvae in the oviposition tank would therefore have to be the result of an oviposition event. The oviposition tank used in the second trial (lasting from Aug. 20 - Sept. 30,1987) was a 0.5 m. deep aquarium prepared with sediment and plants in the same manner as the wading pool, but completely filled with water and supplied with a portable aerator. Plant material was periodically removed from the tanks and examined under a dissecting microscope for signs of larvae, feeding damage, or eggs. Any plants removed were replaced with fresh plant material prepared in the same manner as above.

Plate 5: Rearing tent used to simulate conditions which would promote swarming behaviour of *C. myriophylli*. The tent is suspended from a tree branch approximately 6.5 m high.


5

Figure 5: Diagram of the rearing tent used to simulate swarming conditions and contain resulting swarms of *C. myriophylli*.


PVC frame
6m
tent
oviposition tank
potted poplar
seeding tank

4. Feeding

4.1 Preliminary trials - Trials estimating the optimum number of larvae per meristem were conducted in 4 20 gal. aquaria separated into 4 cells each and a measured stem of milfoil, each with one apical meristem and no axillaries, was planted in each. Randomly, into each cell, were placed 1,2,3, and, as a control, no larvae. After one week the stems were removed and new growth was measured. This process was repeated for 8 replications of each treatment and control. The tips of all meristems (n=32) were then clipped from their stems, simulating natural abscission of the fed-upon portion of the plant. These were planted (16 into the substrate, and 16 placed onto the surface of the substrate to simulate sinking after abscission) in a 10 gal. aquarium prepared with 0.5-1 cm potting soil, covered with 2-4 cm of lake substrate and the aquaria filled with lake water. These aquaria were aerated, and maintained at 20 C and checked at weekly intervals for 2 months.

In evaluating potential host-preferences of *C. myriophylli*, surveys were conducted on native aquatic plant communities in the Okanagan Valley lakes system and the Shuswap River system for any evidence of the presence of larvae or feeding damage. In addition, a 40 gal. aquarium was prepared with lake sediment and water and was planted with a variety of native aquatic macrophytes in combination with *M. spicatum*. Five stems each of *Myriophyllum exalbescens*, *Potamogeton crispus*, *P. amplifolious*, and *Ceratophyllum demersum*, and three stems of *Elodea canadensis* were planted together in an aquarium already containing *M. spicatum*. Twenty larvae were removed from their cases and then placed in the water column of the tank. Tanks were checked at 2 day intervals for 3 weeks (with 20 larvae being added at the beginning of each week) and the plants upon which the larvae became established were noted.

4.2 Suppression of Growth - The amount of growth which was suppressed by the feeding of *C. myriophylli* was assessed using a 2-way Anova design which allowed for replication and complete randomization (Winchester, pers. comm.). Ten 20 gal. aquaria were separated into 8 cells each, each cell was then planted with a stem of milfoil, standardized to 12 nodes in length, each with approximately the same amount of meristematic material to ensure that approximately equal amounts of photosynthetic and meristematic tissue were present on each plant. Four cells were then chosen at random and a larva of *C. myriophylli* was placed on the plant in each of those cells. All plants from 2 aquaria were removed at 2, 4, 6, 8, or 10 day intervals and the new growth of the plant culled off. New growth was assessed as any increase in length over the 12-node starting length or any newly-developed meristematic tissue including axillary tips. The aquaria then were replanted and the new growth was dried at 70 C. for 48 hours. Dried material was then weighed with a Mettler HP10 analytical balance. This was repeated 5 times.

4.3 Host Preference- The feeding of *C. myriophylli* on 12 species of native aquatic macrophyte was tested. This list was compiled by biologists with the B.C. Ministries of Environment and Agriculture and Fisheries and included those native plants thought to be important in the rearing of sportsfish. The suitability of these species as potential food sources for *C. myriophylli* was first tested in isolation (starvation) trials. If any significant feeding activity was noted (which included completion of development by the larvae and successful emergence as adults) then the plant was subjected to a choice trial against *M. spicatum* to see which plant was preferred as a food source.

Starvation trials were conducted in 4 10 gal. aquaria separated into 10 cells each and planted with healthy growing stems of the plant which was to be tested. All plants contained meristematic tissue and were allowed to grow for 2 days to become established in the tank. Larvae of *C. myriophylli* were removed from their cases and placed on each of the plants and monitored at 2 day intervals until they had pupated or were dead. Evidence of feeding activities was apparent as both structural damage to the plant, typical of the feeding activities of *C. myriophylli,* and as larval gut contents. It was possible to evaluate larval gut contents non-destructively since the larvae are translucent when alive and their gut contents, including colour and some structural details, are visible. Choice trials were conducted in aquaria similar to those used in feeding suppression trials. All 8 cells in each of the 4 aquaria were planted with stems of the two plants to be tested. The stems of both plants were in good growing condition and were standardized to 12 nodes in length. Larvae were removed from their cases and placed into the water column, one larva per cell. After 2 days, both plants were examined for feeding damage. Leaving the larvae to feed for a longer period of time than this could have resulted in the insect transferring to the fresh plant after its first choice had been browsed.

4.4 Relocation (Mobility) - These trials were designed to test the ability of *C. myriophylli* larvae to relocate to fresh meristems on other *M. spicatum* plants after completely browsing the one occupied. Four 20 gal. aquaria were divided into 8 cells each, and each cell was planted with a healthy growing stem of E.W.M. Meristems which were occupied by a feeding larva of *C. myriophylli* were collected from the field and culled of all material which may have been edible to the larva and one culled plant tip, containing a larva, was placed into each cell. The aquaria were checked after 3 days for host establishment. A total of 32 larvae were tested.

Larvae which had become established on new plants were allowed to feed until all meristematic tissue had been browsed. At this time stems with fresh meristematic tissue were planted in the same cell. Aquaria were checked daily for the next week and the number of larvae successfully relocating to the new plants noted.

These trials were repeated: 1) using larvae removed from their feeding cases and added to the cells, and 2) by planting stems with occupied meristems, waiting for the existing growing tissue to be browsed and then planting fresh E.W.M., N = 32 larvae for each trial. All stems planted for relocation were approximately the same length and had approximately the same amount of meristematic tissue.The distance between planted stems in the trials reflected the proximity of plants in thick weedbeds and did not exceed 25 cm.

4.5 Temperature Cues - Field observations on the activity of *C. myriophylli* larvae were conducted in the Fall of 1987 as water temperature was rapidly decreasing at approximately 2 C./day, as was daylength. Observations were conducted on SCUBA in the weed beds. Individual cases were opened and the larva inside observed. Larvae also were removed from cases and any swimming motion was observed.

5. Compatibility With Mechanical Treatments

Surveys were conducted 2-5 days and then again 14 days post-harvesting in harvested and adjacent non-harvested sites by swimming, on SCUBA, a straight transect through the weedbed below the level of branching of the plant to avoid bias in the choice of stems examined. Each stem was examined by following it upward and counting the number of meristems present and how many of these were occupied by a larva of *C. myriophylli*. A total of 5 transects were swam in different directions through each weedbed, the first 50 meristems being examined, and the mean numbers of larvae/ 50 meristems were calculated.

The surveyed areas were: 1) Railroad Beach, on the east shore, and Oyama beach, on the north shore of Wood Lake, and the Oyama water intake, 2) Kin Beach and Sproul's Corner, at the tip of Vernon Arm, Okanagan Lake, and the adjacent telephone cable area, and 3) the Rowing Club and Brown's Point, on the Kelowna foreshore of Okanagan Lake, and the telephone cable area to the north.

6. Distribution

To assess the present distribution of *C. myriophylli* in the Pacific Northwest, surveys were conducted over 2 years: 1986) in: Swan Lake, Vernon; the Okanagan lakes/River system and the Columbia River system south to Wenatchee, Wash.; Lake Washington in Seattle; Cultus Lake and Sardis Lake on the lower mainland; Magic Lake on South Pender Island; Cowichan River sloughs, and Long and Diver Lakes on Vancouver Island: and 1987) in: Shuswap Lake (Salmon Arm), Mara Lake and the Shuswap River; Swan Lake north of Vernon; and the Okanagan lakes/River system again.

Surveys conducted by other researchers included: Long Lake (1987) by N.N. Winchester, Champion and Christina Lakes (1986,87) by M. Wallis, the remainder of the Shuswap Lake system (1986-87) by D. Einarson and P. Wright, and Swan Lake in Dawson Creek (1987) by M. Wallis, D. Einarson, P. Wright, and M. Maxnuk.

Dr. D. Oliver, Biosystematics Research Institute, Ottawa, has examined milfoil infestations in Ontario and eastern Canada (Oliver, 1984) as well as samples of insect material from infestations across the U.S. gathered during Balciunas' survey of macroinvertebrates associated with milfoil in that country (Oliver, pers. comm.). Chironomid material also is being requested from Pakistan and Yugoslavia, areas where previous exploratory trips to find natural enemies of E.W.M. have been conducted.

7. Statistical Methods

A multiple regression analysis was conducted to determine if any relationship existed between emergence, temperature and DO_2. One-way ANOVAs were used to determine if there was a significant difference between any of the head capsule groupings obtained from metamorphotype samples and between treatment and control groups during trials to indicate the optimum number of *C. myriophylli* larvae necessary to suppress *M. spicatum* growth. Newman-Keuls multiple comparison tests were conducted for both one-way ANOVAs. A two-way ANOVA was used to analyse the amount of growth suppressed by *C. myriophylli* feeding over time. A Newman-Keuls multiple comparison was conducted to test for differences between groups. A two-way ANOVA was used to test for differences between the populations of C.

myriophylli in harvested and non-harvested areas 2-5 days and 2 weeks after treatment. A Newman-Keuls multiple comparison test was used to indicate which groups differed significantly. T-tests were used to find any significant difference in the number of meristems per plant in each treatment site and its neighbouring control site.

Results of the isolation/starvation host-preference tests were analysed by a 1-tailed binomial test. A two-tailed binomial test was used to analyse the results of the choice trials between *M. spicatum* and *M. exalbescens*. Results of the initial relocation trial, involving placing the insect into the cell with one planted stem of E.W.M. were analysed by a two-tailed binomial test. A one-tailed binomial test was used to analyse results of relocation to a fresh planted stem from one which had been browsed. All analyses followed Zar (1984).

RESULTS

1. Life History

1.1 Emergence - Adult *Cricotopus myriophylli* emerged throughout the summer, beginning in the last week of May in both Ellison and Wood Lakes and continued to the second week of September in 1986 and the third week in 1987 (Figures 6a, 7a, 8a, and 9a). Peak emergence in 1987 lagged behind 1986 by 3-5 days in both lakes. Although there was a small emergence during the week of May 7-14, 1987 in Ellison Lake (Figure 7a). Peak emergence occurred in mid-to late-June in Wood Lake (Figures 8 and 9) and approximately a week later in Ellison Lake (Figures 6 and 7). Sex ratios were not even, the mean ratio of males to females for the two years being 2.38:1 in Wood Lake (Figure 10) and 1.93:1 in Ellison Lake (Figure 11).

A multiple regression analysis showed no significant effect of either DO_2 or temperature on emergence nor any interactive effect between DO_2 and temperature ($0.25 < P$, for Ellison Lake, and $0.05 < P < 0.10$ for Wood Lake). No emergence of adults occurred when water temperature was either below 16 C., or above 25 C. (Figures 6b, 7b, and 8b). During August in both years, there were periods when water temperature exceeded 25 C. and no *C. myriophylli* adults were collected from the traps.

Flight time was charted (Figure 12) only as presence or absence throughout the trapping season since the Malaise trap is only a qualitative, not a quantitative collecting apparatus. Adult *C. myriophylli* were recovered weekly from the Malaise trap from May 28 to September 10, 1987, this extends two weeks later than initial emergence to two weeks before final emergence was monitored by the floating emergence traps. Individuals were still recovered from the Malaise trap during those periods when water temperatures were greater than 25 C., and no emergence was monitored.

1.2 Instar Differentiation - *C. myriophylli* has 4 larval instars (Figure 13). A one-way Anova indicated a significant difference between instars ($P<0.0005$). A Newman-Keuls multiple comparison showed all instars to be distinct.

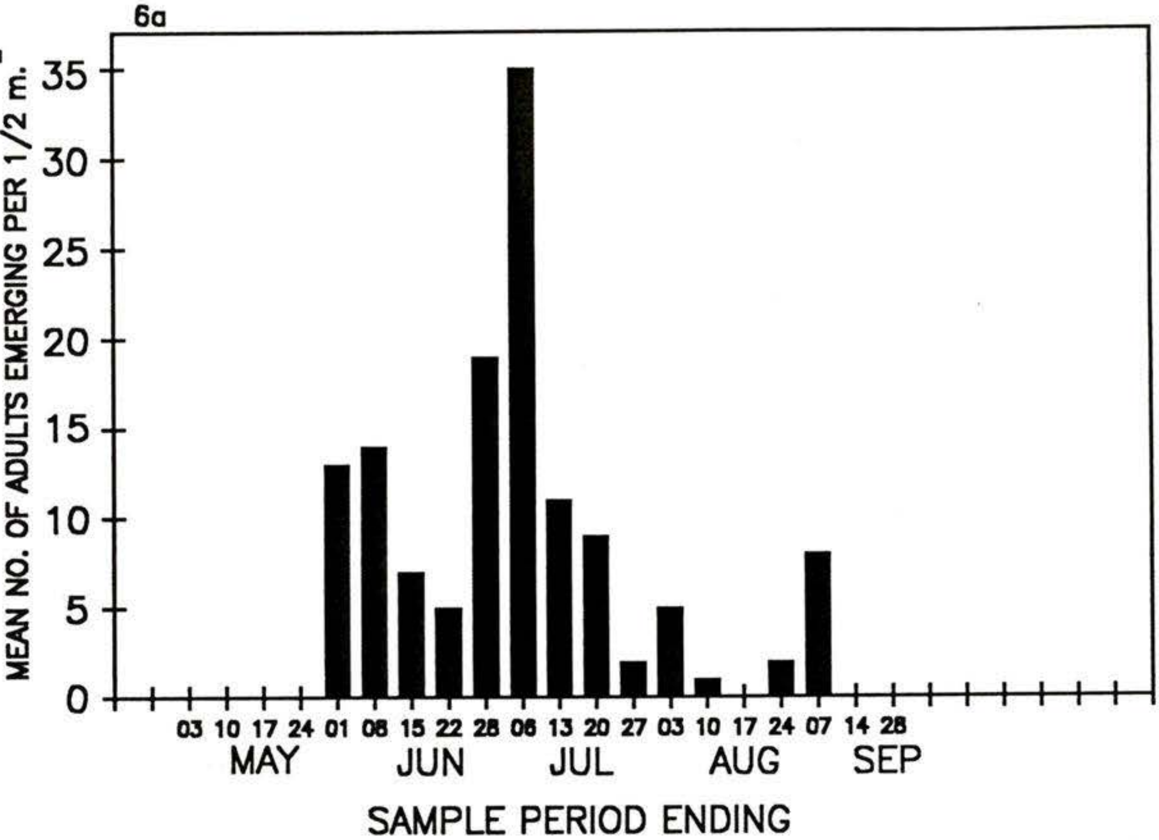
First-instar larvae were collected only from mid-June to mid-August, 1986 (Figure 12). To ensure that this was not a reflection of sampling technique, 1st instar larvae were specifically sought in the summer of 1987 and were recovered only during the same time period as in 1986. Numbers of first instars in the metamorphotype samples taken throughout that period indicated a peak occurrence in the third week of June (June 19, 1986), with smaller emergences, presumably cohorts, preceding and following this peak (Figure 14).

