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THE AUTECOLOGY AND REPRODUCTIVE BIOLOGY
OF MARSILEA VESTITA HOOK. ET. GREV.

by
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B.S., Michigan State University, 1972

Presented in partial fulfillment of the requirements
for the degree of Master of Arts in Botany

THE UNIVERSITY OF MONTANA

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ABSTRACT


The Autecology and Reproductive Biology of Marsilea vestita Hook. et. Grev. (125 pp.)

Director: David E. Bilderback D.E.B.

The optimal conditions for the vegetative and reproductive growth of Marsilea vestita Hook. et. Grev. were correlated with soil moisture content. The heterophylllic and heteroblastic leaf conditions of Marsilea were observed in the field. The lobing of land form leaves was also observed. Germination of completely matured, black sporocarps occurred by the thousands only in June along the shore of Nine-Pipe Reservoir. The viability of sporocarps is not correlated to the environmental conditions in which they developed. The viability of maturing, brown sporocarps was significantly lower than the completely matured sporocarps due to the inability of the sorophore to function properly. A heat treatment of maturing sporocarps enabled the sorophore to work correctly. Freezing the maturing sporocarps before a heat treatment inhibited the sorophore from exuding from the sporocarp. A pH 4.2 affects the viability of the sperm, while a pH 5.8 is optimal for sperm viability. The sporocarp wall of maturing sporocarps is not impervious to the external environment. The maturation process of sporocarps involves a wet condition for initiation, a dry period to enable the sorophore to function properly, and a length of time for sufficient deposition of materials in the sporocarp wall to insure imperviousness to the external environment.
ACKNOWLEDGEMENTS

I wish to thank Dr. James R. Habeck and Dr. John F. Tibbs for serving on my committee and Dr. David E. Bilderback, my committee chairman, for his continuing inspiration, help, and advice.

I also wish to thank Mary Ellen Clayton for typing my thesis and to all of my colleagues on the third floor of the Botany Building for their academic and personal friendships.
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CHAPTER I

INTRODUCTION

The genus Marsilea, an aquatic pteridophyte, has been invaluable as an experimental plant for the study of the morphogenesis of vegetative structures due to their heterophyllic and heteroblastic characteristics (Allsopp, 1951, 1953, 1953a, 1954, 1956, 1959, 1960, 1962, 1963, 1969; Gaudet, 1963, 1964, 1964a, 1965, 1969; Laetsch and Briggs, 1961, 1963; Laetsch, 1962; White, 1966, 1968, 1971). However, little attention has been paid to the ecology of this genus (Bhardwaja, 1955; Gopal, 1970, 1971; Jalan, 1958; Sharma, 1957) and more specifically, even less is known about the reproductive strategy of Marsilea. The complete sexual life cycle of Marsilea has never been studied previously in nature.

Objectives

The first objective of this study was to collect systematic field data on the growth and reproductive conditions of Marsilea vestita Hook. et. Grev. The second objective was to collect qualitative field data on all stages in the sexual life cycle of M. vestita. Thirdly, using laboratory studies, the viability of Marsilea's
reproductive structure, the sporocarp was investigated in relation to the growth conditions of the plant. The fourth and final objective was to conduct laboratory studies investigating factors responsible for the maturation and germination of the sporocarp. Previously, only three studies concerning the maturation of the sporocarp have been reported (Bhardwaja and Sen, 1966; Bloom, 1955, 1961).

Location of Study Area

To accomplish these four objectives, a population of *M. vestita* growing along the shore of a reservoir located on Nine-Pipe National Wildlife Refuge, Charlo, Montana, T.20.N, R.20.W, Sections 27 and 34, longitude 114°07', and latitude 47°27' was selected for this study. The reservoir is located in the Flathead Valley of northwestern Montana. The Mission Mountain Range, rising ten miles to the East, is the main source of water for the reservoir. To the West are low rolling hills (See Figure 1). The elevation of the man-made reservoir is 917.4 m above sea level and has a holding capacity of 18,687.22 km³ (See Figure 2). The water of the reservoir serves a dual purpose, that of irrigating the farmland in the valley and as a nesting area for migrating waterfowl. The water level is regulated accordingly by the refuge management for these purposes.
Figure 1. Location of Nine-Pipe Reservoir in Montana
Figure 2. Location of the study areas at the reservoir.

Figure 3. Detailed map of the study areas.
Study areas

Highway 212

Nine-Pipe Reservoir

Pond study area

Reservoir study area

Adjacent pond study area

Adjacent reservoir study area

Area of large accumulation of Phase 3 sporocarps

Culvert
Flathead valley is a relatively dry intermountain valley with relatively high summer temperatures and low winter temperatures. Climatic data for the valley, recorded at Polson, Montana, approximately sixteen miles north of the reservoir, are summarized in Appendix A.

To obtain the first objective, two study areas were chosen for sampling quantitative data on the reproductive and vegetative characteristics of Marsilea. One study area, designated as the pond study area, is located on the east-facing slope of a pond in the northwest corner of the reservoir, separated from the main reservoir by Highway 212 and connected to the main reservoir by a culvert (See Figures 2 and 3). The other study area, designated as the reservoir study area, is located on a South-facing slope, directly across Highway 212 from the pond study area, also in the northwestern part of the reservoir (See Figures 2 and 3).

Qualitative data was also taken in areas adjacent to the two study areas. These areas are indicated in Figure 3 and were designated as adjacent pond and reservoir study areas.
CHAPTER II

METHODS AND MATERIALS

In order to investigate the autecology of *Marsilea vestita*, a system of transects was imposed on a population growing along the reservoir in the study areas. Two transect lines were constructed perpendicular to the shore line in the pond study area and one transect line was constructed perpendicular to the shore line in the reservoir study area. Two reference stakes were placed for each transect line upslope from the water to allow for re-location of the transect lines throughout the sampling season. The two reference stakes for Transect 1 are located 21.64 m north from the fence line which runs parallel to Highway 212, on the west shore of the pond. The two reference stakes for Transect 2 are located 51.21 m north from the same fence line also on the west shore of the pond. The two reference stakes for Transect 3 are positioned 44.20 m east of the intersection of the dike road and Highway 212.

Zones were constructed along the transect lines parallel to the shore. On June 1 and 4, 1975, a stake was placed at the water level on each transect line and
designated as the upper boundary of zone 4. This zone boundary was 10.97, 4.27, and 2.74 m from the nearest reference stake in Transects 1, 2, and 3 respectively. Each zone of Transects 1 and 2 are 1.22 m wide and 15.2 m long and each zone of Transect 3 is 0.91 m wide and 15.2 m long. Due to the difference in the angle of the slopes in both study areas, the slope in the pond study area being approximately 15° and the slope in the reservoir study area being approximately 25°, the widths of the zones were adjusted to assure that the similarly numbered zones were submerged during the same period of the sampling period. Zones in each transect line were constructed adjacent to each other upslope in relation to zone 4 with the transect line running through the middle of each zone.

Three soil samples were taken from each zone on the sampling trip of October 11, 1975. Two samples were taken from each end of the zone and one from the middle of the zone. A hand shovel was used to take the samples, each with a volume of 10 cm by 10 cm by 10 cm. Each sample was placed in a paper bag, brought back to the University of Montana and left to dry completely at room temperature. After drying, the sample was ground with a mortar and pestle, after removing as much plant material as possible, and put through a 0.991 mm mesh sieve. The material retained in the sieve was placed in a paper bag, weighed,
and recorded and classified as gravel and very coarse sand. The rest of the soil was then put through a 0.246 mm mesh sieve. The material retained was placed in a paper bag, weighed, and recorded and classified as medium to coarse sand. At this point, all of the sporocarps in the soil were removed and counted and stored in a separate bag. The remaining soil, consisting of fine sand to clay was used in the soil analysis of the zones. The soil particles were classified using the Soil Survey Manual, Agriculture Handbook No. 18, U.S. Department of Agriculture. Three samples of fine sand to clay for each zone were combined and mixed and approximately 250 grams of each combined sample were sent to Montana Soil Testing Laboratory, Plant and Soil Science Department, Montana State University, Bozeman, Montana for analysis of pH, organic matter content, nitrate-nitrogen, phosphorus, and soil texture. Samples from zones 1 and 4 of Transects 1, 2 and 3 were sent for analysis the last week in October. Upon consideration of the results of this analysis, samples, prepared by the method previously described, were sent from zones 2 and 3 of Transects 1, 2 and 3 the second week in December to the same soil testing laboratory.

To sample for reproductive and vegetative characteristics of Marsilea, a circular metal quadrat, 9.8 cm in diameter, with an area of 75.4 cm² was placed at random
in each zone ten times for the first sampling period. The number of times the quadrat was placed per zone was modified to five or six times as the growing season progressed and the biomass increased. It was visually obvious where the quadrat had been placed during the previous sampling period and such areas were excluded from future sampling.

The data taken per quadrat consisted of counting the number of sporocarps in the top 1 cm and on the surface of the soil and the number of living leaves present. The various observed types of sporocarps were placed into four phases:

- **Phase 1** - Young green sporocarps, soft sporocarp wall, with abundant white hairs on entire wall surface. Raphe is green.
- **Phase 2** - Maturing light brown sporocarps, hard sporocarp wall, with abundant white hairs on entire wall surface. Raphe is reddish brown.
- **Phase 3** - Matured black sporocarps, hard sporocarp wall, lacking hairs on the wall surface. Raphe is black.
- **Phase 4** - Sporocarps that have begun to deteriorate, or have aborted early in development.

Phase 1, 2, and 3 sporocarps were only counted if they were attached to the plant. Figure 4 illustrates the nature of Phase 2 and 3 sporocarps.
Figure 4.  a. Phase 2 sporocarps.
    b. Phase 3 sporocarps.
Quadrat data also was taken with respect to the angle of the slope beneath the quadrat, the texture of the soil in the quadrat, and the percent coverage of other plants in the quadrat. The aspect of the area of the quadrat was also recorded.

The level of the water was monitored by placing a wooden stake at the edge of the water in the pond of the pond study area. The sampling dates during which the above data was taken are summarized in Table 1. Other sampling dates involved taking qualitative data. The data collected through quadrat sampling was statistically analyzed by determining mean values and constructing confidence intervals around the means. The computer program used is listed in Appendix B.

