Life History Strategies of two Lemnaceae, *Lemna minor and L. trisulca* at Delta Marsh, Manitoba.

by

Alexander L. McIlraith

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CONTENTS

Chapter page

ABSTRACT iii

ACKNOWLEDGEMENTS v

I. LITERATURE REVIEW 1

  1.1 Habitat, taxonomy and range of Lemna 1

  1.2 Morphology, clonal growth and asexual propagation 2

  1.3 Ecological studies of Lemnaceae 5

  1.4 Justification and Objectives of research 7

II. THE NATURE AND ROLE OF SEXUAL REPRODUCTION 8

  2.1 Intoduction 8

  2.2 Materials and methods 9

  2.3 Results and discussion 12

III. INTERACTION AND COMPETITION 32

  3.1 Introduction 32

  3.2 Materials and methods 34

    3.2.1 Site description and environmental measurements 34

    3.2.2 Competition experiments 35

    3.2.3 Transplant experiment 37

    3.2.4 Mat sampling 38

  3.3 Results 39

    3.3.1 Environmental measurements 39

    3.3.2 Competition experiments 39

    3.3.3 Transplant experiment 48

3.3.4 Mat sampling....48

3.4 Discussion....51

SUMMARY....66

LITERATURE CITED....69

ABSTRACT

Lemna minor L. and *L. trisulca* L. flowered abundantly in Delta Marsh, Manitoba. Flowering in *L. trisulca* began in early May, peaked by about June 1 and was finished by early July. The phenology of *L. minor* lagged behind that of *L. trisulca* by about three weeks. Pollen grains and seeds of both species were examined with scanning electron microscopy. Although pollen morphology of the two was similar, *L. trisulca* seed differed from those of *L. minor* in having a logitudinally ribbed surface. Seed - bearing fronds of both species were observed. Seed set varied between years and within sites, and in some sites plants produced no seed. Peak seed densities occurred 3-6 weeks after biomass of flowering plants peaked, indicating that seed may take 3-6 weeks to develop. Seed of both species exhibited dark dormancy, and tolerated drying and freezing. A large *Lemna* seedbank was present at Delta Marsh, but seedlings were not observed in nature even though greater than 95% of seed extracted from sediment was viable. In the laboratory, seeds floated after being dried, and germinated at the water surface. This suggested that drought, followed by rain might liberate seed from sediment and permit it to germinate. It is suggested that the retention of sexuality and tolerance of seed to drought and freezing may function as insurance, allowing some of a clone's genes to survive periods of environmental change.

Replacement series, addition series and 'introduction' experiments were conducted to assess interactions between *L. minor* and *L. trisulca* in the field. In addition, a transplant experiment was conducted to determine whether various sites were capable of supporting a codominant community of *L. minor* and *L. trisulca.* Vegetative biomass trends of natural populations were monitored in three sites. Results suggested that the two species may compete for light and nutrients. One - way competition for light may give *L. minor* a competitive advantage in eutrophic habitats, while nutrient competition may favor *L. trisulca* in sunny oligotrophic habitats. In a sunny eutrophic habitat, *L. trisulca* dominated in spring and fall, while in summer *L. minor* dominated. Dominance patterns, in time and space, of *L. minor* and *L. trisulca* were explained with a resource competition model (for light and nutrients), and by considering environmental factors and life history.

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I have gained much from this two year experience that will never be reflected in a thesis. This time has left me changed. I know and understand some things more clearly, yet there is so much more to understand. I did not do this alone - I treasure the friendships of the people I have known during this time, and the happiness we've shared.