Other larval instars were present throughout the year. Random winter samplings (grab samplings through the ice) indicate that the insect overwinters principally in the 3rd larval instar, although 2nd and 4th instars also are present in winter months. No egg material was recovered from the winter samplings.

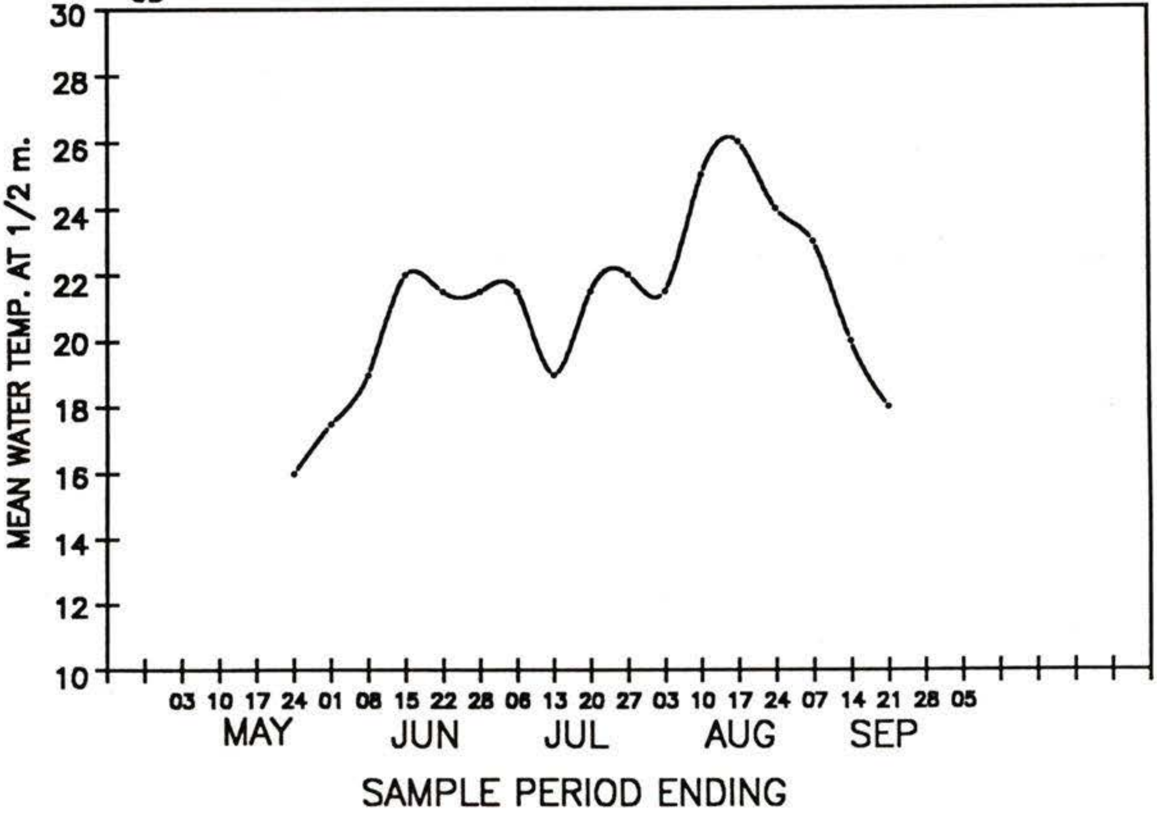
Figure 6a. Emergence of adult *C. myriophylli* per 1/2 m^2 from Ellison Lake sample sites, 1986.

Figure 6b. Water temperature at 1/2 m depth at Ellison Lake sample sites, 1986.

6a


MEAN NO. OF ADULTS EMERGING PER 1/2 m.^{2}
0
5
10
15
20
25
30
35
03 10 17 24 01 08 15 22 28 06 13 20 27 03 10 17 24 07 14 28
MAY
JUN
JUL
AUG
SEP
SAMPLE PERIOD ENDING

6b


MEAN WATER TEMP. AT 1/2 m.
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24
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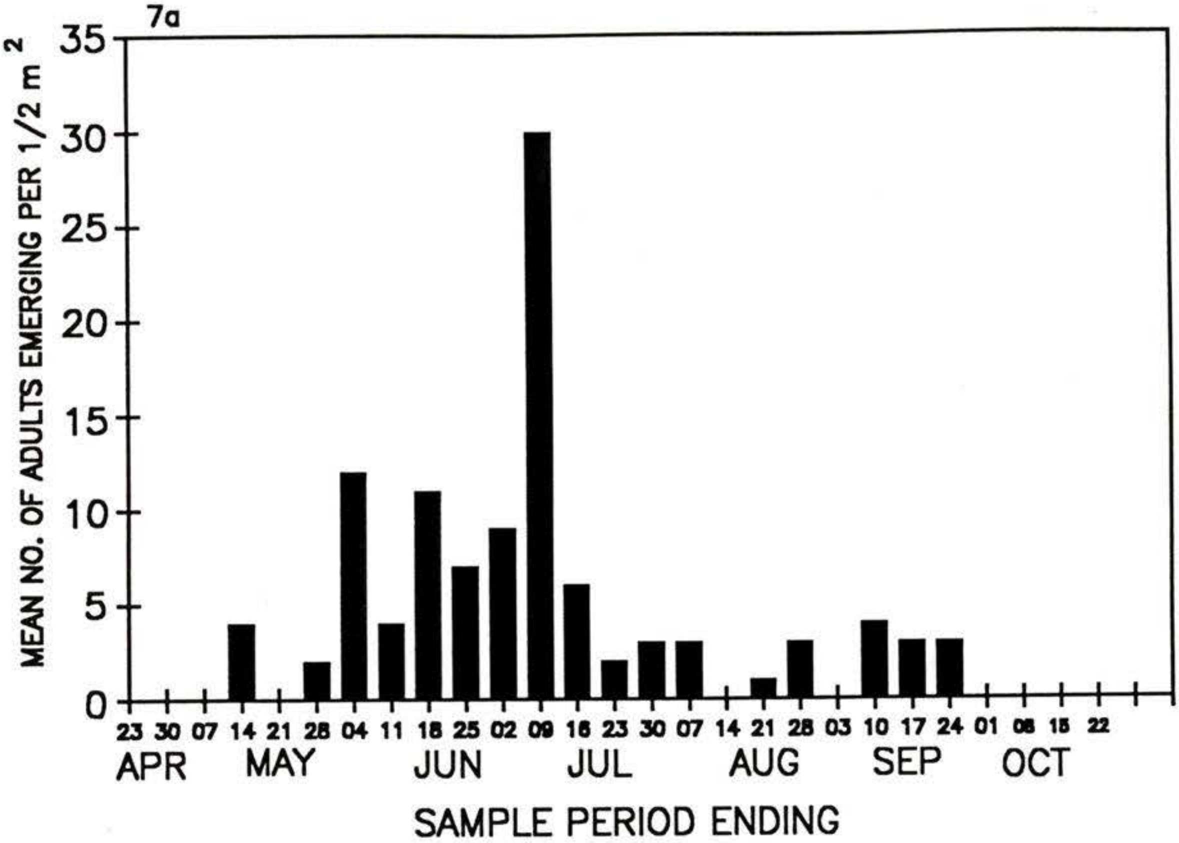
28
30
03 10 17 24 01 08 15 22 28 06 13 20 27 03 10 17 24 07 14 21 28 05
MAY
JUN
JUL
AUG
SEP
SAMPLE PERIOD ENDING

Figure 7a. Emergence of adult C. *myriophylli* per 1/2 m^2 from Ellison Lake sample sites, 1987.

Figure 7b. Water temperature at 1/2 m depth at Ellison Lake sample sites, 1987.

7a


MEAN NO. OF ADULTS EMERGING PER 1/2 m 2
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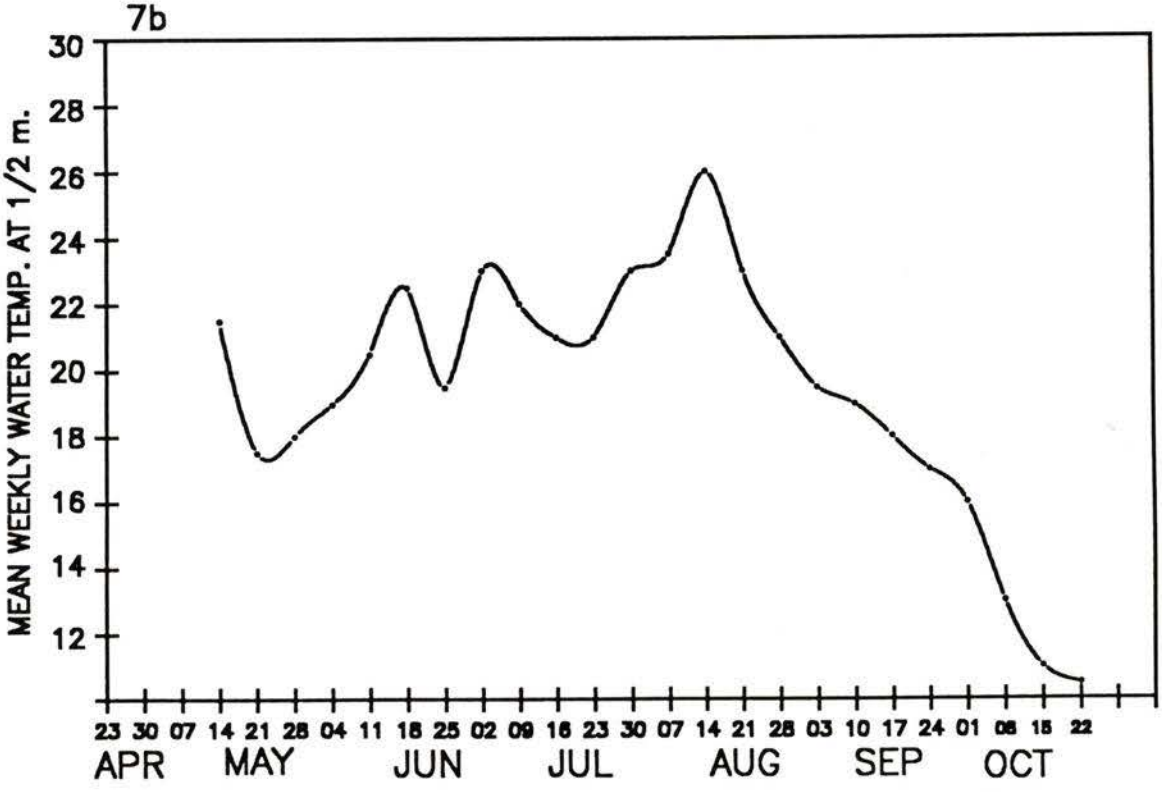
30
35
23 30 07 14 21 28 04 11 18 25 02 09 16 23 30 07 14 21 28 03 10 17 24 01 08 15 22
APR MAY JUN JUL AUG SEP OCT
SAMPLE PERIOD ENDING

7b


MEAN WEEKLY WATER TEMP. AT 1/2 m.
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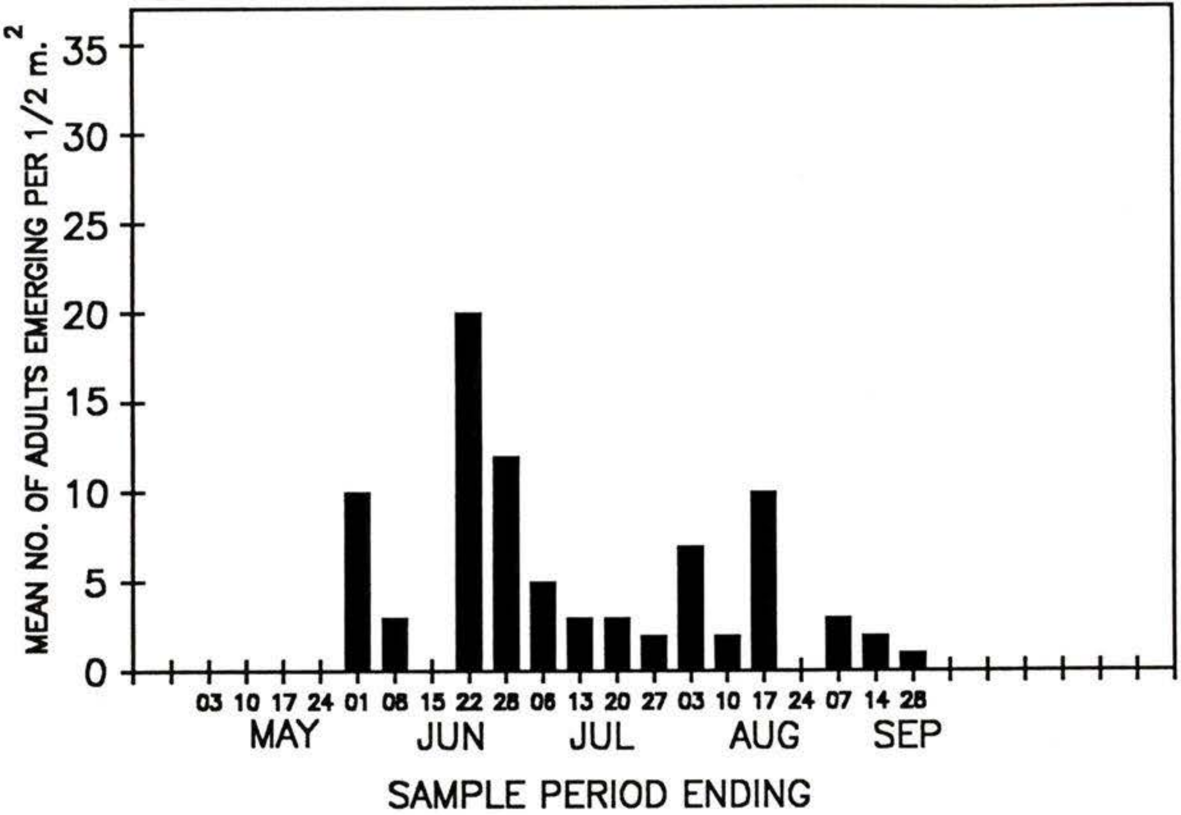
23 30 07 14 21 28 04 11 18 25 02 09 16 23 30 07 14 21 28 03 10 17 24 01 08 15 22
APR MAY JUN JUL AUG SEP OCT

Figure 8a. Emergence of adult *C. myriophylli* per 1/2 m^2 from Wood Lake sample sites, 1986.

Figure 8b. Water temperature at 1/2 m depth at Wood Lake sample sites, 1986.

