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SOME INFLUENCES OF AQUATIC PLANTS ON THE DEVELOPMENT  
AND SURVIVAL OF MOSQUITO POPULATIONS

by

NELLO P.D. ANGERILLI

B.Sc., Simon Fraser University, 1974

A THESIS SUBMITTED IN PARTIAL FULFILLMENT  
OF THE REQUIREMENTS FOR THE DEGREE OF  
DOCTOR OF PHILOSOPHY

in the Department

of

Biological Sciences

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## Abstract

Few mosquito larvae were found in six of nine naturally-occurring ponds containing aquatic vegetation while in three such ponds there were no mosquito larvae. Analyses showed that the numbers of mosquito larvae found were not related to the number of potential predators present, the species composition of aquatic plants present, or any of a number of physical factors measured. However, the species of mosquito larvae present were related to the species composition of aquatic vegetation.

Tests showed that some of the ponds that contained no mosquito larvae were in fact suitable for the development and survival of at least one species of mosquito (Culex pipiens Say).

The results of experiments in covered artificial field ponds using 3 species of aquatic plants and 1 species of alga indicated that mosquito larvae did not always survive to the adult stage in the presence of the plant or alga. The results of these experiments also indicated that the ability of plants to produce these effects is related to plant phenology or to the reactions of the plant to its environment.

The results of experiments using coverless artificial field ponds showed that the presence of the aquatic plants Utricularia minor L., Elodea canadensis Rich. in Michx. and Lemna minor L. discouraged mosquitoes from colonizing the ponds by influencing the site selection behaviour of the ovipositing female. The presence of these same plants both encouraged colonization of the ponds by potential predators of mosquito larvae and facilitated their activities in the pond.

Laboratory tests with extracts of aquatic plants showed some of them to be attractive or repellent to ovipositing mosquitoes and some to be relatively toxic to immature mosquitoes.

The results of laboratory experiments and field observations in natural ponds indicated that some aquatic plants do not impede the activities of certain predators, some aid or facilitate the activities of predators by providing a "working platform" on which they can operate, and some aquatic plants serve as oviposition sites for predatory insects.

Because aquatic plants can influence the mortality and survival of immature mosquitoes and the

egg-laying behaviour of the adults, it may be possible  
to manage aquatic vegetation to reduce the size of  
mosquito populations in some situations..



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## INTRODUCTION

Much effort has been directed towards controlling mosquito populations with the use of chemical pesticides. However, because of increasing resistance by mosquitoes to pesticides it is becoming expedient to re-evaluate previously abandoned lines of research into the control of mosquitoes. One such line is "natural" control, that is, investigations and manipulations of naturally occurring control mechanisms. Certain aquatic plants may be part of these mechanisms.

The number of adult mosquitoes emerging from a given aquatic habitat is related to initial colonization of the habitat by mosquitoes and to larval mortality within the aquatic environment. Information in the literature suggests that certain species of aquatic plants can influence the behaviour and survival of both adult and immature stages of mosquitoes through chemical and physical effects.

### Physical Effects

Plants may reduce wave action of the water and thus provide a stable substrate for oviposition while they may also restrict oviposition by rendering the water surface inaccessible to the ovipositing female

(Matheson 1928, 1930; Matheson and Hinman (1929; Twinn 1931; Sen 1941; Russell 1941; Russell and Rao 1942; Furlow and Hays 1972).

Plants may conceal and protect mosquito eggs, larvae and pupae from predators (Siler 1932, 1933) but they also may provide both an oviposition site for predators and a working platform or scaffold for predators (Angerilli and Beirne 1974).

In 1833 Charles Darwin noted that plants of the genus Utricularia were insectivorous. Many authors have recorded these plants ingesting mosquito larvae both in the laboratory and in the field (Franca 1922; Matheson 1928, 1929, 1939; Twinn 1931; Sen 1941; Angerilli and Beirne 1974) but other authors have found mosquito larvae living among this plant apparently unharmed (Siler 1932, 1933; Currey 1934; Evans and Garnham 1936).

#### Chemical Effects

It is well known that many terrestrial plants produce substances toxic to insects (Heal et al. 1950; Jacobson 1958; Patterson et al. 1975). These substances include nictines, pyrethrins, rotenones, juvenile hormone-like compounds (Bowers et al. 1966;

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Mansingh et al. 1970), anti-juvenile hormone compounds (precocene 1 and 2 discovered by W. Powers, cited by Maugh 1976) and ecdysone-like compounds (Takemoto et al. 1967). Little work however, has been directed towards aquatic plants as sources of such substances. Amonkar and Reeves (1970) found that certain species of Chara (a freshwater alga) release toxic metabolites that inhibit the development of mosquito larvae, while Angerilli and Beirne (1974) in laboratory experiments found two species of freshwater plants, and one species of Chara produced similar effects. A number of other authors have cited Chara spp. as being detrimental to mosquito larvae (Caballero 1919; Matheson 1928, 1929, 1930; Twinn 1931) but MacGregor (1924) found Chara to be devoid of larvicidal properties. Evans and Garnham (1936) found mosquito larvae breeding in a dense growth of Chara sp. and Siler (1932, 1933) suggested that the presence of Chara favoured mosquito breeding. Currey (1934) recorded that Chara under certain conditions, furnished apparently optimum conditions for the nurture of certain species of Anopheles.

The ultimate objective of the present study was to determine if and how aquatic vegetation might be managed as a mosquito preventive or control measure. The immediate objectives were to identify and evaluate

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four plant-insect interactions as follows:

i) effects of aquatic and semi-aquatic plants on the development and survival of mosquito larvae;

ii) effects of aquatic vegetation on the searching behaviour of adult female mosquitoes for oviposition sites;

iii) effects of aquatic vegetation in facilitating the development, survival and activities of predators of mosquito larvae;

iv) effects of phytophagous insects on mosquito-harming plants. This line of investigation was dropped after initial surveys indicated that the incidence of such insects was extremely low in the study area.

PART I. SURVEY AND ANALYSIS OF FIELD SITUATIONS

In laboratory experiments utilizing aquaria containing aquatic plants and mosquito larvae, Angerilli and Beirne (1974) found that certain aquatic plants could affect the development and survival of mosquito larvae physically, chemically and by facilitating the presence and activity of predators of mosquito larvae. This survey of naturally occurring freshwater communities was undertaken to investigate the relationship between the presence or absence of certain aquatic plants and the presence or absence of mosquito larvae and of their predators. Certain physical and chemical factors that might affect these organisms were also measured.

Materials and Methods

Physical and biotic characteristics of 8 freshwater ponds in the lower Fraser Valley of British Columbia were sampled weekly between mid-May and mid-August, 1975. A ninth site in the southern Okanagan was sampled approximately every 3 weeks. The 9 sites were selected from 15 sites surveyed during the summer of 1974.

## Physical Factors

Measurements of the following were made weekly at approximately the same time of day at each site (except at Osoyoos).

i) The concentration of dissolved oxygen in the water was measured using the Winkler technique described by Golterman et al. (1972). A single measurement was made on each of 3 samples taken from approximately 15 cm below the water surface and usually within 1 m from shore. All measurements were made at the site within 15 minutes of when the sample was taken.

ii) Water temperature was measured by suspending the bulb of a mercury-filled glass thermometer approximately 15 cm below the surface of the water and usually not farther than 1 m from shore.

iii) At the time of sampling the condition of the sky was recorded as being one of the following: 1) raining and cloudy; 2) cloudy; 3) mostly cloudy; 4) mostly sunny; 5) bright sun.

## Biotic Factors

i) The abundance of each species of mosquito larvae present was measured weekly by making 5 consecutive sweeps with a fine meshed nylon net (outside frame dimensions: 9.5 by 14 cm) among emergent and submerged vegetation. The net was emptied after each sweep into a small white enamelled pan containing 2 to 5 cm of water. After 5 sweeps the mosquito larvae found were identified to species, counted and their age (instar) was estimated. The contents of the pan were emptied back into the site and the entire process was repeated four more times, yielding a total of 25 sweeps/site/sampling day.

ii) The abundance of each family of organisms known to be predators of mosquito larvae (Jenkins 1964) was determined in the same way as mosquito larvae.

iii) Four New Jersey-type light traps were operated during the sampling period to determine the kind and number of adult mosquitoes present in the vicinity of the study sites. The light traps were located near the sites in Ft. Langley, Pitt Meadows, Richmond and Osoyoos.



The light traps were operated continuously for 10 to 17 weeks on 110V using a 40 watt tungsten filament bulb. In the 1 l collecting chamber which was emptied weekly, an 8 cm resin strip impregnated with dichlorvos was used as a killing agent.

iv) Observations made during the summer of 1974 indicated that the vegetation in and surrounding each of the sites was most abundant in early August. During the first week of August 1975 the aquatic and terrestrial vegetation at each site was given a numerical rating from one of the following categories: 1) dominant; 2) abundant; 3) present; 4) scarce. This information was used to construct a similarity dendrogram (Hummon 1974) for the 9 sites based on aquatic vegetation.

## Results

### Characteristics of Study Sites

All the sites studied were permanent freshwater ponds. Their locations are shown in Figure 1, some of their physical characteristics are listed in Table I and the vegetation of each site is listed in Table 1 in Appendix B.


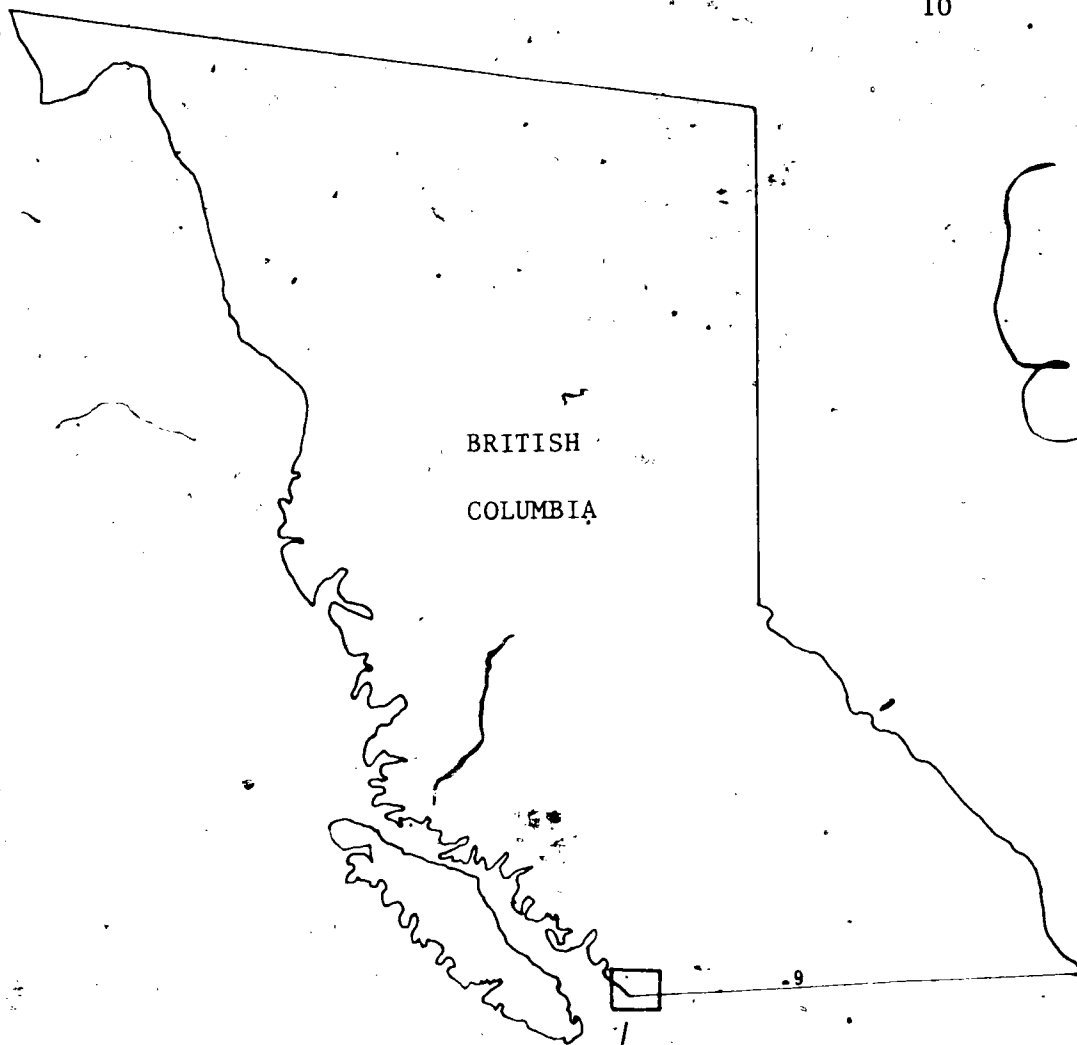


Figure 1. Map of British Columbia and inset of Lower Fraser Valley showing location of nine study sites.



SITES

- 1. Ft. Langley
- 2. Burnaby 1
- 3. Burnaby 2
- 4. Pitt Meadows
- 5. Sheridan Hill 1
- 6. Sheridan Hill 2
- 7. Richmond 1
- 8. Richmond 2
- 9. Osoyoos

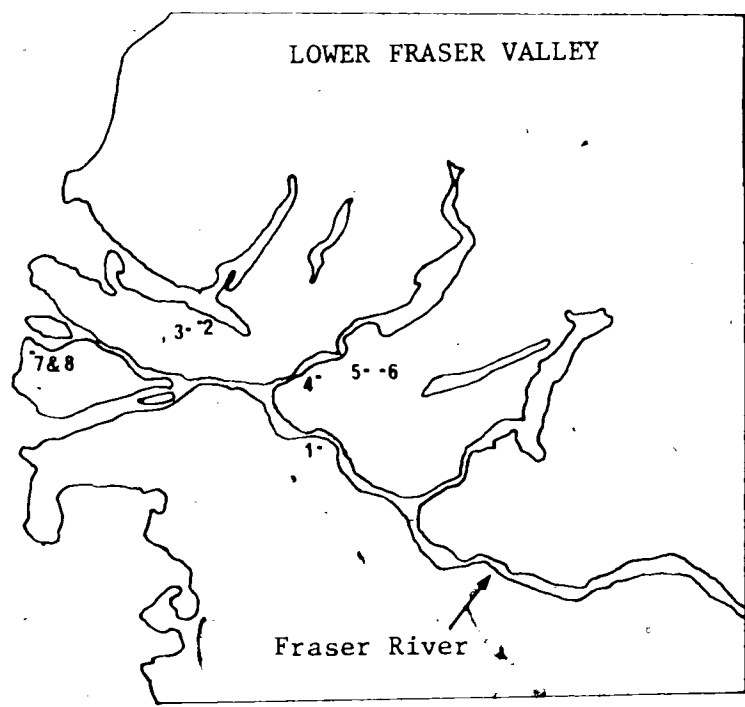


Table 1. Some physical characteristics of the nine field sites and the species of mosquito larvae contained therein.

Name/Location	Size	Shading	Bottom	water clarity	outlets/ inlets	Mosquito Larvae
Ft. Langley	medium	yes	mud	murky brown	none	<u>Anopheles punctipennis</u> <u>Culex territans</u>
Burnaby 1	medium	none	mud	clear	outlet	<u>Culex tarsalis</u> <u>Culex territans</u>
Burnaby 2	small	at times	mud	murky brown	both	<u>Culex territans</u>
Pitt Meadows	medium	none	clay	clear	both	<u>Culex tarsalis</u>
Sheridan Hill 1	small	at times	mud	clear	none	<u>Anopheles punctipennis</u>
Sheridan Hill 2	small	at times	mud	clear	both	<u>Anopheles punctipennis</u>
Richmond 1 and 2	small ditches	none	mud	murky brown	both	none
Osoyoos	large	none	sand/ gravel	clear	none	none

## Mosquitoes and their Predators

There were both quantitative and qualitative differences between the 9 sites surveyed in terms of mosquito larvae, predators of mosquito larvae, and adult mosquitoes.

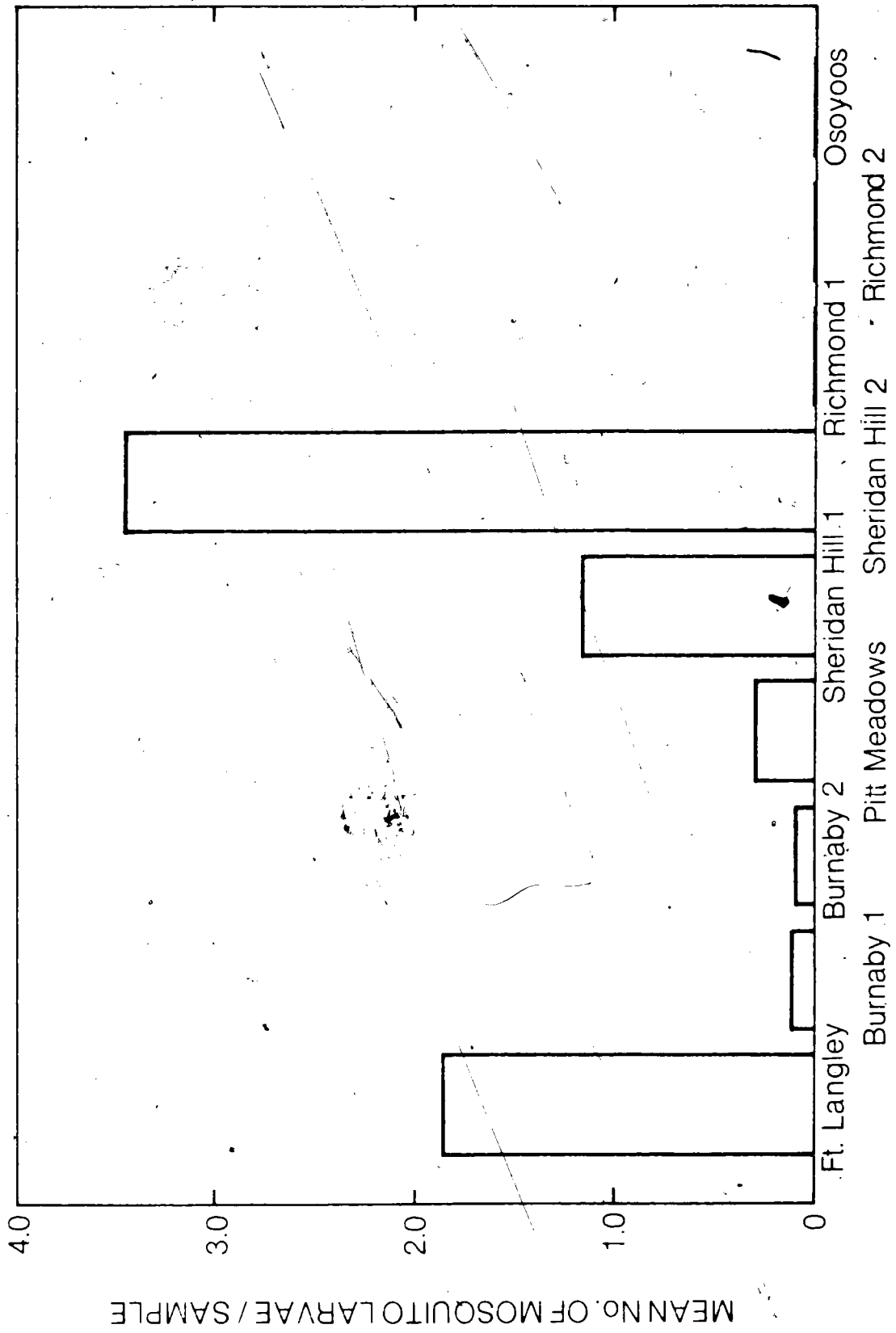
Mosquito larvae were found in only 6 of the 9 sites sampled and were never abundant in those 6 sites (Table 2, App. B and Figure 2). Because the field data were non-normally distributed the Kruskal-Wallis one-way analysis of variance was used and an added variance component was detected ( $H=47.41$ ;  $df=8$ ;  $p<0.001$ ). Larvae were never found in the sites Richmond 1 and 2, Osoyoos and only one site (Sheridan Hill 2) had significantly more mosquito larvae than the other 8 sites (Student-Newman-Keuls procedure,  $p<0.05$ ). Two ponds (Ft. Langley and Burnaby 1) contained 2 species of mosquito larvae of the 3 species found which were Anopheles punctipennis (Say), Culex tarsalis Coquillett, and Culex territans Walker.