Table 1

Sampling Dates for Transects 1, 2, and 3 Involving Quantitative Data Taken on Sporocarps and Leaf Numbers

<table>
<thead>
<tr>
<th>Sampling No.</th>
<th>Transect 1</th>
<th>Transect 2</th>
<th>Transect 3</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Date 1975</td>
<td>Date 1975</td>
<td>Date 1975</td>
</tr>
<tr>
<td>1</td>
<td>June 1</td>
<td>June 2</td>
<td>June 4</td>
</tr>
<tr>
<td>2</td>
<td>June 15</td>
<td>June 15</td>
<td>June 22</td>
</tr>
<tr>
<td>3</td>
<td>August 9</td>
<td>August 9</td>
<td>August 14</td>
</tr>
<tr>
<td>4</td>
<td>August 26</td>
<td>August 26</td>
<td>August 27</td>
</tr>
<tr>
<td>5</td>
<td>September 20</td>
<td>September 20</td>
<td>September 23</td>
</tr>
<tr>
<td>6</td>
<td>October 13</td>
<td>October 13</td>
<td>October 11</td>
</tr>
</tbody>
</table>
During the August 14th sampling trip, quantitative data were taken on the length of petioles and length of the longest attached leaflet and the number of lobes of the leaflets. To do this, a quadrat was placed every 1.22 m along Transect 1 beginning at the reference stake nearest the water. Eight quadrats resulted from this sampling, beginning with Q1 nearest the reference stake to Q8 nearest the water, and the data was statistically analyzed by linear regression using the Stat10.Sta program on the University of Montana's DecSystem 10 unit. The program, modified by J.M. Lang, is listed in Appendix C. Qualitative observations were made on the different leaf forms growing during the summer. Photographs were taken of the submerged, land, and floating form leaves.

To achieve the second objective, observations were made on the number of germinating sporocarps in the field. These data were obtained by walking along the shore of the study and adjacent study areas and counting the number of germinated sporocarps.

On the sampling trip of October 11th, collections of 50-70 Phase 2 and 3 sporocarps from the surface and top 1 cm of soil of each zone in each transect were made. The sporocarps were placed in paper bags, labelled, and stored at room temperature. Experiments were done to test the viability of Phase 2 and 3 sporocarps from different zones.
Eight Phase 2 sporocarps from each zone were tested, except for Transect 1 - zone 2. Only three Phase 3 sporocarps were available for testing from that zone. Eight Phase 3 sporocarps from each zone were tested.

After preliminary experiments to determine the optimal growing conditions for scarified sporocarps, the following experimental design was adopted. Each sporocarp was removed from the paper bag in which it was stored and rinsed with tap water to remove most of the attached soil. The length of the sporocarp was recorded and then a scalpel was used to puncture the side of the sporocarp wall just once. The sporocarp was placed in a 50 ml Erlenmeyer flask containing 30 ml of the incubation medium, adjusted to a pH 5.8 and a piece of aluminum foil was used to cap the top of the flask. The formula used for the incubation medium is as follows:

**Knop Solution - 10 mg / 100 ml**

1. **KNO₃**  
2. **Ca(NO₃)₂ · 4H₂O**  
3. **KH₂PO₄**  
4. **MgSO₄ · 7H₂O**

**Murray and Skoog Iron Supplement - 0.5 ml / 100 ml**

1. **FeSO₄ · 7H₂O**  
2. **NaEDTA**
Nitsch Solution - 0.1 ml / 100 ml

1. H$_2$SO$_4$       0.50 gm/L
2. MnSO$_4$ \cdot 4H$_2$O  3.00 gm/L
3. ZnSO$_4$       0.50 gm/L
4. H$_3$BO$_3$     0.50 gm/L
5. CuSO$_4$ \cdot 5H$_2$O  0.025 gm/L
6. Na$_2$MoO$_4$ \cdot 2H$_2$O  0.025 gm/L

The flasks were then placed in a Percival growth chamber with a 24 hour photoperiod with 12 hours of light. A constant temperature of 24 ± 1°C was selected. The light intensity in the growth chamber was 170 ft-c. The sporocarps of all experiments were incubated for six days. It was determined that all megagametophytes and sporophytes that develop in culture are detectable after three days (Mahlberg and Baldwin, 1975). Upon removal from the growth chamber, the number of megaspores, megagametophytes, and sporophytes were counted per flask. The contents of the flask were emptied into an empty glass petri dish that had a grid drawn on the bottom with a black wax pencil to facilitate the counting. The counts were all made under a dissecting microscope. The megaspore is a small oval structure with a very slight protuberance at the apical end where the endospore wall is the thinnest. The megagametophyte has a definite protuberance at the apical end extending through the endospore wall. The protuberance is unpigmented in the early development of the megagemeto-
phyte. In later stages, a cushion of cells surrounds the protuberance and is either dark brown or green. It could not be determined with the dissecting microscope whether the megagametophyte had been fertilized or not. A sporophyte was recorded if the first leaf arising from the cushion of cells, or calyptra, was greater than 1 mm in length. The few aborted megaspores were counted with normal megaspores. The number of megaspores at all stages of development that were retained within the sporocarp wall also was recorded. The Kruskul-Wallis test, and the Multiple Comparisons Test, non-parametric tests, were used to analyze data of samples with equal variances at a significance level of 0.05 and are listed in Appendices D and E, respectively. The Kolmogorov-Smirnov test, also a non-parametric test, was used to analyze data with unequal variances at a significance level of 0.05 unless indicated otherwise.

Further experiments were conducted to explain the observed differences in viability of sporocarps from different zones. All following experiments have the same design as previously described for the viability experiments. Sixteen Phase 2 sporocarps were placed in a glass petri dish with distilled water and placed in a freezer for two weeks, and 80 Phase 3 sporocarps were placed in a petri dish with distilled water and placed in a freezer
for four weeks. The Phase 2 sporocarps were from Transect 1-zone 4 and Transect 3-zone 4. The Phase 3 sporocarps were from Transect 1-zones 1, 2, 3, and 4. Phase 2 and Phase 3 sporocarps were removed from the freezer after two and four weeks, respectively and placed in incubation medium for six days.

The other eight Phase 2 sporocarps that had been frozen for two weeks and allowed to thaw were placed in a dry petri dish and put in an oven at 60°C for 12 hours. After this heat treatment, each sporocarp was incubated in the growth chamber following the previously described experimental procedure.

For further clarification of the developmental system of the sporocarp, eight Phase 2 sporocarps from Transect 2-zone 4 were removed from their paper bag and placed in a dry petri dish and put in an oven at 60°C for 12 hours. They were then scarified and incubated in a growth chamber, following the usual experimental procedure.

One additional experiment involving sporocarp viability was conducted. Eight Phase 2 sporocarps from Transect 3-zone 4 were removed from their paper bag and each was cracked with a pair of pliers. The sporocarp wall and its exposed contents were placed in Erlenmeyer flasks containing incubation medium and placed in a growth chamber.
A preliminary experiment investigating the effect of acidity on the viability of sporocarps was done. Eight Phase 3 sporocarps from the adjacent reservoir study area were scarified and placed in the incubation medium with a pH 4.2. The same two non-parametric tests used on the data from the viability studies, were similarly implemented on the above data.

Of the 80 Phase 3 sporocarps that had been frozen for four weeks, the remaining 72 were allowed to sit in petri dishes containing the melted distilled water at room temperature. Observations were made on sporocarp germinations without previously having been scarified with a scalpel. Data were taken after two months.

Another experiment was set up to test the natural germination of Phase 3 sporocarps compared with the natural germination of Phase 2 sporocarps. Twenty-one Phase 3 sporocarps from Transect 2-zone 4 and Transect 1-zone 4 and twenty-one Phase 2 sporocarps from Transect 1-zone 4 and Transect 2-zone 4, that had previously been stored in paper bags, were placed in glass petri dishes containing distilled water. Data were taken on the number of germinated sporocarps after a five month period.
CHAPTER III

OBSERVATIONS AND RESULTS

Physical Characteristics of the Study Areas

Slope and Soil Characteristics. Transects 1 and 2 occur on an east-facing slope in a pond study area, and Transect 3 occurs on a south-facing slope in the reservoir study area. The slope of the pond study area is approximately 15° while the slope of the reservoir study area is about 25°.

Soil sample analysis, listed in Table 2, shows that the pH of the soil samples from the zones in Transect 1 were similar, all within a range of 7.2 to 7.5. The pH of the soil samples from the zones in Transect 2 were in the range of 6.6 to 7.1, while pH of the soils from Transect 3 ranged from 7.9 to 8.3.

The percent of organic matter present in the soil samples was high for all the zones of Transect 1 and all of the zones tested in Transect 2. All zones had readings greater than 6.0%. The percent organic matter content of the soils from Transect 3 were low, with readings less than 4.0%. Qualitative interpretation (i.e. high) of the
Table 2

Soil Analysis Data

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<thead>
<tr>
<th>Transect</th>
<th>Zone</th>
<th>pH</th>
<th>Percent organic matter</th>
<th>Nitrate-nitrogen ppm</th>
<th>Phosphorus ppm</th>
<th>Soil texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>7.5</td>
<td>6.01 high</td>
<td>1.44</td>
<td>6 vv1</td>
<td>clay loam</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>7.3</td>
<td>&gt;6.56 high</td>
<td>2.49</td>
<td>29 vl</td>
<td>loam</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>7.2</td>
<td>&gt;6.56 high</td>
<td>1.97</td>
<td>26 vl</td>
<td>loam</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>7.3</td>
<td>6.42 high</td>
<td>1.44</td>
<td>11 vl</td>
<td>loam</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>6.9</td>
<td>10.00 high</td>
<td>4.12</td>
<td>2 vv1</td>
<td>loam</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>6.6</td>
<td>-</td>
<td>3.03</td>
<td>24 vl</td>
<td>loam</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>6.8</td>
<td>-</td>
<td>3.57</td>
<td>29 vl</td>
<td>loam</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>7.1</td>
<td>9.78 high</td>
<td>.93</td>
<td>24 vl</td>
<td>clay loam</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>7.9</td>
<td>4.00 low</td>
<td>.93</td>
<td>11 vl</td>
<td>clay loam</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>8.0</td>
<td>-</td>
<td>1.44</td>
<td>26 vl</td>
<td>loam</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>8.3</td>
<td>-</td>
<td>3.03</td>
<td>21 vl</td>
<td>clay loam</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>8.0</td>
<td>3.40 low</td>
<td>1.44</td>
<td>6 vv1</td>
<td>loam</td>
</tr>
</tbody>
</table>

1 vv1 = very, very low
2 vv1 = very low
percent organic matter readings was determined by the soil testing laboratory where the samples were sent for analysis.