8a


MEAN NO. OF ADULTS EMERGING PER 1/2 m.^{2}
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15
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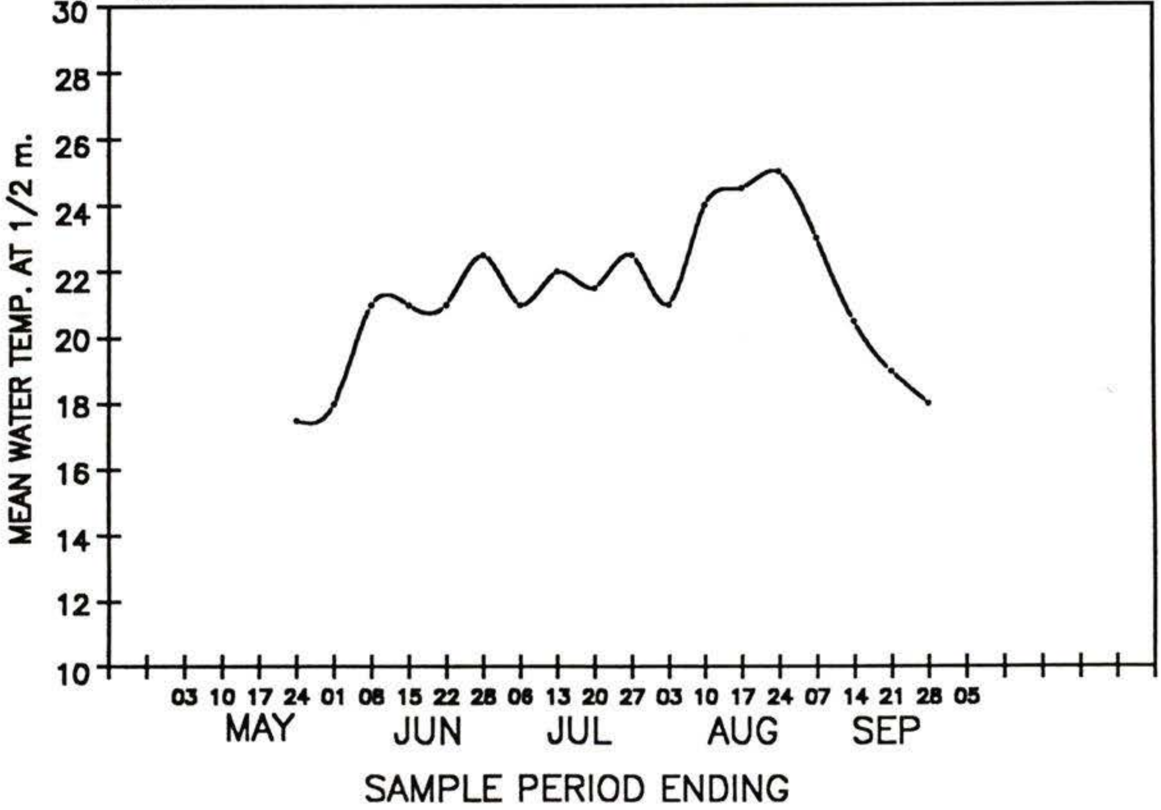
03 10 17 24 01 08 15 22 28 06 13 20 27 03 10 17 24 07 14 28
MAY
JUN
JUL
AUG
SEP
SAMPLE PERIOD ENDING

8b


MEAN WATER TEMP. AT 1/2 m.
30
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16
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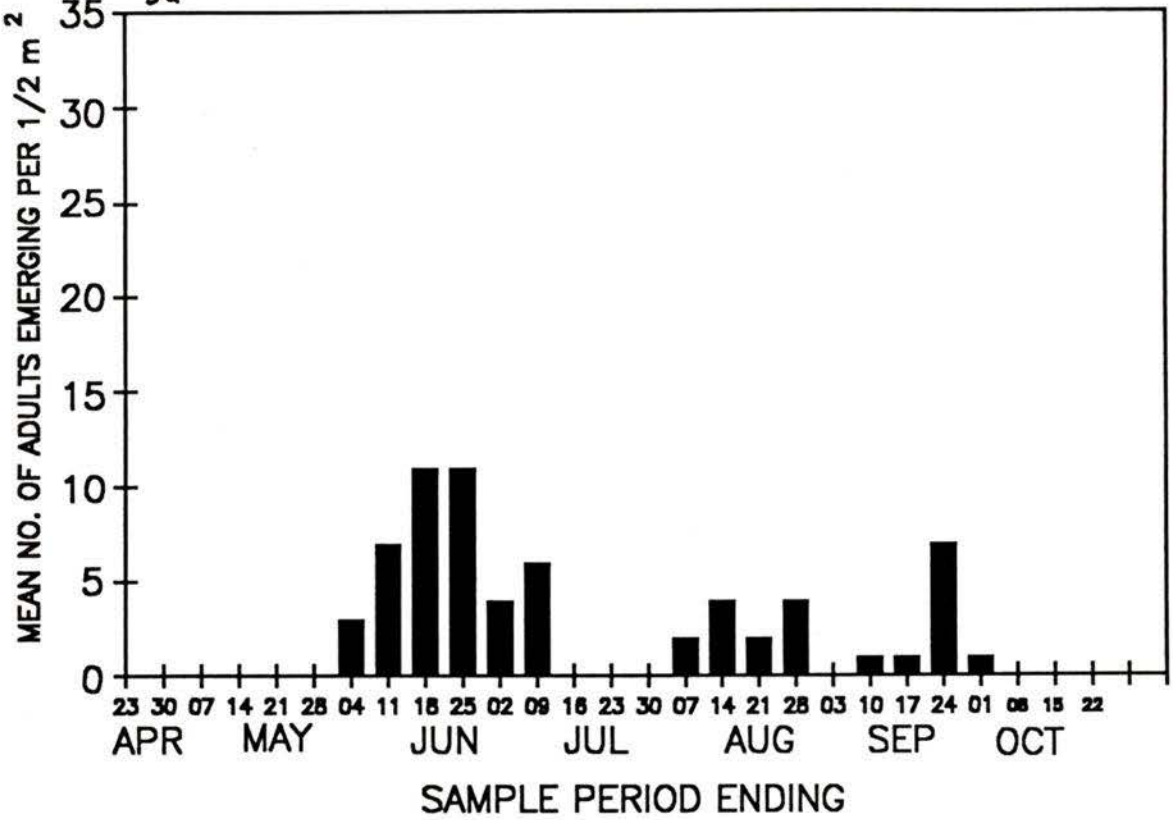
03 10 17 24 01 08 15 22 28 06 13 20 27 03 10 17 24 07 14 21 28 05
MAY
JUN
JUL
AUG
SEP
SAMPLE PERIOD ENDING

Figure 9a. Emergence of adult C. *myriophylli* per 1/2 m^2 from Wood Lake sample sites, 1987.

Figure 9b. Water temperature at 1/2 m depth at Wood Lake sample sites, 1987.

9a


MEAN NO. OF ADULTS EMERGING PER 1/2 m 2
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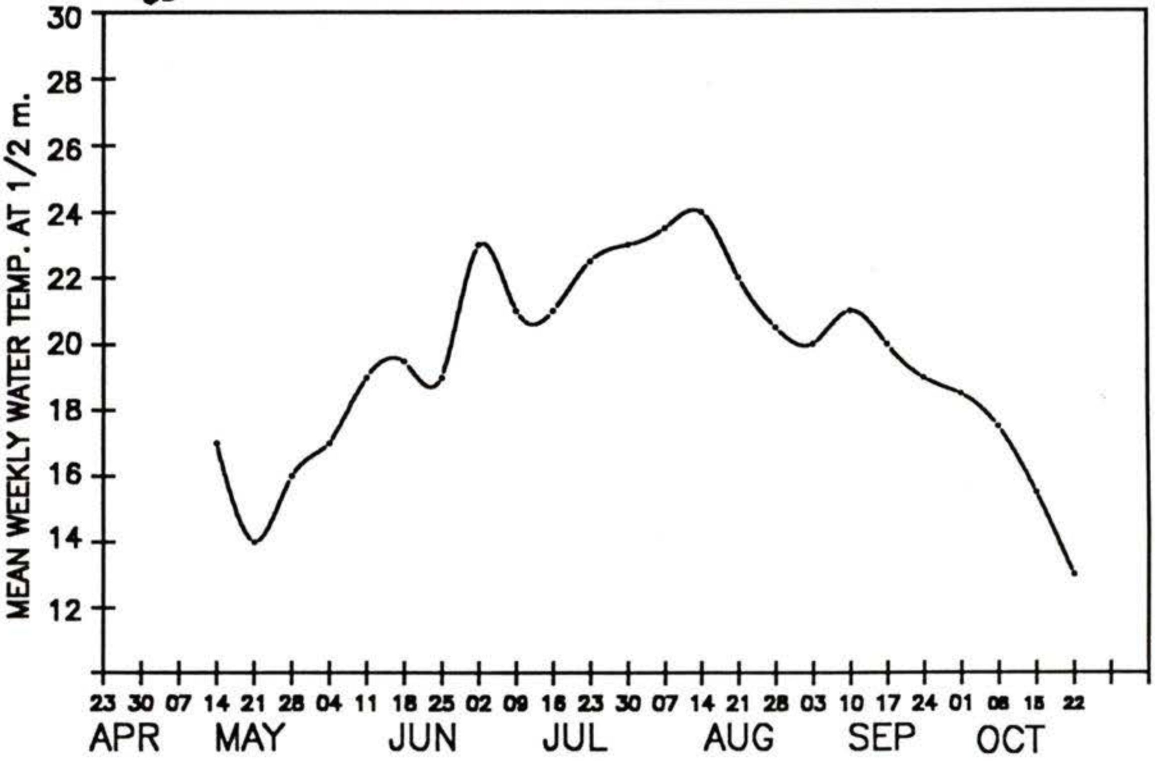
25
20
15
10
5
0
23 30 07 14 21 28 04 11 18 25 02 09 16 23 30 07 14 21 28 03 10 17 24 01 08 15 22
APR MAY JUN JUL AUG SEP OCT
SAMPLE PERIOD ENDING

9b


MEAN WEEKLY WATER TEMP. AT 1/2 m.
30
28
26
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22
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18
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14
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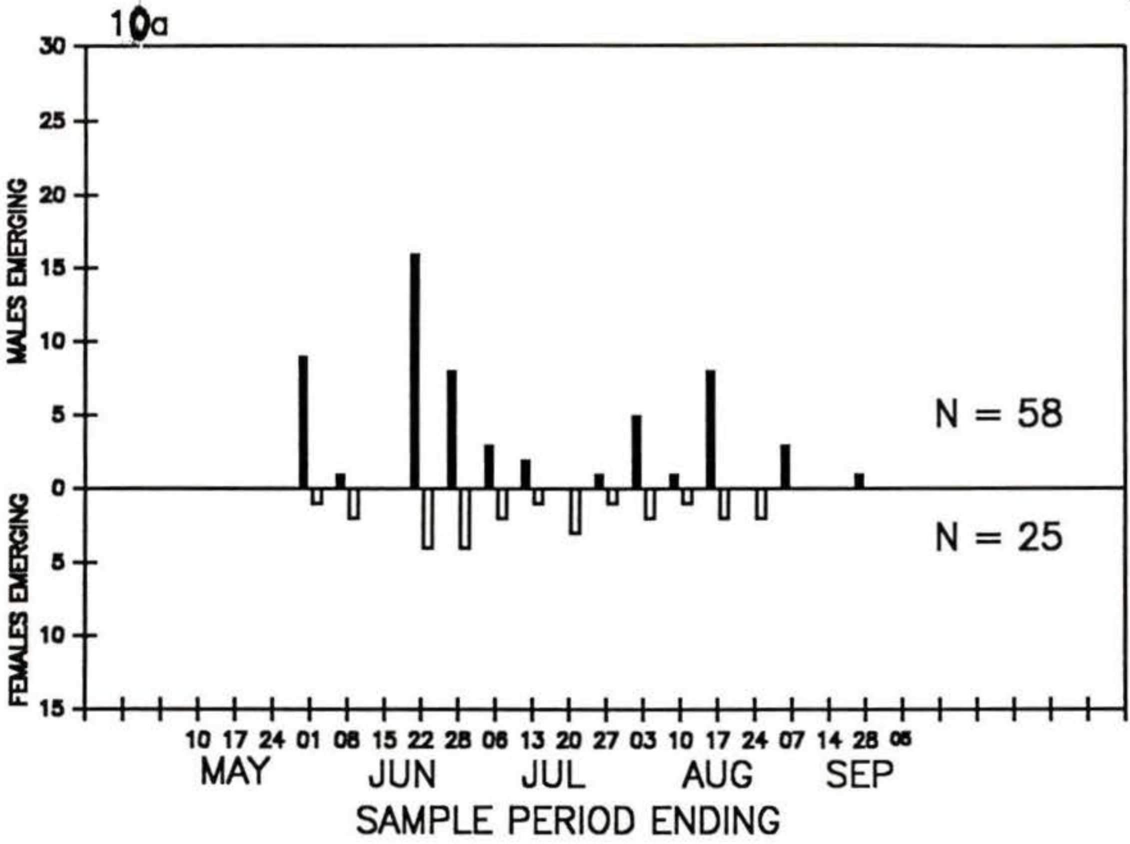
23 30 07 14 21 28 04 11 18 25 02 09 16 23 30 07 14 21 28 03 10 17 24 01 08 15 22
APR MAY JUN JUL AUG SEP OCT

Figure 10a. Emergence of adult *C. myriophylli* (males above, females below) from Wood Lake, 1986.

Figure 10b. Emergence of adult *C. myriophylli* (males above, females below) from Wood Lake, 1987.

10a


30
25
20
15
10
5
0
5
10
15
MALES EMERGING
FEMALES EMERGING
N = 58
N = 25
10 17 24 01 08 15 22 28 06 13 20 27 03 10 17 24 07 14 28 06
MAY
JUN
JUL
AUG
SEP
SAMPLE PERIOD ENDING