There did not appear to be any relationship between the presence or absence of mosquito larvae and any of the characteristics listed in Table I.

Table II. Species and numbers of adult mosquitoes taken in light traps.

Location	Species	Number Caught	Period of Operation
Sheridan Hill	<u>Aedes vexans</u>	29	28 May 1975
	<u>Anopheles punctipennis</u>	7	to
	<u>Culex tarsalis</u>	2	28 August 1975
	<u>Culiseta impatiens</u>	8	
	<u>Culiseta sp.</u>	1	
	total	47	
Ft. Langley	<u>Aedes vexans</u>	70	8 May 1975
	<u>Anopheles punctipennis</u>	4	to
	<u>Culex pipiens</u>	11	28 August 1975
	<u>C. territans</u>	7	
	<u>Culiseta inornata</u>	2	
	unidentified	15	
total	109		
Richmond	<u>Aedes vexans</u>	1	7 June 1975
	<u>Culex pipiens</u>	250	to
	<u>C. tarsalis</u>	2	15 August 1975
	<u>Culex sp.</u>	1	
	<u>Culiseta inornata</u>	6	
total	260		
Osoyoos	<u>Aedes triseriatus</u>	1	19 May 1975
	<u>A. vexans</u>	2	to
	<u>Aedes sp.</u>	1	6 September 1975
	<u>Culex tarsalis</u>	49	
	<u>Culiseta inornata</u>	38	
	<u>Culiseta sp.</u>	3	
	<u>Mansonia perturbans</u>	1	
total	95		

Figure 2. Mean number of mosquito larvae per sample found in each of nine study sites during sampling period.





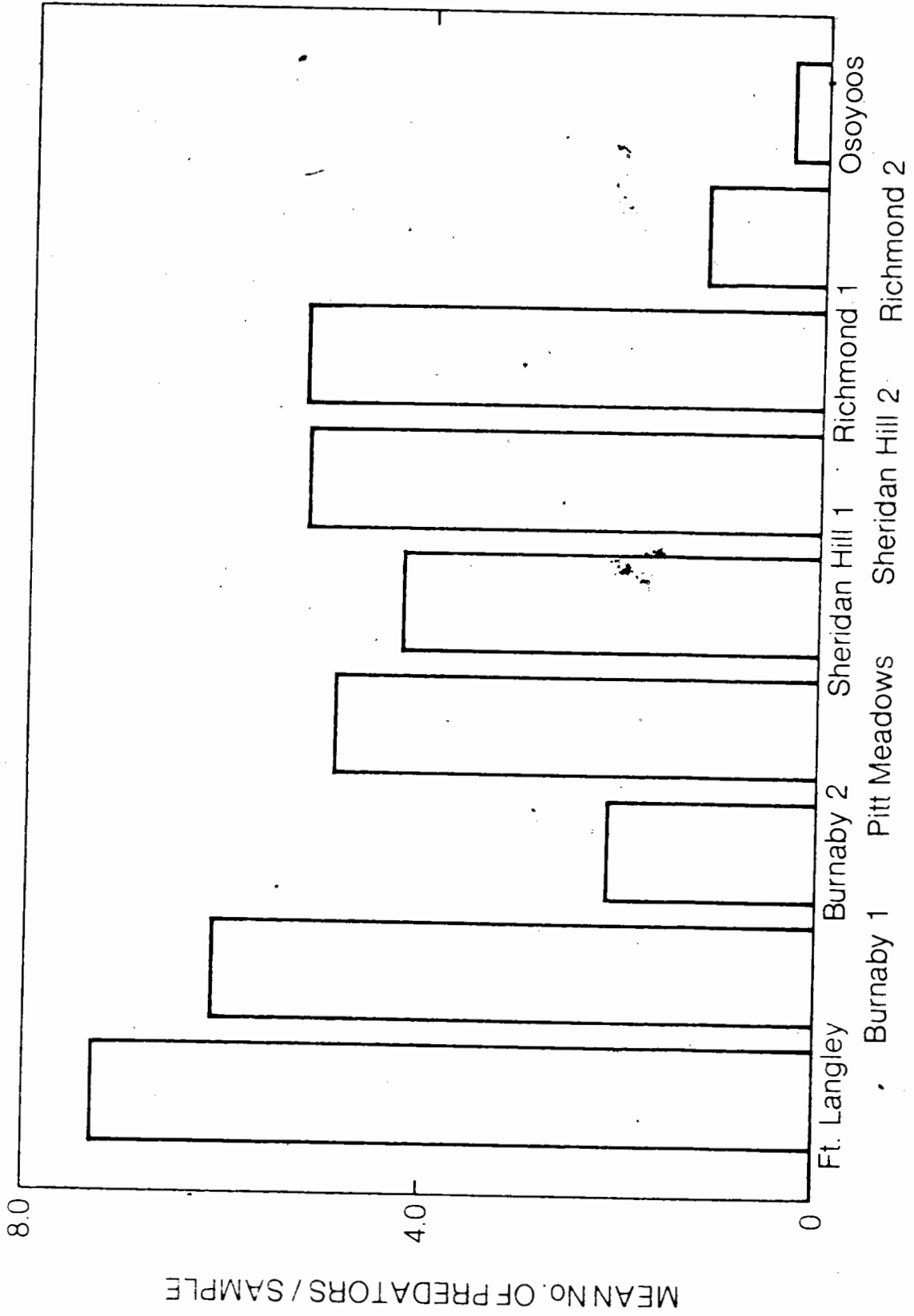
Nine families of organisms known to be predators of mosquito larvae (Jenkins 1964) were found. The percentage composition by family at each site is shown in Table 3 (App. B), and the mean data for each site are summarized in Table 4 (App. B), and Figure 3. An analysis of variance was performed on the data which were transformed to  $x' = \text{square root}(x+0.05)$  and differences between means were tested with the Student-Newman-Keuls (SNK) procedure (Table 4, App. B).

Nine mosquito species were taken in the light traps. Four species were taken by the Sheridan Hill and Richmond traps, and 5 species in the Osoyoos and Ft. Langley traps. The numbers and species caught by each trap are shown in Table II.

The following variables were used in a stepwise multiple regression analysis (MRA) (program BMD02R, Dixon 1973): i) dissolved oxygen concentration of the water; ii) water temperature (at the time of sampling); iii) sky condition; iv) mosquito larval abundance (3 species); v) predator abundance (11 groups); vi) SHA'.

Variable sets iv and v were transformed to  $x' = \text{square root}(x+0.5)$ . SHA' was calculated by comparing SH' for the 9 field sites to a hypothetical

Figure 3. Mean number of predators per sample found in each of the nine study sites during the sampling period.



site containing equal quantities of all the species of vegetation found in the other 9 sites.

The MRA did not produce a useful predictive model for either mosquitoes or predators. When the total numbers of mosquito larvae sampled were pooled and considered as a single dependant variable the strongest relationship found was between the number of mosquito larvae and the amount of dissolved oxygen in the water. The multiple correlation coefficient (R) was equal to 0.32 ( $F=43.53$ ;  $df=1,383$ ;  $p<0.001$ ). This value squared (0.1021) indicates that 10.21% of the variance of "mosquito" is accounted for or explained by the variable "dissolved oxygen". After all significant ( $p<0.05$ ) variables had been entered into the equation a total of 24.17% of "mosquito" variance was accounted for. When the 11 groups of predators were pooled and considered as a single independant variable ("predator") it accounted for only 1.48% ( $R=0.12$ ;  $F=26.48$ ;  $df=3,381$ ;  $p<0.001$ ) of the variance of "mosquitoes". "Dissolved oxygen" still had the strongest relationship with "mosquito" ( $R=0.32$ ) and the total variance accounted for by all the variables was 18.13%.

Using the dependant variable "predator" the independant variable with the highest R value was SHA'

( $R=0.29$ ;  $F=34.58$ ;  $df=1,383$ ;  $p<0.001$ ) which accounted for 8.28% of the variance of "predator". The next strongest relationship was with "dissolved oxygen" ( $F=23.00$ ;  $df=2,382$ ;  $p<0.001$ ) which accounted for 2.47% of the variance of "predators". After all variables had been entered 17.39% of "predator" variance was explained.

The data (with no transformations) were further analyzed by non-parametric correlation (Spearman Correlation Coefficients, Nie et al. 1975). These analyses confirmed the results of the MPA as follows:

- i) "mosquito" was positively correlated with "dissolved oxygen" and "predator" ( $r=0.33$  and  $r=0.18$ ;  $p<0.001$ ) which means that as the dissolved oxygen content or the number of predators present increased, the number of mosquito larvae present increased;
- ii) if the 3 mosquito species are considered separately only *A. punctipennis* is significantly ( $p<0.001$ ) correlated with "dissolved oxygen" ( $r=0.35$ ) and "predator" ( $r=0.21$ ).
- iii) "predator" was positively correlated with both "SHA" and "dissolved oxygen" ( $r=0.30$  and  $r=0.23$ ;  $p<0.001$ ).

"Predator" was also used to calculate a Pearson Product-Moment correlation coefficient (Nie et al. 1975) using the diversity index  $H'D$  (Lloyd et al.

Figure 4. Similarity (SH') dendrogram for species composition and abundance of aquatic plants in the nine study sites. Also shown are the species of mosquito larvae found in each site.

Anopheles punctipennis \*

Culex tarsalis °

Culex territans +



(1968) for the aquatic vegetation present as the independent variable. The correlation was significant ( $r=0.72$ ;  $p<0.05$ ) and indicated that the absolute number of predators increased as aquatic vegetation species diversity increased.

#### Mosquito Species and their Relation to Plant Species

The similarity dendrogram shown in Figure 4 was calculated for the aquatic vegetation of the sites using Hummon's (1974) similarity index. The index measures similarity in biota over space or time using procedures based on species composition.

Figure 1 indicates that there are 3 groups of ponds. The Osoyoos pond stood alone and contained no mosquitoes. The second group consisted of Pitt Meadows, Burnaby 1 and Burnaby 2. Culex tarsalis larvae and C. territans larvae were found in this group of ponds. Ft. Langley, Sheridan Hill 1, Sheridan Hill 2, Richmond 1 and Richmond 2 formed the third group. C. territans larvae and Anopheles punctipennis larvae were found in this group of ponds.



## Discussion

There are 3 possible reasons why mosquito larvae were rare in or absent from the ponds studied in this survey.

Predation, parasitism and competition may have limited or reduced the numbers of mosquito larvae present in the ponds. Though competition and parasitism were not measured, predation was measured to a certain degree and did not appear to affect mosquito numbers. Even though the sampling period was probably not long enough to reveal predator-prey oscillations in individual ponds, it is significant that no predator-prey relationship was detected between ponds. That is, those ponds that contained high numbers of predators did not necessarily contain low numbers of mosquitoes. This result is not unusual; Ray (1974), after an extensive review of the literature concerning predator-prey relationships among aquatic insects, concluded that there does not seem to be any good evidence that invertebrate predators appreciably reduce field populations of mosquito larvae. However, measuring predation in natural populations and attempting to characterize the dynamic interactions between predators and prey is an overwhelming task (Picklefs 1973). Therefore, the possibility that

predation was an important contributing factor to limiting larval mosquito populations in this study cannot be discounted.

It is possible that some of the sites studied were chemically or physically unsuitable for the growth of mosquito larvae. That is, larvae resulting from any eggs laid are unable to survive because of the presence or absence of certain substances (e.g. toxic compounds, essential growth factors) or because of harsh physical characteristics (e.g. wave action, temperature extremes). It is probably not possible to determine and then measure the myriad of factors of this type that influence larval survival. For this reason, I chose to measure directly the suitability of various field sites for larval survival by measuring the survival rate of an introduced population of mosquito larvae. This study is described in a subsequent section.

Certain species of aquatic vegetation are known to have detrimental effects on the development and survival of mosquito larvae (e.g. Angerilli and Beirne 1974). It is possible then that in some of these ponds aquatic plants were a source of mosquito larval mortality..

Alternatively, it is possible that mosquito larvae were rare or absent from the ponds studied because ovipositing female mosquitoes did not select them as oviposition sites. Support for this idea is provided by the fact that only a small proportion of the adult mosquito species found in an area (as measured by light traps) were represented by larvae in the sites.

Initially, the female mosquito must find an aquatic site to lay her eggs in and it appears that the mosquito uses visual and olfactory cues to locate and select potential sites (Gillett 1971; Osgood 1971). Once the eggs are laid, survival of the species is dependant on survival of the larvae. Therefore, if an inappropriate oviposition site is selected by the adult, the survival of the larvae and species is jeopardized. Natural selection would then tend to favour those mosquitoes choosing oviposition sites suitable for larval survival. It is possible that site selection behaviour is influenced by aquatic vegetation or effects produced by aquatic vegetation present in a site. That is, certain species of plants may present behavioural cues to female mosquitoes that are correlated with one of the following situations:

i) There are organisms or physical-chemical conditions in the pond that are beneficial. That is, they aid in the development and survival of mosquitoes:

ii) There are organisms or physical-chemical conditions in the pond that have detrimental effects on the development and survival of mosquitoes;

iii) The composition of the community or physical-chemical factors is such that the potential for survival and development of mosquitoes lies somewhere between I and II.

There would be negative selection pressure for those mosquitoes responding to cues correlated with situation II and positive or neutral selection pressure for those mosquitoes responding to cues correlated with situations I and III.

The habitat range of a species, that is, the number of different kinds of habitats it can occupy, must be limited to those habitats which all contain a whole series of common factors which are present within certain limits. If the presence or absence of certain aquatic plants is a measure or correlate of the limits of these factors, it is also possible that this presence or absence could affect mosquito oviposition site selection. Moreover, plant presence or absence may also influence the number of eggs laid in a particular site. If this is true, then one would expect to find different types and numbers of mosquito

larvae in ponds containing different kinds of aquatic vegetation. The results of this study as represented by the dendrogram (Figure 4) indicate that there is a relationship between aquatic plant species composition ( $H'$ ) and the species of mosquito larvae found in aquatic communities. This relationship could be used to facilitate identification of suitable and unsuitable larval habitats by noting which aquatic plants are present or absent. Because aquatic plants are not such transient inhabitants of freshwater habitats as mosquito larvae, it may be possible to distinguish between suitable and unsuitable larval habitats by merely noting which aquatic plants are present or absent.

The results of this study also showed that with increasing aquatic vegetation species diversity (as measured by  $H'$ ) there were more predators of mosquito larvae. This effect should increase the probability of there being present a sufficient number of predators that are capable of limiting larval mosquito populations. This kind of pond should be identifiable on the basis of vegetation and could be excluded from the list of potential mosquito breeding sites requiring the application of chemicals for mosquito control. And, again because aquatic vegetation is easier to sample than mosquitoes or their predators, such sites could be

identified after a shorter sampling period than would otherwise be required.

In summary, it appears that aquatic plants influence natural mosquito populations in a number of ways, but because of the complexity of the interactions occurring in natural ponds the nature of these influences is probably best studied under artificial situations as described in the following sections.

## PART II. FIELD EXPERIMENTS

### A. SUITABILITY OF FIELD SITES FOR LARVAL SURVIVAL

#### Materials and Methods

This experiment, which involved placing mosquito larvae into cages in field sites was designed to test the suitability of those sites for mosquito larval survival.

The cylindrical cages (dimensions: H=22 cm, D=8.5 cm) were constructed of plastic screening with petri dishes for ends and each cage was topped with an emergence chamber (Figure 5). The cages were immersed to a depth of about 15 cm of water and fastened to bamboo stakes fixed in the substrate. To prevent the cages from becoming completely submerged by rising water levels each cage was equipped with two plastic foam "floats" (Figure 6).

Five cages were placed into each site. Each contained 20 6-day-old Culex pipiens Say larvae. In the laboratory, 8 cages were placed into two 19 l glass aquaria, 4 cages in each. Each aquarium contained fifteen cm of dechlorinated tap water and a larval food supply consisting of Tetramin (Kraftwerke) and Baker's


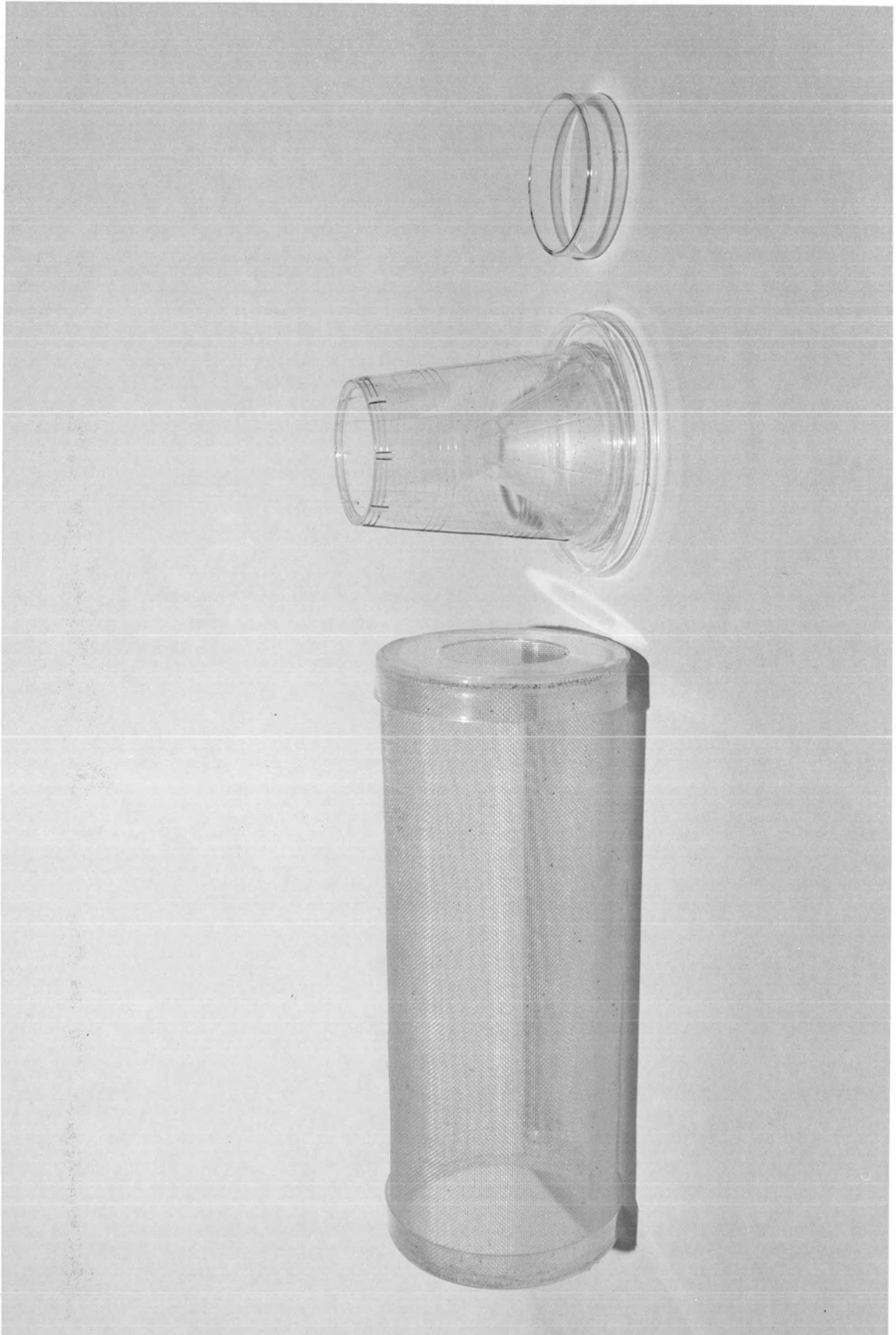


Figure 5. Cage and emergence chamber used in determination of suitability of field sites for larval survival.