LIST OF TABLES

Table page

2.1: Survival of overwintered propagules. 26

2.2: Site temperatures (°C) for overwintering experiment. Temperatures for levels above the sediment, the ice - snow interface, and air (overnight low) are listed. 27

3.1: Mean water pH, and conductivity in *Lemna* sites. 40

3.2: Amount of shading in *Lemna* sites. 41

3.3: Pure stand densities in replacement series experiments. 44

3.4: Mean frond ratio (Output/Input) in addition experiment. 46

3.5: Mean frond ratio (Output/Input) in introduction experiments. 47

3.6: Mean dry weight per quadrat in transplant experiment. 49

LIST OF FIGURES

Figure page

2.1: Flowering fronds of *L. minor* and *L. trisulca*.______ 13

2.2: Seasonal patterns of flowering biomass in Mixed ditch.______ 15

2.3: Seasonal patterns of *Lemna* flowering biomass in Minor pond, and in Trisulca pond.______ 16

2.4: Scanning electron micrograph of *L. minor* pollen grain.______ 18

2.5: Scanning electron micrograph of *L. minor* and *L. trisulca* seeds.______ 20

2.6: Seasonal patterns of *Lemna* seed number in Mixed ditch.______ 21

2.7: Seedlings of *L. minor* and *L. trisulca*.______ 24

3.1: Ratio diagrams for replacement series experiments in Mixed ditch.______ 42

3.2: Ratio diagrams for replacement series experiments in Minor pond.______ 43

3.3: Seasonal patterns of *Lemna* dominance in Mixed ditch.______ 50

3.4: Seasonal patterns of *Lemna* dominance in Minor pond and Trisulca pond.______ 52

3.5: Hypothetical net growth curves for *L. minor* and *L. trisulca*.______ 57

Chapter I

LITERATURE REVIEW

1.1 HABITAT, TAXONOMY AND RANGE OF LEMNA

Extensive reference lists for the family Lemnaceae (duckweeds) can be found in Hillman (1961), Landolt (1980) and Kandeler (1985). Duckweeds are floating aquatic plants closely related to the Araceae (Meheshwari and Kapil, 1963), and fossil evidence for the family dates back to at least the Tertiary (Sternberg, 1924). *Lemna* is found mostly in still, slow moving, or stagnant waters, where eutrophic conditions tend to encourage the growth of large populations (Hillman, 1961). In North America, members of the family occur in waters with pH ranging from 6.4 to 10.4, specific conductivities from 17 to 4400 $\mu S \cdot cm^{-1}$ (Landolt, 1981), and in both heavy shade and full sun (Hillman, 1961). This study is concerned with *L. minor* and *L. trisulca*. The former floats at the surface, while the latter is submerged.

When attempting to identify Lemnaceae, Landolt's (1983) key is the most useful. Landolt (1975) suggests that *L. minor* s. l. should be divided into *L. minor* L. and *L. turionifera* Landolt s. str. *L. turionifera* tends to have 3-7 papules on its upper surface, to produce turions (see below) and to have red pigmentation on the underside of fronds. *L. minor* s. str. rarely has distinct papules, does not produce turions and is unpigmented. Landolt (1981) indicates that *L. turionifera* occurs in Manitoba while *L. minor* s. str. does not. Clones of *L. minor* s. l. isolated from Delta Marsh, Manitoba (50°

11'N, 98° 23'W) fit the description of *L. turionifera* s. str. while one clone isolated north of Powerview, Manitoba (50° 34'N, 96° 10'W) by Dennis Brown (Technical Services, Manitoba Department of Natural Resources) fits the description of *L. minor* s. str. Dudley (1987) showed that *L. minor* L. clone 6591, collected by Landolt (1957), could produce turions and suggested that observed differences between *L. minor* s. str. and *L. turionifera* may depend on growing conditions. Although clone differences exist, there is doubt that differences are sufficient to justify Landolt's (1975) division of *L. minor* L. For this reason, I use the binomial *L. minor* L. in the sense of *L. minor* L. s. l. There appears to be no such confusion over the naming of *L. trisulca* L.

The Lemnaceae have a world-wide distribution (Landolt,1984). Both *L. minor* and *L. trisulca* have a circumpolar distribution, with *L. trisulca* having a slightly extended northern limit. Both have been recorded in Africa and Australia, but *L. trisulca* has not been recorded in South America (Landolt, 1975, 1984).

1.2 MORPHOLOGY, CLONAL GROWTH AND ASEXUAL PROPAGATION

Although the Lemnaceae reproduce sexually (see Chapter 2), they are best known for their prolific clonal growth and propagation. Information in this section is from Hillman (1961) unless otherwise specified. The basic structural units in Lemnaceae are 'fronds' which are leaflike but also possess stem characteristics like lateral meristems.