10b

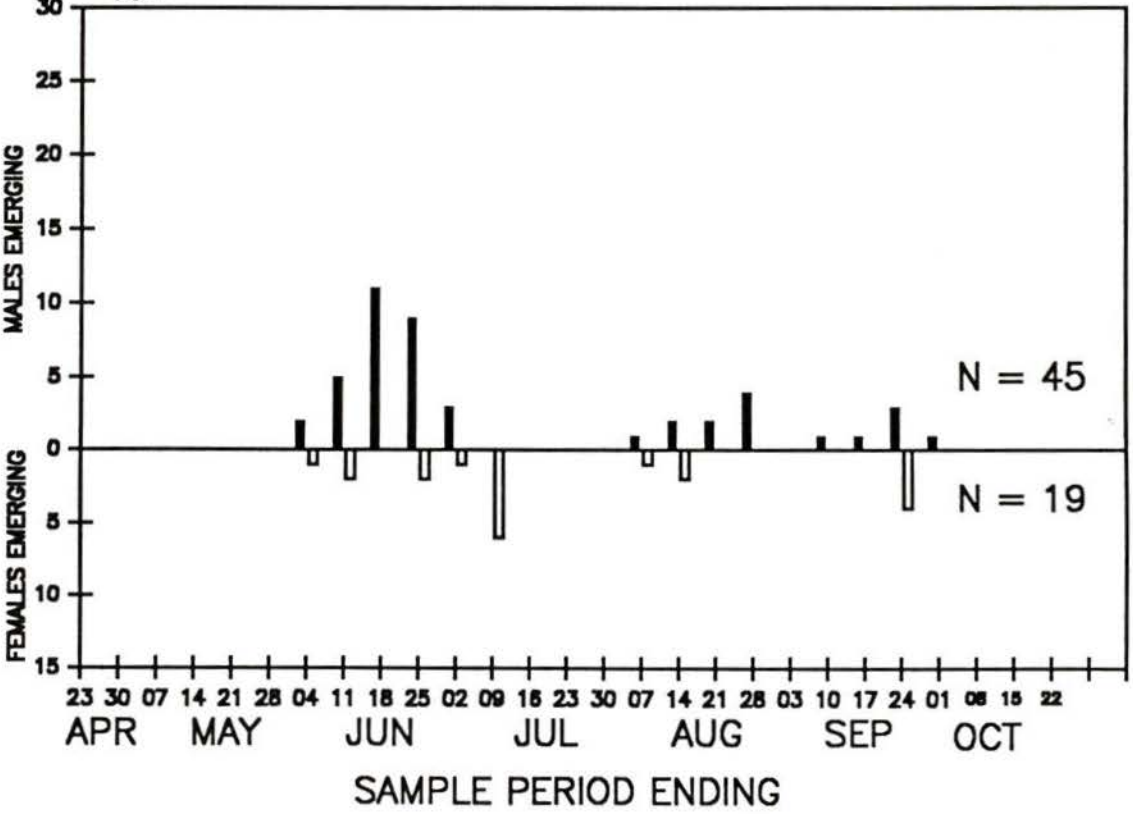
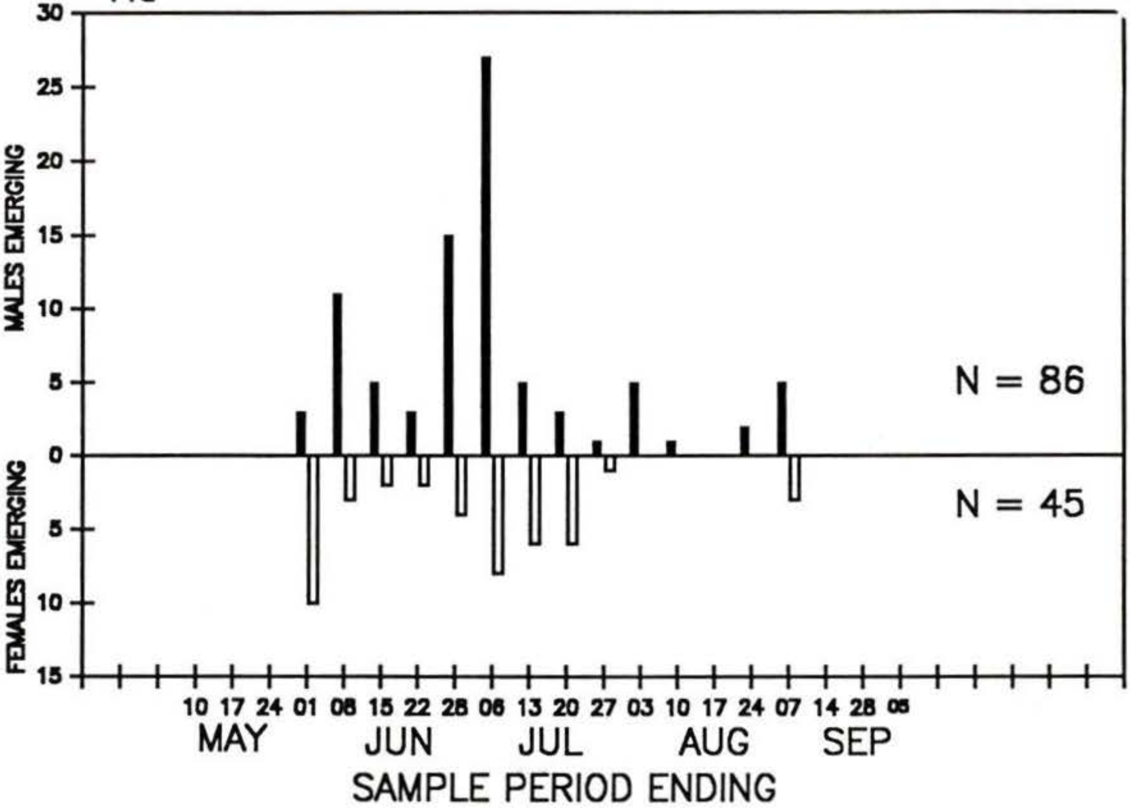

30
25
20
15
10
5
0
5
10
15
MALES EMERGING
FEMALES EMERGING
N = 45
N = 19
23 30 07 14 21 28 04 11 18 25 02 09 16 23 30 07 14 21 28 03 10 17 24 01 08 15 22
APR
MAY
JUN
JUL
AUG
SEP
OCT
SAMPLE PERIOD ENDING

Figure 11a. Emergence of adult *C. myriophylli* (males above and females below) from Ellison Lake 1986.

Figure 11b. Emergence of adult *C. myriophylli* (males above and females below) from Ellison Lake 1987.

11a


30
25
20
15
10
5
0
5
10
15
MALES EMERGING
FEMALES EMERGING
N = 86
N = 45
10 17 24 01 08 15 22 28 06 13 20 27 03 10 17 24 07 14 28 06
MAY
JUN
JUL
AUG
SEP
SAMPLE PERIOD ENDING

11b

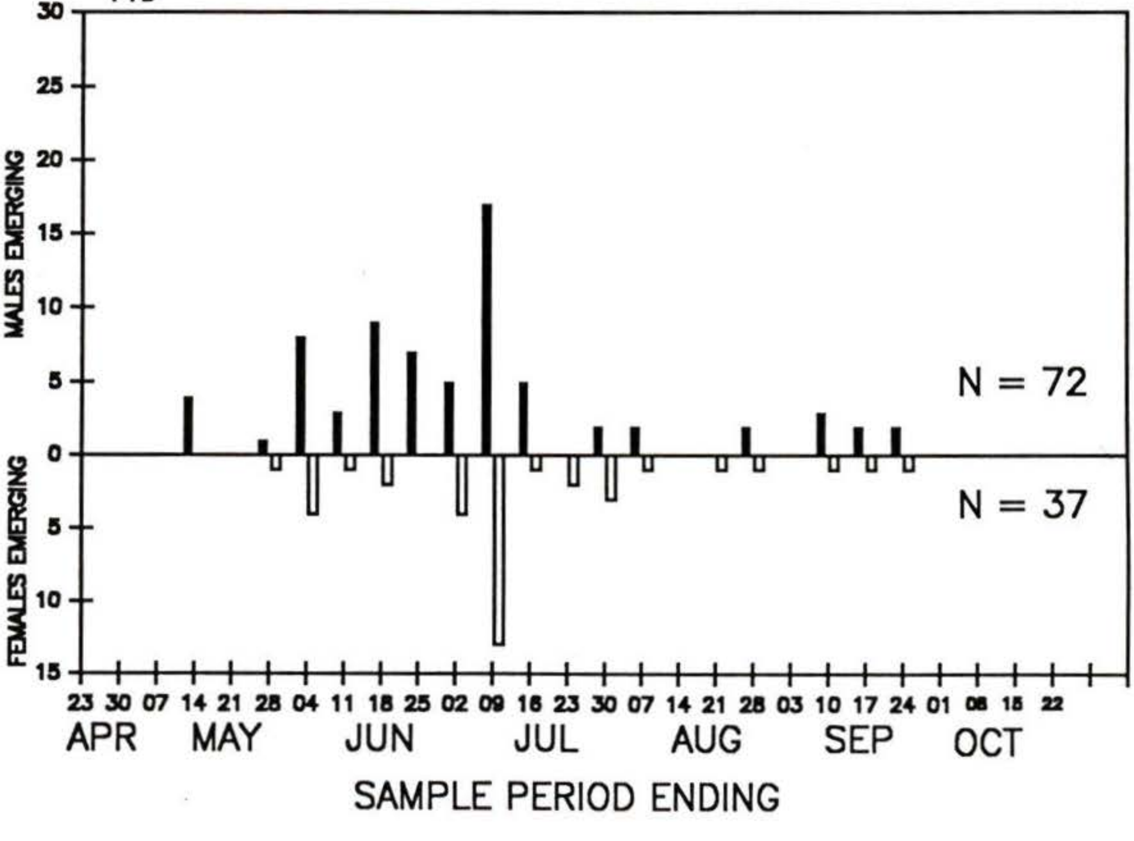

30
25
20
15
10
5
0
5
10
15
MALES EMERGING
FEMALES EMERGING
N = 72
N = 37
23 30 07 14 21 28 04 11 18 25 02 09 16 23 30 07 14 21 28 03 10 17 24 01 08 15 22
APR
MAY
JUN
JUL
AUG
SEP
OCT
SAMPLE PERIOD ENDING

Figure 12. Temporal presence of *C. myriophylli* life cycle stages. Numbers indicate total individuals recovered from samples taken once per month.

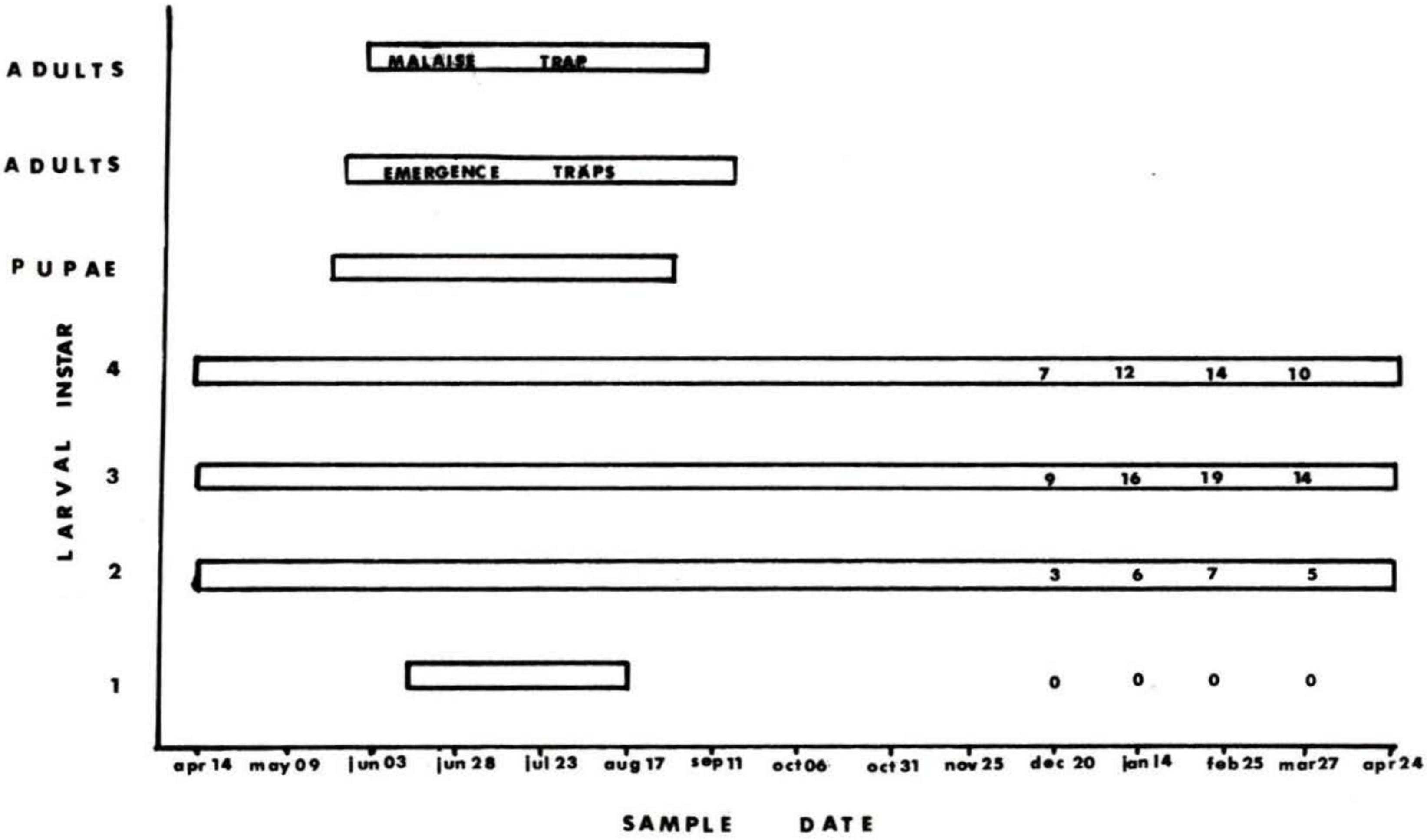

ADULTS
MALAISE TRAP
ADULTS
EMERGENCE TRAPS
PUPAE
LARVAL INSTAR
4
7 12 14 10
3
9 16 19 14
2
3 6 7 5
1
0 0 0 0
apr 14
may 09
jun 03
jun 28
jul 23
aug 17
sep 11
oct 06
oct 31
nov 25
dec 20
jan 14
feb 25
mar 27
apr 24
SAMPLE DATE

Figure 13: Head capsule length vs. head capsule width of larval *C. myriophylli*. Groupings represent larval instars. Some points represent more than one measurement, bars represent 95 % C.I.