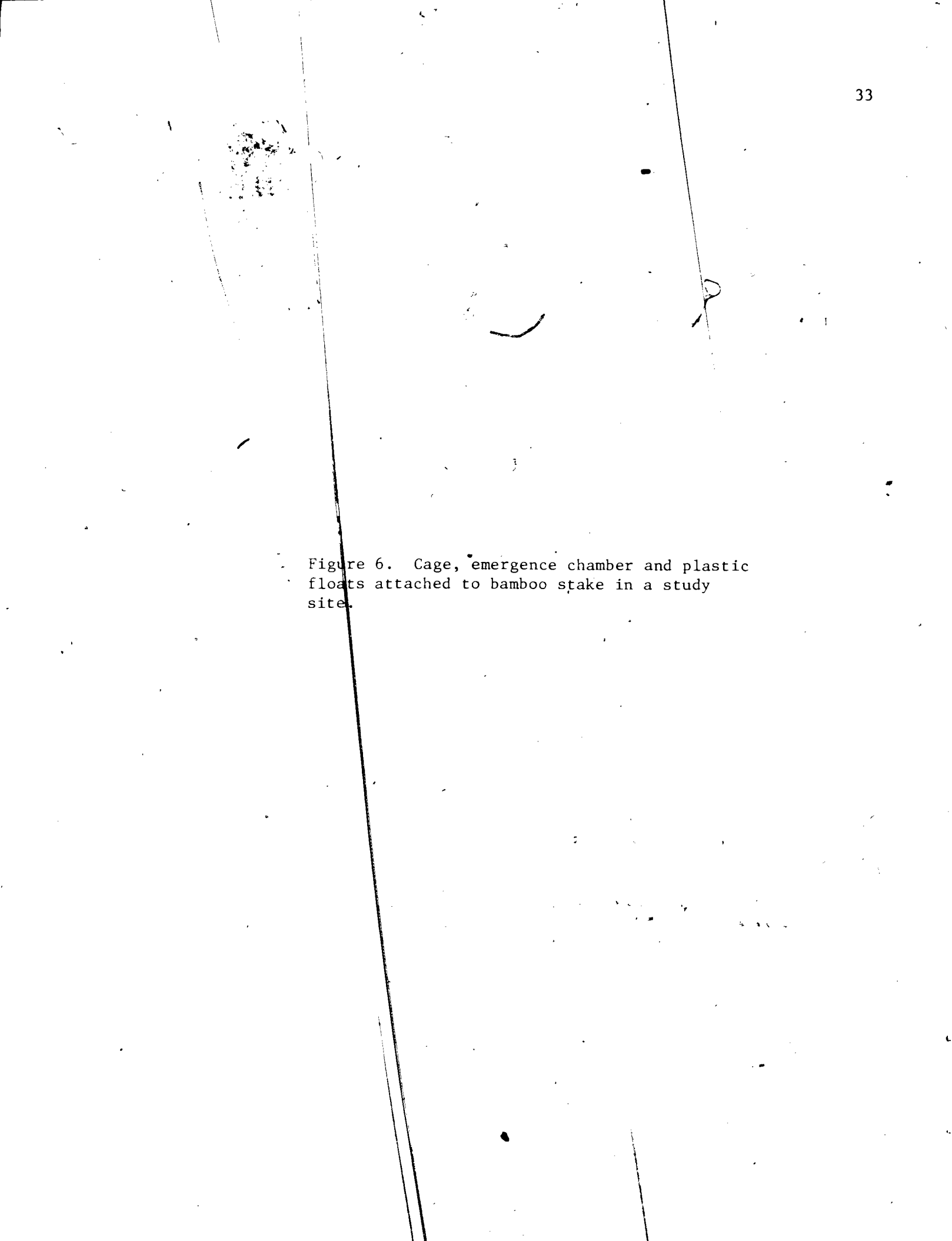


Figure 6. Cage, emergence chamber and plastic floats attached to bamboo stake in a study site.



yeast. Twenty-five 6-day-old laboratory-reared C. pipiens larvae were placed into each cage. The number of adults present in the emergence chamber was counted after a minimum of 16 days had elapsed since the beginning of the experiment.

To determine the effect on larval survival of transportation to the field sites one batch of larvae destined for a control site (C1) was transported along with those larvae destined for field sites and then brought back to the control site. The larvae placed into the second control site (C2) were placed directly there from the rearing tray.

The entire experiment was performed 3 times during 1976. The first run of the experiment began on 14 June and ended on 10 July; the second run began on 10 July and ended on 26 July, and the third run began on 26 July and ended on 12 August. The following procedural differences existed between runs:

i) On the first run only one control was used (C2);

ii) On the second and third runs the pond Pitt Meadows was not used because it was flooded. The site Richmond 2 was used.

## Results and Discussion

The results of these experiments are shown in Table III as mean values for the percentage of adults emerging.

The percentage data were transformed to  $x' = \arcsin$  square root ( $x$ ) and an analysis of variance was performed on each run of the experiment. The transformed means within each experiment were compared using the SNK procedure.

Analysis of variance of data from the first experiment indicated that there was a significant ( $p < 0.001$ ) added variance component between sites for the number of mosquitoes emerging. The SNK results suggest that none of the sites was as suitable as the laboratory control site for the survival of C. pipiens larvae to the adult stage. This experiment was conducted during the period 10 June, 1976 to 10 July 1976 and the weather was unseasonably cool and cloudy. This weather factor would not only affect the survival of the mosquito larvae but also qualitative and quantitative aspects of their food supply.

Table III. Number of adult Culex pipiens emerging from cages in selected ponds.\*\*

Location	Time in Place (days)	Mean (percent)* ± 1 standard deviation	
Run 1	26		
Ft. Langley		39.0	(16.0) a
Burnaby 1		5.0	(5.0) b
Pitt Meadows		9.0	(17.5) a
Sheridan Hill 1		2.0	(4.5) a
Sheridan Hill 2		10.0	(7.9) a
Control 2		78.0	(14.4) c
Run 2	16		
Ft. Langley		21.2	(8.5) a
Burnaby 1		1.0	(2.2) d
Pitt Meadows		22.5	(18.9) a
Sheridan Hill 2		64.0	(7.4) c
Granville 2		42.0	(10.4) bc
Control 1		37.0	(20.0) ab
Control 2		55.0	(15.4) bc
Run 3	17		
Ft. Langley		6.2	(4.8) abc
Burnaby 1		0.0	(0.0) a
Pitt Meadows		3.0	(4.5) ab
Sheridan Hill 2		17.0	(16.0) bcd
Granville 2		38.7	(18.9) d
Control 1		24.0	(10.8) cd
Control 2		24.0	(9.8) cd

\*Means followed by the same letter (within runs) are not significantly different (Student-Newman-Keuls Procedure, p less than 0.05).

\*\*Five cages/pond, 20 larvae/cage. Four cages/control, 25 larvae/cage.

The analysis of variance of the second experiment indicated that there was a significant ( $p < 0.001$ ) added variance component between sites for the number of adult mosquitoes emerging. The SNK procedure revealed that with one exception (Burnaby 1) the field site results were not significantly different from those of the control sites. Furthermore there was no significant difference between the results obtained in C1 and results obtained in C2. Therefore, in only one pond during the time period 10 July to 26 July 1976 (Burnaby 1) can the absence of naturally-occurring mosquito larvae (i.e. Culex pipiens) be attributed to either the presence of toxic compounds or the absence of essential growth factors. This of course does not rule out the possibility that other factors (e.g. predation, non-selection by ovipositing females) are responsible for the absence of mosquito larvae in this pond, but the results do indicate the suitability of the other 4 sites for mosquito survival.

The analysis of variance of the results of the third experiment revealed that there was a significant ( $p < 0.001$ ) added variance component between sites for the number of adult mosquitoes emerging. The results of the SNK procedure indicate that the results from all but two of the field sites (Burnaby 1 and Pitt Meadows) were not significantly different from those of the



control sites. And again there was no significant difference between the results obtained in the two control sites.

It appears, then, that in the sites Ft. Langley, Pitt Meadows (sometimes), Sheridan Hill 1 and Richmond 2, C. pipiens larvae can survive to the adult stage at least as well as under laboratory conditions and that the absence of naturally occurring C. pipiens larvae is not due to the presence of toxic compounds or the absence of essential growth factors. Absence of C. pipiens from the sites Burnaby 1 and Pitt Meadows could be due to the presence of toxic compounds or the absence of essential growth factors. These two sites are closely related in terms of SH' for species composition of aquatic vegetation and were the only two ponds surveyed containing C. tarsalis larvae. This may indicate that there are some basic environmental or biological similarities between the two ponds which can be indicated by the presence or absence of aquatic plants.



B. SOME EFFECTS OF AQUATIC VEGETATION ON THE DEVELOPMENT AND SURVIVAL OF MOSQUITO LARVAE IN ARTIFICIAL POOLS.

Previous experiments in laboratory aquaria have indicated that certain plants can influence the development and survival of mosquito larvae (Angerilli and Peirne 1974). This experiment was designed to test further, effects of some of these plants under more natural conditions. The experiment was repeated 3 times in order to determine some of the effects of varying climatic conditions on plant efficacy.

#### Methods and Materials

Twelve one square metre artificial ponds were built on the Pestology Site of the University of British Columbia Research Forest, Maple Ridge, B.C. The site used was a grassy meadow surrounded by a deer proof fence. The ponds were located in the middle of the meadow in a 5 by 7 m rectangle, each pond being 1 m from its nearest neighbour (Figure 7).

Each pond consisted of 5 by 20 cm douglas fir boards lining the perimeter of a square, flat, level bottomed pit. Black, 4 mil polyethylene sheet plastic was used to line the frame and pit to create a water

holding pool. The ponds were filled with 15 to 20 cm of water pumped from a nearby well and then a thin layer of washed white sand was placed on the bottom. Ten grams of garden fertilizer (analysis: 10-6-4) were added to each pond to serve as an inorganic nutrient source.

On top of each pond was an emergence cage of black anodized aluminum window screening in the shape of a four-sided pyramid approximately one metre in height. At the top of the pyramid was a removable chamber (Figure 8) in which any mosquitoes that emerged from the water became trapped.

#### Experiment 1, Early Summer

On 24 June 1975 2 species of aquatic plants and 1 species of alga were placed into 3 ponds each while 3 plantless ponds served as controls. All treatments were assigned randomly. The species used were: i) Chara globularis, an alga, collected in May 1975 from Osoyoos and was reared in laboratory aquaria until use. ii) Lemna minor, collected from Richmond on 24 June 1975. iii) Utricularia minor, collected from Burnaby on 23 June 1975.

All plant material was thoroughly rinsed with


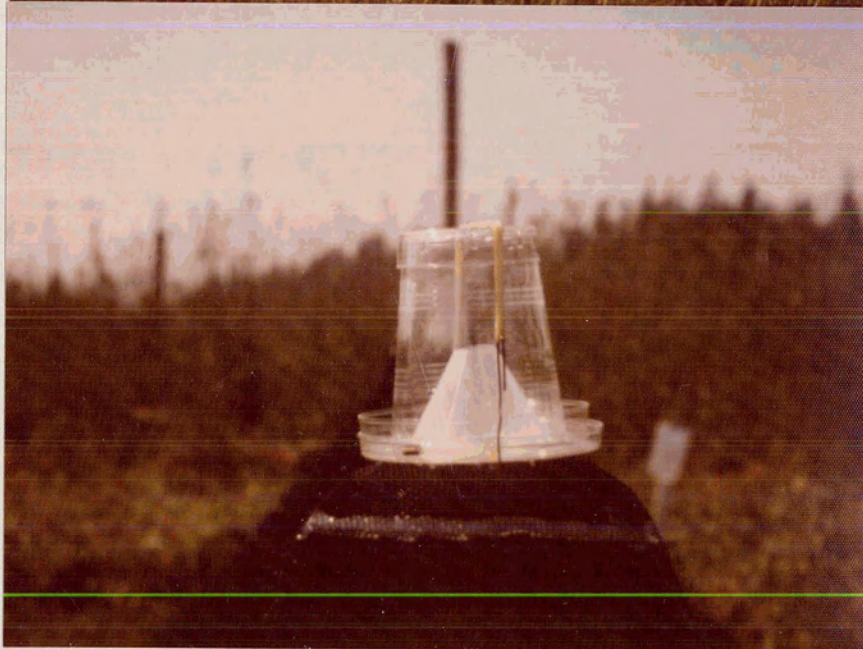


Figure 7. Covered artificial ponds.

Figure 8. Emergence chamber on top of screen pyramid covering artificial pond.



running tap water before being placed into the ponds in order to remove non-plant material (e.g. potential predators ~~and~~ their eggs). Also, samples of each plant were retained in the laboratory to determine what kinds of organisms might still emerge from them.

On 29 June 1975, 100, 6-day-old, laboratory-reared Culex pipiens larvae were placed into each of the ponds along with a baseline meal consisting of 10 g of tetramin and 0.5 g of baker's yeast. Emerging adult mosquitoes were counted weekly.

#### Experiment 2, Mid-summer

All 12 ponds were drained, cleaned and refilled on 22 July 1975. U. minor and L. minor were reused. C. globularis died during experiment 1 so additional material was collected (from Osoyoos), cleaned, and placed into 3 artificial ponds on 28 July 1975. Mosquito larvae were added to the ponds on 29 July. All other procedures were as in experiment 1.

#### Experiment 3, Early mid-summer

The ponds were cleaned and refilled on 11 July 1976 and the following plants were used: i) Flodea canadensis, collected from Vancouver on 19 July 1976;

ii) L. minor, collected from Vancouver on 10 July 1976; iii) U. minor, collected from Burnaby on 10 July 1976.

Mosquito larvae were added to the ponds on 12 July. All other procedures were as in the previous experiments.

### Results and Discussion

The results of the 3 experiments are summarized in Table IV and shown in Figure 9.

#### Experiment 1

The data from the first experiment were transformed to  $x' = \log(x+1)$  and the analysis of variance of these transformed data indicated a significant ( $p < 0.01$ ) added variance component. Comparison of means with the SNK procedure revealed that there were significantly fewer ( $p < 0.05$ ) adult mosquitoes emerging from ponds containing L. minor than from plantless ponds (controls) or from ponds containing U. minor. There was no significant difference between the number of mosquitoes emerging from ponds containing L. minor and ponds containing C. globularis and there was no difference between ponds containing C. globularis, U.

Table IV. Mean number of adult mosquitoes that emerged from artificial ponds ( $\pm$  1 standard deviation).

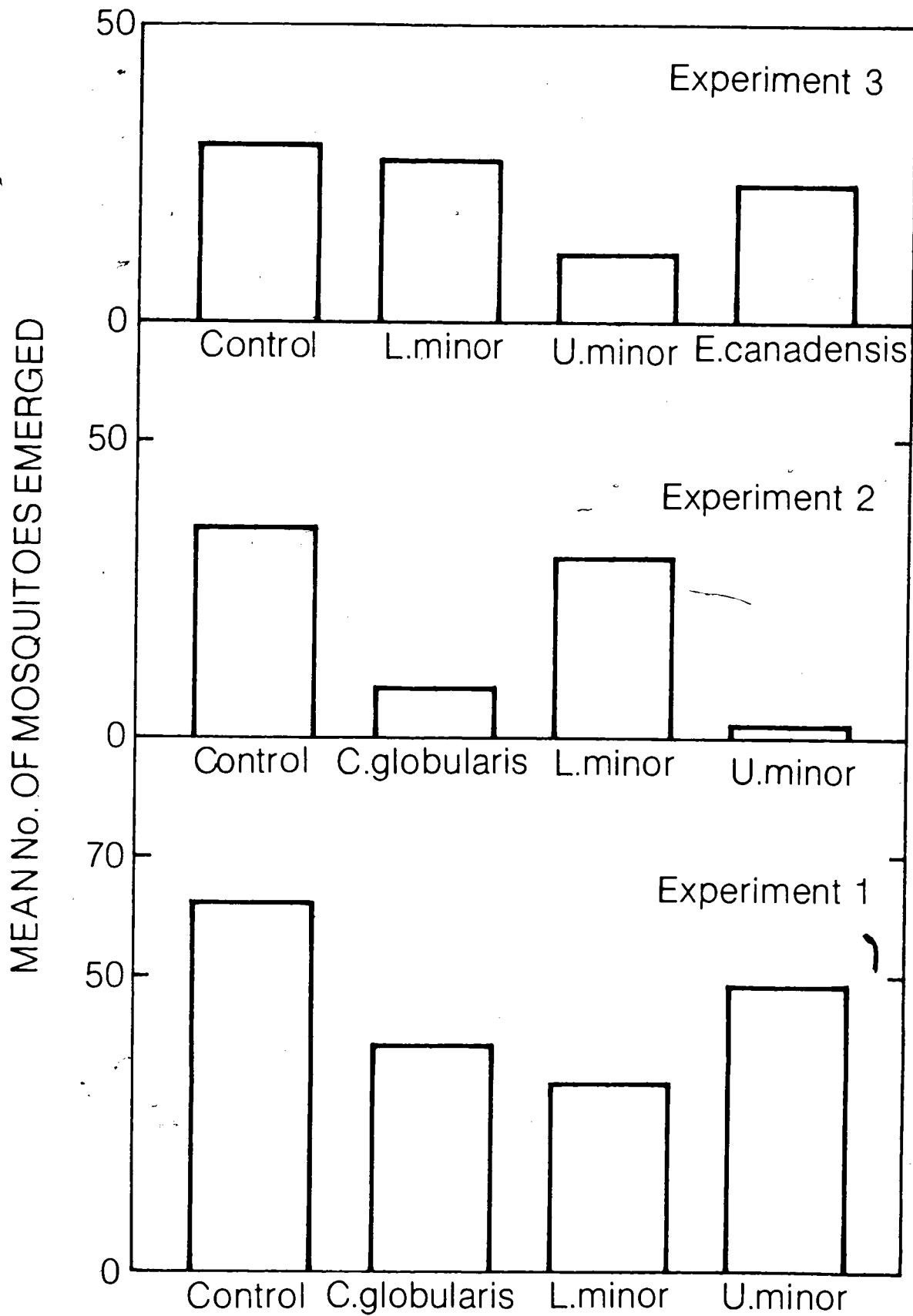
Treatment	Experiment 1 <sup>a</sup>	Experiment 2 <sup>a</sup>	Experiment 3 <sup>a</sup>
Control	62.67 (7.57) a	35.67 (6.66) a	30.00 (1.73) a
<u>Chara globularis</u>	38.33 (16.44) ab	8.67 (6.36) b	-
<u>Lemna minor</u>	32.00 (19.92) b,	30.33 (3.21) a	27.33 (15.95) a
<u>Utricularia minor</u>	48.67 (9.45) a	2.50 (3.53)*b	11.67 (12.22) a
<u>Elodea canadensis</u>	-	-	23.33 (3.51) a

<sup>a</sup>Results followed by the same letter (within experiments) are not significantly different (Student-Newman-Keuls Procedure, p less than 0.05)

\*During experiment 2 one of the ponds containing U. minor became inoperative and the results from it are not used.