Nitrate-nitrogen concentrations were similar for all zones on each transect. In Transect 1, the readings ranged from 1.44 to 2.49 ppm; in Transect 2, they ranged from 0.93 to 4.12 ppm; and in Transect 3, they ranged from 0.93 to 3.03 ppm.

Determination of phosphorus in the soil samples was also done. In Transect 1, zone 1 had a very, very, low reading of 6 ppm, while the other three zones had very low readings, between 11 and 29 ppm. The data for Transect 2 was similar. In Transect 3, zones 1, 2, and 3 had readings that were very low, lying between 11 and 26 ppm, while zone 4 had a very, very low reading of 6 ppm. Qualitative interpretation (i.e. very, very low) of phosphorus readings was determined by the soil testing laboratory where the samples were sent for analysis.

The soil texture for each soil sample listed in Table 2 is based on analysis of just the clay to fine sand partition of the total soil samples taken from the reservoir and pond study areas. Two other partitions, gravel to very coarse sand and medium to coarse sand were weighed and recorded in Table 3. The weights are all given with respect to a constant volume of 3000 cm$^3$, which is the combined volume of the three soil samples taken per zone.
Table 3

Amount of Different Sized Soil Particles Per Combined Soil Samples

<table>
<thead>
<tr>
<th>Transect No.</th>
<th>Zone No.</th>
<th>gravel-very coarse sand (g/3000 cm³)</th>
<th>medium-coarse sand (g/3000 cm³)</th>
<th>total weight (g/3000 cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>47.0</td>
<td>12.0</td>
<td>59.0</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>52.9</td>
<td>10.8</td>
<td>63.7</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>213.5</td>
<td>52.0</td>
<td>265.5</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>42.7</td>
<td>14.7</td>
<td>57.4</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>19.3</td>
<td>6.4</td>
<td>25.7</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>5.7</td>
<td>2.4</td>
<td>8.1</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>14.1</td>
<td>11.4</td>
<td>25.5</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>250.5</td>
<td>38.4</td>
<td>288.9</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>288.2</td>
<td>52.2</td>
<td>340.4</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>270.3</td>
<td>26.9</td>
<td>297.2</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>131.5</td>
<td>108.6</td>
<td>240.1</td>
</tr>
</tbody>
</table>
The amount of the gravel to very coarse sand partition in samples from Transect 3 was considerably higher than in Transect 1 and 2 for all zones. The medium to coarse sand partition in samples from Transect 3 was also higher than the samples from Transect 1 and 2.

The number of sporocarps collected from the three combined soil samples from each zone was quite high (Table 4). Over 90% of the sporocarps were Phase 3 sporocarps, some of them buried to a depth greater than five centimeters.

In summary, Transect 3 consists of soil that is very gravelly and low in organic matter with a pH averaging about 8.0. Transects 1 and 2 comprise an area that is composed mainly of sand and clay particles and is very high in organic matter with a pH averaging about 7.0.

Cover of Other Plant Species. Throughout the growing season there was an observed difference of percent of other plant species between zones of each transect. There were no other plant species in zone 4 of all three transects (Table 5). Moving upslope, there was a higher percent of other plant species growing with Marsilea in all of the transects.

Water Levels. The water level, in relation to the water level marker in the pond study area, was recorded
### Table 4

**No. of Sporocarps Collected From Combined Zone Soil Samples**

<table>
<thead>
<tr>
<th>Transect No.</th>
<th>Zone No.</th>
<th>No. of sporocarps/3000 cm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>148</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>278</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>275</td>
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<tr>
<td>1</td>
<td>4</td>
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<td>2</td>
<td>4</td>
<td>363</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>144</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>126</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>180</td>
</tr>
</tbody>
</table>

### Table 5

**Percent Coverage of Other Plant Species Per Quadrat After July Submergence**

<table>
<thead>
<tr>
<th>Transect No.</th>
<th>Zone No.</th>
<th>Percent coverage of other plant species</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>80-100</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>40-60</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>10-20</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>80-100</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>40-60</td>
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<tr>
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<td>3</td>
<td>10-20</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>40-70</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>40-60</td>
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<td>3</td>
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<td>10-20</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>
from May 10, 1975 to April 17, 1976 (Table 6). The degree of flooding of each zone of the three transects is presented in Table 7.

Several ponds near the reservoir, created by glacial activity and not connected to the reservoir, were checked for the presence of Marsilea. No Marsilea was found growing in these ponds, which were mainly surrounded by Typha latifolia. The water level of these ponds depended entirely on precipitation and possibly underground springs, while the water level of the reservoir was regulated by the refuge management.

Vegetative Growth Conditions

The number of leaves per quadrat were counted at specified sampling dates from the beginning of June to the middle of October (Figures 5, 6, and 7). Some indication of the vegetative reproductive success of Marsilea can be gathered from this data. The dotted lines connecting the means signify that the zone was under water during that time period, and the solid lines signify that the zone was free of water. All zones of all transects were covered with water by the end of June and remained covered until the beginning of August.

Leaf numbers were similar for Transects 1 and 2. Zones 1 and 2 of these transects had a constant number of leaves throughout the summer. In zone 3 on Transect 1,
Table 6
Readings on Water Level Marker

<table>
<thead>
<tr>
<th>Date</th>
<th>Level on water level marker (cm)</th>
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</thead>
<tbody>
<tr>
<td>April 20</td>
<td>&lt; 26.3</td>
</tr>
<tr>
<td>May 10</td>
<td>26.3</td>
</tr>
<tr>
<td>May 17</td>
<td>26.3</td>
</tr>
<tr>
<td>May 26</td>
<td>13.0</td>
</tr>
<tr>
<td>June 1</td>
<td>0</td>
</tr>
<tr>
<td>June 15</td>
<td>0</td>
</tr>
<tr>
<td>June 22</td>
<td>5.0</td>
</tr>
<tr>
<td>June 24</td>
<td>31.0</td>
</tr>
<tr>
<td>July 1</td>
<td>138.0</td>
</tr>
<tr>
<td>July 12</td>
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</tr>
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<td>July 27</td>
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</tr>
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<td>October 11</td>
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<td>October 19</td>
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<tr>
<td>December 14</td>
<td>58.0</td>
</tr>
<tr>
<td>1976 April 17</td>
<td>115.0</td>
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Table 7
Area of Transects Covered by Water Throughout Sampling Season

<table>
<thead>
<tr>
<th>Date 1975</th>
<th>Zone 1</th>
<th>Zone 2</th>
<th>Zone 3</th>
<th>Zone 4</th>
</tr>
</thead>
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<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>5/10</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>A*</td>
</tr>
<tr>
<td>5/17</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>A</td>
</tr>
<tr>
<td>5/26-6/22</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>A</td>
</tr>
<tr>
<td>6/24</td>
<td>T</td>
<td>T</td>
<td>.25A</td>
<td>A</td>
</tr>
<tr>
<td>7/1-7/27</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>8/9-10/11</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>10/19</td>
<td>T</td>
<td>T</td>
<td>.80A</td>
<td>A</td>
</tr>
<tr>
<td>12/14</td>
<td>T</td>
<td>.90A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>4/17/76</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
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<table>
<thead>
<tr>
<th>Date 1975</th>
<th>Zone 1</th>
<th>Zone 2</th>
<th>Zone 3</th>
<th>Zone 4</th>
</tr>
</thead>
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<td>4/20</td>
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<td>T</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>5/10</td>
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<td>T</td>
<td>T</td>
<td>A</td>
</tr>
<tr>
<td>5/17</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>A</td>
</tr>
<tr>
<td>5/26</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>.50A</td>
</tr>
<tr>
<td>6/1-6/15</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>6/22</td>
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<td>T</td>
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<td>6/24</td>
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<td>T</td>
<td>.25A</td>
<td>A</td>
</tr>
<tr>
<td>7/1-7/20</td>
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<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>7/27</td>
<td>T</td>
<td>T</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>8/9-10/11</td>
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<td>T</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>10/19</td>
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<td>T</td>
<td>.80A</td>
<td>A</td>
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<tr>
<td>12/14</td>
<td>T</td>
<td>.90A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>4/17/76</td>
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<td>A</td>
<td>A</td>
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</tr>
</tbody>
</table>

*A = aquatic phase
T = terrestrial phase
Table 7 (con't.)

Transect 3

<table>
<thead>
<tr>
<th>Date</th>
<th>Zone 1</th>
<th>Zone 2</th>
<th>Zone 3</th>
<th>Zone 4</th>
</tr>
</thead>
<tbody>
<tr>
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<td>T</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>5/10-5/26</td>
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<td>T</td>
<td>T</td>
<td>A</td>
</tr>
<tr>
<td>6/1-6/15</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>6/22</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>.50A</td>
</tr>
<tr>
<td>6/24</td>
<td>T</td>
<td>T</td>
<td>.25A</td>
<td>A</td>
</tr>
<tr>
<td>7/1-7/20</td>
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<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>7/27</td>
<td>T</td>
<td>T</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>8/9-10/11</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>10/19</td>
<td>T</td>
<td>T</td>
<td>.80A</td>
<td>A</td>
</tr>
<tr>
<td>12/14</td>
<td>T</td>
<td>.90A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>4/17/76</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
</tbody>
</table>

*A = aquatic phase

T = terrestrial phase
Figure 5. Production of leaves in Transect 1 throughout the sampling season.
Figure 6. Production of leaves in Transect 2 throughout the sampling season.
Figure 7. Production of leaves in Transect 3 throughout the sampling season.
there was a significantly greater number of leaves at the beginning of the sampling season than in zones 1 and 2. After a month of submergence in July, the number of leaves dropped significantly and remained constant throughout the rest of the season. In zone 4 of Transects 1 and 2, the production of leaves rose sharply in the two weeks before the July submergence and dropped as sharply directly after the water level dropped. However, unlike the other zones where there was a constant production throughout the rest of the sampling season, the number of leaves per quadrat in zone 4 of Transect 1 increased significantly into September. In October they finally decreased. The number of leaves in zone 4 was significantly higher in August and September than in zones 1 and 2.