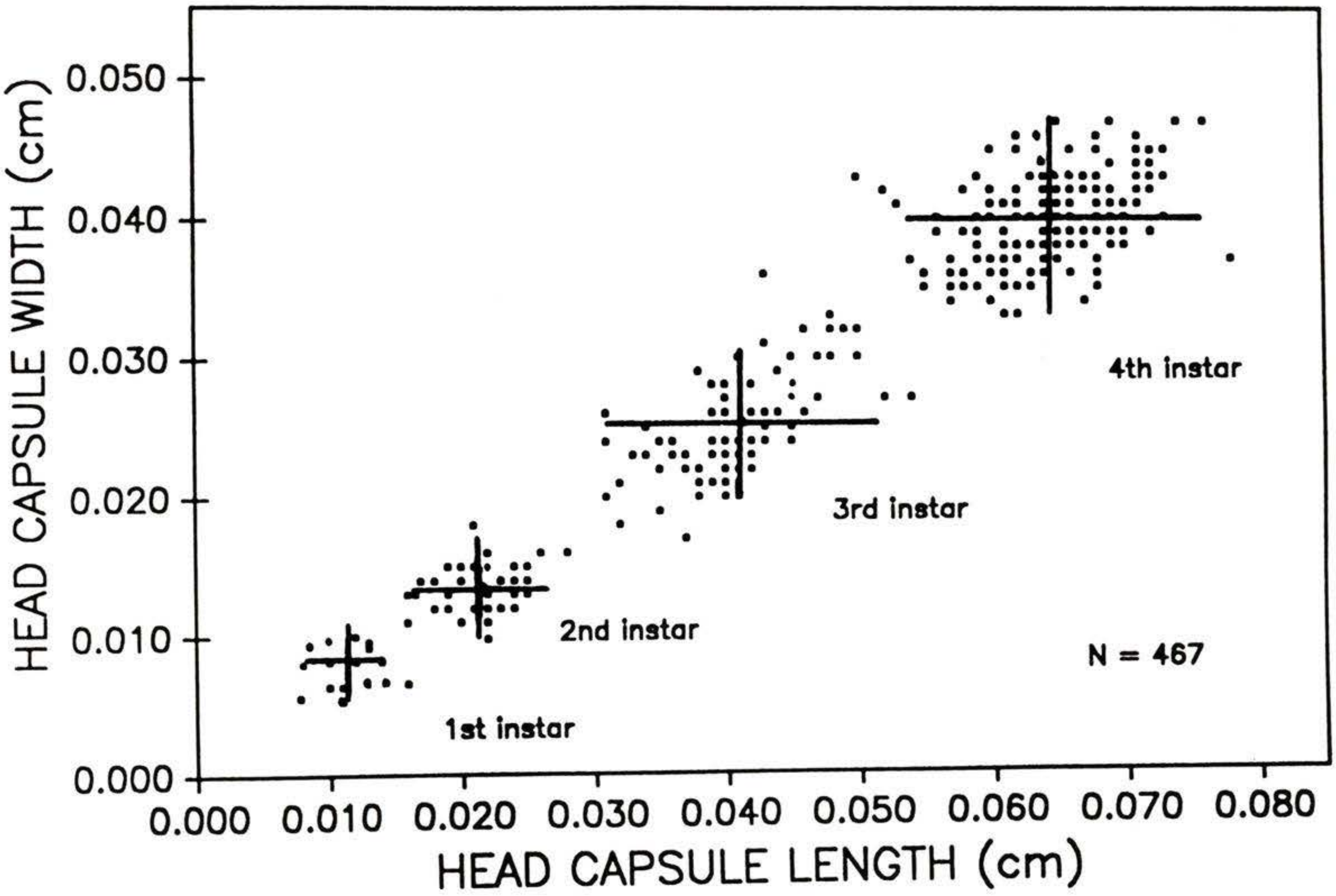
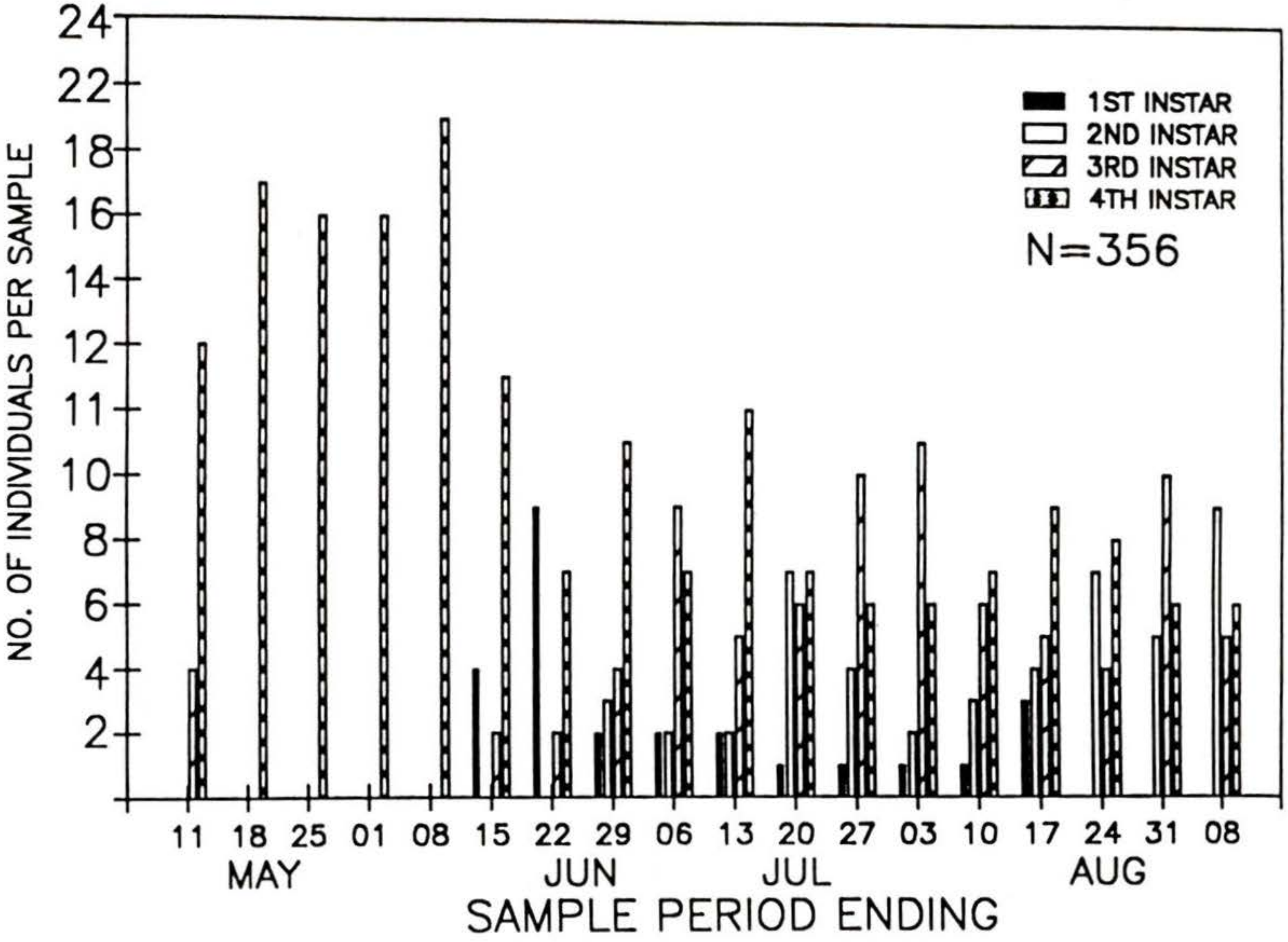

HEAD CAPSULE WIDTH (cm)
0.000
0.010
0.020
0.030
0.040
0.050
HEAD CAPSULE LENGTH (cm)
0.000
0.010
0.020
0.030
0.040
0.050
0.060
0.070
0.080
1st instar
2nd instar
3rd instar
4th instar
N = 467

Figure 14: Occurrence of larval instars in weekly and monthly morphometric samples, summer, 1986.


24
22
18
16
14
12
11
10
8
6
4
2
NO. OF INDIVIDUALS PER SAMPLE
■ 1ST INSTAR
☐ 2ND INSTAR
▨ 3RD INSTAR
▥ 4TH INSTAR
N=356
11
18
25
01
08
15
22
29
06
13
20
27
03
10
17
24
31
08
MAY
JUN
JUL
AUG
SAMPLE PERIOD ENDING

2. Reproduction and Rearing

Swarming observations commenced June 10, 1986. Reports from early investigators stated that *C. myriophylli* swarms formed only above trees and, therefore, occurred approximately 10-15 m in the air (Kangasniemi and Oliver, pers. comm.). A *C. myriophylli* swarm observed on June 21, 1985 (at 20:45 hrs.) formed above a picnic table 1 m in height. Many swarms were subsequently observed forming over low bushes and other structures less than 3 m high.

In late June, swarming generally began at approximately 19:45-20:00 hours and continued throughout dusk until just before nightfall (21:30-22:00 hours). As summer proceeded and daylight lengthened and then shortened, the timing of the swarms in relation to available light remained the same, beginning at early dusk and breaking up before dark.

All swarms sampled were composed only of males. One female was recovered from aerial net sampling.

At the conclusion of the first rearing tent trial (July 29, 1987) a check of the oviposition tank revealed very heavy feeding damage to the plants. Approximately 92% of the meristems exhibited feeding damage. However, only 3 live larvae (two 2nd. instar, one 3rd. instar) and 9 dead larvae were found. There also were larval cases on the plants but none contained pupal exuviae. Neither were any exuviae found floating on the surface. The temperature of the pool was then taken with a thermometer and found to be 35 -37 C.

At the conclusion of the 2nd. rearing-tent trial (Sept 30/ 87.), a check of the oviposition tank revealed no damage to the plants. Plants were examined but no eggs were found. However, stable mating swarms of *C. myriophylli* were observed forming above the uppermost poplar branches on Aug. 28 and 29.

3. Feeding

3.1 Preliminary Trials - Mean growth rates of plants with 1, 2, 3 or no *C. myriophylli* larvae feeding on them were charted (Figure 15). A one-way Anova indicated there was a highly significant difference between the growth rate of *M. spicatum* plants with 1, 2, or 3 larvae feeding on them and plants without ($P << .0005$). A Newman-Keuls multiple comparison indicated no significant difference in the growth rate of plants with 1, 2, or 3 larvae feeding on them. Observations on the feeding larvae indicated that one individual could crop a meristem in 3-5 days.

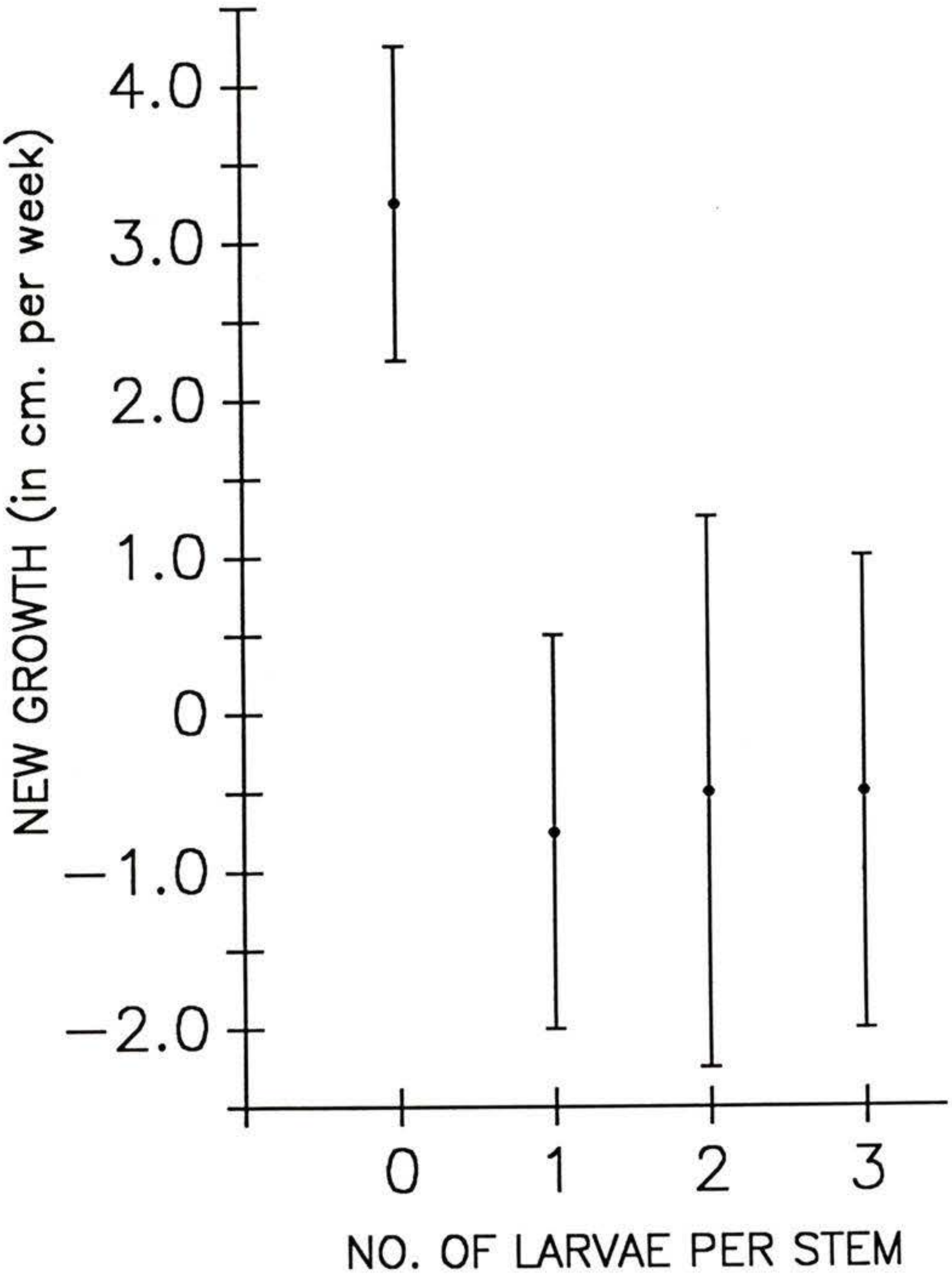
It was observed that usually only one larva becomes established on each apical tip, although this did not always preclude a second, or even a third, from becoming established on the same stem. Usually additional larvae were located on either an axillary meristem or on the stem itself, just below a meristem.

None of the browsed meristems which were planted grew or developed in any way to establish a new plant. The meristems of the non-fed upon tips planted in the same aquarium subsequently grew well, indicating that conditions for growth were suitable in the testing aquaria.

Surveys on native weed beds conducted to detect possible feeding by *C. myriophylli* indicated no evidence of the presence of the insect on any of the plants examined other than species of *Myriophyllum*. This observation was reinforced by the trials involving a variety of native plants placed together in an aquarium with and without *M. spicatum*.

Other than *M. exalbescens*, none of the native plants in the aquarium showed any sign of feeding damage. Even in the absence of *M. spicatum*, only *M. exalbescens* showed any feeding damage or presence of larvae.

Figure 15: Mean new growth of *M. spicatum* plants (in cm per week) with 0, 1, 2, or 3 *C. myriophylli* larvae feeding on them. Vertical bars represent 95 % C.I.

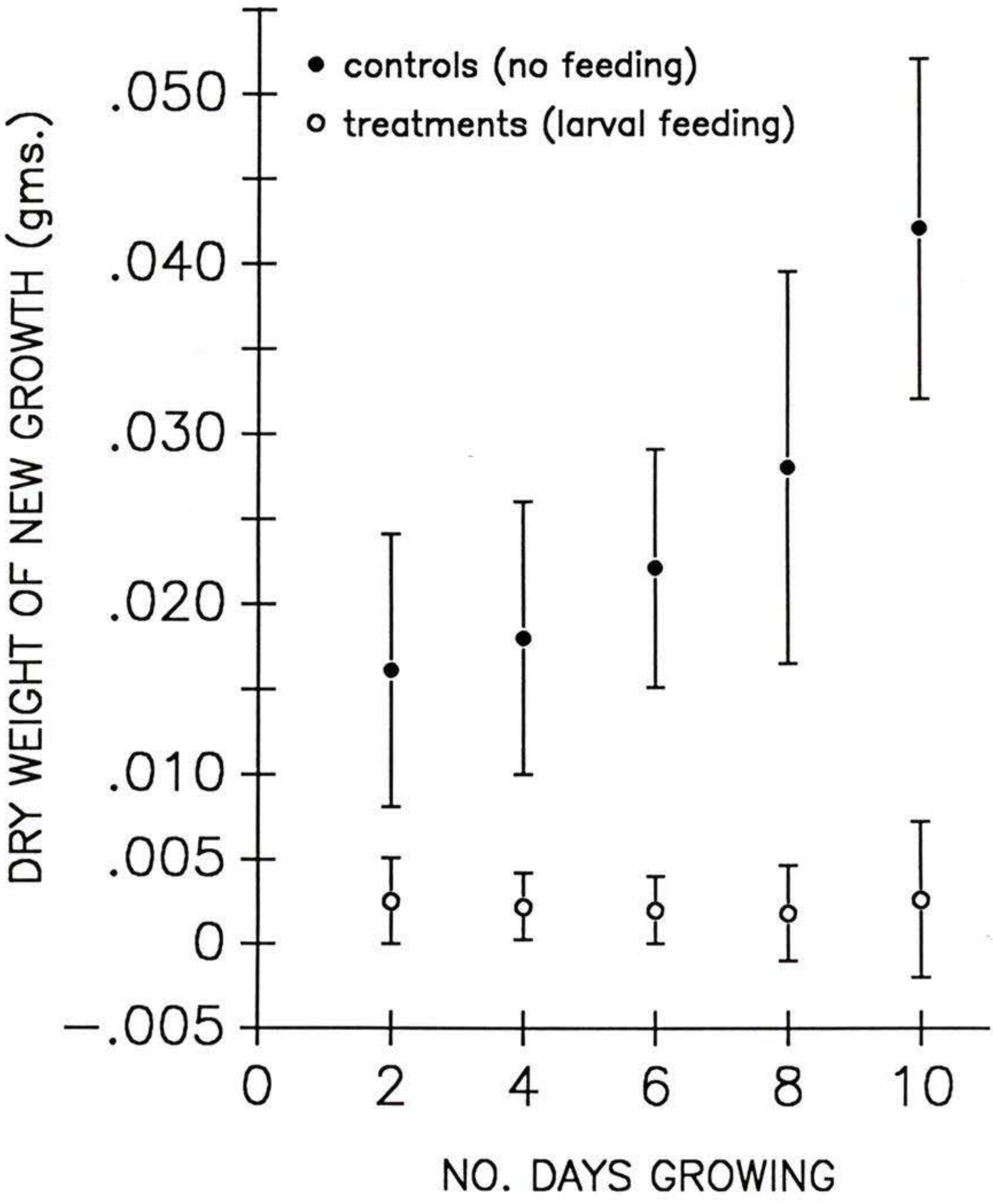

NEW GROWTH (in cm. per week)
4.0
3.0
2.0
1.0
0
−1.0
−2.0
0
1
2
3
NO. OF LARVAE PER STEM

3.2. Suppression of Growth - A two-way anova indicated a highly significant difference ($P < .0005$) in the growth rates of fed upon and non-fed upon individuals of *M. spicatum* (Figure 16). The effect of time was also highly-significant ($P < .0005$). The interaction effect was also found to be significant ($P < .025$). There was no significant difference in the amount of new growth in all fed-upon groups through time. While there was no significant difference in the amount of new growth of non-fed upon groups at day 2 and 4, there was a significant difference in new growth of non-fed upon plants between all other time intervals.