Figure 9. Total mean number of adult mosquitoes emerging from covered artificial ponds during experiments one, two, and three.





minor, or no plant.

### Experiment 2

The analysis of variance of the results of experiment 2 also indicated a significant ( $p < 0.001$ ) added variance component between treatments. The SNK procedure indicated that the means fell into two groups that were significantly different ( $p < 0.05$ ) from each other. The mean numbers of mosquitoes that emerged from control ponds and from L. minor ponds form one group whereas the other is formed by the smaller means of C. globularis ponds and U. minor ponds.

### Experiment 3

The results of experiment 3 did not contain a significant added variance component between treatments.

In general, the results of these experiments are similar to those of previous laboratory studies (Angerilli and Beirne 1974) and field experiments (e.g. MacGregor 1924) in that the plants sometimes do and sometimes do not reduce adult emergence.

## Influences of Lemna minor

The number of adult mosquitoes that emerged from ponds containing L. minor remained relatively constant between all 3 experiments. But there was no significant difference between the numbers of mosquitoes emerging from L. minor ponds and from control ponds during experiments two and three. The plant normally covered the entire surface of the water during all three experiments, so that, if total surface coverage and consequent larval suffocation is the mechanism by which L. minor causes larval mortality (Bradley 1932; Furlow and Hays 1972), this constant larval mortality between experiments was to be expected. Because L. minor forms a surface mat it would tend to filter environmental influences such as temperature fluctuations. As a result, mosquito larvae in L. minor ponds would experience a different microclimate than mosquito larvae in control ponds and the effects of L. minor induced mortality would be masked by a higher average survival rate in L. minor ponds as compared with control ponds.

If a plant-produced chemical is responsible for larval mortality (Angerilli and Beirne 1974) then the L. minor results (as compared with control results) may be explained on the basis of phenological changes in the

plant and consequent differential "chemical" production rates. That is, because weather influences on plant growth are not constant, the types and amounts of compounds produced by the plant will vary, and larval mortality will vary. However, because of the "sheltered environment" effect discussed above, this experiment failed to distinguish between the effects of L. minor "protecting" mosquito larvae and the effects of L. minor harming mosquito larvae.

#### Influences of Chara globularis

As mentioned in the materials and methods section, C. globularis did not survive the rigours of transplanting and deteriorated rapidly during the first experiment. If C. globularis harms mosquito larvae by releasing some chemical substance into the water (Angerilli and Beirne 1974), then the low larval mortality that occurred in C. globularis ponds is not surprising. C. globularis grew vigorously during the second experiment and larval mortality was high (a mean of 91.33%). It appears then, that under the appropriate conditions C. globularis can cause larval mortality. The exact environmental conditions required for C. globularis to grow and produce anti-larval chemicals are not precisely known but would need to be determined before C. globularis could be

used in any mosquito control programme. It would be particularly important to determine if the active growing period of C. globularis coincides with the occurrence of natural populations of mosquito larvae. However, if these two events do not coincide it might still be possible to utilize the plant in mosquito control programmes by culturing it and extracting the insecticidally-active compounds. This possibility is explored in a later section of this dissertation.

#### Influences of Utricularia minor

Because U. minor causes larval mortality by ingesting larvae with its bladder-like appendages, the level of larval mortality in U. minor ponds must be related to the number of bladders present on the plant (each bladder can consume only one larva at a time). U. minor normally sheds its bladders when stressed, for example during transplanting. In experiment 1 of this series the low level of larval mortality occurring in U. minor ponds can be explained by the absence of bladders due to shedding. During the second experiment, by which time numerous new bladders had been produced, the plant apparently caused considerable larval mortality. Larval mortality was also high in U. minor ponds during experiment 3 but not significantly

high relative to control ponds.

It is possible that the control pond results of this experiment were anomalous and due to unusual weather conditions, the effects of which were moderated in ponds with plants. For example, larval food supplies were probably low in plantless ponds compared to plant-filled ponds because of weather-related reduced productivity in plantless ponds (e.g. fewer algae) and relatively constant supplies of plant-produced detritus in plant-filled ponds.

#### Influences of Flodea canadensis

Flodea canadensis also caused considerable (but not significant) larval mortality in the third experiment but there are no other field experiments to compare this result with for repeatability.


The effect of artificial growing conditions on plant function, particularly the production of insecticidal compounds, is unknown in all of these experiments. It is possible that the production of such compounds is a response to environmental stress or that the production of such compounds is curtailed under stressful growing conditions. Therefore, to determine the true ability of a plant to affect

mosquito larvae in a natural situation, the plant must be grown in conditions that simulate the habitat range of the plant.

Two general conclusions are indicated by the results of these three experiments:

i) that the plants tested can cause larval mortality under field conditions; and

ii) that the level of larval mortality caused by a plant is related to phenological and environmental responses of the plant.



C. SOME EFFECTS OF AQUATIC PLANTS ON THE  
COLONIZATION OF ARTIFICIAL POOLS BY MOSQUITOES AND  
THEIR PREDATORS.

The results of the field survey suggested that mosquito presence in, or absence from some ponds might be due, in part, to the oviposition behaviour of the female mosquito. Furlow and Hays (1972) found that certain aquatic plants could affect the species composition of mosquitoes colonizing small pools of water. The following study was designed to measure the effects of some aquatic plants:

- on the species and numbers of mosquitoes colonizing small pools of water;
- on the kinds and number of potential mosquito predators colonizing these pools; and
- on other factors in the aquatic environment that might affect mosquito larvae.

#### Materials and Methods

Sixteen one-square-metre artificial ponds were built on the Pestology site of the University of British Columbia Research Forest in Maple Ridge, B.C.



The ponds were located in a grassy field surrounded by a deer-proof fence. The minimum distance between adjacent ponds was 3 m while the maximum distance was 5 m.

The ponds were constructed and treated identically as those used in the previous section except that they did not have covers on them.

One of 3 species of aquatic plant was added to each of 4 ponds. Four plantless, but otherwise identical ponds served as controls. The following plants were used:

- i) Elodea canadensis, collected near Ft. Langley;
- ii) Lemna minor, collected from an ornamental pool, Vancouver;
- iii) Utricularia minor, collected in Burnaby.

Treatments were assigned randomly and were made on 14 July 1976. The following measurements and observations were made on each pond at weekly intervals and at the same time of day +/- 2 hrs (except the last 3 which were biweekly) starting on 21 July 1976.

- The number of mosquito egg rafts on the surface of the pond was counted by direct observation.

- The number of mosquito larvae in the water was estimated by taking 5 samples with a one litre, rectangular, white-enamelled dipper.

- The number of potential predators of mosquito larvae was counted by direct observation, at times aided by a plexiglas-bottomed plastic cylinder which was used to peer through dense vegetation covering the surface of some ponds.

- Water temperature was measured with a mercury-filled glass thermometer.

- The pH of the water was measured with a Fisher Accumet portable pH-meter.

- Dissolved oxygen content of the water was measured with a portable dissolved oxygen meter (Yellow Springs Instrument Company).

- The amount of particulate matter (detritus + organisms) suspended in the water was estimated by taking a 25 ml water sample, adding 1 ml of Lugol's

solution as a preservative, and then in the laboratory counting on a haemocytometer the particles contained in two 10 microlitre subsamples.

An additional 5 ml water subsample was filtered through a Nuclepore membrane filter having a pore diameter of 1.0 micrometres. The filter was mounted on a scanning electron microscope (SEM) stub, coated with gold, and then observed and photographed with a SEM. The photographs were then used to determine if qualitative differences in particulate matter existed between ponds.

### Results

The largest number of mosquito larvae was found in the control (plantless) ponds. The mean number of larvae found there during the 11 week observation period was 40.21. Ponds containing Utricularia minor and Elodea canadensis had moderate numbers of mosquito larvae (11 week means of 8.41 and 5.75 respectively). Ponds containing Lemna minor had negligible numbers of larvae with a mean of 0.17.

The mean number of mosquito egg rafts found followed a pattern similar to that of the number of mosquito larvae. The largest number of rafts was found

in control ponds with a mean of 3.25. Ponds containing U. minor and E. canadensis had moderate numbers of egg rafts with 0.87 and 0.45 respectively and L. minor had negligible numbers of egg rafts with 0.09.

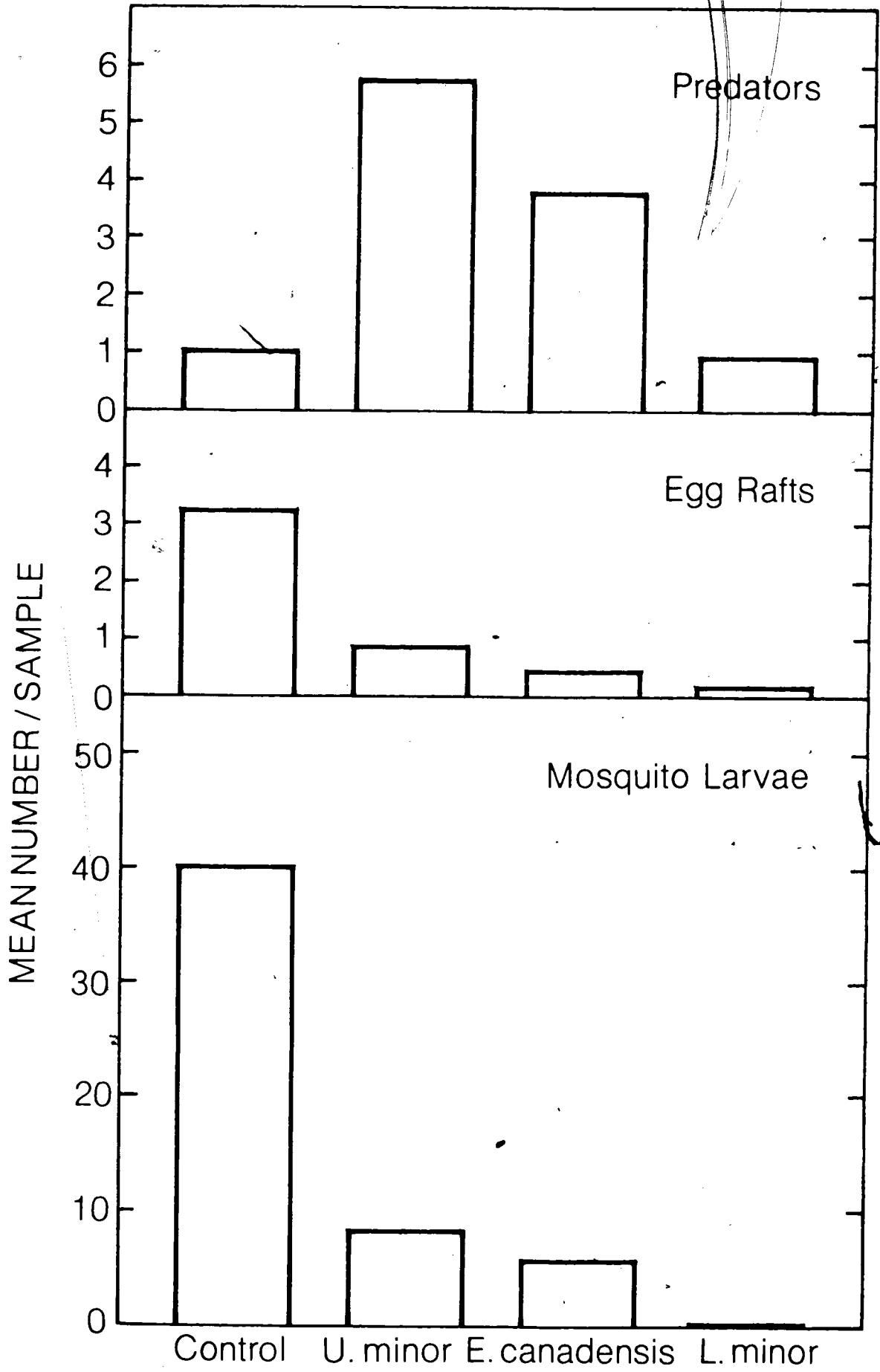
Overall there was a very strong correlation between the number of mosquito larvae and the number of egg rafts found ( $r=0.63$ ;  $p<0.001$ , using data transformed to  $x'=\log(x+1)$ ).

The number of potential predators of mosquito larvae found in the ponds followed a different pattern. The largest number of predators was found in ponds containing U. minor (11 week mean of 5.77), the next largest number in E. canadensis ponds (3.80) and the lowest numbers in the control and L. minor ponds (1.03 and 0.91 respectively).

All of the above results are summarized graphically in Figure 10.

Analysis of variance and subsequent comparison of means using the SNK procedure indicated the following. (All data were transformed to  $x'=\text{square root}(x+0.5)$ ). There was a significant added variance component ( $p<0.001$ ) between treatments for the number of mosquito larvae, the number of mosquito egg rafts, and the

Figure 10. Mean number of mosquito larvae per sample, mosquito egg rafts per sample and predators of mosquitoes per sample found in coverless artificial ponds during sampling period.



number of potential predators of mosquito larvae. The SNK procedure revealed the following relationships, where means not joined by a horizontal bar are significantly different ( $p < 0.05$ ).

#### Mosquito Larvae

L. minor	E. canadensis	U. minor	Control
x = 0.77	<u>1.85</u>	<u>2.21</u>	5.50

#### Mosquito Egg Rafts

L. minor	E. canadensis	U. minor	Control
x = 0.76	<u>0.91</u>	<u>1.00</u>	1.68

#### Predators

L. minor	Control	E. canadensis	U. minor
x = <u>1.05</u>	<u>1.12</u>	1.91	2.30

The weekly mean numbers of mosquitoes, mosquito egg rafts and predators are shown graphically for each treatment in Figures 11, 12 and 13. These figures

Figure 11. Mean number of mosquito larvae by treatment for each week of sampling of coverless artificial ponds.



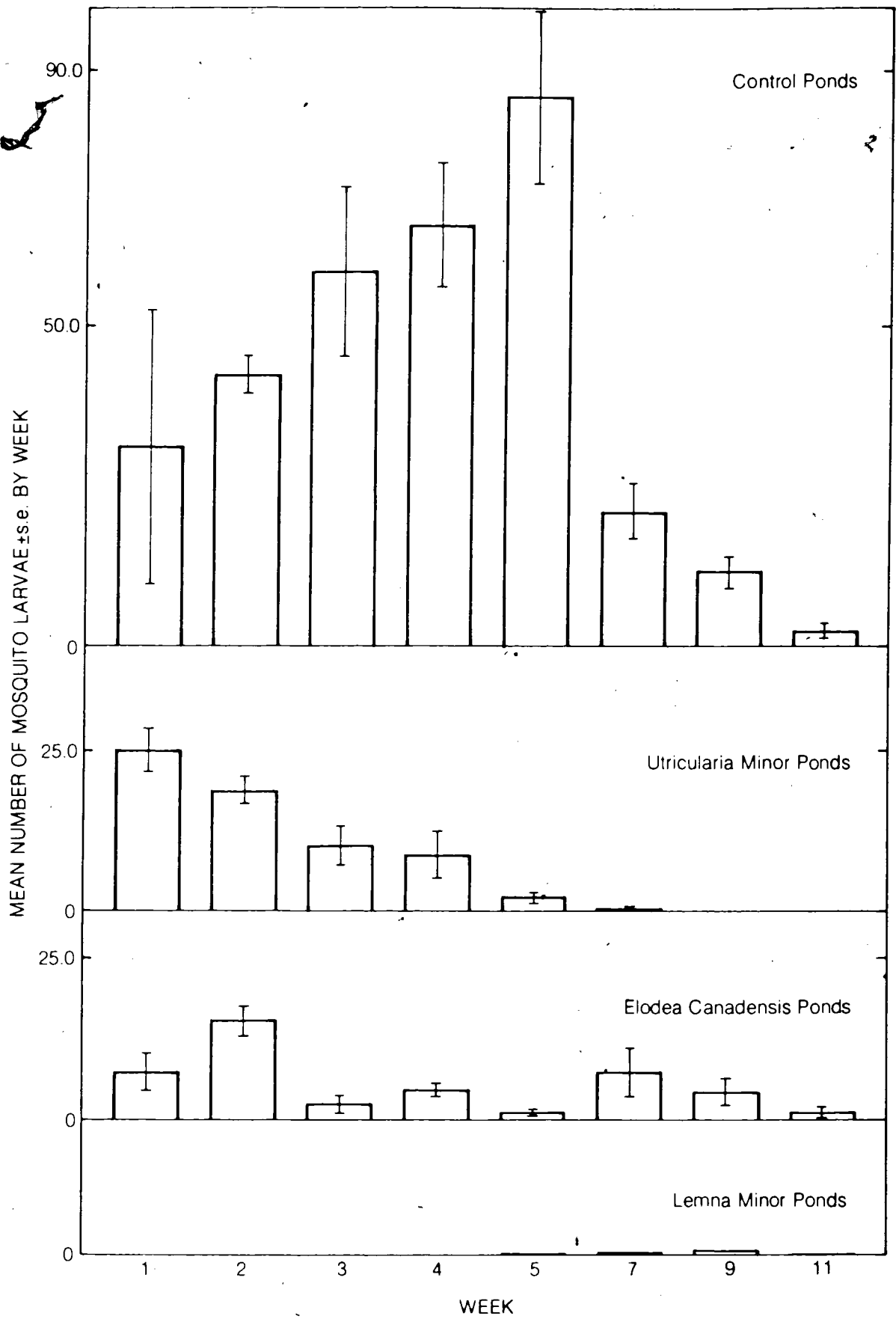


Figure 12. Mean number of mosquito egg rafts by treatment for each week of sampling of coverless artificial ponds.