In zones 1 and 2 on Transect 3, initially there were very few leaves in early June, however, they did increase in number before the July submergence. In zone 2, the leaves increased significantly after the submergence, but began to drop in number by late August. Zone 3 began the summer with more leaves, but production remained constant until the middle of September when it began to drop. Production was still high in zone 3 during August when the leaves were dying in zones 1 and 2. In zone 4, the number of leaves present was higher in the beginning of the summer than in zones 1 and 2 and increased significantly
in late August where production remained constant into October. There was no drop in production in zone 4 on Transect 3 in October like there was in zone 4 of Transects 1 and 2.

*Marsilea* began vegetative growth sometime before June in zones 3 and 4 of all transects and in early to mid-June in zones 1 and 2 of all three transects. The production of leaves remained constant during the July submergence in zones 1 and 2 of Transects 1 and 2. The number of leaves decreased significantly during the flooding in zones 3 and 4 of Transect 1 and zone 4 in Transect 2, but zone 3 in Transect 2 remained constant. In Transect 3, the number of leaves remained constant through submergence in all zones except zone 2, where there was a significant increase immediately after the water receded than before the flooding. Production began to cease in early October for all zones except zone 4 in Transect 3. Growth in this zone continued into October.

Observations were made in the field on different leaf forms of *Marsilea*. The three types of leaves, land form, floating form, and submerged form, were all observed at Nine-Pipe Reservoir. Figures 8 and 9 illustrate these forms, respectively. All three forms were found in the same area, often arising from the same rhizome. A juvenile leaf form was also observed (Figure 9a). Figure 10 shows
Figure 8. Floating Form Leaf.

Figure 9. a. Juvenile Bifid Leaf. (4x)
   b. Submerged Form Leaf. (4x)

Figure 10. a. Floating Form Leaf.
   b. Land Form Leaf.
a comparison of the floating form and the land form with respect to color and pubescence. The floating form has very few hairs on the surface and is much lighter green than the land form. The floating form also has a waxy leaf surface whereas the land form leaf does not.

The first floating form leaves were observed in mid-July during the period of high water. The submerged form leaves were noticed in late July after the water had receded down to zone 4. The land form leaf was always present in all zones.

On the July 27th sampling trip, quadrats were placed along Transect 1 and the length of the petiole of each leaf as well as longest leaflet attached to that petiole was measured. The data was analyzed using the previously mentioned Basic computer program for linear regression analysis (Figure 11).

The equation of the regression line is \( Y = 0.136 + 0.078x \) with an \( r^2 \) value of 0.78. The length of the petiole correlated well with the length of the leaflet. There was no relationship between the leaflet size and the zones. Some of the land form leaves were lobed (Figure 12). The number of leaves that had one or more lobes were also counted per quadrat (Table 8). Only the quadrats nearest the water had lobed leaves.
Figure 11. Regression on petiole length and leaflet length.
Figure 12. Lobed leaflets on Land Form Leaves.
Table 8
Number of Lobed Land Form Leaves Per Quadrat on July 27, 1975

<table>
<thead>
<tr>
<th>Quadrat No.</th>
<th>Lobed Leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1</td>
<td>0</td>
</tr>
<tr>
<td>Q2</td>
<td>0</td>
</tr>
<tr>
<td>Q3</td>
<td>0</td>
</tr>
<tr>
<td>Q4</td>
<td>0</td>
</tr>
<tr>
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Sexual Reproductive Growth Conditions

Phase 1 Sporocarps. Data on Phase 1 sporocarps are represented in Figures 13, 14 and 15. As with the number of leaves produced, Transects 1 and 2 were similar in the production of Phase 1 sporocarps. In zone 1 of both Transects 1 and 2, there were very few Phase 1 sporocarps at the beginning of the season, and there was no significant increase or decrease in numbers throughout the summer. In zone 2 of both transects, the data is similar to zone 1, but qualitatively there seemed to be more sporocarps present initially in June. In zone 3 of Transect 1, there was a significantly larger amount of Phase 1 sporocarps at the beginning of the season than in zone 1 or 2 of that transect, however, the number of Phase 1 sporocarps remained constant throughout the summer. Zone 3 of Transect 2 had significantly fewer Phase 1 sporocarps June 1 than zone 3
Figure 13. Production of Phase 1 sporocarps in Transect 1 throughout the sampling season.
Figure 14. Production of Phase 1 sporocarps in Transect 2 throughout the sampling season.
Figure 15. Production of Phase 1 sporocarps in Transect 3 throughout the sampling season.
of Transect 1, but there was a sharp increase in Phase 1 sporocarps in the following two weeks. After the July submergence, there was significantly less Phase 1 sporocarps per quadrat in that zone. The number then remained relatively constant throughout the rest of the sampling season.

In zone 4 of both transects, there were no Phase 1 sporocarps present at the beginning of June. In two weeks there was a definite increase in production until the beginning of July. There qualitatively appeared to be a decrease in number of Phase 1 sporocarps after the water receded at the end of July. There was a significantly larger amount of Phase 1 sporocarps in zones 2 and 3 during the first part of August than in zone 4 of Transect 2. However, during the last half of August, there was a significant increase in the number of Phase 1 sporocarps in zone 4 of Transect 2.

In zone 1 of Transect 3, there were very few Phase 1 sporocarps June 1 and no increase was observed until late August. The number remained constant into October. Qualitatively, this zone seemed to be contributing more Phase 1 sporocarps than the similar zones of the other transects. Zone 2 of Transect 3 is almost identical to zone 1 of that transect, except there was a significant increase in numbers in mid-August with a decrease in numbers beginning in mid-October. Zone 3 is similar to zones 1 and 2 of that
transect. The number of Phase 1 sporocarps is practically zero before the water covered the zone in July. In mid-August there was a significant increase, which remained constant until a decrease in numbers occurred in mid-October.

Zone 4 of Transect 3 is different from all of the other zones on that transect and from zone 4 of the other transects. There were no Phase 1 sporocarps by June 1 and no increase in numbers before the water rose in July. After the water receded, there was a significant increase in numbers of Phase 1 sporocarps. The number remained constant even into mid-October. There was a significantly higher number of Phase 1 sporocarps in zone 4 of Transect 3 than in all the other zones of the transect during September and October.

In general, if there was an increase in number of Phase 1 sporocarps in Transects 1 and 2 it usually occurred before the zones were covered with water in July. An increase in number of sporocarps in Transect 3 occurred after the July submergence. This is in contrast to the data on vegetative growth. The number of leaves increased before the water covered the zones in July, or remained constant, but there was never a noticeable increase in vegetative growth after the water receded, except in zone 2 of Transect 3.
Phase 2 and 3 Sporocarps. Transects 1 and 2 were similar with respect to vegetative growth and Phase 1 sporocarps. They are different with respect to Phase 2 and 3 sporocarp numbers (Figures 16, 17, and 18). Zone 1 in Transect 2 had a significantly higher number of Phase 2 and 3 sporocarps on June 1 than zone 1 in Transect 1. However, the numbers remained constant in both transects for that zone.

In zone 2 of Transects 1 and 2, there was a significantly higher number of Phase 2 and 3 sporocarps than in zone 1 in August and September, but zone 2 of Transect 2 also had a significantly higher number of these sporocarps than of zone 2 on Transect 1 in early June. In zone 2 of Transect 1 there was a significant increase of these sporocarps during August, but there was a constant number throughout August in zone 2 of Transect 2.

There is a difference in zone 3 of the two transects. They began the season with about the same number of Phase 2 and 3 sporocarps, but in Transect 1 there was a significant increase during late August that remained constant into October. In Transect 2, there was no noticeable increase throughout the season. In both transects, zone 3 had a significantly larger amount of sporocarps during most of the sampling dates than of zones 1 and 4 of those transects.
Figure 16. Production of Phase 2 and 3 sporocarps in Transect 1 throughout the sampling season.
Figure 17. Production of Phase 2 and 3 sporocarps in Transect 2 throughout the sampling season.
Figure 18. Production of Phase 2 and 3 sporocarps in Transect 3 throughout the sampling season.
Zone 1

Zone 2

Zone 3

Zone 4

Number of Phase 2 and 3 Sponcarps/Quadrat

Transect 3
Zone 4 of both transects were similar. They both had fewer sporocarps present at the beginning of the season than in zone 3 of both transects and the number remained constant throughout the season.

The zones of Transect 3 were not similar to the zones of Transects 1 and 2. Zone 1 had a significantly larger number of Phase 2 and 3 sporocarps at the beginning of the season than of the other similarly numbered zones in other transects, but like these other zones, the number of sporocarps remained constant throughout the season. Zone 1 also had a significantly larger number of sporocarps than the other zones of the transect on June 4.

Zone 2 of Transect 3 had a significantly lower number of Phase 2 and 3 sporocarps than of zone 2 in Transect 2 in June and the number remained constant through the season.

Zone 3 of Transect 3 had significantly fewer numbers of sporocarps than zone 3 in Transect 2 in June and late August and September and the number remained constant through the season.

In zone 4 of Transect 3, there were significantly fewer sporocarps than in zone 4 of Transect 2 in early June, but there was a significant increase in the number present by mid-October.

There was an increase in the numbers of Phase 2 and 3 sporocarps in zones 2 and 3 of Transect 1 after submergence and in zone 4 of Transect 3 in October. In all of
the other zones, the number remained constant throughout the season.

The ratio of the number of Phase 2 sporocarps to the number of Phase 3 sporocarps on the last sampling date for each transect is given in Figure 19. At the beginning of the season, there were no Phase 2 sporocarps. Qualitatively, the first Phase 2 sporocarps were observed in August. The ratio is constant for all zones in Transect 1 and is always less than 1. The same is true for Transect 2. In Transect 3, the ratio was not significantly different for zones 1, 2, and 3, but zone 4 had a significantly higher ratio of Phase 2 sporocarps to Phase 3 sporocarps than in zones 1 and 2.

Phase 4 Sporocarps. The number of Phase 4 sporocarps in zone 1 of all three transects is low at the beginning of the season and remains constant throughout the season (Figures 20, 21, and 22).

In zone 2 of all the transects, the number is still low at the beginning of the season and remains constant throughout the season.

In zone 3 of Transects 1 and 3, there is an increase in Phase 4 sporocarps from June to late August and late September, respectively. The number remained constant in Transect 2.