Most meristematic tissue of the apical tip was totally culled by the larvae within 2-3 days. Unless axillary meristems subsequently developed, there was no growing tissue present after this.

More non-fed upon plants finished the trials with extant axillary meristems than fed upon plants. This observation was later verified by a 2x2 contingency table ($P << .001$).

Figure 16: Effect of *C. myriophylli* larval feeding on new growth of *M. spicatum*. Treatment plants have one *C. myriophylli* larvae per stem, control plants have none.


● controls (no feeding)
○ treatments (larval feeding)
DRY WEIGHT OF NEW GROWTH (gms.)
.050
.040
.030
.020
.010
.005
0
−.005
0
2
4
6
8
10
NO. DAYS GROWING

3.3 Host Preference - Of all the native plants tested in isolation as potential food sources for *C. myriophylli* (Table 1), the only species fed upon was *Myriophyllum exalbescens*, a native milfoil. There was limited feeding on *Potamogeton natans* by 2 larvae. Their gut contents, however, were brown, not green, indicating that they had been feeding on the dead tissue at the leaf edges and not the healthy meristematic tissue present. Although the larvae were found on the leaf of *P. natans*, no larval cases were present on the plant. There was a limited amount of case-building activity on *Ranunculus aquatilis* (3 larvae) but the guts of all individuals were empty and no feeding damage was observed on the plant. In addition, the cases were constructed entirely of silk without incorporation of any plant material. None of the larvae on native plants other than *M. exalbescens* survived to pupation.

A two-tailed binomial-test did indicate a preference for feeding on *M. spicatum* over *M. exalbescens* (22 of 32 larvae fed on *M. spicatum*, P = 0.035) There was, however, a preference for pupating on *M. exalbescens* (P = 0.035) over *M. spicatum*.

Table 1: Native aquatic macrophyte species tested in isolation (starvation) as suitable food sources for *Cricotopus myriophylli* (- denotes no activity, + denotes presence of activity, -+ indicates some isolated activity, + + denotes considerable activity).

Species Tested	**Case Building**	**Feeding**
Elodea canadensis	-	-
Ranunculus aquatilis	- +	-
Ceratophyllum demersum	-	-
Potamogeton crispus	-	-
P. pectinatus	-	-
P. zosteriformis	-	-
P. natans	-	- +
P. amplifolius	-	-
Nuphar polysepalum	-	-
Nymphaea odorata	-	-
Lemna minor (floating)	-	-
Myriophyllum exalbescens	+	+ +

3.4 Relocation - Larvae relocated to *M. spicatum* plants under lab and field conditions. Larvae removed from their cases swam to the nearest plant and became established there. Larvae from culled meristems placed into the aquarium, relocated to planted stems. A two-tailed binomial test indicated that this was significant ($P < .00005$). New plants were placed in those cells which held relocated larvae. A significant number of larvae relocated to these fresh plants ($P < .00005$, one-tailed binomial test). A significant number of larvae removed from their cases and placed into the cells became established on planted *M. spicatum* stems ($0.25 < P < 0.05$, one-tailed binomial test). All of the larvae on planted *M. spicatum* stems relocated to fresh stems when they had exhausted the occupied stem's source of meristematic tissue.

3.5 Temperature Cues - Larvae were observed feeding during the autumn of 1987 when water temperatures were 10 C. (Oct. 12). Feeding activity increased above 13 -15 C. and was most active between 18 - 25 C.

4. Compatibility With Existing Control Techniques

T-tests indicated no significant difference in the number of meristems per *M. spicatum* stem in control and harvesting sites. Numbers of larvae/meristem were decimated by harvesting but rebuilt over time (Table 2). T-tests indicated a significant difference between population levels post-harvesting and 2 weeks later in all 6 survey locations ($P < .0005$ in all cases). Population levels of the 2 treated sites and 1 control site were significantly different 10 weeks post-harvesting ($.025 < P < .05$, one-way ANOVA, in all 3 cases). Subsequent Newman-Keuls multiple comparisons showed no significant difference between the two treated sites. Of the larvae recovered after the 2 week interval, many were 3rd and 4th instars.

Table 2: Effects of mechanical harvesting on populations of *Cricotopus myriophylli*.

	Mean No. Larvae/Meristems After Harvesting (2-5 days)		Mean No. Larvae/Meristems After Harvesting (2 weeks)
Kelowna Foreshore			
Rowing Club	9/50		27/50
Brown's Point	7/50		28/50
Cable Area (control, no harvesting)		39/50	
Wood Lake			
North Shore	5/50		29/50
East Shore	11/50		29/50
Intake (control, no harvesting)		45/50	
Vernon Arm			
Kin Beach	6/50		31/50
Sproul's Corner	7/50		29/50
Cable Area (control, no harvesting)		42/50	

5. Distribution

Surveys conducted in *M. spicatum* infestations throughout the Pacific Northwest revealed that *C. myriophylli* larvae were present only in the Okanagan Valley lakes system; Swan Lake in Vernon; Cultus Lake and Sardis Pond; some ponds and sloughs in the lower mainland; and Long Lake on Vancouver Island (reported in 1987). In addition it has also been recently recorded from Magic Lake and the Cowichan River system (Winchester, pers comm., 1988).

In eastern Canada , *C. myriophylli* has been reported from Burleigh Falls, Peterborough Co.; Chaffey Locks, Leeds Co.; Grenadier Is., St. Lawerence Islands National Park; Lake Opinicon, Leeds Co.; Squaw Islands, St. Law. Is. Nat. Pk.; and St Lawerence River, E. of Tremont Island, St. Law. Is. Nat. Pk. (Oliver, 1984).

DISCUSSION

1. Life History

1.1 Emergence - Floating traps were used rather than a submerged type because they were readily available and inexpensive. It also has been suggested that floating traps are more accurate in estimating the emergence of insects from aquatic macrophyte beds than non-floating traps (Johnson and Mulla, 1982). The clear plastic windows functioned well in attracting the insects to the top of the trap. This has also been recorded when comparing clear and opaque funnel traps (Daniel *et al.*, 1985).The first adult emergence of 1986, in both lakes, was from 24-26 May and first instar larvae were first collected in that year on 17-19 June. Mating, oviposition, and hatching, therefore, must all occur within 2 1/2 - 3 weeks, the time period from initial emergence to the occurrence of 1st instars. Furthermore, as there is protandry in emergence, males emerging before females by a period of 2-4 days, it is likely that the length of the egg stage is approximately 2 weeks.

The temporal occurrence of 1st instar larvae and the length of the egg stage indicate that the final successful oviposition event of the year must occur during early August. Although duration of the egg stage in most chironomids is unknown it is felt that it may be dependent upon temperature and perhaps related to the overall length of the life cycle (Oliver, 1971). If developmental time for the egg is influenced by temperature, then duration of the egg stage may be decreased, and the last successful oviposition of the year may occur as late as the 2nd week of August. In addition, the presence of first instars for only two months, with a peak occurrence in late June, indicates that the insect is univoltine.

Overwintering larvae feed and begin development as water temperature increases in the spring. There appears to be a minimum temperature which triggers pupation. Partial evidence for this lies in the fact that no emergence was recorded when water temperatures were below 16 C. Although light levels also may be involved, this factor probably is of secondary importance. Both temperature and light have been recorded as influencing emergence of several species of chironomids (Corbet, 1964; Fischer and Rosin, 1968). In May, 1987, unseasonably warm temperatures raised the water temperatures of shallow Ellison Lake to 20 C. in early May. A small emergence of males was seen, but when air temperatures dropped rapidly, water temperatures also decreased to 14 -15 C. No further emergence was recorded until water temperatures rose above 16 C. once more, despite the fact that daylight hours would have been increasing throughout the period of lowering water temperatures. The fact that no emergence was monitored when water temperatures at the sample sites rose above 25 C indicates this may be a maximum threshold above which emergence does not occur. Although adults were recovered from the Malaise trap during this period these individuals may have emerged from a different area of the lake where water temperatures may not have been this high.

Multiple regression analyses indicated that temperature and DO_2 levels had no effect on the rate of emergence. This is understandable when one considers that the daily temperatures in the littoral zone of these lakes vary considerably during the summer months. Mean summer air temperatures in the Okanagan are warm, but drop significantly during overcast or rainy periods.

Water temperatures will, correspondingly, drop a few degrees, although not much more as these lakes represent a considerable heat sink. Any aquatic insect species in these circumstances must, therefore, be plastic in its emergence habits.

DO_2 levels are more variable than temperature in *M. spicatum* weedbeds. Significantly different levels have been recorded in a weedbed at different times of the same day as the photosynthetic activity of *Myriophyllum spicatum* increases the dissolved oxygen in the surrounding water to supersaturation during daylight hours (Newroth, pers. comm.). This is evident when observing the plants underwater. Long trails of bubbles issue from the plants and float surfaceward. Correspondingly, there is an associated drop in DO_2 levels at night when photosynthesis stops.

The ratio of emerging males to females was not equal for either lake sampled. In both lakes sex ratios approached 2:1 (males:females). The argument proposed by Fisher (1958) that it is most advantageous for an individual to produce a 1:1 sex ratio of offspring is generally accepted when both sexes experience equal mortality and require equal parental investment to be produced (Trivers and Hare, 1976). A skewed sex ratio indicates that there is either greater mortality or greater parental investment involved in the creation of one sex. Although the females of *C. myriophylli* are slightly larger and might, therefore, represent a greater parental investment, they have only a single clutch of approximately 200 eggs ($x = 189.25$, $s = 7.72$, from 10 females examined). This sexual dimorphism in size may be a contributing factor in the skewed sex ratio, but it is doubtful that this could account for twice the cost of production of a male. A much larger contributing factor is likely a greater mortality experienced by the males. Not only do they emerge before the females, and thus are exposed to terrestrial and aerial predation for a longer period of time before mating, but they are the sex that forms the mating swarms. Swarms are almost entirely composed of males, with females being attracted, perhaps by the shape of the swarm (Lindeberg, 1964) or by flight tone (Saweda and Hall, 1980) and entering only long enough to be mated. These swarms are highly visible since they are meant to be a method of mating orientation for the species, and are undoubtedly very attractive to aerial predators such as birds and odonates. Indeed, considerable predation of this sort on *C. myriophylli* swarms was observed. Various insectivorous birds, large dragonflies, mostly Aeshnidae and Libellulidae, and on one occasion even a bat were observed preying on individuals in the swarm.

There is abundant literature dealing with predation of chironomid larvae. The majority of aquatic predation that was observed was due to damselfly nymphs (Odonata: Zygoptera). This agrees with Menzie's (1981) estimate of predation of Cricotopus sylvestris inhabiting M. spicatum plants in New York. Two sources of invertebrate predation mentioned in the literature but not observed were predation on larvae by water-mites (Paterson, 1970) and planaria (Ali and Mulla, 1983). While these were not directly observed in the field or the lab, there were a number of mite species and planaria present in many of the plant and insect samples.

As the larvae feed in their cases and leave only to relocate to a fresh food source they may not suffer extensive predation. This may be why the population is expanding even though the single clutch size is relatively small (x=189).

1.2 Instar Differentiation - Traditionally, head capsule lengths and widths have been used to differentiate chironomid larval instars (Czeczuga, *et al.*, 1968; Oliver, 1971). This tradition has been confirmed by studies which indicate that these are the only reliable measurements which can be taken for identification from chironomid larvae (Berg, 1950; Czeczuga *et al.*, 1968; McCauley, 1974). Although there are several other areas on the larval body which are sclerotized, such as the parapods, they are either too small to measure accurately or are soft enough that some malformation occurs when they are preserved (McCauley, 1974).

Although Oliver (1984) published a complete description of Cricotopus myriophylli, identification of the larva is difficult if it is temporally close to ecdysis to the next stadium. This is due to the wearing of the mentum by feeding. The larvae are identified as Cricotopus sylvestris according to Oliver and Roussel's descriptions (1983) but Oliver (1984) has pointed out that C. myriophylli lacks many features of this species, notably the shape of the mentum (Figure 20) and the presence of setal brushes on the first 7 abdominal segments (Figure 20) rather than the first 6 as in C. sylvestris. These features, in combination with habitat and habits, are the best taxonomic features to use in identifying the larvae of this insect. However, occasionally the setal brushes stick together in the alcohol preservative and appear as a single seta, a taxonomic feature of the genus Psectrocladius. When larvae are in the first 2 instars, it is sometimes difficult to separate these setal brushes. Identification is then dependent on the shape of the mentum the insect's dental code, the location and relative size of the mental 'teeth'.

It has been reported that 1st and 2nd instar larvae are often difficult to differentiate with head capsule measurements due to their small size (McCauley, 1974). This explains the close grouping of those instars on Figure 14. Similarily, the sexual dimorphism in size of adults is reflected in the broad group which represents the 4th instar larvae when the sexual dimorphism in size of male and female chironomids first becomes apparent (Ford, 1959; Hilsenhoff, 1966). The wider range of head capsule lengths and widths of 4th instar larvae in Figure 14, despite a larger sample size than the other instars, reflects the size difference in the sexes. The greater sample sizes of the later instars reflects their presence through a greater portion of the year. As first instars are present only during the mid-summer months, and 2nd instars are present in greatest proportions during this time, they make up a much smaller percentage of the overall, yearly sample.