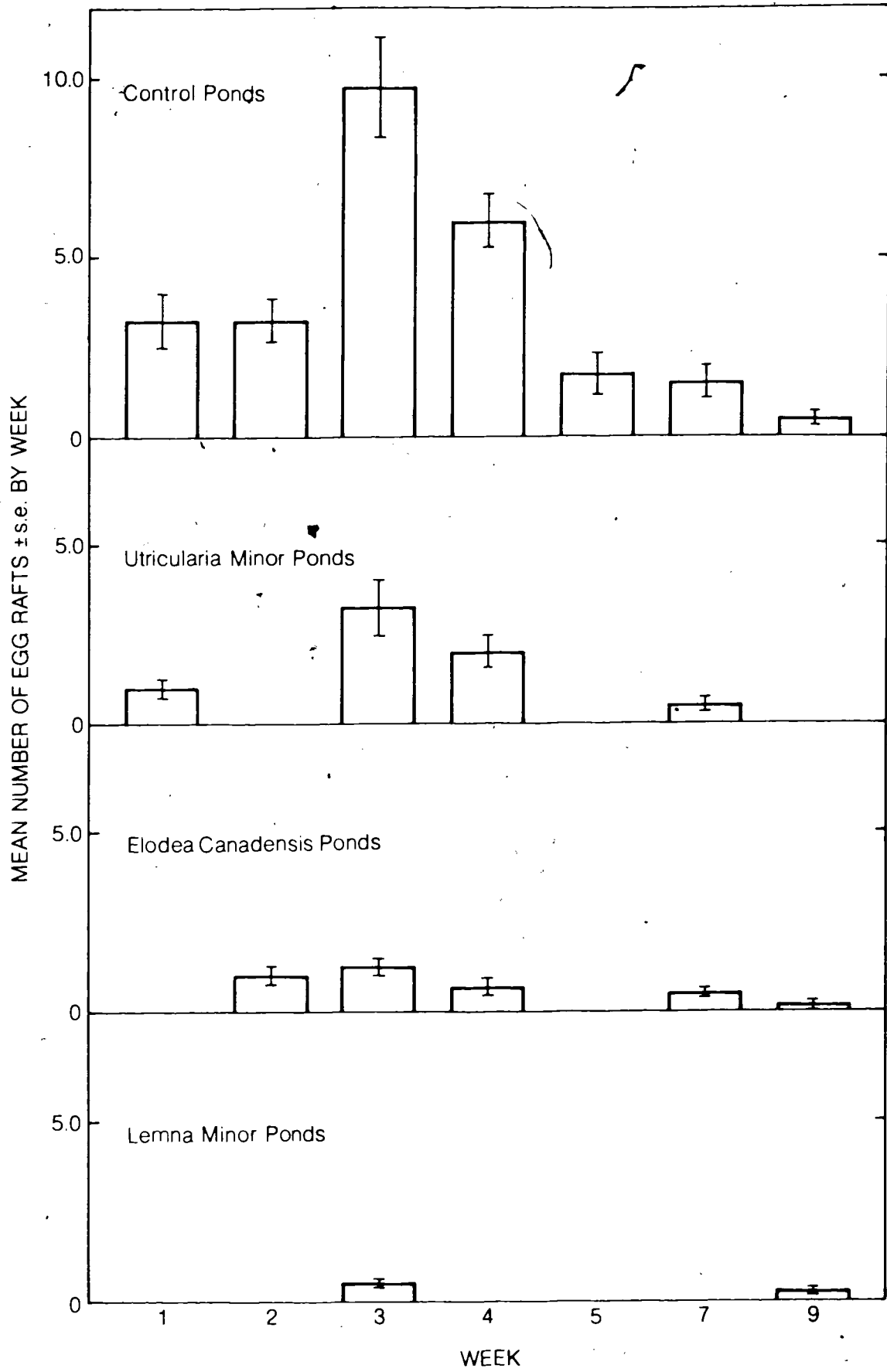
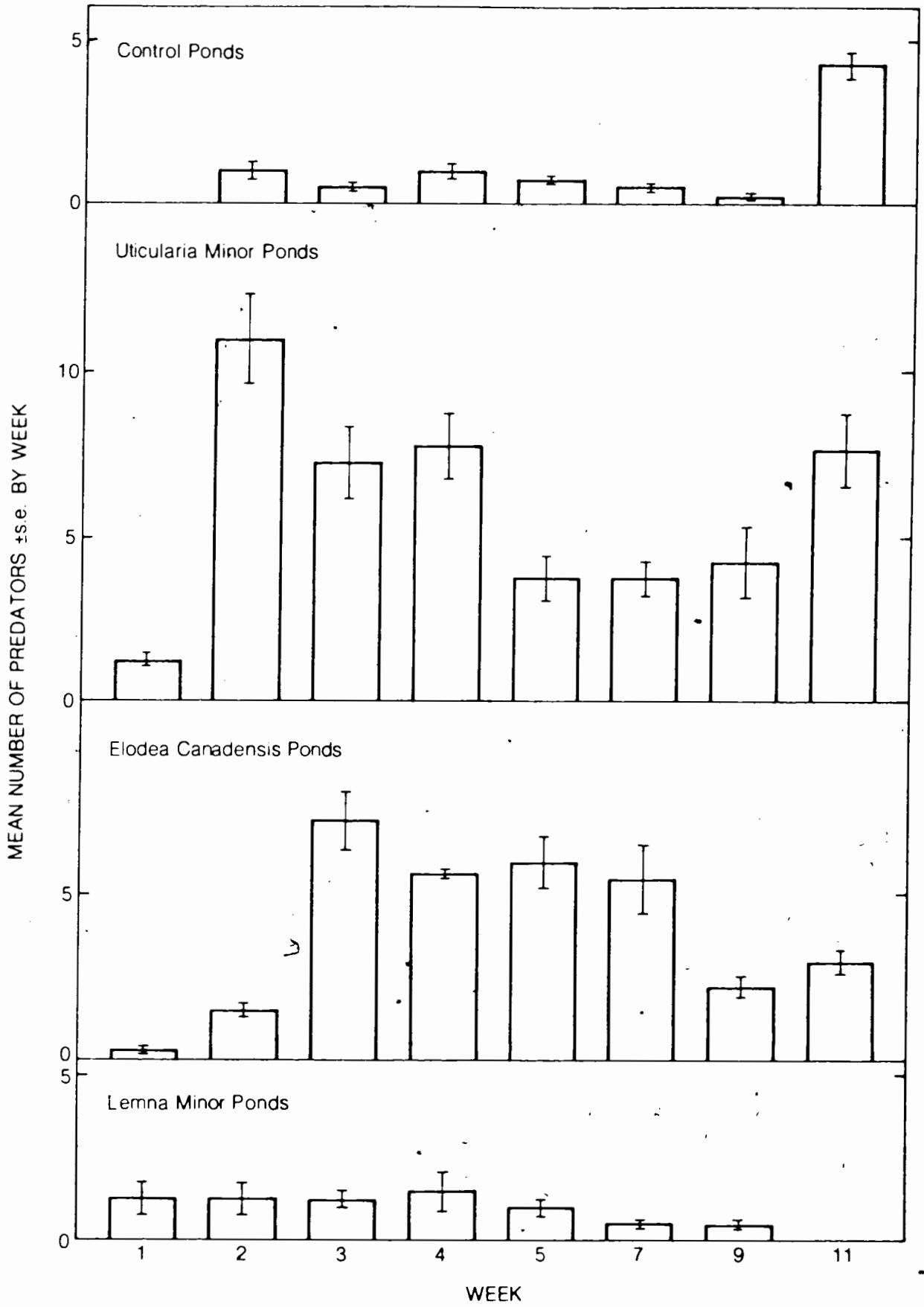


Figure 13. Mean number of predators of mosquito larvae by treatment for each week of sampling of coverless artificial ponds.



indicate that there were definite temporal differences in the occurrences of mosquito larvae and predators between treatments. Peaks in mosquito egg raft numbers although different in magnitude appear to occur simultaneously in all treatments.

Water temperature, pH and relative amounts of particulate matter also differed between treatments. There was no significant difference between treatments for the dissolved oxygen content of the water.

The differences, which were determined by analysis of variance and subjected to the SNK procedure ( $p < 0.05$ ), were as follows:

Water Temperature (degrees C) ( $F=6.45$ ;  $p < 0.001$ )

L. minor	E. canadensis	Control	U. minor
x= <u>16.59</u>	<u>17.47</u>	<u>17.77</u>	18.70

pH ( $F=3.86$ ;  $p < 0.01$ )

L. minor	E. canadensis	Control	U. minor
x= <u>5.80</u>	<u>6.32</u>	6.60	<u>6.83</u>

Particulate Matter (F=15.82; p<0.001)

L. minor	E. canadensis	U. minor	Control
x= 38.46	78.58	83.91	85.42

Dissolved Oxygen (F=2.31; p<0.07)

Means (f/- standard error) for particulate matter and water temperature are shown in Figure 14.

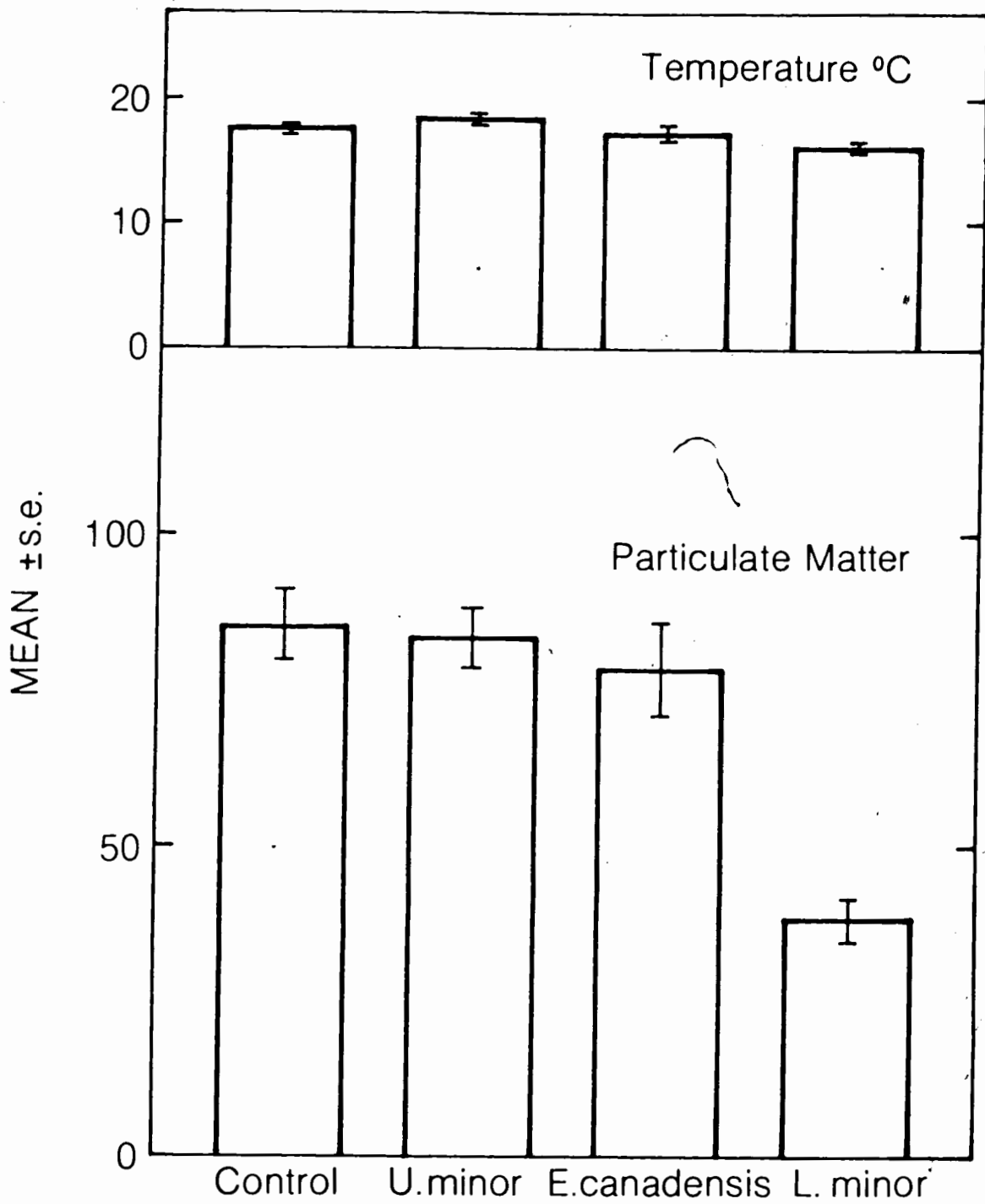
These data were subjected to stepwise multiple regression analysis (SPSS subprogram regression, Nie et al. 1975) to determine the relative importance of the measured variables to the number of mosquito larvae present.

When all treatments were considered simultaneously and the dependant variable was the number of mosquito larvae (transformed to  $x' = \log(x+1)$ ) regressed against all other variables the single most important predictor variable was the number of eggs, which accounted for 39.6% of the variance of mosquito numbers. When all other variables are added to the equation 46.5% of the variance of mosquito numbers is accounted for. The number of eggs present is not strongly related to any of the variables measured (total variance accounted for

Figure 14. Mean water temperature and particulate matter content of coverless artificial ponds during sampling period.



ρ



= 10.3% when physical factors entered).

When the 4 treatments are considered individually it appears that different factors are important in influencing the variance of mosquito numbers.

The number of Notonectidae present explained the largest amount of mosquito number variance in the plantless ponds (35.3%,  $r=-0.60$ ) and was strongly negatively correlated with mosquito numbers. When all variables were entered into the regression equation 54.5% of mosquito number variance was accounted for. When all the predators were combined into a single variable (PREDALL) and a regression was performed using mosquitoes as the dependant variable, the variable EGGS accounted for 30.8% of mosquito variance and PREDALL accounted for only 3.8%. The total explained variance in this run was 42.8%. The number of egg rafts present was strongly correlated with the number of mosquito larvae present ( $r=0.555$ ;  $p<0.001$ ).

The number of mosquito larvae present in ponds containing U. minor was correlated with the number of larval Dytiscidae present ( $r=0.537$ ;  $p<0.001$ ) and 28.8% of mosquito variance was explained by this variable. The variable DISSOLVED OXYGEN explained 30.4% of the variance of mosquito numbers and was negatively

correlated ( $r=0.51$ ;  $p<0.001$ ). After all the variables were added to the equation 78.3% of mosquito variance was explained. If PREDALL was used in the equation it only explained an insignificant amount of mosquito variance while DISSOLVED OXYGEN accounted for 25.5%. After all significant ( $p<0.05$ ) variables were entered into the regression equation 58.2% of the variance of mosquito numbers was explained. EGGS accounted for 20.6% of this variance.

In ponds containing E. canadensis the variable Notonectidae (NOTON) accounted for 14.9% of the variance of mosquito numbers and was negatively correlated ( $r=-0.386$ ;  $p<0.01$ ). Water temperature was the next most important variable explaining 9.3% of mosquito variance and then PARTICULATE MATTER explaining 10.5% but negatively correlated ( $r=-0.296$ ;  $p<0.05$ ). After all variables were entered into the equation only 37.3% of the variance of mosquito numbers was accounted for. When PREDALL was used in the equation, EGGS explained the greatest amount of mosquito variance (10.4%) and was positively correlated with MOSQUITO ( $r=0.32$ ;  $p<0.05$ ). After all variables were added to the equation only 30.1% of mosquito variance was accounted for. The variable PREDALL accounted for 8.8% of this and was negatively correlated with MOSQUITO ( $r=-0.29$ ;  $p<0.05$ ).

After all variables for L. minor ponds were entered into the regression equation 16.2% of mosquito variance was accounted for. Eggs accounted for 7.3% of this variance. In subsequent runs of this analysis using various combinations of the measured variables, a substantial amount of mosquito number variance was unaccounted for.

It appears then that, in addition to the variables measured, mosquito larval numbers are influenced by some factor or factors not measured. It is also possible that some of the variance not accounted for in the regression analyses is due to sampling errors. Furthermore, certain factors that can affect mosquito larvae and adults are either unobservable or unquantifiable. For example, although mosquito numbers did not appear to be related to the amount of particulate matter present (particulate matter can be assumed to be the larval food supply) they may be related to the kinds of particulate matter present in different ponds.

Tables 5 and 6 (App. B) summarize the differences between ponds in terms of mosquito species and predator species.

Water samples taken on 21 July, 4 August and 29 September were examined using the SEM technique described previously. The identifiable objects found were detritus, bacteria and algae. The origin of the detritus could not be determined and the bacteria were not identified beyond that category. The algae present were: Ankistrodesmus sp. Corda, Scenedesmus sp. Meyen, both in the Class Chlorophyceae; Navicula sp. Bory, a diatom (Bacillariophyceae) and two unidentified kinds. The first unidentified organism was unicellular and because of its green colour and quarter-moon-like shape is probably of the Chlorophyceae and for purposes of this analysis was termed "luna". The second unidentified species was colonial and was termed "cp".

Bacteria were the dominant organisms in the control ponds on all 3 days for which water samples were examined. Scenedesmus sp., Ankistrodesmus sp. and luna were present but rare.

Bacteria were also the dominant organisms in Utricularia minor ponds. In the second sample observed however, Ankistrodesmus sp. was dominant in one of the 4 ponds but was then replaced by bacteria in the third sample as the dominant organism. Luna, Navicula sp. and cp were present but never dominant.

Detritus was most abundant in 3 of the 4 Flodea canadensis ponds in the first sample. Bacteria were dominant in the fourth pond. In the second sample Ankistrodesmus sp. was dominant in 3 of the 4 ponds while bacteria were dominant in the fourth pond (a different pond from above). Ankistrodesmus sp. and bacteria were dominant in two ponds each in the third sample. Luna and Scenedesmus sp. were also present but not until the second sample.

In the first sample of Lemna minor ponds Navicula sp. were dominant in two of the ponds, detritus in one and Ankistrodesmus in the fourth pond which also contained Scenedesmus sp. Bacteria were dominant in two ponds and detritus was dominant in the other two ponds in the second sample. Cp was present in one of the ponds in the second sample. In the third sample Ankistrodesmus sp. was dominant in 3 ponds and bacteria were dominant in the fourth. Cp was present in all 4 ponds and was the only other organism present.

## Discussion

As the number of mosquito larvae found in a given site was largely a consequence of the number of mosquito egg rafts laid in that site, the results of this study provide further experimental evidence that the restriction of certain species of mosquito larvae (in this case Culex pipiens, Culiseta inornata and Anopheles punctipennis) to certain breeding places is largely a result of a behavioural response of the ovipositing female mosquito (Buxton and Hopkins 1927; Muirhead-Thompson 1940; Wallis 1954). The results of this study also provide evidence to support the suggestion that aquatic plants can influence the oviposition behaviour of adult female mosquitoes. The mechanism by which this occurs could be direct through visual, olfactory or gustatory means or indirect through an influence on other physical or chemical aspects of the aquatic environment or on other organisms and their subsequent effect on the environment.

The factors affecting mosquito oviposition can be grouped as physical phenomena or as chemical phenomena.

## Physical Effects

In laboratory studies some water temperatures inhibit oviposition by Aedes aegypti L. and C. pipiens (B. Gillespie, per. comm.) but Muirhead-Thomson (1940) found that temperature had no effect on oviposition by Anopheles minimus in the field. In the present study the 4 treatments fell into 3 groups with respect to statistical similarity of water temperature. The first and warmest group consisted of U. minor ponds and plantless ponds - these ponds also contained the largest mean numbers of mosquito egg rafts and hence the largest mean numbers of mosquito larvae. The second group consisted of plantless and E. canadensis ponds. The third and coolest group was formed by E. canadensis and L. minor - these ponds contained the lowest mean numbers of egg rafts and the lowest mean numbers of mosquito larvae. The low mean temperatures encountered in L. minor ponds are not surprising as the plant normally covered the entire water surface and therefore probably acted as an insulator and reflector of both transmitted and radiant heat.

It would be to the mosquito's advantage to select a warm oviposition site rather than a cool one because larval development should proceed much faster in the warmer site. This would occur for two reasons. Faster



larval metabolism and a larger food supply to fuel it should be present in the warm site relative to the cool site. Quick larval development leading to earlier achievement of reproductive age would confer an advantage to those mosquitoes selecting warm rather than cool oviposition sites.