In zone 4, the number of Phase 4 sporocarps was low in June and remained low throughout the season in Transects
Figure 19. Ratio of Phase 2 sporocarps to Phase 3 sporocarps in Transects 1, 2, and 3 on the last sampling date.
Figure 20. Production of Phase 4 sporocarps in Transect 1 throughout the sampling season.
Figure 21. Production of Phase 4 sporocarps in Transect 2 throughout the sampling season.
Figure 22. Production of Phase 4 sporocarps in Transect 3 throughout the sampling season.
In Transect 2, there was an increase in Phase 4 sporocarps in late September. In general, there were initially few Phase 4 sporocarps in all zones of all transects and this number remained constant, except in zone 3 of Transect 1, zone 4 of Transect 2, and zone 3 of Transect 3, where there was a noted significant increase sometime after the July flooding.

There were two types of aborted sporocarps: Phase 2 sporocarps that began to deteriorate and Phase 1 sporocarps that stopped growth soon after initiation. Qualitative observations showed that the first type occurred mainly after mid-August on wet soils. The second type occurred mostly in zones upslope on dry soils.

**Qualitative Field Observations on the Sexual Life Cycle**

Inferences can be made on the time of initiation and maturation of the sporocarps in nature from the quantitative data previously reported. The germination of sporocarps of *Marsilea* was observed in the field. The time and numbers of germinating sporocarps were recorded and appear in Table 9. All of the observations of germination from May 3 through June 24 occurred in the water within a range of 1.22 m from shore. Data from Table 9, combined with data from Table 7 on the area of transects covered by water throughout the sampling season, provides information on which zones contained germinating sporocarps. The study
Table 9

Number of Observed Sporocarps Germinating in the Field

<table>
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<th>Date</th>
<th>Pond study area</th>
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<th>Reservoir study area</th>
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<td>97</td>
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<td>&gt;100</td>
<td>&gt;300</td>
<td></td>
<td></td>
</tr>
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area zonation pattern was extended to the adjacent study areas to enable reporting the area of germinations in them.

The earliest noted germination occurred on May 3 in zone 4. The maximum number of germinations occurred in the third week of June, just before the water covered the entire area during the month of July. All observed germinations occurred in zones 3 and 4. Figure 23 shows many germinated sporocarps from the area of large accumulation of Phase 3 sporocarps (Figure 3).

Only Phase 3 sporocarps germinated from May 3 to June 24. There were also Phase 1 sporocarps present at that time, but at that point in their development they are unable to germinate. It could not be determined what percent of the germinating sporocarps were attached to rhizomes. There are many unattached Phase 3 sporocarps
Figure 23. Germination of sporocarps in the field in water.

a. Extended sporophore with attached sori.

Figure 24. Accumulation of Phase 2 sporocarps on the shore of the reservoir.
along the shoreline of the adjacent reservoir study area. Figure 24 shows a portion of the area of large accumulation of Phase 3 sporocarps (Figure 3) covered by thousands of Phase 3 sporocarps.

On October 11, when all zones had been free of standing water since the beginning of August, three germinations were observed in zone 1 of Transect 1. It could not be determined whether these germinating sporocarps were Phase 2 or Phase 3 sporocarps.

No new sporophyte plants, resulting from sporocarp germination, were observed during the sampling season.

**Laboratory Studies on Sporocarp Viability**

The data on the viability of sporocarps are given in Figures 25, 26, and 27. There was no significant difference in the percentage of megagametophytes or sporophytes formed from Phase 3 sporocarps that had developed in different zones in any of the three transects using the Kruskul-Wallis Statistical test. There was also no significant difference in the percentage of megaspores retained within the sporocarp wall of Phase 3 sporocarps, using the Kolmogorov-Smirnov statistical test. If megaspores were retained within sporocarps, it was mainly due to the fact that the sporophore remained within the sporocarp walls. Some megaspores were freed from the sporocarp
Figure 25. Percent megagametophytes formed from Phase 2 and Phase 3 sporocarps.
Figure 26. Percent sporophytes formed from Phase 2 and Phase 3 sporocarps.
Figure 27. Percent megaspores retained in Phase 2 and Phase 3 sporocarps after scarification.
without the exudation of the sorophore, but not many. There was no significant difference between the percent of megagametophytes and the percent of sporophytes formed from Phase 3 sporocarps of all the zones in all of the transects.

The results were similar for Phase 2 sporocarps. Using the Kruskul-Wallis test, there was no significant difference in the percentage of sporophytes formed from sporocarps of different zones. Similarly, there was no difference in the percent of retention of megaspores within Phase 2 sporocarp walls from different zones of all the transects.

Using the Kolmogorov-Smirnov test, there was a significantly higher percent of megagametophytes from Phase 2 sporocarps collected from zones 1 and 2 than from zones 3 and 4 of each of the transects. There was a significantly higher percent of megagametophytes than sporophytes in Phase 2 sporocarps from zones 1 and 2 of all the transects. There was no difference between the percent of megagametophytes and the percent of sporophytes formed in zones 3 and 4 of Transects 1 and 2, but there was a significant difference from those formed in zones 3 and 4 of Transect 3.

A striking difference exists between the data from Phase 3 and Phase 2 sporocarps. There is a significantly
higher percent of megagametophytes and sporophytes formed from Phase 3 sporocarps than from Phase 2 sporocarps. The average percent of megagametophytes from Phase 3 sporocarps was 49.8 ± 3.9%. The average percent of megagametophytes from Phase 2 sporocarps was 8.8 ± 2.4%. The average percent of sporophytes from Phase 3 sporocarps was 44.6 ± 4.1%. The average percent of sporophytes from Phase 2 sporocarps was 1.2 ± 1.1%. There was also a significantly higher percent of megaspores retained within the Phase 2 sporocarps than in the Phase 3 sporocarps.

**Laboratory Studies on the Maturation and Germination of Sporocarps**

The data from experiments involving freezing and heat treatments of sporocarps are represented in Figures 28 and 29. Phase 2 sporocarps that had been frozen for two weeks and then treated with heat in an oven at 60°C for 12 hours were not significantly different than Phase 2 sporocarps that had just been frozen for two weeks, using the Kruskal-Wallis test. Using the Kolmogorov-Smirnov test, there was a significantly lower amount of megaspores retention with Phase 2 sporocarps that had been frozen for two weeks and then treated with heat than with Phase 2 sporocarps that were untreated. Untreated Phase 2 sporocarps from zone 1 of Transect 1, chosen randomly from the viability study, were used as a comparative control.
Figure 28. Freezing and heat treatments on Phase 2 sporocarps.
Number of sporocarps
Figure 29. Freezing and acid effects on Phase 3 sporocarps.
Phase 2 untreated

Phase 2 heat treated

Phase 3 untreated

Phase 3 frozen

Phase 3 acidic

Number of sporocarps
Phase 3 sporocarps that had been frozen for four weeks were not different from untreated Phase 3 sporocarps. Untreated Phase 3 sporocarps from zone 1 of Transect 1 were chosen randomly from the viability study and used as a control.

Phase 2 sporocarps that had just been given a heat treatment were significantly different from untreated Phase 2 sporocarps and not significantly different from untreated Phase 3 sporocarps, using the Kolmogorov-Smirnov test. There was a significantly lower percentage of megaspores retained and a significantly higher percentage of sporophytes formed in the heat treated Phase 2 sporocarps than in the untreated Phase 2 sporocarps. However, there was no difference in the percentage of megagametophytes formed.

There was a significantly higher percent of megagametophytes and sporophytes formed and a significantly lower percent of megaspore retention in Phase 2 sporocarps treated with heat than with Phase 2 sporocarps frozen for two weeks and then treated with heat, using the Kolmogorov-Smirnov test.

There was a significantly higher percent of sporophytes formed from megaspores coming from Phase 2 sporocarps that had been cracked with pliers to expose all of the megaspores, than from untreated Phase 2 sporocarps, using the
Kolmogorov-Smirnov test at a significance level of 0.28. There was no difference in the percent of viable megaspores formed.

The acidity of the incubation medium had an effect on the percent of sporophytes formed (Figure 29). In incubation medium with pH 4.2, there were significantly fewer sporophytes formed than in incubation medium with pH 5.8, but there was no difference in the percent of megagametophytes or the percent of retention of megaspores using the Kruskul-Wallis test.

Seventy-two Phase 3 sporocarps were placed in water for two months, unscarified, after four weeks of freezing. Only one of these sporocarps germinated and produced many sporophytes.

Of the 21 Phase 2 sporocarps placed in petri dishes with distilled water for five months, only two of them germinated, but no sporophytes were formed. Of the 21 Phase 3 sporocarps placed in petri dishes with distilled water for five months, none of the sporocarps germinated. There was considerable amounts of fungi in the petri dishes containing Phase 2 sporocarps, but little fungal activity was observed in the petri dishes containing Phase 3 sporocarps.
CHAPTER IV

DISCUSSION AND CONCLUSIONS

Effects on Vegetative Growth of Marsilea

There was no significant difference in the total number of leaves produced throughout the sampling season in Transects 1, 2, and 3. Although Transects 1 and 2 were on an east-facing slope and Transect 3 was on a south-facing slope, the low height of the embankment cancelled any significant effect due to the amount and intensity of light received by the plants.

There were three edaphic factors that were different between the pond study area and the reservoir study area. Transects 1 and 2 had soils with a high percent of organic matter, a pH 7.0, and a low content of gravel to very coarse sand, while Transect 3 had soils with a low percent of organic matter, a pH 8.0, and a high content of gravel and very coarse sand. These factors did not directly effect the number of leaves produced during the sampling season between transects. Gopel (1971), using eight populations of five different Indian species of Marsilea, showed that in all populations, the soil organic matter
promoted growth considerably. However, only the five individual species of *Marsilea* were grown in different soils. In nature, *Marsilea* must compete with other plant species. This factor would interfere with the effect of organic matter on the growth of *Marsilea*.

There was no difference in percent cover of other plant species in zone 2 of all the transects. The same was true for zones 3 and 4, and there was no difference in the number of leaves of *Marsilea* between transects in similar numbered zones of 2, 3, and 4. There was a higher percent cover of other plant species in zone 1 of Transects 1 and 2 than zone 1 of Transect 3, but the number of leaves of *Marsilea* present throughout the growing season was the same for zone 1 in all of the transects. The percent cover of other plants did not affect the number of leaves present. Soils with higher percent organic matter do favor higher plant production.