Fronds are iterated functional units or modules (Harper, 1977). New 'daughter' fronds are produced by lateral meristems located in pockets found at the narrow end of the 'mother' frond. The narrow end of a daughter frond is called proximal (close to the mother frond) while the wide end is called distal (far from the mother frond). Often, several generations of daughters remain attached to the mother and thus form 'colonies'. A given clone consistently produces a daughter first from a pocket on one side (+ pocket), then the other (- pocket). When they occur, flowers are in the minus pocket.

Almost the whole plant is chlorenchymatous. In floating forms the upper epidermis is cutinized and contains stomata, while the lower epidermis lacks these characteristics. Rudimentary vascular tissue occurs in both fronds and roots. Roots, which are thought to be adventitious, lack root hairs, have a root cap and contain chloroplasts. Evidently, roots have no significant role in nutrient uptake (Muhonen *et al.* 1983); the submerged surface of the frond serves this purpose (Hillman, 1961). Roots may, however, function to tangle plants together or to keep them upright.

Growth rate, which is exponential under non-limiting conditions, is thought to be best determined by counting fronds (Hillman, 1961). Datko *et al.* (1980) provide a detailed characterization of growth patterns in *Lemna paucicostata.* During exponential growth, a stable distribution of different frond stages (daughter generations) is attained and is independent of the initial stage distribution. Since a stable stage distribution develops, experiments involving *Lemna* may be started with fronds of a heterogeneous initial stage distribution (Datko *et al.*,1980).

Although fronds and frond colonies may serve as propagules, specialized fronds may be produced. Many aquatic plants overwinter in the form of dormant buds or 'turions' which serve as propagules (Vintéjoux, 1982). In the Lemnaceae, turions are fronds that have small intercellular spaces, high cellular starch content, roots which are short or lacking, and in the case of *Spirodela polyrrhiza* (and often *L. minor*), much red pigment. They are heavy and sink to the bottom. Turions were first noted in *Spirodela polyrrhiza* and later in *L. minor, L. gibba* and *Wolffia* (Hillman, 1961). *L. trisulca* does not appear to produce turions, although it becomes pigmented in the fall.

Most information on turion behavior is based on studies of *Spirodela polyrrhiza* because it is best known, but *L. minor* turions behave similarly. Conditions of short day length, nutrient deficiency and low temperature appear to encourage turion formation (Sibasaki and Oda, 1979; Malek and Oda, 1980; Jungnickel, 1986). Turions are often dormant when produced and may require a period of after-ripening (Jungnickel, 1986) or cold treatment (Jacobs, 1947) before they germinate. They also normally require light to germinate (Newton et al., 1978). Turion germination occurs unevenly in time (Sibasaki and Oda, 1979), but this heterogeneity can be reduced by subjecting turions to mild osmotic stress (Malek, 1981). When turions begin to germinate, each develops a gas bubble which carries it to the surface. Turions break through the surface film, are held at the surface by surface tension, and produce fronds under suitable conditions (Jacobs, 1947).

1.3 ECOLOGICAL STUDIES OF LEMNACEAE

In studying Minnesota Lemnaceae, Jacobs (1947) found that *L. trisulca* was "the most uniformly distributed species", but was easily overlooked when hidden by floating species. Monospecific stands of *L. minor* were found in both heavily shaded and unshaded sites. *L. minor* and *L. trisulca* often coexisted, with *L. trisulca* appearing to dominate in spring before the canopy of *L. minor* closed. Monospecific stands of *L. trisulca* were uncommon. *L. minor* produced turions starting in early August. Jacobs (1947) believed that Lemnaceae were most likely to be dispersed by running water, aquatic mammals and floods. Waterfowl during fall migration were considered potential but poor dispersal vectors. Flowering in both species was observed.

Lawalrée (1961), Den Hartog (1964) and Rejmánková (1975a) reported abundant flowering in *L. minor, L. trisulca* and *L. gibba* respectively. The first two authors were mainly interested in pollination mechanisms. No studies have attempted to determine the extent and importance of flowering and seed set in a *Lemna* population.