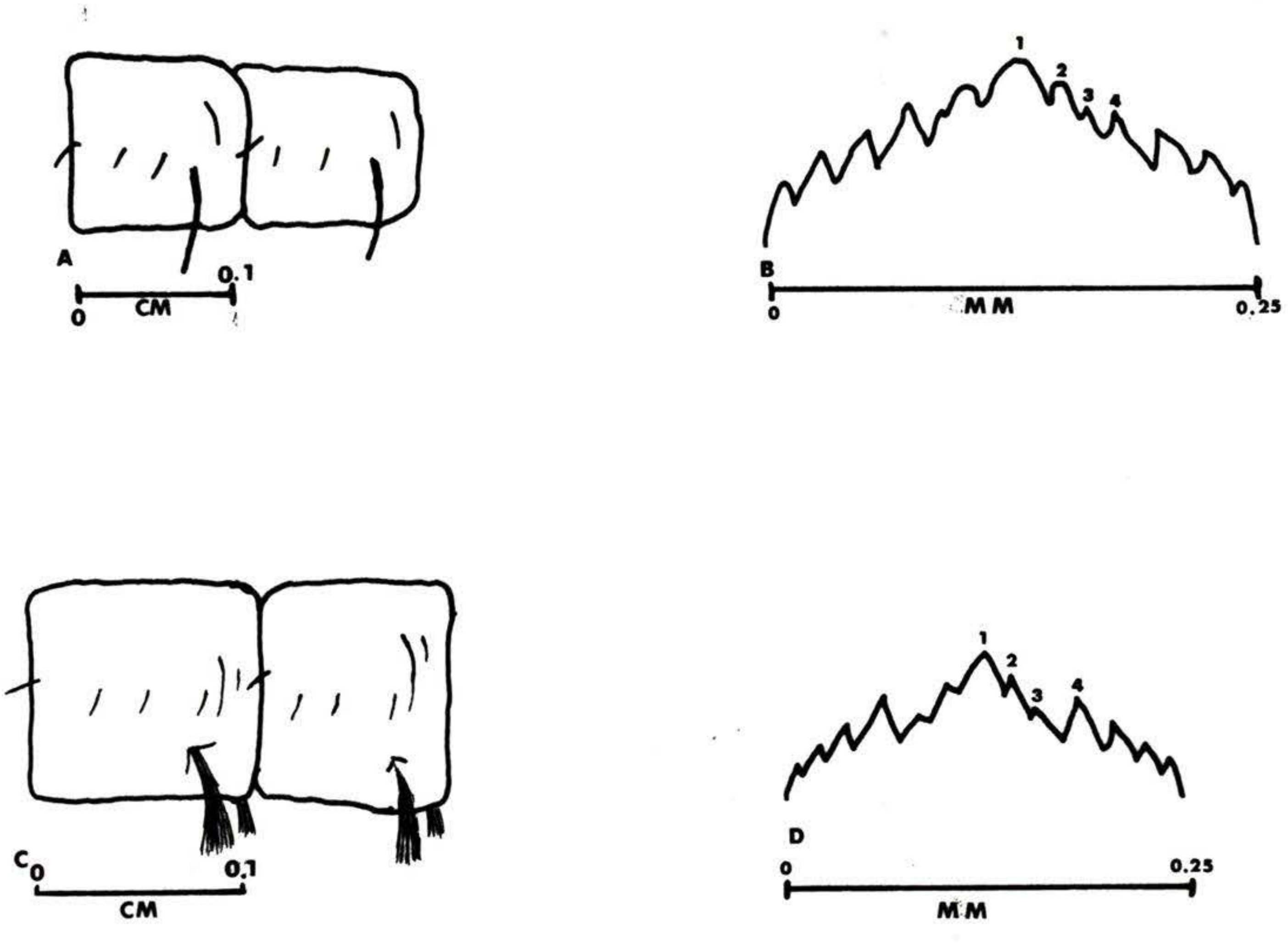
First instar larvae from eggs, taken from a female in the wild, have been examined by Oliver (pers. comm.). These early stadia are difficult to find in the field and were not found *in situ* until June, 1985 in Vernon Arm. These instars were most frequently found in the apical leaflets of healthy *M. spicatum* plants. Occasionally, 1st instars could be found in the occupied cases of later instars. Larvae of *Cricotopus* species have been reported as being phoretic (Vinikour and Anderson, 1981). When found alone, they did not appear to construct cases but instead bored into the soft meristematic tissue located at the plant's tip. Round tunnels filled with frass were found; the end of each was usually occupied by a 1st instar larva. The first instar is small, approximately 1/4 cm long, and its mouthparts are large enough to feed only on the newest meristematic tissue of the plant. Indeed, of those recovered from the plant, all of the individuals had bright green gut contents. The gut contents of later instars, while also green, often had a reddish colour due to feeding on the apical leaflets and sub-meristematic stem. If a meristem was occupied by a later instar, the growing tissue would be fed upon by the larger of the two larvae. First instar larvae found in the cases of older instars may be feeding on the remains of meristematic tissue or other plant material which the younger instar's mouthparts could not directly damage.

Larvae in feeding trials with a water temperature of 21 C. and a photoperiod of 15:9 (L:D) completed the development of each stadium in 10-14 days. As these lab conditions are reflections of natural water temperatures and photoperiods during mid-summer, it can be assumed that larvae in the field are developing at a similar rate. First instars are not found in the field after mid-August. This, with the timing of emergence, confirms the estimate that the latest that oviposition can occur is in early August.

C. myriophylli overwinters primarily in the 3rd instar, this is very important from the perspective of its use as a biocontrol agent. The insect probably does not develop during winter months, as feeding at this time is rare in temperate species (Oliver, 1971). If the insect is not feeding through the winter, it may replenish energy stores depleted by overwintering activity before moulting to the 4th instar. In the 4th instar, it must feed to prepare for metamorphosis, emergence and adult activities, since the adults have reduced mouthparts and do not feed.

Larvae taken from the field in winter were observed feeding on plants in the lab within 12 hours at 20 C and a 10 hour photoperiod. Whether this treatment breaks diapause or the larvae are merely opportunistic and begin to feed when conditions are favourable is, at present unknown. When sampled in the field during winter months, overwintering larvae were present in their cases with the ends closed with plant material. This behaviour has been observed in other species of Chironomidae (Armitage, 1970).

Figure 17: a) Abdominal segment 1 of *Psectrocladius sp*. showing single seta. b) prementum of *C. sylvestris*; note the rounded appearence of the center tooth (# 1)and the shallow gap between the 3rd and 4th lateral teeth. c) abdominal segment 1 of *Cricotopus myriophylli* showing setal brush on abdominal segments (on segments 1 to 7 in *C. myriophylli* and 1 to 6 in *C. sylvestris*) d) prementum of *C. myriophylli*; note the large, pointed center tooth and the deep gap between the 3rd and 4th lateral teeth.


A
0
CM
0.1
B
1
2
3
4
0
MM
0.25
C
0
CM
0.1
D
1
2
3
4
0
MM
0.25

2. Reproduction and Rearing

A nematoceran swarm generally forms on a vertical object that impedes the line of flight, which is usually upwind. As males join the swarm, they move to the part of the swarm where the female is expected to enter, the entry site usually being species specific. This causes the swarm to move about. However, as the individual males 'mark' on vertical objects, the swarm never rises above the height of the marker as the individual males attempt to keep their position relative to it. Therefore, when the swarm has reached the top of the marker, it becomes stable and does not elevate any further. However, the males in the swarm, still moving for preferred position, cause the swarm to move in a motion which is reminiscent of 'tumbling'. This tumbling motion causes the swarm to rise and fall constantly by about 1/2 m.

Early observation of swarming adult *C. myriophylli* by B.C. Ministry of Environment researchers noted that the swarms usually formed above trees. It was then thought that heights of 10 m or more were necessary to stimulate swarming. B.C. Ministry of Environment researchers placed a 3 M. tall rearing tent in a greenhouse at the Vernon campus of Okanagan College and this was filled with male *C. myriophylli* adults collected from swarms in the wild (Wallis, pers. comm.). and the behaviour of these insects was observed. Some excitation in flight behaviour was noted at dusk, but no actual swarming occurred. However, no vertical objects for marking of swarms or tanks containing E.W.M. for oviposition were placed in the tent and the greenhouse had only small windows which opened to permit a limited flow of air which may have hampered swarm orientation. The swarms observed in the 6 m tall rearing tent indicate that these problems have been overcome and swarming can occur in the tent.

Recovery of adults from the Malaise trap throughout the summer indicates that *C. myriophylli* are capable of swarming for this entire period and observations of swarming in the rearing tent confirm this. Swarming is believed to be initiated by low light levels and influenced by several other environmental conditions, such as humidity, wind, and temperature (Downes, 1969; Titmus, 1980; Thornhill and Alcock, 1983). It is necessary, therefore, that the tent be placed in such a position that natural light is available and that there is an adjacent body of water. Many aquatic species of nematocerous Diptera have been observed to swarm only near water or damp patches of soil where the humidity is higher than that of the surrounding area (Downes, 1956, 1969; Oliver, 1971; Savolainen and Syrjamaki, 1971). It is unknown whether this is a physical requirement for swarming or is merely more expedient for the insects' to mate there rather than expend energy to moving from the emergence site. In any case, constructing the rearing facilities in such a way that light, humidity and temperature can be controlled naturally during summer is common sense.

The swarming behaviour observed in the field consisted of all the males, apparently oriented in the same direction (windward), jockeying for position. That is, they would move about in the swarm displacing other males, who in turn would displace others. The entire swarm moved slowly up and down, but was stable relative to the marker. Occasionally a lone insect would enter the swarm, and several seconds later, a lone insect would emerge again. Ten of these emerging insects were caught and examined by aerial net and all were identified as females. These were dissected and found to contain eggs ($x=189.25$, $s=7.72$, for 10 females examined). This is at least circumstantial evidence that they were prepared to mate.

That the body of the swarms contained only males is not unusual. In most nematocerans this is the case, with females entering only for mating and then leaving. Swarming is a method whereby the sexes are brought together for reproduction. Depending on the species, mating can occur on the wing, or a male will grasp a female when she enters the swarm and fly off with her to a suitable place where mating can occur (Downes, 1969). In several species swarming is required before mating and this may be the case with *Cricotopus myriophylli*.

Only 3 live and 9 dead larvae were recovered at the completion of the first rearing tent trial; however, the amount of damage to the plants in the oviposition tank must have been due to a far greater number of larvae. Approximately 85% - 90% of the meristems showed heavy feeding damage. The fact that any larvae were present in the oviposition tank, considering the source and treatment of the E.W.M., is evidence that an oviposition event had taken place. The high temperature and corresponding low DO_2 levels must have killed the other larvae which sank into the sediment. Larvae in the lab appeared to be sensitive to low DO_2, dying if the dissolved oxygen dropped rapidly, as occurred during several early sampling attempts. This agrees with other reports dealing with *Cricotopus* larvae (Bacon and Neff, 1982). While some samples of sediment were examined for larvae, none was found. This is not surprising considering the surface area of the sediment was approx. 1.8 m^2, more than sufficient area for 1st and 2nd larval instars to be overlooked. In addition, a stable mating swarm was observed in the tent during the second trial, although no larval or egg material was recovered. The fact that no oviposition occurred late in the summer corresponds with the absence of 1st instar larvae in the field after mid-August. Oviposition and successful development of the egg stage is influenced by environmental factors such as water temperature, light levels, and humidity. These conditions will have to be accurately controlled if an artificial rearing system is to be devised and operated through the winter months.

As both swarming and an oviposition event occurred, it must be concluded that conditions in the rearing tent were suitable for reproduction. Certain conditions, however, did not allow for the survival of the larvae. These conditions can be manipulated to be more favourable.

3. Feeding

3.1 Preliminary trials - One larva can eat all the meristematic tissue of a planted milfoil stem, thereby inhibiting growth. This happens so rapidly that there is no significant difference in the new growth of stems with 1,2, or 3 larvae feeding on them. The rapidity with which one larva can strip a meristematic region completely, well within the time period required to complete one stadium, implies that each larva must require more than one meristem to complete development. In addition, this means that from a biocontrol point of view, there is no advantage to having more than one larva per meristem when the insects are introduced. This indicates that fewer larvae will be necessary for introduction than were originally estimated (Kangasniemi, pers. comm.).

It is important to note in these feeding trials that pieces of E.W.M. which had been fed upon did not develop into new plants when placed into aquaria. This is indeed surprising when it is considered *M. spicatum* can produce a new plant from a 1 cm. long fragment if it contains a node (B.C. Ministry of Environment, 1981). As asexual fragmentation is considered the most important method of reproduction and dispersal, the fragmentation of stems fed-upon by larvae was initially considered a possible problem. This is not the case.

The failure to produce new plants may be due to the fact that any plant fragments that broke away were so thoroughly browsed that, even though containing a node, no photosynthetic material was present. In addition, naturally formed fragments have a lower concentration of total nonstructural carbon (TNC) than do artificially caused fragments and so are more likely to produce new plants (Kimbel, 1982). Kimbel also reports that naturally caused fragments overwinter more successfully than artificially caused fragments, which may indicate that fragments caused by the late seasonal feeding of insect control agents will not lead to further spread of *M. spicatum*.

There was undoubtedly stress placed on the plant by the feeding activity of the insect as well. This stress is sometimes evidenced by brown, necrotic patches surrounding the area fed upon. These necrotic patches failed to produce new growth either while still attached to the plant or after the patch had fragmented and the detached fragment become established on the bottom of the aquarium. As all fed-upon fragments which were used in the trials contained nodes, and planted fragments from healthy plants grew well, it must be concluded that fed-upon fragments don't readily establish new plants.

Despite surveys conducted throughout the Okanagan Valley lakes and Shuswap Lake systems on native aquatic plants, no evidence was found of the presence or even feeding damage, of *C. myriophylli* larvae. Even in those few areas where a number of native aquatic plant species were present along with *M. spicatum*, none of the native non-milfoil species showed any evidence of feeding damage. The only native species surveyed which showed any feeding damage was *M. exalbescens*, but only in areas where it was growing in proximity to *M. spicatum*. None of the other native species planted in the aquaria together were fed upon by *C. myriophylli*. This, with the results of the field surveys, indicates a strong preference for feeding on milfoils (this was confirmed with later starvation and choice trials between *M. spicatum* and a number of these native plants). This indicates *C. myriophylli* may be an introduced species. If the insect is native and has a strong preference for feeding on milfoils, why is it not feeding on native milfoils in areas where *M. spicatum* has not yet been introduced?

3.2 Suppressing Growth - *C. myriophylli* larvae can suppress the growth of *M. spicatum* in the lab. The suppression of growth and flowering seen in the Okanagan weedbeds in the early 1980's and the localized control still seen in some of those weedbeds is good evidence that this also is possible in the field. Plate 6 shows a healthy, apical stem of *M. spicatum*, and plate 7 shows the same stem after 3 days of feeding activity by a larva of *C. myriophylli*. After becoming established, larvae construct cases and begin to feed. Once all meristematic tissue has been removed from the plant, no new growth occurs. The suppression of growth in this manner indicates that insects can prevent growth and it may be possible to hold the plant's growth back to subsurface levels. The requirements for such a proposition, now that some success has been achieved with the rearing tent, would include establishing the optimum time of introduction.

The cropping of the apical meristem does appear to stimulate the development of axillary buds as in other plants (Salisbury and Ross, 1978). When axillary buds developed on plants that had larvae established on them, however, the larvae cropped these secondary buds as soon as this material was available. Since all apical meristematic material had been browsed within 3 days, the larvae had little to feed upon at the apex of the plant and readily relocated to the fresh growing tissue on the axillary buds. In fact, if no additional growing tissue was supplied, in the form of axillary meristems or fresh material added to the cell, the larvae would begin to starve in 12-14 days despite the rest of the plant still being available. This confirms early observations that the insect feeds only on meristematic tissue and adjacent portions of the plant (Kangasniemi and Oliver, 1983). Furthermore, this rapid rate of feeding confirms that the insects must require more than one meristem to complete development of every instar. This cropping of axillary buds when apical material is not available has obvious benefits with regard to control programs.

Plate 6: Healthy stem (apical portion) of *M. spicatum*.

Plate 7: Same stem after 3 days of feeding by a *C. myriophylli* larva. Note the presence of a larval case at the right end of the stem and the discolouration (necrosis of tissue) surrounding the fed upon area.