The cover of other plant species was indirectly correlated with the number of *Marsilea* leaves between zones of individual transects. Zone 1 of all the transects had the highest percent cover of other plant species than the other zones of the transect and also had the lowest number of *Marsilea* leaves than the other zones. The fact that cover of other plant species did not effect the number of leaves of *Marsilea* in similar numbered zones, but did
effect the number of leaves of *Marsilea* between zones of each transect shows that another factor must have been directly affecting the vegetative growth of both *Marsilea* and other plant species.

All three transects ran downslope perpendicular to the shoreline of the study areas. Zone 1, an area farthest upslope, had significantly fewer leaves present than all of the other zones in Transect 1, and zone 1 of Transect 2 had fewer leaves present than zones 3 and 4 in that transect in early June and mid- and late August. This indicates that *Marsilea* may have a requirement for a certain level of moisture to prosper vegetatively. By mid-September, there was a significantly higher number of leaves present in zone 4, the area nearest the standing water, than in the other zones of Transects 2 and 3. Zones 1, 2, and 3 became deficient in a factor necessary for high vegetative reproduction. This was probably sufficient soil moisture. All zones were equally receptive to precipitation during the season, but the rate of runoff and seepage was higher in areas upslope. Gopal (1970) also found that significant moisture was required for the optimal growth of *Marsilea*. On the other hand, zone 4 had significantly fewer leaves during the earlier part of the sampling season than in mid-September. Soil moisture content must have been too high for optimal vegetative
growth. Thus, there was an optimal range of soil moisture content, above and below which vegetative reproduction declines.

The production of leaves remained constant during the July submergence in zones 1 and 2 of Transects 1 and 2, but decreased significantly during the flooding in zones 3 and 4 of Transect 1 and zone 4 of Transect 2. Gopal (1970) found that although a sufficient soil moisture was required for optimal growth, that none of the species or populations he studied could endure submergence for an extended length of time. Zones 3 and 4 were submerged longer than zones 1 and 2, and the depth of standing water was greater in zones 3 and 4. This accounts for the fact that there was a decrease in leaf production in zones 3 and 4 and not in zones 1 and 2 after the July submergence.

*Marsilea* was not found in the ponds surrounding the reservoir, which were mainly surrounded by *Typha latifolia*. *Typha* did not grow in the study areas due to the fact that the soils were too dry for too long during low water level (Curtis, 1959). This suggests that *Marsilea* requires an environment of fluctuating water levels, that combined with a dryness factor, excludes the competition of other plant species. The presence of *Marsilea* was not documented until four years after the man-made reservoir was formed in 1929. This also supports the theory that *Marsilea* requires fluctuating water levels.
The heteroblastic and heterophyllic characteristics of *Marsilea* were expressed during the sampling season. The land, submerged, and floating forms were documented. The land form was present in all zones during all sampling periods. The floating form was observed in mid-July during the period of high water. This corresponds to the experiments of Gopal (1970) showing the presence of elongated petioles of leaves initiated under water. He found that mature land form leaves did not change to the floating form upon submergence. The submerged form leaves were also observed in the same area as the floating form. Gaudet (1968) found that at greater depths of water, greater than a depth conducive to floating forms, that the plants produced short floating leaves which did not reach the water surface and eventually produced submerged forms. This explains the observation of both types of these leaves in the same area which was progressively subjected to greater depths of water throughout July. Allsopp (1963) related the different forms to nutritional levels, while Gaudet (1963, 1965) implicated light quality as a source of differences. Further studies by Looi (1967) have shown that increased concentrations of CO₂ effected the leaf forms. The causes seem to be interrelated and more complex than originally thought.
It was found that the length of the petiole correlated well with the length of the longest leaflet of the land form leaves. There could be an internal relationship between the controls involving the meristematic regions of the leaf. No relationship was found between the size of the leaflets and the different zones, although a previous study (Gopal, 1970) has shown that the leaflet area increased with increasing soil moisture level.

Lobing of the leaves was observed in the quadrats near the standing water during the season after the water had receded in late July. Lobing of leaflets also has been observed by Gopal (1970), who attributed this phenomenon to a decrease in soil moisture content. The lobed leaves observed in this study occurred in relatively wet areas. According to Gupta (1962), the first appearance of crenations or lobes in the leaflets on the plants was different for the xerophytic and hydrophytic species of Marsilea grown under identical ecological conditions. Gopal (1970) found the distinction between hydrophytic and xerophytic species of Marsilea unclear, and believed that it was only the range of adaptability that varied within certain limits. He chose to call the five species that he investigated helophytes, possessing adaptibility both to the water-logged and extreme drought conditions. M. vestita could also be classified as a helophyte, growing
neither in extremely arid conditions or extremely wet conditions, but adaptable to both extremes and growing optimally in the middle of these extremes.

Effect on the Sexual Reproductive Growth of Marsilea

Phase 1 Sporocarps. In zones 1, 2, and 3 of Transect 1 and zones 1 and 2 of Transect 2, there was no increase in Phase 1 sporocarps before the July submergence. There was also no increase in leaf production in these zones. However, in zone 4 of both transects, there was a significant increase in Phase 1 sporocarps and leaf production. When conditions were favorable for vegetative growth in Transects 1 and 2, they were also favorable for initiation of sporocarps.

In Transect 3, when the leaves were being produced before the submergence, there was no production of sporocarps. In zones 2, 3, and 4, there was a significant increase in Phase 1 sporocarps after the submergence, when the leaf production was constant.

The soil characteristics did not play an important role in that production of Phase 1 sporocarps. During the sampling season, the production of Phase 1 sporocarps in Transect 3, which had different soil conditions than Transects 1 and 2, was similar to that in Transects 1 and 2.
The cover of other plant species indirectly affected the production of Phase 1 sporocarps. Where there was a higher percent cover of other plant species, there was a corresponding decrease in percent cover of Marsilea. There was less production of Phase 1 sporocarps in zone 1 of all transects than of zone 3 of Transects 1 and 2 in June and zone 3 and 4 of Transect 3 in August. Zone 1 always had a higher percent cover of other plant species than the other zones. Where there was a low production of vegetation by Marsilea there was also a low production of Phase 1 sporocarps.

The fact that Phase 1 sporocarp production increases significantly after the July submergence would indicate two possibilities: (1) after the flooding there was a sufficient increase in soil moisture content to favor initiation of sporocarps, or (2) that the sudden change in soil moisture content stressed the plants, somehow causing initiation of sporocarps. The first possibility is probably correct because Phase 1 sporocarps were initiated before the submergence in Transects 1 and 2 where a sudden change in soil moisture was not observed.

As the season progressed, there was a decrease in production of Phase 1 sporocarps in the zones away from the water and an increase in production of Phase 1 sporocarps in the zones closer to the water. This would
support the theory that there was an optimal soil moisture regime for the production of Phase 1 sporocarps.

**Phase 2 and 3 Sporocarps.** Zone 3 of both Transects 1 and 2 had a significantly higher percent of Phase 2 and 3 sporocarps than zone 1 and 4 of those transects at the beginning of the sampling season and during most of the subsequent sampling dates. In Transect 3, though, zone 1 had a higher percent of Phase 2 and 3 sporocarps than the other zones of that transect at the beginning of the sampling season and during most of the following sampling dates. These results could not be correlated directly with the soil characteristics. All of the zones in each of the transects were similar to each other with respect to soil characteristics.

The percent cover of other plant species with the soil texture data would explain the differences in zone numbers with respect to numbers of Phase 2 and 3 sporocarps. In zone 1 of Transects 1 and 2, the percent cover of other plant species was significantly higher than that of zone 1 of Transect 3 due to the higher organic matter content in Transects 1 and 2. *Marsilea* simply was outcompeted vegetatively and reproductively in zone 1 of Transects 1 and 2. Although the soil in the zones of Transect 3 was low in organic matter content and high in gravel content, thus having a low water holding capacity, higher plant species
could survive in zone 1 which was also less effected by the fluctuating water level. The presence of the higher plant species increased the water holding capacity of the soils in zone 1 and provided a higher level of humidity in the environment near the soil surface. These conditions would be favorable for the reproductive growth of *Marsilea* with respect to Phase 1 sporocarps early in the season and later in the season the zone would have soils dry enough to promote maturation of the Phase 1 sporocarps to Phase 3 sporocarps, which requires a period of dryness. Zone 4 in Transect 3 would have relatively wet soil later in the season near the standing water when the sporocarps needed dry soils for maturation. Zones 2 and 3 may have been too dry. Although the sporocarp needs a dry environment to mature, it is possible that a small amount of moisture is necessary in aiding the deposition of sporocarp wall material to insure imperviousness to the environment.

The water level, together with the soil texture, was responsible for the differences that effected the accumulation of Phase 2 and 3 sporocarps. Conditions favorable for the production of Phase 1 sporocarps were different than conditions favorable for the production of Phase 2 and 3 sporocarps. Zone 4 in Transect 3 was more conducive for the production of Phase 1 sporocarps after the July
submergence than the other zones in that transect, but it was zone 1 where the greatest number of Phase 2 and 3 sporocarps were observed. Although many more new sporocarps were produced late in the season near the water, these sporocarps did not develop to the point where they could survive the following winter and spring. The soil moisture was sufficient for the initiation of sporocarps, but it was possibly too high for the maturation of sporocarps. This suggests that there is a point in the development of the sporocarp when a dry environment is necessary.

In zone 3 of Transect 1 and zone 4 of Transect 3, there was a significant increase in the number of Phase 2 and 3 sporocarps during mid-October, but apparently there was no accumulation of matured sporocarps. Data would have to be taken in June of 1976 on the number of Phase 2 and 3 sporocarps per quadrat to prove conclusively that there was no significant increase in the population of Phase 2 and 3 sporocarps in the zones from year to year. However, the fact that the average number of Phase 2 and 3 sporocarps per quadrat in early June in zone 3 of Transect 1 and zone 4 of Transect 3 were 12 + 4 and 2 + 2 sporocarps, respectively, and that this accumulation has taken place for over forty years would indicate that there were very few sporocarps being added to these populations
from year to year. Most of the sporocarps produced in zone 4 of Transect 3 during early fall did not survive the winter. It is possible that some of the sporocarps could have been washed away due to wave action, particularly on the shore of the reservoir.