Landolt (1957) conducted ecophysiological studies on Lemnaceae collected from across North America, including one clone from Delta Marsh (*L. minor* I, 6853). He compared species and clones on the basis of growth response to varying nutrient concentration, light intensity and temperature. He found that some species, including *L. minor*, flowered under conditions of high light intensity and water temperature.

The initial study of competition among free floating aquatics was conducted by Clatworthy and Harper (1962). They found that competition occurred between Lemnaceae, and between Lemnaceae and *Salvinia*. Outcomes of competition between pairs of species could not be predicted from growth parameters determined in monoculture (such as final yield, arithmetic and exponential growth rates) alone. Although not explicitly stated, shading ability was suggested as one important factor involved in determining competitive outcomes.

McLay (1974) examined environmental factors limiting the distribution of *L. perpusilla* in a California lake. Plants did not occur in open water because wind and wave action quickly moved them towards shore. In dense stands of submerged macrophytes, they grew poorly because of the high pH induced by macrophyte photosynthesis. Dense mats of *Lemna* developed in sheltered openings in the emergent macrophyte border of the lake. Densities were low within dense emergent macrophyte stands.

Rejmánková (1973,1975a) studied seasonal dynamics of growth in *L. gibba*, relating relative growth rates in the field to light and temperature. Rejmánková (1975b) made one of the first attempts to explain the distribution of *Lemna* (*L. gibba* and *L. minor*) based on considerations of environmental factors (e.g. water nutrient status), plant morphology and interspecific competition. Rejmánková (1975a) suggested that it was *L. gibba*'s ability to overtop *L. minor* that allowed it to dominate in eutrophic waters. Keddy (1976) studied competition for light in *L. minor* and *L. trisulca*, and found that *L. minor* could inhibit its own growth (by shading itself) more than it could limit *L. trisulca*. *L. trisulca* was able to grow when shaded by *L. minor*. He used biogeographical theory to predict that *L. minor* should occur more frequently in water bodies on the basis of its superior dispersal and establishment ability, and equal or greater competitive ability (for light only).

Landolt and Wildi (1977) examined communities of Lemnaceae in the southwestern U.S.A., using multivariate techniques to establish relationships between water nutrient status and community composition. Dissolved nitrogen and magnesium were correlated with differences in species distribution patterns. Climatic factors (aridity and temperature patterns) and competition were also deemed important in explaining distribution patterns.

Although there is a large amount of literature available on other aspects of *Lemna* biology, such as physiology, embryology and development (Landolt,1980), this material is beyond the scope of this study.

1.4 JUSTIFICATION AND OBJECTIVES OF RESEARCH

Sexual reproduction of *L. minor* and *L. trisulca* in nature, and the effect of competition between the two species on their dominance patterns is poorly understood. This study was undertaken to: 1) obtain a better understanding of natural sexual reproduction in *L. minor* and *L. trisulca* and 2) develop an understanding of the factors, particularly interspecific competition, which influence dominance patterns of these species in space and time.

Chapter II

THE NATURE AND ROLE OF SEXUAL REPRODUCTION

2.1 INTRODUCTION

Harper (1977) defines reproduction as the development of a new individual from a single cell, and growth as the production of a new individual from a multicellular meristem. For this reason, I use 'clonal growth' to refer to the production of frond colonies, and 'propagation' for the fragmentation of colonies or production of turions from meristems in *Lemna.*

Little is known about sexual reproduction in *Lemna.* It is commonly suggested that flowers rarely occur (Arber, 1920; Hillman, 1961; Keddy, 1976) and that seed set is poor (Caldwell, 1899). At Delta Marsh, Manitoba, however, *Lemna minor* L. and *Lemna trisulca* L. flowered consistently and profusely throughout the period of this study. This chapter describes the sexual life history, and documents flowering and fruiting phenology of *Lemna* at Delta Marsh. The role of the seed habit in *Lemna* has not been previously assessed. To begin to assess this, a field experiment was conducted to determine whether or not seeds survived better than asexual propagules after overwintering at different water depths. The role of the sexual reproduction and the seed habit in the life history of the two species is discussed.