Light and background colour also appear to play a role in mosquito oviposition site selection. Muirhead-Thomson (1940) found that gravid An. minimus preferred to oviposit in shade which was normally provided by a "thick grassy edge" of a typical breeding place. Belton (1967) found that artificial pools lined with black as opposed to translucent (which appeared white to yellowish) polyethylene film received more than three times as many egg rafts of C. restuans (Theo.) and that artificial illumination at night of either type of pool with an intensity similar to that received during the day prevented oviposition. Oviposition sites with dark backgrounds are preferred by a number of mosquito species (Rates 1940; Kennedy 1942; Lund 1942). It is also known that ovipositing mosquitoes respond differentially to the colour of the oviposition medium and that spectral sensitivity varies from species to species (Williams 1962; Snow 1971; Yap 1975). The presence of plants in the artificial ponds used in this experiment would certainly alter the

reflectance and colour of the ponds and render them visually different to the gravid female mosquito. This would be particularly true in the case of L. minor because it normally covered the entire water surface. One effect this plant in particular might have had was to make the pond "invisible" to mosquitoes. A completely green water surface in the middle of a green grassy field might be invisible to most organisms lacking a high degree of visual acuity. As an aside, this plant was collected from an ornamental pond from which it had to be removed because so many people thought it was a lawn and tried to walk on it. If colour is important for oviposition site selection in mosquitoes, it may, in some cases be because of the contrast between the colour of the water and the colour of the site's surroundings.

It is also possible that gravid adult mosquitoes are sensitive to highly plane-polarized light such as that reflected from water-surfaces. If this were the only means by which such mosquitoes find potential oviposition sites then L. minor and other surface-covering plants would tend to make water surfaces invisible. Unfortunately this effect was not tested for in this experiment.

## Chemical Effects

Most laboratory studies of mosquito oviposition have concentrated on the chemistry of the water because it is believed to be the most likely factor to affect the resulting larvae. A series of chemical compounds have been found which are attractants or oviposition stimulants in the laboratory.

Some of these compounds are: inorganic salts (Wallis 1954; Hudson 1956; Salama and Ata 1972); organic compounds such as methane gas (Gjullin et al. 1965); alkyl carbonyl acids, ketones or esters when 9 atoms long and branched (Ikeshoji and Mulla 1975); plant phenolics derived from creosote (Ikeshoji 1975); bacterial intermediate metabolites of fatty acids such as capric acid and pelargonic acid (Maw 1970); unidentified naturally occurring compounds in breeding and/or holding waters of mosquito larvae and pupae (Hudson and McIntock 1967; Ikeshoji and Mulla 1970; Bentley et al. 1976); unidentified compounds from alfalfa infusions (Hazard et al. 1967; Lewis et al. 1974); and an egg-associated pheromone (Osgood 1971).

Plants could affect mosquito oviposition behaviour chemically in 3 ways:

i) They could produce chemical compounds that are attractive, stimulative or repellent;

ii) they could produce intermediate compounds which when metabolized by other organisms (e.g. bacteria) become attractive, stimulative or repellent;

iii) they could compete for such intermediate compounds and/or other essential growth compounds and prevent other organisms from producing attractive, stimulative or repellent compounds.

Hasler and Jones (1949) found that dense growths of certain aquatic plants in large artificial ponds had a significant inhibiting effect on the growth of phytoplankton and rotifer populations. Embury (1928), investigating fish culturing techniques in large artificial ponds obtained much denser cultures of plankton, crustacea, midges, mosquitoes and mayflies in ponds without higher plants when compared with ponds with plants. He believed that plants have two functions that tend to limit the production of food animals in two ways:

- i) by preventing the warming-up of bottom water, which retards the processes of decomposition; and
- ii) by competing with phytoplankton for nutrients.

Previous laboratory experiments (Angerilli, unpublished data) showed that certain aquatic plants could significantly alter the cation content of the water they were growing in. It is quite probable that plants alter other inorganic chemical properties of their growth medium. Also, in the present study there were significant differences between treatments in terms of pH and particulate matter. Moreover, there were also definite qualitative differences between treatments in terms of particulate matter, the most striking of which was the domination of bacteria in plantless ponds. Although it is generally agreed that pH has little if any effect on developing larvae, I would expect that different kinds of particulate matter, which serves as larval food, should affect larval development. That is, certain kinds of particulate matter provide an optimal diet for mosquito larvae while other kinds provide subsistence or worse conditions. There may be ovipositional cues associated with the gourmet fare as a result of natural selection. It is possible, then, that the plants in this study operated either directly or indirectly through chemical

mechanisms to produce degrees of attractiveness, repellency or neutrality with respect to mosquito oviposition behaviour. That is, they may have produced conditions unsuitable for the growth of bacteria or other organisms capable of producing oviposition attractants; they may have produced conditions suitable for the growth of organisms capable of producing oviposition repellents or they may have produced oviposition repellents or other kinds of behavioural cues directly.

It appears that the presence or absence of plants also affected the size of predator populations in the ponds. Although some of the predatory organisms found in the ponds may have originated from eggs attached to or inserted into the plants, the time of occurrence and age of a great many of the predators found suggested that they or their ancestors were attracted to the ponds. However, though I was not able to detect a significant relationship between the size of predator populations and the size of mosquito populations, there may be a negative relationship in natural situations. It would be interesting to remove all or part of the vegetation from a mosquito-free, predator-rich, naturally occurring pond and observe the subsequent secondary succession during the recovery of the system. I would predict a crash of the predator population and

the development of a mosquito population. The control of aquatic weeds by chemical, physical, or biological methods could then, result in the appearance or enlargement of mosquito populations. The importance of an aquatic plant to mosquito breeding, either alone or in conjunction with other aquatic plants should therefore be evaluated before attempts are made to eliminate it from an aquatic habitat.

### PART III. LABORATORY INVESTIGATIONS

#### A. INFLUENCES OF PLANT EXTRACTS ON THE SURVIVAL OF MOSQUITO LARVAE.

Information gathered in previous experiments of my own and others indicates that certain aquatic plants could affect mosquito larvae by releasing toxic compounds into the aquatic environment. The objective of the following study was to screen two kinds of extracts of various species of aquatic plants for the presence of toxic compounds.

#### Materials and Methods

Seven species of freshwater plants and one freshwater alga were collected during the summer of 1974, fresh-frozen at -56 degrees C, freeze-dried and then reduced to a powder in a blender. The powdered plant material was extracted with pentane and then methanol in a Soxhlet extractor for 24 h/batch/solvent. The solvents were removed from the extracts with a rotary evaporator. The residues of the methanol extractions were resuspended in 100 ml of distilled water and stored at 4 degrees C. The residues of the pentane extractions were resuspended in 25 ml of a 1% solution (by volume) of the emulsifier Tween 80 then diluted



with 75 ml of distilled water and stored at 4 degrees

C.

The extracts were tested for toxicity against 6-day old laboratory reared larvae of Culex pipiens and Aedes aegypti by placing 10 larvae into 50 ml of plant extract in distilled water solution. Each extract was tested over a range of concentrations consisting of 0, 1, 2, 5 or 10 percent (by volume) plant extract. A small amount of Tetramin was added to each of the three replicates for each treatment. Covers were placed over the containers (100 ml glass beakers) to minimize evaporation. Larval mortality was counted after 24 h.

#### Results and Discussion

The data collected from each of these experiments were analyzed in the following manner, for each test of extract/species:

- i) the mean percentage mortality occurring in the control containers (i.e. those containers having no plant extract) was subtracted from all the test results yielding a corrected mortality; ii) the corrected percentage mortality caused by the highest concentration (10%) was added to the corrected percentage mortality caused by the lowest

concentration; iii) the value produced by operation (ii) was then multiplied by a concentration correction factor which was calculated by dividing the total dry weight of each plant by 33.9 (the lowest dry weight value used, belonging to E. canadensis). This step was necessary because the total dry weights of the plants extracted were not equal; iv) an analysis of variance was performed on each set of data (a set consisting of the results of one extract type vs one mosquito species) and differences between means were tested for with the Student-Newman-Keuls procedure.

The results are summarized in Table V.

A number of differences between data sets were tested for with one-tailed t-tests with the following results:

- methanolic extracts caused higher mortality in C. pipiens than in A. aegypti ( $p < 0.05$ );
- pentane extracts caused about the same mortality between the two species;
- pentane extracts caused higher mortality in C. pipiens than did methanolic extracts;

Table V. Mean corrected percentage larval mortality ( $\pm$  1 standard deviation) caused by plant extracts. Concentration correction factors for each plant are also shown.

Solvent/Plant	Corrected Percentage* Mortality		Correction Factor
	<u>Aedes aegypti</u>	<u>Culex pipiens</u>	
<u>Methanol</u>			
<u>Callitriche palustris</u>	7.35 (0.12) bc	9.49 (0.40) d	3.1
<u>Ceratophyllum demersum</u>	3.53 (0.00) a	8.31 (0.39) cd	1.6
<u>Chara globularis</u>	3.03 (0.10) a	3.03 (0.10) a	10.1
<u>Elodea canadensis</u>	7.66 (1.65) bc	12.67 (1.00) e	1.0
<u>Lemna minor</u>	5.49 (0.23) ab	5.27 (0.24) b	4.0
<u>Nymphaea tuberosa</u>	4.41 (0.74) a	2.68 (1.71) a	3.1
<u>Ranunculus aquatilis</u>	8.35 (2.16) c	7.33 (0.79) c	1.2
<u>Utricularia minor</u>	3.49 (0.70) a	7.38 (0.00) c	1.1
<u>Pentane</u>			
<u>Callitriche palustris</u>	98.00 (3.46) d	75.00 (3.46) d	
<u>Chara globularis</u>	10.00 (0.00) a	11.67 (3.51) a	
<u>Elodea canadensis</u>	3.33 (5.77) a	0.00 (0.00) a	
<u>Lemna minor</u>	7.00 (5.00) a	2.33 (2.52) a	
<u>Nymphaea tuberosa</u>	32.33 (8.50) b	45.00 (0.00) b	
<u>Ranunculus aquatilis</u>	19.63 (19.63) c	100.00 (17.21) c	
<u>Utricularia minor</u>	6.00 (10.39) a	0.00 (0.00) a	

\*Means followed by the same letter (within cells) are not significantly different (Student-Newman-Keuls Procedure, p less than 0.05).

The concentrations of the plant extracts used in this study were very high. For example, the lowest concentration of plant material in a 10% test solution was 1.78 gram-equivalents or 35,700 ppm for U. minor solutions. All the other plant solutions were more concentrated and therefore it would be unrealistic to assume that any of the plant extracts in an unrefined state as they were in this study are potential "natural" insecticides. The study does indicate however, that some of the plants are potentially toxic and that under certain conditions sufficient quantities of certain compounds may be liberated into the environment by some plants to cause mortality of some organisms. This indication is supported by the fact that different extract types (pentane or methanol) caused different levels of mortality and that the two mosquito species responded differently. Similar results were obtained by Cabellero (1919) when investigating some effects of different species of Chara on mosquito larvae. Amonkar (1969) tested various species of algae for toxic effects on mosquito larvae and found that toxic metabolite production was not constant but that there were peaks in production. Experiments of my own (Angerilli 1973) have indicated that actively growing plants sometimes do and sometimes do not produce toxic effects on mosquito larvae.

Failure to detect highly toxic compounds in the present experiments may be because some or all of the plants were collected at a time when toxic compounds were not present or present only in small quantities.

This is not unreasonable as there is mounting evidence that many secondary plant compounds, which are here presumed to be responsible for larval mortality, are not present in a plant at all times (Seigler and Price 1976)..

It is also possible that the techniques used failed to extract toxic compounds or that the extraction processes destroyed such compounds. Further investigation by a natural products chemist is warranted here as well as a study of the decay products of such plants.

## B. INFLUENCES OF PLANT EXTRACTS ON MOSQUITO OVIPOSITION BEHAVIOUR.

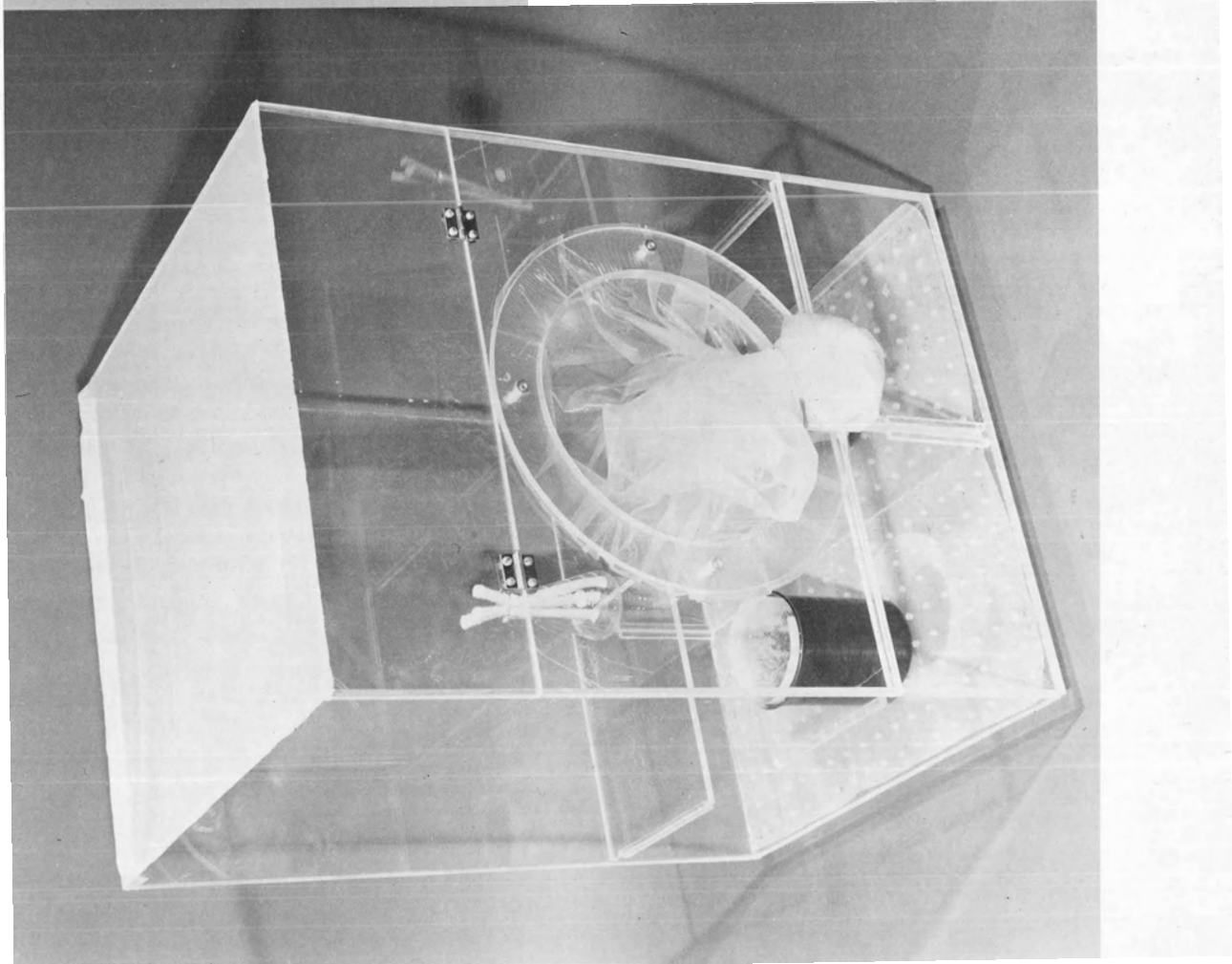
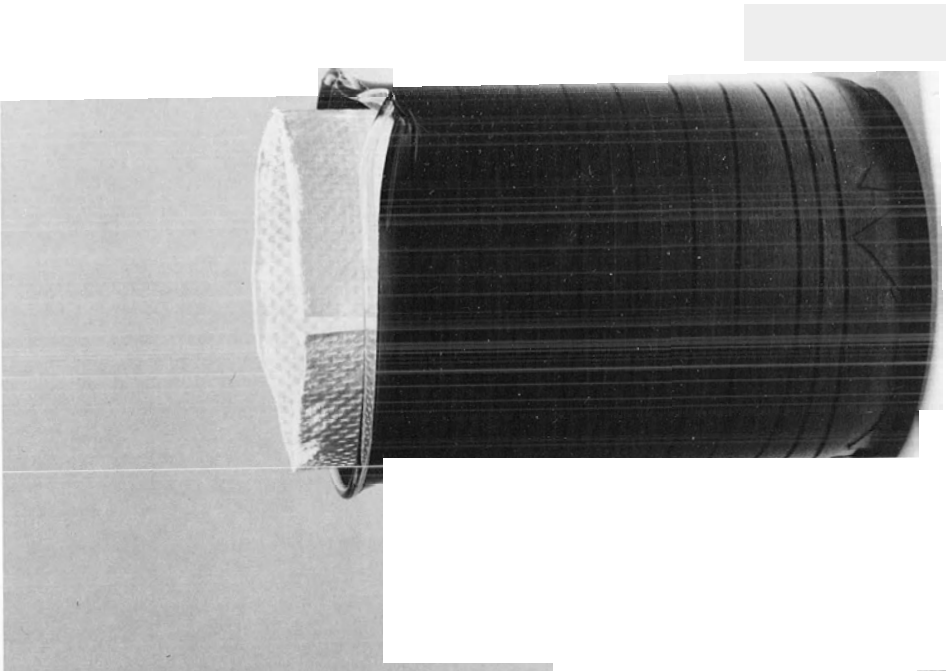
Insect behaviour is known to be influenced by plant-emitted substances (Gupta and Thorsteinson 1960; Fraenkel 1969; Yammamota et al. 1969). It is then, not unreasonable to suspect that some species of mosquitoes might use olfactory cues provided by aquatic vegetation to find or avoid potential oviposition sites. The objective of the following study was to measure the ability of methanolic extracts of a number of species of aquatic plants to influence mosquito oviposition behaviour.

### Materials and Methods

The methanolic plant extracts previously described were tested for their ability to attract or repel ovipositing A. aegypti in an olfactometer.

The olfactometer used in these experiments was a large box or "open arena" design. It did not use forced air (Figure 15). The box was constructed of plexiglas on all sides except the top which was mesh. The bottom was drilled with quarter inch holes on one inch centres and covered with mesh. The outside dimensions of the box were 46 by 46 by 61 cm with a

Figure 15. Olfactometer and beaker used as oviposition site.





volume of 0.13 cubic metres. The only obstructions inside were a 15 cm high vertical strip of plexiglas along the middle of the bottom extending from front to back (the divider) and also a 15 cm wide horizontal strip of plexiglas extending from side to side at the extreme rear of the box (the shelf) and about 15 cm from the bottom of the box. The box itself rested on cork rings with a 4 cm space between the bottom of the box and the bench top. All olfactometer experiments were done at room temperature and relative humidity, with a photoperiod of at least 16 h light, provided by a mixture of fluorescent tubes and natural light.

Twenty-five A. aegypti females, laboratory-reared from the same batch of eggs and between 7 and 10 days old were released into the olfactometer. A guinea pig, shaved and mechanically-restrained, was immediately provided as a blood meal source by placing it on the shelf of the olfactometer. After one to one and one-half hours, during which most of the mosquitoes had engorged, the guinea pig was removed and a 10% sucrose solution was made available. Four to five days later two 1000 ml pyrex beakers wrapped with black vinyl tape were provided as oviposition sites. Each beaker was situated in the middle of the area on each side of the divider and contained 150 ml of either distilled water or an aqueous solution of methanolic plant extract.

The extract concentration was equal to the LD50 of that extract for 6 day old A. aegypti larvae. A. aegypti oviposit on damp to moist substrates rather than directly on a water surface so the inside of each beaker was lined with a paper towel which acted as a wick. To insure similarity of wicks between sites one paper towel was cut in half, with one half going to each beaker. The beakers were removed after four to five days and the eggs laid on the towelling were counted. Each plant extract was tested three times and each time the position of the test beaker was switched with the control beaker to eliminate any biasing effects of the olfactometer.