There was a significantly larger number of Phase 2 and 3 sporocarps in zone 2 of Transect 3 than of zone 4 of Transect 3 in June. This is related to the fact that the largest number of Phase 1 sporocarps occurred in early August in zone 2 of Transect 3. Early August production would give the Phase 1 sporocarps the necessary environmental conditions (i.e. sufficient length of time or dryness before the temperatures drop or the water rises) to insure winter survival and addition to the population of Phase 3 sporocarps in the spring.

**Phase 4 Sporocarps.** There was no direct correlation between the production of Phase 4 sporocarps and soil characteristics. In zones 1 and 2 of all the transects, there were few abortions initially and the rate remained constant throughout the season. If there was an increase in abortions, it occurred in zone 3 or 4 of all the transects in late August or September.

There is no direct correlation between cover of other plant species and the number of Phase 4 sporocarps. In the zones where there were few Marsilea plants and many
other plants, with a corresponding low number of Phase 1 sporocarps and low accumulation of Phase 2 and 3 sporocarps, there also were few Phase 4 sporocarps.

The zones nearest the water had the highest amount of aborted sporocarps late in the season. Abortions were of two types: Phase 2 sporocarps that began to deteriorate and Phase 1 sporocarps that stopped growth soon after initiation. Qualitative observations showed that the first type of aborted sporocarps occurred mainly after mid-August in zones with wet soils. The second type occurred most often in zones upslope where the soils were dry. Apparently, there was a point in development where the sporocarp required moisture to continue growth and that there was also a point in development where the sporocarp must have a relatively dry environment.

From Figures 20 and 21, it is apparent that there is usually a significantly larger number of Phase 4 sporocarps present in zone 3 than in zone 1 of both Transects 1 and 2. It was zone 3 of both transects that has significantly larger numbers of Phase 1, 2, and 3 sporocarps than zone 1 of these transects during many of the sampling dates. This intermediate zone, with respect to water level, soil texture, and cover of other plant species, is the optimal zone for the initiation of sporocarps and accumulation of mature sporocarps, and it also had the
highest level of aborted sporocarps. The amount of vegetative growth in zone 3 was also larger than in zone 1 of Transects 1 and 2.

In Transect 3, the zone of highest production of Phase 1 sporocarps was not the zone that had the largest accumulation of Phase 2 and 3 sporocarps for reasons previously discussed. There were few Phase 1 sporocarps produced in zone 1 which had a significantly larger number of Phase 2 and 3 sporocarps than the other zones at the beginning of the season, but there were also few Phase 4 sporocarps throughout the season. The sporocarps that are initiated in zone 1 of Transect 3, although these were few, exist in an environment which favors their complete development. This environment was characterized by an optimal range of soil moisture, regulated by the water level, the soil texture, and the cover of other plant species.

Qualitative Field Observations on the Sexual Life Cycle

The maximum number of germinating sporocarps occurred in mid-June in water in zones 3 and 4. All germinating sporocarps were Phase 3 sporocarps. The sporocarp walls of Phase 3 sporocarps were very hard and impervious to the external environment. The sporocarps could remain viable for years (Allsopp, 1952). In normal germinations,
the sporophore must imbibe water to expand and rupture the sporocarp wall, liberating the megaspores and microspores before fertilization occurs. The mechanism which enables water to enter the sporocarp in nature has not been determined. Bold (1967) felt that bacteria may play an active role in scarifying the sporocarp wall. Another possibility could be fungal activity. The scarification process which may entail several years must be completed during the winter to enable sporocarps to germinate in the spring. At this time, they only needed to be immersed in water for germination to occur. All of the scarified sporocarps germinated at the same time of the year. No germinations in water were observed while the water was receding in early August or when the water rose again in mid-October. The sporocarps observed germinating in the fall on damp soil could have been Phase 2 sporocarps. The sporocarps may not have been impervious to the environment, and the sorophore could have imbibed enough water to open the sporocarp wall. There were very few of these germinations in the fall.

The germinations in water were only observed in zones 3 and 4, but the following week after these observations, the water covered the entire transect such that observations in zones 1 and 2 were impossible. There is no reason to believe that sporocarps did not germinate in all zones.
It was impossible to tell if new sporophytes were produced from fertilization resulting from the germinations of the sporocarps.

**Laboratory Studies on Sporocarp Viability**

There was no difference in the percent of viable megaspores or sporophytes formed from Phase 3 sporocarps that had developed in different zones in any of the transects or in the percent retention of megaspores within the sporocarp wall. Environmental conditions do not have to be optimal for the initiation and maturation of sporocarps. If the sporocarp matures completely, the percent of viable megaspores and sporophytes will be the same for different sporocarps regardless where they developed. It has been reported (Bloom, 1955) that in Phase 3 sporocarps, megaspores sometimes develop normal gametophytes, but will not produce sporophytes. This suggested to Bloom that microspores appear to be more susceptible to aging effects than megaspores. This was not observed in this study.

In Phase 2 sporocarps, regardless of the zone in which they developed, the percent of sporophytes formed was the same and the percent retention of megaspores was similar. Sporocarps similar to each other in their developmental stage all produce the same amount of sporophytes and retain the same percent of megaspores. There
was a higher percentage of viable megaspores formed from Phase 2 sporocarps that developed in zones 1 and 2 of all the transects than in zones 3 and 4. The difference was not great, but it could reflect the fact that Phase 2 sporocarps from zones 1 and 2 were relatively more mature than the Phase 2 sporocarps from zones 3 and 4. The highest production of Phase 2 sporocarps from zones 3 and 4 occurred late in the season, while the highest production of Phase 2 sporocarps from zones 1 and 2 generally occurred in the middle of the season.

There was a significantly higher percent of viable megaspores formed than sporophytes from Phase 2 sporocarps in zones 1 and 2 of all the transects. This could be explained in three ways: (1) The microspores remained within the sporocarp wall thus preventing fertilization, (2) The microspores were retained within the sporocarp wall until they developed into microgametophytes before they were able to leave the sporocarp. It has been found that the release of the sperm is inhibited in a high concentration of sporocarp contents, until the sperm finally die within the microgametophyte (Bilderback, personal communication). It has also been found that when microspores are added to a population of megagametophytes that had developed for 12 hours, the percent of sporophytes formed decrease significantly from when the microspores
and megaspores were released together (Mahlberg and Baldwin, 1975). (3) The microspores were too immature at that point in development to form microgametophytes.

There was a significantly higher percent of viable megaspores and sporophytes formed and a significantly lower percent of megaspore retention in Phase 3 sporocarps than in Phase 2 sporocarps. The reasons for these differences will be discussed in the following section under maturation and germination of sporocarps.

The average percent of viable megaspores from Phase 3 sporocarps was 49.8 ± 3.9%, and the average percent of sporophytes formed was 44.6 ± 4.1%. These percentages corresponded to the values found by Mahlberg and Baldwin (1975) working with M. vestita. They found that the average percent of megagametophytes formed was 59.5 ± 6.3%, and the average percent of sporophytes formed was 38.5 ± 7.3%. They also found that there was little apogamy or parthenogenesis in M. vestita. The average percent of viable megaspores from Phase 2 sporocarps was 8.8 ± 2.4%, and the average percent of sporophytes formed from Phase 2 sporocarps was 1.2 ± 1.1%. These significantly lower percentages also have been noted by Bloom (1955).
Laboratory Studies on the Maturation and Germination of Sporocarps

Differences between the viability of Phase 3 and Phase 2 sporocarps has been observed. Experiments were done to determine what factors were responsible for these differences. If the Phase 2 sporocarps were treated with heat at 60°C for 12 hours, germination proceeded normally as with Phase 3 sporocarps, with comparable percent of viable megaspores and sporophytes. This heat requirement has been observed by Bloom (1955) and Bhardwaja and Sen (1966). Bhardwaja and Sen (1966) found that fresh sporocarps treated with heat at 65°C for 24 hours had the optimal percent of fertilization and that the percent of fertilized megagametophytes decreased with a heat treatment of only 54 hours.

If Phase 2 sporocarps are frozen for two weeks before the heat treatments, they were not significantly different from untreated Phase 2 sporocarps. This indicates that the sporocarps in nature must reach maturity before they are subjected to constant freezing temperatures. However, heat treatment is not the only factor involved in the maturation process of sporocarps. Bloom (1961) treated sporocarps with heat and then stored them over the summer. He then retreated some with heat and left some untreated. He found a weight loss of 7.9% in the group of sporocarps
retreated with heat. He found that the unretreated sporocarps were less viable than the retreated sporocarps. Even after a heat treatment, Phase 2 sporocarps can take up moisture. Thus, it appears that along with a heat treatment, the sporocarp wall must change to become impervious to the environment, possibly by extra deposition of materials in the sporocarp wall.

It was found that upon cracking Phase 2 sporocarps and releasing the entire contents into the incubation medium, that there was an increase in the percent of sporophytes when compared to untreated Phase 2 sporocarps, although no increase in viable megaspores was observed. Apparently, the microspores and megaspores are mature in the Phase 2 sporocarps, but there is a failure in the sorophore system. The sorophore in Phase 3 sporocarps consists entirely of dead cells which imbibe water in order to break open the sporocarp wall (Bilderback, personal communication). The heat treatment must allow the sorophore to detach from the inner sporocarp wall and exude normally.

Rice and Laetsch (1967) found that the viability of sperm was affected by heat. This study showed that the viability of sperm was also affected by acidity. With an incubation medium of pH 4.2, there were significantly fewer sporophytes formed than in an incubation
medium of pH 5.8. There was no difference in the percent of viable megaspores or the percent megaspore retention.

The mechanism controlling germination is not known. Freezing Phase 3 sporocarps for four weeks and placing them in water for two months produced no results. One sporocarp germinated, but that was probably due to fungal or bacterial activity which sufficiently scarified the sporocarp.

Although the experiment in which 21 Phase 3 sporocarps and 21 Phase 2 sporocarps were placed in water for five months produced no information on factors involved in germination, it provided evidence to support the fact that Phase 2 sporocarp walls are not impervious to the environment. A lot of fungal activity was noticeable on the walls of Phase 2 sporocarps, but not on the walls of Phase 3 sporocarps. This would suggest that the sporocarp was being utilized by the fungi. In fact, the cells of the sorophore are filled with carbohydrates containing the sugars, rhamnose and arabinose (Bilderback, personal communication).
Quantitative and qualitative field data and quantitative data from laboratory experiments have provided information on the autecology and reproductive biology of Marsilea vestita Hook. et. Grev.