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3.3 Host Preference - Other than Kangasniemi and Oliver's (1983) original noting of the insect and Oliver's (1984) subsequent description, there is no mention in the literature regarding the feeding preferences of *C. myriophylli*. Zwolfer and Harris (1971) stated that considerable time can be saved if, rather than testing each important crop plant that the control organism is going to have access to, the feeding habits of the organism and its most closely related species could be examined. Oliver (1984) has stated that *C. myriophylli* belongs in the *Cricotopus sylvestris* group. In many rice producing areas of the world, *C. sylvestris* is considered a pest, often referred to as the 'Rice Midge' or 'Rice Seed Midge' (Berczick, 1979; Gigarick, 1984). The propensity of this species for attacking rice also may extend to its close relative *C. myriophylli*. Although rice is not grown as a crop in B.C., it is important in some areas where *M. spicatum* is a problem. Therefore, before *C. myriophylli* can be exported as a biocontrol agent to any of these areas, its feeding activities on rice must be tested.

The very strong evidence for feeding preference of *C. myriophylli* on *M. spicatum* over native aquatic plant species, and the actual inability of the insect to feed on most of these leads to the conclusion that the larvae are feeding-dependent on milfoils. Indeed, a suitable common name for this species could be the 'Milfoil Midge'. As the feeding stage of *C. myriophylli* is an obligate aquatic, no terrestrial plants were tested for suitability as possible hosts. None of the native aquatic plant species tested in isolation showed any evidence of feeding damage. This is even more convincing when one considers that, during starvation, insects will often feed on plants which, in the field, would not normally be suitable as food sources. For this reason, it is sometimes considered that starvation trials are too stringent, and thus a positive result in one should not necessarily preclude an insect's use as a biocontrol agent (Zwolfer and Harris, 1971). The demonstration of feeding-dependence on milfoils under these conditions is strong evidence for the safety of introducing *C. myriophylli* into areas where it is not already present.

Even when case-building activity was seen on native plant species, cases did not include any plant material. This lack of suitability of native plants as either food or construction material for cases is not understood. Phytochemical attraction of *C. myriophylli* to E.W.M. is possible as *M. spicatum* has been reported as producing allelochemicals which repel certain species of nematocerans (Dhillon *et al.*, 1982). These chemicals may serve as attractants to other species of Diptera, such as *C. myriophylli*. Some native plants may not being structurally suited for *C. myriophylli* to use as case material, it is interesting to consider that although no plant material was utilized, there was case-building activity seen on *Ranunculus aquatilis*, which has fine stems and submerged leaves, similar to the milfoils.

It was not surprising that *M. exalbescens* proved suitable as a potential food source for *C. myriophylli. M. exalbescens* is very closely related to *M. spicatum* and the two are often so morphologically similar that they can be separated taxonomically only by using phytochemical techniques (Ceska, 1977; Ceska and Ceska,1986). The acceptability of *M. exalbescens* as a food source for *C. myriophylli* gives rise to two important considerations. First, it is possible that populations of *C. myriophylli* could feed on beds of *M. exalbescens* during periods when *M. spicatum* might be in decline or unavailable. A resident population of *C. myriophylli* would, therefore, survive these periods and a total reintroduction would not be necessary, although augmentation of existing stocks may be required. These forms of 'refugia' during periods of target-plant decline are important to biocontrol agents (Harris, 1972). Second, the suitability of *M. exalbescens* as a food source but the larvae's preference for *M. spicatum* may be considered secondary evidence that the insect may have been introduced. Although many native insects have shifted from a native plant food source to an introduced, related plant species, *Leptinotarsa decemlineata* for example, these insects were well distributed across the +original, native plant's range. This is not the case for *C. myriophylli*. Although it has been found feeding on *M. exalbescens* in the field, it has not been collected in areas where *M. spicatum* is not present. Also, *C. myriophylli* has not been found in those areas which support large populations of *M. exalbescens* but where *M. spicatum* has not yet been introduced. The feeding dependency of *C. myriophylli* on milfoils means that there should be a minimal effect of this species on native aquatic plants and thus little objection to the introduction of the insect to various areas of the province. Concern over introductions of foreign insects into fresh water systems was initially anticipated from provincial and federal fisheries departments, mainly because many native aquatic macrophytes are considered important in the production of sports fish, although detailed research in this area is required. Feeding dependency on milfoils could, in fact, be beneficial to native plants. With the introduction of large numbers of *C. myriophylli* and the subsequent decline of *M. spicatum* populations, native plant species would have a competitive advantage, especially early in the growing season, and could once again become established. This would facilitate a return to original aquatic plant communities. Since the introduction of *M. spicatum* into B.C., the numbers of species present in these communities have decreased. *M. spicatum* has the ability to grow quickly and shade out the native plants (Aiken *et al.*, 1979).

Consequently, the only native species present are those that either become quickly established after a milfoil bed has been treated by tillage, such as *Potamogeton crispus*, or those species, such as *Elodea canadensis*, which can tolerate lower light intensities and become established at a greater depth than does *M. spicatum*. In either case, these native species are not very successful, being either too deep to be robust, or eventually being outstripped by the rebound growth of *M. spicatum* and thus, once again, becoming overshaded.

3.4 Relocation of Larvae - Larval ability to relocate was demonstrated in the field when it was shown that insects removed from their cases would swim in a characteristic wriggling motion until coming in contact with a new plant. This orienting behaviour also was observed in virtually all larvae disturbed from their cases. Swimming did not appear to be random or pointless but, rather was directed. Orientation toward the plant was observed. After an initial period of wriggling which didn't change the larva's position (presumably 'finding its bearings'), it set off in the direction of a plant stem. This also was observed when placing the larvae into the cells used in the starvation trials (which did not hold *M. spicatum*). Attraction to plant stems may, therefore, be visual instead of chemical since no distinction between milfoils and non-milfoils was seen.

Field observations of relocation were confirmed with lab experiments. Larvae were seen to relocate when either the food source was depleted or when removed from their cases. The short length of time necessary to crop a meristem and their ability to move to axillary meristems when the apical tip was browsed anticipated this result. More larvae relocated when the whole case was placed in the cell than when larvae removed from their cases were deposited. These observations are especially convincing when the trial involving planted stems, with cases attached, is considered. All of those larvae involved which did not pupate relocated when their food source was depleted. This trial most accurately reflects natural conditions and shows that larvae are well able to find additional sources of food, provided they don't have to search too far. The ability and the ease with which larvae relocate shows that they are able to browse more than one meristem/instar. This has implications in control programs since it supports the earlier supposition that fewer larvae will be required for control than was originally estimated (Kangasniemi, pers. comm.).

3.5 Temperature Cues - *M. spicatum* is capable of growth at a minimum temperature of 10 C. The observations that larvae of *C. myriophylli* are still active and feeding at this temperature are, therefore, very significant. One of the limiting factors in this control program will be the time of inoculation. The activity of chironomid larvae is usually dependent upon their response to low light levels and temperatures (Oliver, 1971). If the insects are active early enough in the year, they may be introduced before *M. spicatum* begins its rapid growth spurt. Feeding at this time will prevent the plant from reaching the surface and may decrease the growth and depth of the characteristic thick mat of vegetation. Feeding activity increases as the water temperature rises. In addition to an increase in larval feeding, developmental time decreases (Oliver, 1971). In spring, as water temperature rises and *M. spicatum* growth increases, larval feeding rates also increase. If the numbers of larvae are sufficient, they should suppress the growth of the plant. Most larvae at this time of year are 3rd and 4th instars when sexual dimorphism becomes apparent and the need for the larvae to store energy, for pupation and adulthood, is greatest. This is reflected by the feeding rates of larval instars of various chironomid species, which have been shown to feed more heavily during later larval instars (Konstantinov, 1958, MacKey, 1977).

4. Compatibility With Existing Control Techniques

The technique of harvesting removes the apical meristems of *M. spicatum* by cutting and collecting plant material from the surface. Continued growth is from axillary meristems and from newly developed shoots that grow from the root crowns which develop and send new stems to the surface. The cropping of the uppermost portions of the plant not only removes the habitat of *C. myriophylli*, but also, many larvae as well. The process of harvesting aquatic plants is not perfect. Rarely does it result in perfectly overlapping swaths and, consequently, large patches of uncut or half-cut weeds are sometimes left. Surveys of harvested areas did not include these patches as they were few in number and the population of *C. myriophylli* was much higher in them. They were, therefore, not representative of the population after harvesting. However, they do indicate that some insects will survive this mechanical treatment. Recovery of insects on fully-cut weeds, though quite few in number, also indicates that larvae can survive harvesting. Probably these individuals were either already established on the plant below the maximum depth of the cutting blade or relocated there after being disturbed by the harvester.

The population levels of *C. myriophylli* in weed beds contiguous to treated areas was equal to control area populations. In addition, 3rd and 4th instar larvae were recovered after the 2 week period, which is not long enough for these stadia to have developed from the egg. This indicates that insects, like the weeds, re-establish in harvested areas through immigration from neighbouring, untreated areas. This raises the possibility that heavy insect damage in these untreated areas might decrease this source of reinfestation of milfoil into harvested areas. If the population of *C. myriophylli* could be augmented in these untreated areas to a level where the impact from their feeding stressed the plant enough to keep it from surfacing, this would decrease the potential for artificial fragmentation caused by human activities such as boating, waterskiing, and other activities.

5. North American Distribution of *C. myriophylli*

The distribution of *C. myriophylli* is limited and patchy in North America and in British Columbia. I suspect that the species was introduced, perhaps with *M. spicatum* plants at some point. The fact that in B.C. it has only been found in those areas where *M. spicatum* has been introduced lends credence to this hypothesis. Although able to feed on native milfoils, this has not been reported in any area where *M. spicatum* has not yet been introduced. If the insect is native and fed upon native milfoils before *M. spicatum* spread to the province, it would probably have spread to most areas in the province which support healthy populations of these native aquatic plants. This does not appear to be the case, even in areas which are geographically quite close and have human traffic in the form of boats being transferred from one lake to another.

The insect may have been distributed throughout B.C.with E.W.M. on boats and trailers. The 1987 discovery of *C. myriophylli* in Long Lake in Nanaimo was puzzling considering that it was absent from this lake in 1986. A possible explanation lies in the fact that Long Lake is utilized by an active water-skiing club as a practice site. In the summer of 1986 the provincial waterskiing championships were held in Ellison Lake one of the sample areas for this study and the site of one of the healthiest populations of *C. myriophylli* in the province. The team from Nanaimo is known to have taken their own boat, to have used it in Ellison Lake, and upon their return to Vancouver Island, to have returned the boat to Long Lake. This method of distribution follows that of *M. spicatum* in that the most common method of introduction of any aquatic weed is by human activity. The flight abilities and behaviour of *Cricotopus myriophylli* are typical of chironomids, which fly well post-emergence, to enable them to reach shore, but fly poorly later in life (Oliver, 1971). Flight, therefore, is an unlikely method for wide distribution of this species. In addition, while larvae do swim well enough to relocate to fresh meristems, their typical wriggling form of locomotion could not account for much of an expansion in distribution.

Besides the recent introduction to Long Lake, the distribution of the insect in the Okanagan Valley is expanding. In 1987, larvae were recovered in areas from which it had not been present in 1985-86. Even in the first year of this study (1985) larvae of *C. myriophylli* were found in areas of the Okanagan Lake where Ministry of Environment researchers recorded it as absent in 1982-83 (Wallis, pers. comm.). At the end of the 1987 field season no groups of *M. spicatum* plants, even small patches, that were examined failed to reveal *C. myriophylli* larvae. It is likely that the population in Ontario is also expanding. It now has also been tentatively identified in areas other than those listed in the original reports (Oliver, pers. comm.).

CONCLUSIONS

The evidence from feeding experiments testing the amount of E.W.M. growth that the insect can suppress, in combination with the observed localized control of E.W.M. by *Cricotopus myriophylli*, indicates that this species has potential for success as a biocontrol agent under certain conditions. Furthermore, the results of the host-suitability trials indicate that this species is safe for introduction anywhere in British Columbia. There should be little concern over any possible negative impact on native aquatic macrophytes. The insect's habit of denuding the plant of meristematic tissue, and thereby suppressing growth, lends itself to a variety of strategies including its introduction post-harvesting to suppress further growth, and introduction to untreated areas bordering mechanically-treated sites to decrease the rate of reinfestation. However, it also suggests that there may be limits to the application of this insect. It is estimated that it would be of little use to introduce *C. myriophylli* into an untreated *M. spicatum* patch after early summer, when the plant has begun to develop axillary buds, since there would be too much meristematic material for the insects to browse completely. The growth of the plant would outstrip feeding damage of the larvae and the plants would continue to be a nuisance.

Introductions of *C. myriophylli* should be conducted in the early spring before the development of lateral growth by the plant. The larval stage is recommended as the ideal stage to use in seeding new locations since it is easier to manipulate than either the eggs, which require special conditions to develop which might not be present at that time of year, or the adults, which are prone to desiccation and, therefore, difficult to transport. The later instars are probably the most effective for introduction at this time of year since it more closely reflects the natural situation, in that most larvae present at that time of year are 3rd and 4th instars, and they have a greater feeding rate. Introductions into areas scheduled to be mechanically treated in the near future should be avoided due to the negative effect treatment has on *C. myriophylli* populations. However, introductions into neighbouring, untreated areas during fall and spring rototilling and cultivation treatments should be considered.

It would be wise to acclimatize larvae to lake conditions before introduction. Furthermore, it must still be ascertained how many larvae are necessary for an introduction to be a success, establishing a population large enough to be capable of damaging the weeds. To this end, trials should be conducted to establish the densities of *C. myriophylli* larvae necessary for control purposes.

Since *C. myriophylli* may be an introduced species, classical biocontrol procedures may work in this situation. There is a good chance that this control will become self-replicating in areas into which it is introduced. Subsequent augmentations to the existing population may occasionally be necessary to raise population levels to a point where the insects can damage the plant. However, repeated restocking of entire populations into areas will probably be unnecessary.

Further research is necessary to perfect artificial rearing methods. Since swarming occurs as long as light levels and air temperatures are suitable, it will likely be the egg stage which is the limiting factor in this activity. Optimum water temperatures and/or light levels necessary for egg development must, therefore, be defined. Suggestions have been made of a greenhouse-like structure which will provide adequate control of air temperature and humidity (Bartnik, pers. comm.). Indeed, this is the method whereby weevils are raised for the control of alligatorweed (Cofrancesco, 1984). If that plan is followed in this project, the greenhouses must contain vertical objects, preferably poplar trees, for marking by aerial swarms and must also provide for the aeration and temperature control of oviposition tanks.

Continued research also is necessary on other species which may be useful as biological control organisms. As has been stated, while *C. myriophylli* does show considerable promise in certain circumstances, there are occasions when it will not be completely successful by itself. This is true of any single biocontrol agent. Most authors now believe that a complex of species is necessary to control a pest successfully. This is also the opinion of other researchers in the field of biocontrol of E.W.M. Spenser and Lekic (1974), citing the 25 insect species found feeding on E.W.M. in Pakistan and the 16 species in Yugoslavia, stated that the reason E.W.M. may not be a problem species in its home range is that the plant is under greater environmental pressures than in N. America where only one or two insects are feeding on it. Some of those insect species which should be examined have been referred to. Many species mentioned in the literature are considered worthwhile for study but very few are pursued.

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Title of Thesis
EVALUATION OF *CRICOTOPUS MYRIOPHYLLI* OLIVER (DIPTERA: CHIRONOMIDAE) AS A POTENTIAL BIOCONTROL AGENT FOR EURASIAN WATER MILFOIL, *MYRIOPHYLLUM SPICATUM* L.

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Aug 31/88.