In addition to the tests with methanolic plant extract, one series was run using water taken from an aquarium containing actively growing Chara globularis. The control site contained distilled water.

### Results and Discussion

The results of these experiments are given in Figure 16 which shows the percentage of the total number of eggs laid in the test and the control solutions. These data are also shown in Table VI which contains the raw data for each experiment. Table VI shows the results of a Chi-square analysis of the

Figure 16. Mean percent of total eggs laid in methanolic plant extract solutions vs mean percent of total eggs laid in distilled water.

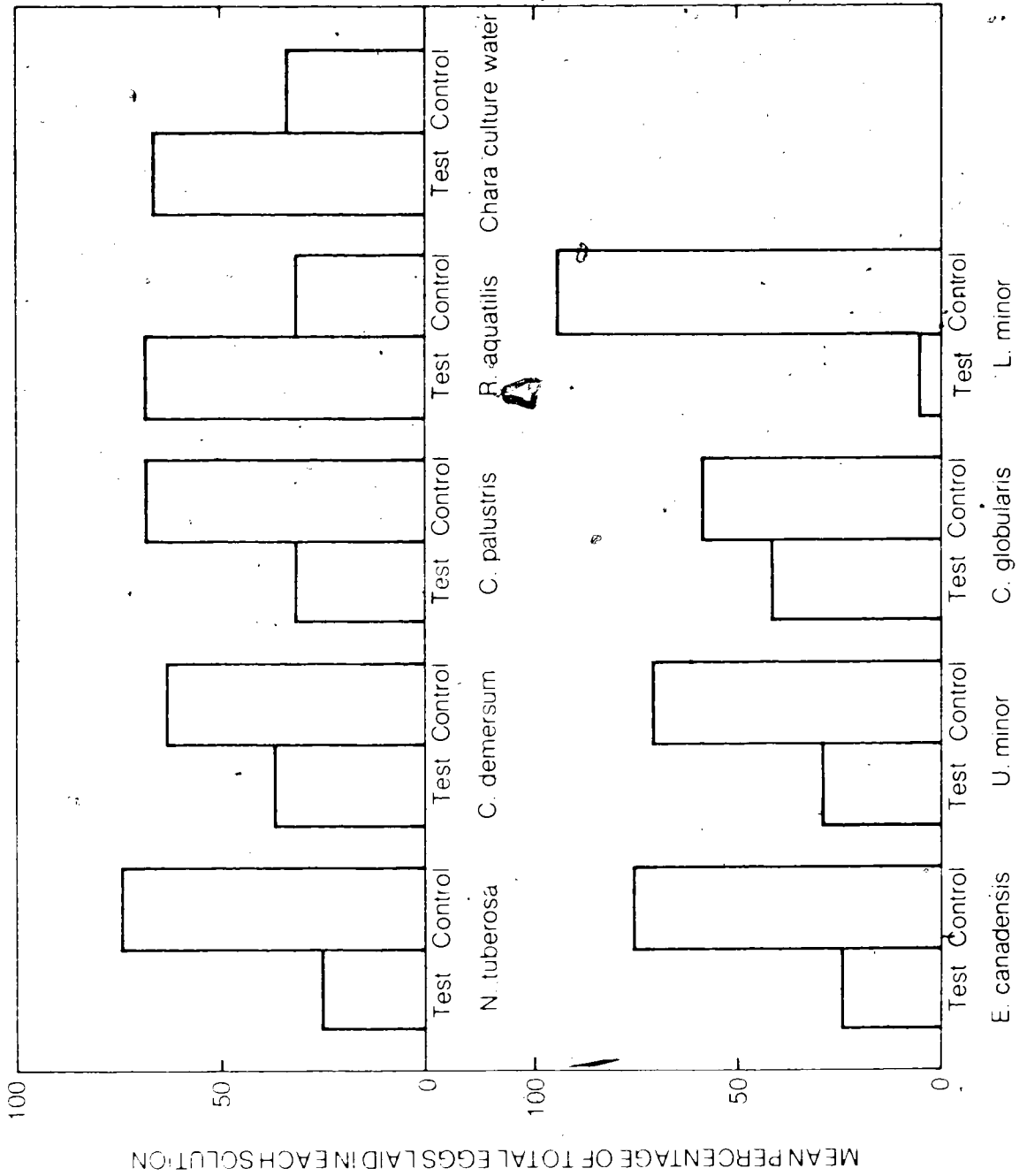


Table VI. Mean number of eggs laid and mean percentage of eggs laid in solutions of methanolic plant extract and in distilled water. Also shows results of Chi-square comparison.

Treatment	Results		Chi-square
	Mean number	Percentage	
<u>Elodea canadensis</u>	328	24.1	26.81**
distilled water	900.33	75.8	
<u>Ranunculus aquatilis</u>	901.66	68.8	14.21**
distilled water	441	31.1	
<u>Utricularia minor</u>	641.33	29.3	17.11**
distilled water	1073.67	70.7	
<u>Chara globularis</u>	528.67	41.1	3.13
distilled water	781.67	58.8	
<u>Lemna minor</u>	38.33	5.5	79.10**
distilled water	2317.67	94.5	
<u>Nymphaea tuberosa</u>	220	25.3	24.37**
distilled water	652	74.7	
<u>Ceratophyllum demersum</u>	352.67	36.5	7.29**
distilled water	461.67	63.5	
<u>Callitriche palustris</u>	351	31.4	13.88**
distilled water	636.67	68.6	
C. globularis culture water	1327.50	61.8	11.30**
distilled water	789.50	33.2	

\*\*Significant at p less than 0.01 if it is expected that there would be 50% of the total eggs laid in the distilled water and 50% in the test solution.

number of eggs laid in test versus control solutions. It was assumed that if the test solutions did not influence the site selection behaviour of the ovipositing female then each site should contain 50 percent of the total number of eggs laid during the test.

In tests using extract solutions of E. canadensis, U. minor, L. minor and N. tuberosa, C. demersum and C. palustris, a significantly larger proportion of the total number of eggs was laid in the distilled water of the control beaker. Therefore, relative to distilled water, these potentially toxic solutions were repellent. There is no evidence to suggest that distilled water is either attractive or repellent, so that it must be concluded that the test solutions were repellent and not merely lacking an oviposition stimulant. The results of this experiment do not indicate whether the repellent effect is produced by naturally occurring compounds or whether the effect is due to compounds produced by other organisms using the test solution as a growth medium as was found by Ikeshoji et al. (1975). In either case, the compounds are probably water soluble (because they were soluble in methanol) and may leach out of the growing plants. Alternatively, they leach only out of broken or damaged plants. The presence of these

compounds and associated organisms in some cases, may explain the absence of mosquito larvae from some bodies of water where these plants occur.

In tests using methanolic extracts of R. aquatilis and of C. globularis culture water, a significantly larger proportion of the total number of eggs laid was found in the beakers containing the test solutions. In these cases it is not possible to distinguish between oviposition attractant effects and oviposition stimulant effects. The sources of the responsible compounds may be the same as for the above repellent compounds. In the case of the C. globularis culture water, the attractive compound (or its precursor) must have leached out of the plant into the water. This finding is contrary to that of Matheson and Hinman (1929) who found that mosquitoes would not oviposit in water containing actively growing C. fragilis (= globularis).

There was no significant difference between the proportion of eggs laid in control beakers and the proportion laid in beakers containing solutions of C. globularis extract. There are a number of possible explanations for the different results obtained with methanolic extracts of C. globularis and with water in which it was growing:

- for phenological reasons the field-collected C. globularis (extracted with methanol) did not contain the oviposition attractive or stimulative compounds or their precursors;

- these compounds, if present in the field-collected C. globularis, were destroyed during the extraction process or the process failed to extract them;

- other organisms growing in the C. globularis culture tanks were responsible for the production of the oviposition attractive and/or stimulative compounds. One, some or all of these factors may have been involved in this experiment.



C. SOME INFLUENCES OF AQUATIC VEGETATION ON  
PREDATION ON MOSQUITO LARVAE.

It is often assumed that in situations where predators might influence larval mosquito populations that the presence of aquatic vegetation will impede the actions of predators. For example, Hazelrigg (1974) concluded that notonectids could be useful as predators in mosquito control programs but that field releases of the predator should be avoided if the habitats contain floating algae or debris and profuse emergent vegetation that obstructs most of the nectonic zone where prey and predator most often interact.

Although this may hold true for notonectids and some other predators, my own experience suggests it is not true for all potential predators of mosquito larvae. During field and laboratory studies I have observed that dytiscid beetle larvae, because of their poor swimming abilities, fail to capture mosquito larvae unless anchored to some sort of substrate which more often than not, was a plant. Dytiscid larvae were frequently seen to ambush mosquito larvae which were grazing on algae and debris on an aquatic plant. Hydra were found suspended below individual L. minor plants and also lining the stems and branches of C. palustris, and because Hydra are essentially

non-motile, plants have (in these cases) allowed them to exploit the full spatial dimensions of their habitat and thereby increase the probability of random encounters with prey.

Moreover, the results of the open artificial pond experiments discussed previously, suggested that predators are more abundant in certain plant-filled waters than in plantless waters.

The objectives of the following two studies were, then:

- to determine the incidence of aquatic vegetation as oviposition sites for potential predators of mosquito larvae; and

- to measure the effect of aquatic vegetation on the rate at which a general predator could find and consume mosquito larvae.

a. Aquatic Vegetation as Oviposition Sites of Predators

## Materials and Methods

During the late spring and summer of 1974, 1975 and 1976 various species of aquatic vegetation were collected from the field and examined in the laboratory to see if the eggs of potential predators of mosquito larvae were present. The surveys of 1974 and 1975 gave preliminary results that led to the survey of 1976 which was done in the following manner.

Approximately every two weeks, 6 litres of each of ten species of aquatic vegetation were collected from a number of sites in the Fraser Valley. Because of limited facilities, not every species was collected on each occasion. The plants were transferred into glass beakers containing 1800 ml of dechlorinated tap water in the laboratory, and examined immediately for the presence of clinging organisms. The plants were examined at irregular intervals during the following two weeks to see if predators of mosquito larvae emerged.

## Results and Discussion

During the preliminary surveys of 1974 and 1975 of aquatic plants as oviposition sites for mosquito predators, Dytiscidae and Notonectidae had emerged from

Nymphaea tuberosa and Utricularia minor.

In the 1976 survey (Table VII), out of 10 plant species collected three had 2 families of predacious insects emerge from them, 3 other species of plants had 1 family of predators, and 4 species did not produce any organisms known to be predacious. The four families of predators found were Zygoptera and Anisoptera (which are sub-orders of the Odonata), Dytiscidae (Coleoptera) and Notonectidae (Hemiptera).

On numerous occasions adult Zygoptera and Anisoptera were observed ovipositing on or into aquatic vegetation. Zygoptera normally oviposit into slits cut by the ovipositor in the stems and leaves of aquatic or semi-aquatic plants. Their eggs are therefore endophytic. The Anisoptera lay their eggs freely in the water or attach them to aquatic plants. Their eggs are therefore exophytic. Dytiscid oviposition is usually endophytic but notonectid oviposition can be either exophytic or endophytic. In all four families there is then, a certain dependance on aquatic vegetation. In fact, a great number of aquatic insect families known to be predators of mosquito larvae and newly emerged ovipositing adults lay their eggs on or in aquatic and semi-aquatic vegetation (Table 7, App. B). Insects whose eggs are endophytic are probably .

Table VII. Aquatic plants from which predators of mosquito larvae emerged.

Plant	Collection Site			
	Ft. Langley	Burnaby 1	Pitt Meadows	Sheridan Hill 2 Richmond 2
collected 27/5/76				
<u>Callitriche palustris</u>	-	Dytiscidae	-	Dytiscidae
<u>Nymphaea tuberosa</u>	-	-	Dytiscidae	-
<u>Utricularia minor</u>	-	Dytiscidae	-	-
collected 10/7/76				
<u>C. palustris</u>	-	-	-	Zygoptera
<u>Ceratophyllum demersum</u>	-	Anisoptera	-	-
<u>Elodea canadensis</u>	Dytiscidae	-	-	-
<u>Potamogetan foliosus</u>	Notonectidae	-	-	Notonectidae
collected 26/7/76				
<u>E. canadensis</u>	Zygoptera	-	-	-
<u>P. foliosus</u>	-	-	-	Zygoptera

more selective in terms of the plant species used than insects whose eggs are exophytic.

## b. Effects of Vegetation on Predation Rate

## Methods and Materials

Three sub-adult male and two sub-adult female fathead minnows (Pimephales promelas Rafinesque) were used as predators. They were obtained from a commercial supplier and then maintained in laboratory aquaria under a 16 h light/8 h dark lighting schedule. When not participating in feeding experiments they were fed unlimited tetramin daily.

A randomly selected mixture of laboratory-reared second and third instar Aedes aegypti L. were used as prey.

All experiments were carried out in 19 l glass aquaria (dimensions: 20 by 26 by 41 cm) filled to the brim with water that was filtered and aerated continuously. There was a thin layer of washed white sand on the bottom of each tank. There was one fish in each tank and all fish were visually isolated from each other.

Four series of experiments were carried out in this study, each series tested a different shape or type of aquatic vegetation for its effects on predation

rate. The four types tested were: (see Figure 17)

- 1) large leaf submerged vegetation:
- 2) evenly dispersed reeds:
- 3) small leaf submerged vegetation:
- 4) clumped reeds.

The tests were conducted by placing vegetation into two randomly selected aquaria at 0800 hrs of the first day of testing for that form. The fish were starved for two days before the first day of testing. At 1400 hrs testing was started by presenting 30 A. aegypti larvae to each fish and then measuring either the time required by each fish to consume all the larvae or counting the number of larvae remaining after ten minutes. Predation rate was calculated as elapsed time divided by the number of larvae consumed. Each vegetation shape was tested for 5 consecutive days.

#### Results and Discussion

Table VIII shows the results of the series of experiments by indicating the predation rate for each fish on each day of testing.



Figure 17. The four plant shapes used in predation rate investigations.

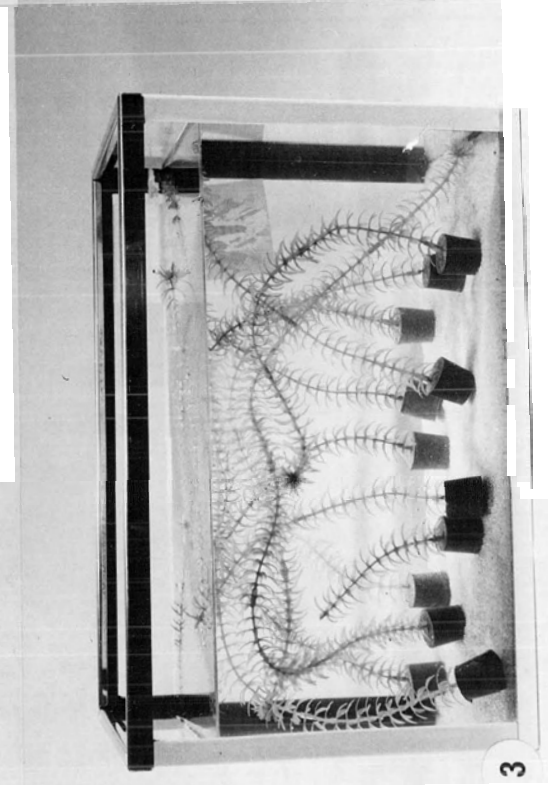
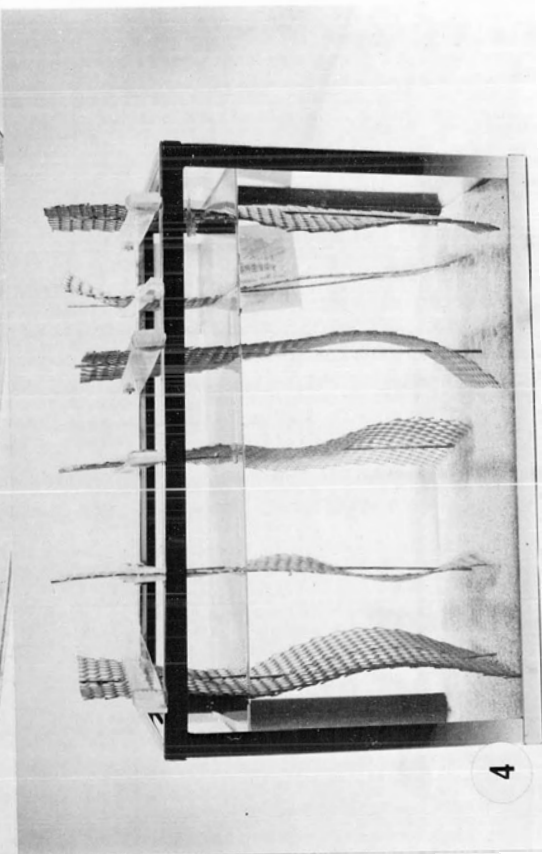


Table VIII. Rate (time/larva in minutes) at which Fathead Minnows captured and consumed mosquito larvae with aquatic vegetation present or absent.

Fish	Day					Mean ( $\pm 1$ s.d.)
	1	2	3	4	5	
<b>Large Leaf Submerged Vegetation</b>						
1	0.11	0.22	0.17	0.25	0.36	0.22 (0.09)
2*	0.33	0.21	0.24	0.36	0.20	0.27 (0.07)
3*	0.28	0.21	0.14	0.10	0.40	0.23 (0.12)
4	0.26	0.31	0.11	0.12	0.09	0.18 (0.10)
5	0.22	0.33	0.18	0.37	0.29	0.28 (0.08)
mean $\pm$	0.24	0.26	0.17	0.24	0.27	
s.d.	0.08	0.06	0.05	0.13	0.12	
<b>Small Leaf Submerged Vegetation</b>						
1*	0.30	0.13	0.24	0.33	0.33	0.27 (0.08)
2	0.21	0.26	0.28	0.12	0.21	0.21 (0.06)
3	0.29	0.34	0.26	0.33	0.36	0.31 (0.04)
4*	0.17	0.21	0.23	0.33	0.15	0.22 (0.07)
5	0.36	0.26	0.36	0.20	0.28	0.29 (0.07)
mean $\pm$	0.27	0.24	0.27	0.26	0.27	
s.d.	0.08	0.08	0.05	0.10	0.09	
<b>Evenly Dispersed Reeds</b>						
1*	0.11	0.15	0.14	0.25	0.12	0.15 (0.06)
2	0.33	0.24	0.21	0.20	0.32	0.26 (0.06)
3*	0.12	0.17	0.29	0.36	0.33	0.25 (0.10)
4	0.22	0.16	0.17	0.19	0.28	0.20 (0.05)
5	0.23	0.34	0.36	0.29	0.18	0.25 (0.07)
mean $\pm$	0.20	0.21	0.23	0.26	0.25	
s.d.	0.09	0.08	0.09	0.07	0.09	
<b>Clumped Reeds</b>						
1*	0.26	0.26	0.20	0.20	0.20	0.22 (0.03)
2	0.21	0.34	0.42	0.36	0.36	0.34 (0.08)
3*	0.09	0.32	0.20	0.19	0.21	0.20 (0.08)
4	0.36	0.18	0.11	0.14	0.16	0.19 (0.10)
5	0.34	0.24	0.18	0.19	0.22	0.23 (0.06)
mean $\pm$	0.25	0.27	0.22	0.22	0.23	
s.d.	0.11	0.06	0.12	0.08	0.08	

\*Vegetation present in tank containing these fish.