Vegetatively, Marsilea has a requirement for a certain amount of soil moisture, intermediate between dry and water-logged conditions. The leaves can not endure submergence for an extended length of time. The fluctuating environment of man-made reservoirs is a necessary element in the survival of the species. Marsilea was not found in the ponds surrounding the reservoir where the water does not fluctuate dramatically as in the reservoir.

Marsilea exhibited its heterophyllic and heteroblastic leaf conditions in the field. The land form was always present. The floating and submerged forms occurred when the water covered the transects in July. The juvenile bifid leaf was observed along with the adult quadrifid leaf. During August, lobing of the land form leaves was observed in zones near the standing water. This was due to a change in the soil moisture content.
When conditions were favorable for the vegetative growth, they were also favorable for the production of Phase 1 sporocarps. The conditions favorable for the initiation of sporocarps were not favorable for the maturation of sporocarps. Sporocarps need a relatively high soil moisture content to develop initially, but a relatively low soil moisture content to mature.

The maximum number of germinated sporocarps occurred in mid-June in water. All of the germinated sporocarps, which were Phase 3 sporocarps, germinated at the same time of the year, during the month of June. They completed the scarification process during the winter months possibly due to fungal or bacterial activity.

There was no difference in the viability of the Phase 3 sporocarps that had developed in different zones of different transects. The average percent megagametophytes formed from Phase 3 sporocarps was $49.8 \pm 3.9\%$ and the average percent of sporophytes formed was $44.6 \pm 4.1\%$.

In Phase 2 sporocarps, regardless of the zone in which they developed, the percent sporophytes formed per sporocarp was the same. There was a higher percent of megagametophytes formed from Phase 2 sporocarps that developed in zones 1 and 2 than in zones 3 and 4. This could reflect the fact that Phase 2 sporocarps from
zones 1 and 2 were more mature than the Phase 2 sporocarps from zones 3 and 4.

There was a significantly higher percent of megagametophytes formed than sporophytes from Phase 2 sporocarps. There are three possibilities: (1) The microspores remained within the sporocarp wall, thus preventing fertilization. (2) The microspores were retained within the sporocarp wall until they developed into microgametophytes. This also would prevent fertilization due to the inactivation of the sperm or the loss of receptiveness of the egg. (3) The microspores were too immature at that point in development to form microgametophytes.

Phase 2 sporocarps were not ready for germination in the fall of the season that they were produced. The percent megagametophytes and sporophytes formed were significantly lower than that of Phase 3 sporocarps due to the fact that there was a significantly higher percent of megaspores retained in the Phase 2 sporocarp walls than in Phase 3 sporocarp walls. The average percent of megagametophytes formed in Phase 2 sporocarps was 8.8 ± 2.4% and the average percent of sporophytes formed was 1.2 ± 1.1%.

If Phase 2 sporocarps were treated with heat at 60°C for 12 hours, germination proceeded normally as with Phase 3 sporocarps, with comparable percent viability.
If Phase 2 sporocarps are subjected to freezing temperatures before a heat treatment, germination is inhibited, and percent viability is comparable to untreated Phase 2 sporocarps. A heat treatment is not the only factor in sporocarp maturation. There must be a sufficient deposition of materials in the sporocarp wall to insure imperviousness to the environment.

The viability of sperm is affected by acidity. At a pH 4.2, viability is significantly lower than at a pH 5.8.

Mechanisms controlling germination are not known. From germination studies, there was evidence to support the fact that Phase 2 sporocarp walls are not impervious to the environment. A large amount of fungus grew on the walls of Phase 2 sporocarps but not on the walls of Phase 3 sporocarps.
LITERATURE CITED


Climatology of the United States, Series 86: 11-21, Climatic Summary of the United States, Supplement for 1951-1960, MONTANA.


# APPENDIX A

Climatic Data from Polson, Montana

## TOTAL PRECIPITATION

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1^From Climatography of the United States, Series 86 11-21
### Appendix A (con't.)

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APPENDIX B

COMPUTER PROGRAM FOR COMPUTING MEANS AND CONFIDENCE INTERVALS

1700 READ X
1800 IF X=999999 THEN 2300
1900 LET S=S+X
2000 LET S1=S1+X*X
2100 LET N=N+1
2200 GO TO 1700
2300 LET M=S/N
2400 LET V=(N*S1-S*S)/N/(N-1)
2500 LET S3=SQR(V)
2600 LET S4=SQR(V/N)
2700 PRINT "NUMBER", "SUM OF SQ.", "M", "VAR"
2800 PRINT N, S, S1, M, V
2900 PRINT
3000 LET Q=S3/M
3100 PRINT "STD. DEV.", "STD. ERR. M", "COEFF. V."
3101 PRINT S3, S4, "", Q
3102 IF N=96 THEN 3108
3103 IF N=91 THEN 3112
3104 IF N=5 THEN 3114
3107 GOTO 3300
3108 T=1.9854
3111 GOTO 3227
3112 T=1.9867
3113 GOTO 3207
3114 T=2.7764
3115 GOTO 3207
3200 PRINT S3, S4, "", Q
3207 LET G1=S4*T+M
3208 LET G2=M-S4*T
3209 PRINT G1, M, G2
3300 PRINT
3500 READ X
3600 IF X=999999 THEN 3950
3700 LET S=0
3800 LET S1=0
3900 LET N=0
3940 GOTO 1900
3950 STOP
APPENDIX C

Computer Program for Computing Regression Lines

250 READ N
260 FOR I = 1 TO N
270 READ X, Y
280 LET X1 = X1 + X
290 LET Y1 = Y1 + Y
300 LET X2 = X2 + X*X
310 LET Y2 = Y2 + Y*Y
320 LET Z = Z + X*Y
330 NEXT I
340 LET S1 = N*X2 - X1*X1
350 LET S2 = N*Z - X1*Y1
360 LET B = S2/S1
370 LET Y3 = Y1/N
380 LET X3 = X1/N
390 LET B1 = Y3 - B*X3
400 LET N1 = N - 1
410 LET N2 = N1 - 1
420 LET S3 = (Y2 - Y1*Y3 - B*S2/N')
430 LET S4 = S3/N2
435 LET S5 = N*Y2 - Y1*Y1
440 PRINT "NUMBER = "N,", "MEAN OF X = "X3/N,", "OF Y = "Y1/N
450 PRINT "Y-INTERCEPT = "B1
460 PRINT "SUM-OF-SQUARES", "TOTAL", Y2
470 PRINT "", "MEAN", Y3*Y1
480 PRINT "", "SLOPE", B*S2/N
490 PRINT "", "RESIDUAL", S3
500 PRINT "STANDARD DEVIATIONS"
510 PRINT "", "X", SQR(S1/N’/N1)
520 PRINT "", "Y", SQR((Y2 - Y1*Y3)/N1)
530 PRINT "", "ERROR", SQR(S4)
540 PRINT "", "Y-ERROR", SQR(S4/N)
550 PRINT "", "SLOPE", SQR(S4/S1*N)
560 PRINT "F-RATIO FOR SLOPE = " B*S2/N/S4
570 PRINT "R5=" R5
575 PRINT "R6=" R6
580 STOP
APPENDIX D

Computer Program for the Kruskul-Wallis Test

700 FOR T=1 TO C
710 FOR I=G(T-1)+1 TO G(T)
720 FOR L=1 TO M-1
730 IF X(I)<<K(L) THEN 760
740 Z=Z+R(L)
750 GOTO 770
760 NEXT L
770 NEXT I
780 D'(T)=Z
790 Z=0
800 NEXT T
810 FOR T=1 TO C
820 E=E+((D'(T)+2)/B(T))
830 NEXT T
840 H=((12/(N*(N+1)))*E)-(3*(N+1))
850 H1=H/(1-(H2/(N^3-N)))
860 PRINT "H"=";H1
861 PRINT"REJECT H0 IF H"=";D1;";ALPHA="E1
870 FOR T=1 TO C
880 FOR I=T+1 TO C
890 P(T,I)=ABS(D'(T)/N1-D'(I)/N1)
900 NEXT I
910 NEXT T
920 FOR T=1 TO C
930 FOR I=T+1 TO C
940 PRINT "P(";T;",";I;">";P(T,I)
950 NEXT T
960 NEXT I
970 NEXT T
980 READ U
990 S=U*(SQR((C*(C*N1+1))/12))
1000 PRINT "S="S
1010 PRINT
1020 FOR L=1 TO M-1
1040 NEXT L
COMPUTER PROGRAM FOR THE MULTIPLE COMPARISONS TEST

```
1 DIM A(500),X(500),K(500),J(500),R(500)
2 DIM B(18),G(18),P(18,18),D(18),CIS(18)
3 READ N1,C
4 DIM BIS(50)
5 FOR I=1 TO C
   6 B(I)=N1
   7 NEXT I
8 N=N1*C
9 FOR T=1 TO C
   10  NEXT T
11 READ D1,E1
12 FOR I=1 TO N
13  READ X(I)
14  A(I)=X(I)
15  NEXT I
16 FOR I=1 TO N=1
17 FOR K1=I+1 TO N
18 IF A(I)<=A(K1) THEN 110
   19 F=A(I)
   20 A(I)=A(K1)
   21 A(K1)=F
   22 NEXT K1
23 NEXT I
24 L=1
25 FOR I=1 TO N
26 IF A(I)=A(I+1) THEN 330
   27 J(L)=I
   28 K(L)=A(I)
29 L=L+1
30 NEXT I
31 M=L
32 FOR L=1 TO M-1
33 IF J(L)=J(L-1)=1 THEN 580
34 FOR I=0 TO (J(L)-J(L-1))-1
35 Q=(J(L)-I)+Q
36 NEXT I
37 R(L)=Q/(J(L)-J(L-1))
38 H2=H2+(J(L)-J(L-1))(3-(J(L)-J(L-1)))
39 Q=0
40 GOTO 590
41 R(L)=J(L)
42 NEXT L
43 FOR I=1 TO C
44  O=O+B(I)
45  G(I)=O
46  NEXT I
47 FOR T=1 TO C
```

APPENDIX E