The data were analyzed for differences in predation rates between fish and between days (but within treatments) by using a two-way analysis of variance without replication. There were no significant added variance components ( $p < 0.05$ ), that is, none of the vegetation shapes facilitated or impeded the rate at which the fish consumed mosquito larvae.

It was assumed in this experiment that the presence of plant shapes would affect predation rate by impeding the movement of the predator or impeding the accessibility of the prey. The results of this experiment do not disprove either of these two assumptions directly. They do, however, suggest that other factors may have been involved. For example, it is possible that while one or both of the above assumptions were in effect, the plants assisted the predator by making the prey more visible. That is, the light coloured prey were more visible against the darker coloured plant background, so that prey in plant-filled tanks were easier to find than prey in plantless tanks. This visibility factor could then decrease the amount of time required by the predator to find and consume prey, relative to the time required by a predator in a plantless tank to perform the same tasks.

Observations on the fish during the feeding experiments indicated that the presence of plant shapes caused the fish to perform a more systematic search of the tank. More new space appeared to be searched per unit time in plant-filled tanks than in plantless tanks where prey-searching movement appeared to be random and predators frequently moved through the same space back and forth without searching new space. As the prey were distributed randomly throughout the space, systematic searching may have led to more frequent predator encounters with prey because new or previously unsearched space was being explored.

The fathead minnow is a robust fish that reaches a maximum length of 90-100 mm and has a distribution that includes southern Canada, the entire United States and northern Mexico (McMillan and Smith 1974). In previous laboratory studies (Dixon and Brust 1970) the fathead minnow showed considerable potential as a predator of mosquito larvae. This study suggests that the fathead minnow could be used as a mosquito control agent in breeding sites that contained some kinds of vegetation without a loss of efficiency. Furthermore, because of its climatic and environmental tolerances (McMillan and Smith 1974) the fish should be useful as a general purpose bio-control agent for mosquitoes in permanent ponds in most of southern Canada.

## CONCLUDING DISCUSSION

This study, like most investigations in science, leaves many questions unanswered, and has, in fact, probably raised more questions than it has answered. This is particularly true as the work was a very broad overview of plant/mosquito relationships with no emphasis on detail in any one area. The reason for this is twofold: one, over the last 80-90 years there has been considerable interest in plant/mosquito interactions, but never a comprehensive study of this sort, in which I have attempted to integrate a number of different facets of these interactions; and two, my outlook on ecology is such that I believe only "big picture" approaches will advance our understanding of many ecological phenomena such as the one I have studied here.

A number of questions arise from this investigation. Although mosquito larvae were never abundant in the kinds of ponds surveyed in this study, I was not able to develop any good explanation for why this was the case. The low numbers of mosquito larvae present could not be explained by the presence of predators or by the physical factors measured. However, I believe there is convincing evidence to support the hypothesis that the presence of certain

plants in the water affects the oviposition behaviour of adult female mosquitoes either directly or indirectly, and that this accounts for the absence or rarity of mosquito larvae in many situations.

Precisely how the plants do this is an area that deserves to be explored, and will hopefully yield some interesting and useful results. For example, there appears to be considerable potential here for the development of either oviposition repellents or attractants as alternatives to using pesticides for the control of mosquitoes.

Although there is still no strong evidence to suggest that insect predator populations are important in controlling mosquito populations, predator impact on mosquito population dynamics should not be discounted. Considering the apparent affect of plants on predator populations and their behaviour as predators, there should be further research into the exact nature of these interactions. This could be particularly important in two different kinds of situations: In some cases it might be possible to enhance the habitat of predators through selective management of aquatic vegetation; In other cases, aquatic weed control may lead to the appearance or enlargement of mosquito populations because of the destruction of predator habitat and/or the removal of plants that are repellent

to mosquitoes.

This study has shown that aquatic plants can cause the death of mosquito larvae, prevent oviposition by adult mosquitoes, encourage colonization by predators, and facilitate predator activities.. It remains to be shown if and how these interactions can be used to control mosquito populations by managing aquatic plant populations.



## SUMMARY OF MAIN CONCLUSIONS

1) The species composition of aquatic plants can be a useful indicator of the species of mosquitoes that may potentially breed in an aquatic habitat and could therefore serve as an "early warning" system in mosquito control programmes.

2) Aquatic plants influence the oviposition site selection behaviour of ~~mosquitoes~~ mosquitoes and their predators. :

3) Aquatic plants do not normally impair and sometimes facilitate the ability of mosquito predators to capture mosquito larvae.

4) Aquatic weed control could lead to the appearance or enlargement of mosquito populations because of the interactions between plants and insects mentioned above.

## Appendix A

## Mosquito Rearing Techniques

Aedes aegypti L.

Eggs of Aedes aegypti were obtained from a colony maintained by the Department of Biological Sciences, Simon Fraser University. When larvae were required, the eggs were placed into a 20 x 6.5 cm glass preparation dish containing distilled water, 1.0 g of finely ground Tetramin and 0.1 g of finely ground baker's yeast. The culture was then maintained at room temperature until larvae of the required age were available.

Culex pipiens

Larvae of Culex pipiens were obtained from a breeding site in Richmond, British Columbia in July, 1974 and then maintained in the laboratory until adults emerged. These adults were then used to develop a laboratory colony from which egg rafts could be obtained when required. The colony was maintained in a cage (42 x 87 x 45 cm) at room temperature, relative humidity and in a light/dark cycle of 16 h/8 h. Larvae were contained in a water-filled preparation dish (20 x

6.5 cm) to which distilled-water was added periodically. The larvae were fed 1.0 g of finely-ground Tetramin and 0.1 g of finely-ground baker's yeast twice per week. A mechanically - restrained, shaved guinea pig was placed in the cage over-night once per week as a blood meal source for the adult females. A 125 ml erlenmeyer flask containing a 10% sucrose solution and three dental cotton wicks was available continuously in the cage as an energy source for adult female and male mosquitoes. When larvae were required, egg rafts were removed from the surface of the rearing container and placed into identical, but previously unused containers and left until larvae of the appropriate age were available.

Appendix B. Supplementary Data.

Table 1. Species and relative abundance of aquatic vegetation in the nine field sites.

Location	Plant Species	Abundance *
Ft. Langley	<u>Elodea canadensis</u> Rich. in Michx.	1a
	<u>Potamogetan foliosus</u> Raf.	1a
	<u>Nuphar polysephalum</u> Engelm.	3
Burnaby 1	<u>Nymphaea tuberosa</u> Paine.	1
	<u>Potamogetan natans</u> L.	2
	<u>Utricularia minor</u> L.	2
	<u>Ceratophyllum demersum</u> L.	2
	<u>Callitriche palustris</u> L.	3
	<u>Lemna minor</u> L.	4
Burnaby 2	<u>Nymphaea tuberosa</u>	1
	<u>Utricularia minor</u>	2
	<u>Lemna minor</u>	3
	<u>Potamogetan foliosus</u>	4
Pitt Meadows	<u>Elodea canadensis</u>	1
	<u>Nymphaea tuberosa</u>	2
	<u>Nuphar polysephalum</u>	3
	<u>Potamogetan natans</u>	3
	<u>Ceratophyllum demersum</u>	3
Sheridan Hill 1	<u>Potamogetan foliosus</u>	1
	<u>Myriophyllum sp.</u>	2
	<u>Utricularia minor</u>	2
	<u>Elodea canadensis</u>	2
	<u>Ranunculus aquatilis</u> L.	3
	<u>Callitriche palustris</u>	3
Sheridan Hill 2	<u>Potamogetan foliosus</u>	1
	<u>Nitella sp.</u>	2
	<u>Lemna minor</u>	3
	<u>Callitriche palustris</u>	3
	<u>Ranunculus aquatilis</u>	3
	<u>Myriophyllum sp.</u>	4

(cont'd)

Table 1. Species and relative abundance of aquatic vegetation in the nine field sites. (continued)

Location	Plant Species	Abundance
Granville 1	<u>Lemna minor</u>	1
	<u>Elodea canadensis</u>	3
	<u>Callitriche palustris</u>	3
	<u>Potamogetan foliosus</u>	3
Granville 2	<u>Lemna minor</u>	2
	<u>Callitriche palustris</u>	2
	<u>Elodea canadensis</u>	2
	<u>Potamogetan foliosus</u>	3
Osoyoos	<u>Chara globularis</u> Desv. (an alga)	1

\* Relative abundance was scored as follows:

1. dominant
2. abundant
3. present
4. scarce.

Table 2. The mean number and the number of each species of mosquito larvae found in each of the nine field sites.

Site	Total number of <u>Anopheles punctipennis</u>	Total number of <u>Culex tarsalis</u>	Total number of <u>Culex territans</u>	Mean*
Ft. Langley	78	-	15	1.86a
Burnaby 1	-	1	-	0.12a
Burnaby 2	-	-	4	0.09a
Pitt Meadows	-	15	-	0.30a
Sheridan Hill 1	59	-	-	1.18a
Sheridan Hill 2	173	-	-	3.46b
Richmond 1	-	-	-	0.00a
Richmond 2	-	-	-	0.00a
Osoyoos	-	-	-	0.00a

\*Means followed by the same letter are not significantly different (Student-Newman-Keuls Procedure, p less than 0.05).

5





Table 4. Mean number of predators found in each of the nine field sites per sampling day.

Site	Mean $\pm$ standard error*	
Ft. Langley	7.32	0.87 c
Burnaby 1	6.14	0.84 c
Burnaby 2	2.13	0.45 abc
Pitt Meadows	4.90	0.60 abc
Sheridan Hill 1	4.22	0.60 abc
Sheridan Hill 2	5.20	0.54 bc
Richmond 1	5.24	1.06 abc
Richmond 2	1.20	0.27 ab
Osoyoos	0.35	0.13 a

\*Means followed by the same letter are not significantly different (Student-Newman-Keuls Procedure,  $p$  less than 0.05).

Table 5. The species of mosquito larvae present in each of the coverless artificial ponds at the time of sampling.

Treatment	Week										
	1	2	3	4	5	7	9	11			
Control 1	Ca	Ca	Ca, Cx	Cx, Ca	Cx, Ca	Cx, Ca	Cx, Ca	Cx	Ca		
2	Ca	Ca	Ca, Cx	Ca, Cx	Ca, Cx	Ca, Cx	Ca, Cx	Ca, Cx	Ca, Cx		
3	Ca	Ca, Cx	Ca, Cx	Ca, Cx	Ca, Cx	Ca, Cx	Ca	Ca	Ca		
4	Ca	Ca	Ca, Cx	Cx, Ca	Ca, Cx	Ca, Cx	Ca	Ca	-		
<u>Utricularia minor</u>											
1	Ca	Ca	Ca	-	-	-	-	-	-		
2	Ca	Ca	-	-	-	-	-	-	-		
3	Ca	Ca	Ca, Cx	Cx, Ca	Cx, Ca	Ca	-	-	-		
4	Ca	Ca, Cx	Ca, Cx	Cx	Ca, Cx	-	-	-	-		
<u>Elodea canadensis</u>											
1	-	Ca	-	nf	nf	Cx, Ca	Ca, Cx	Ca, Cx	Ca, Cx		
2	-	Ca, An	Ca	Ca, Cx	Ca	-	-	-	-		
3	Ca	Ca	Ca, Cx	Cx, Ca	Cx	Cx	-	-	-		
4	-	Ca	Ca, Cx	Ca, Cx	-	-	-	-	-		
<u>Lemna minor</u>											
1	-	-	-	-	Ca, Cx	Cx	Cx, Ca	Ca	Ca		
2	-	-	-	-	-	-	-	-	-		
3	-	-	-	-	-	-	-	-	-		
4	-	-	-	-	Ca	-	-	-	-		

Ca - Culiseta inornata

Cx - Culex pipiens

An - Anopheles punctipennis

- relative abundance is indicated by order of occurrence

nf - pond inoperative

Table 6. The families of predators present in each of the coverless artificial ponds at the time of sampling.

Treatment	Week										
	1	2	3	4	5	7	9	11			
Control 1	-	Da	-	-	Da	Da	-	-	Da	-	N, Da
2	-	-	-	Dl	Da	-	-	-	-	-	N
3	-	-	Da	Da	Da	-	-	-	-	-	Dl, N
4	-	N, Da, G	G	-	-	Da	Da	Da	-	Da	N, Da
<u>Utricularia minor</u>											
1	N	N, Dl, Da, G	Z, N	Z, N, G,	N, Z	N, Da	Z	Z, N			Z, N
2	N, Da	Dl, N	N, Da	N, Z	N, Z, Da	N, Z	Z, N, Da	Z, N, Da			Z, N, Da
3	-	Dl, Da	Dl	Dl, Da	-	-	-	-			N, Da
4	N, Da	N, Da	N, Da, G	N, Da	N, Z, A	N	N, Da	N, Da			nf
<u>Elodea canadensis</u>											
1	-	N, G	N, Da	nf	nf	Da, Z	-	Da			Da
2	Da	n, Da	N	Dl, N, G	Dl, Z, N	Z, N, Da, G	N, Da	N, Da			N, Da
3	-	-	Dl, N, Da	N, Dl	N, Dl	N	N	N, Da			N, Da
4	-	Da	Da, Dl	N, Da, Dl, G	Dl, G, Z	N, Da, G, Z	N, G	N, Da			N, Da
<u>Lemna minor</u>											
1	-	-	Dl	-	-	Da	-	-			-
2	-	-	-	-	Dl	Dl	Dl	-			-
3	Dl	Dl	Dl, Da	Dl, Da	Dl, Da	-	Da	-			-
4	-	-	Da	-	-	-	-	-			-

-relative abundance is indicated by order of occurrence

N - Notonectidae  
 Da - Dytiscidae (adult)  
 Dl - Dytiscidae (larva)  
 G - Gerridae  
 Z - Zygoptera  
 A - Anisoptera  
 nf - pond inoperative

Table 7. Predaceous aquatic insects recorded in the literature as using aquatic vegetation for oviposition sites.

Predator (Order:Family)	Egg Location (exophytic or endophytic)
Coleoptera:	
Dytiscidae*	both
Gyrinidae	exophytic
Noteridae	exophytic
Hemiptera:	
Belostomatidae*	exophytic
Corixidae	exophytic
Gerridae	exophytic
Hebridae	exophytic
Hydrometridae	exophytic
Mesoveliidae	endophytic
Nepidae*	endophytic
Notonectidae*	both
Odonata:	
sub-order: Anisoptera*	exophytic
sub-order: Zygoptera*	endophytic

\*these organisms were found in at least one of the field sites surveyed.

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\* references followed by RAEP were seen only in abstract form in the Review of Applied Entomology, Series B.

## CURRICULUM VITAE

ANGERILLI, Nello P.D.

## SPECIAL INTERESTS

Medical and veterinary entomology, pest management, and freshwater ecology.

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## PERSONAL

Born 15 May 1952: Trail, British Columbia, Canada.  
Age: 25; 6'2": 170 pounds; married (no children); health excellent.

## EDUCATION

Glenmerry Elementary School, Trail, B.C. 1958 to 1960  
J.L. Webster Elementary School, Trail, B.C. 1960 to 1965  
Trail Junior Secondary School, Trail, B.C. 1965 to 1967  
J.L. Crowe Senior Secondary School, Trail, B.C. 1967 to 1970

## Awards

Award for Outstanding Musicianship 1967  
Award for Outstanding Musicianship 1969

University

B.Sc. (Biological Sciences) from Simon Fraser University. 1973  
Cumulative Grade Point Average: 3.89 (4 point scale) in Biological Sciences.

## Awards

Government of British Columbia Scholastic Award 1972  
Government of British Columbia Scholastic Award 1973,  
Simon Fraser University Open Scholarship 1973

## Curriculum Vitae

ANGERILLI, Nello P.D.

## EDUCATION (cont'd)

Ph.D. Graduate Student, Department of Biological Sciences, Simon Fraser University. Expected completion date, August 1977.

## Awards

Simon Fraser University Graduate Scholarship, tenable for one year (1975-76) but awarded for a second year (1976-77) and awarded to students who have demonstrated outstanding scholastic achievement and who show further high potential in their studies.

## Committee Membership

Faculty of Science Undergraduate Curriculum Studies Committee. Student Representative. 1971-72

Senate Undergraduate Curriculum Studies Committee. Student Representative. 1972

Chairman, Departmental Summer Employment Programme Committee. Committee of 2 students and 2 faculty whose duties were to evaluate faculty grant applications (for student employment) and disburse funds to selected applicants. 1976

Departmental Undergraduate Curriculum Studies Committee. Graduate Student Representative. 1975-77

## SPECIAL COURSES

"Mosquitoes, their biology, control and sensory organ morphology."  
Held at the University of Alberta, Edmonton, Alberta. A regional short course at the advanced graduate level for selected graduate students who are registered at any one of the Western Universities. Included a practical course on the use of the Scanning Electron Microscope as a tool for research on mosquitoes and other biting flies.

## Curriculum Vitae

ANGERILLI, Nello P.D.

## WORK EXPERIENCE

Teaching Assistant, Department of Biological Sciences, Simon Fraser University in the following courses:

- 1) Introduction to Biology (Bisc. 102) 1974 and 1975
- 2) Plant Ecology (Bisc. 404) 1974
- 3) Insect Biology (Bisc. 317) 1975
- 4) Histochemical Techniques (Bisc. 448) 1976

Research Assistant (undergraduate), Department of Biological Sciences, Simon Fraser University. Duties included laboratory and field work involving locating and testing freshwater aquatic plants for possible effects on mosquito larvae. Development of testing methods; plant extraction and bioassay for juvenile hormone-like compounds; and analysis of water for various chemical (inorganic) and physical properties. 1972 to 1973

Vacuum operator and maintenance worker. Zinc Roasters, Cominco Ltd. Trail, B.C. Summer 1972

Mechanic (light duty) and gas station attendant. Banff, Alberta. Summer 1970

Musician (semi-professional) touring East and West Kootenay Districts of British Columbia. 1968 to 1970

## SOCIETY MEMBERSHIPS

Canadian Society of Zoologists  
 Entomological Society of Canada  
 Entomological Society of British Columbia  
 Canadian Nature Federation

## PUBLICATIONS OR ADDRESSES TO SCIENTIFIC MEETINGS

Entomological Society of British Columbia. Vancouver, B.C. "Influence of some freshwater plants on the development and survival of mosquito larvae." Spring 1973

Entomological Society of Canada and the Entomological Society of Alberta. Joint Meeting, Banff, Alberta. "Influences of some freshwater plants on mosquito larvae in British Columbia." Fall 1973

## Curriculum Vitae

ANGERILLI, Nello P.D.

## PUBLICATIONS OR ADDRESSES TO SCIENTIFIC MEETINGS (cont'd)

Northwest Mosquito and Vector Control Association. Fourteenth Annual Meeting, Vancouver, British Columbia. "Biological Control and Mosquito Populations." Fall 1974

Fifteenth International Congress of Entomology. Washington, D.C. "Some effects of aquatic vegetation on mosquito population ecology." Summer 1976

Angerilli, Nello P.D.C. and Bryan P. Beirne. 1974. Influences of some freshwater plants on the development and survival of mosquito larvae in British Columbia. Canadian Journal of Zoology 52:813-815.

## OTHER INTERESTS

Music, downhill and cross-country skiing, hiking, photography, sailing, home-brewing and wine-making.

## REFERENCES

Dr. Bryan P. Beirne, Professor of Pest Management. Director of the Pestology Centre. Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada.

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