2.2 MATERIALS AND METHODS

Observations were made primarily at the University of Manitoba Field Station (Delta Marsh)(50° 11'N, 98° 23'W) in 1986-87. The mean January temperature at Delta is -19.8°C±2.9SD, and the mean July temperature 19.1°C±1.2SD (Environment Canada, n.d.). The area averages 1600 degree days (above 5.5°C) and 125 frost free days (Weir, 1983). The study was conducted at three sites:

Mixed ditch supported *Lemna minor* and *L. trisulca* in approximately equal proportions. This site is an L-shaped drainage ditch which is constricted at the bend into a north-south arm and an east-west arm. It is 3-4m wide and its depth varies between 0.3m and 1.0m as marsh water flows in and out through a connection with the marsh. Sediment is highly organic silty - clay with some fine sand. The site is bordered by *Salix interior* to the north and by *Typha* spp. and *Phragmites australis* in other directions. Shading is mostly limited to the edges.

Minor pond was dominated by *L. minor*. and has no direct connection with the marsh. It is 2m wide, 15m long and its depth varies between 0.1m and 0.8m. Sediment is similar to that described above. This site is heavily shaded in summer by *Acer negundo*, *Salix interior* and *Populus deltoides*.

Trisulca pond was dominated by *L. trisulca*. It is located about 35km from the University of Manitoba Field Station. The surrounding area is marginal farmland and aspen parkland. Surface deposits of calcareous glacial till are evident. Sediments have a high proportion of stone and appear to be much less organic than the other two sites. The dugout is well sheltered by *Salix* spp. on all sides, is deep (>3m) and has a large area of relatively unshaded water. *Spirodela polyrrhiza* was present but rare.

An overwintering experiment was conducted in 1986 in the Mixed ditch and Minor pond. Seeds were obtained from Mixed ditch sediment collected with an Eckman Grab (sample area=232cm^2) in September 1986. Sediment was washed through 1.6mm mesh and 0.3mm mesh sieves, and seeds removed with forceps from organic material caught by the fine mesh. *L. trisulca* fronds and *L. minor* turions were collected one week prior to freeze-up from the Mixed ditch and placed in plastic containers. Fifteen seeds and fronds of *L. trisulca* and 25 seeds and turions of *L. minor* were placed into each of twenty-four 250mL Nalgene bottles filled with wet silica sand (the different number of propagules used for the two species reflects difficulty in obtaining sufficient numbers of *L. trisulca* propagules). An 8mm hole was drilled in bottle caps and covered with 0.3mm nylon mesh to permit water exchange. All material was stored at 4°C until bottles were placed in the field.

In the first week of November, three wooden stakes were placed in the two sites in water 80cm deep. Bottles were assigned randomly to stakes and were attached with stainless steel wire at 0cm, 25cm, 50cm or 75cm above the bottom.

Thermistor probes were attached to a fourth stake at each site (one probe per level). These were encased in 13mm copper pipe (to prevent ice and muskrat damage to cables). Temperatures were taken approximately every 21 days with a YSI telethermometer at each level and at the ice -

snow interface. The overnight low air temperature was obtained from University Field Station weather records.

Bottles were removed in late April, 1987 as the ice thawed, and propagules were extracted from the sand with feather forceps. They were first placed into 125mL Ehrlenmeyer flasks which contained 50mL of Woods Hole MBL growth medium (Nichols, 1973). Flasks were plugged with foam stoppers. Dense algal growth occurred and hindered germination, so propagules were transferred to 50mL distilled water where they subsequently germinated well. Flasks were placed in a controlled environment chamber under continuous illumination (ca. $200 \mu E \cdot m^{-2} \cdot sec^{-1}$ P.A.R) at 25°C. Flasks were checked periodically, and seedlings, germinated turions and living *L. trisulca* fronds counted and removed. Turions and fronds which had no trace of greenness were considered to be dead. Flasks were monitored until late August, at which time only a few ungerminated seeds remained. These were assumed to be either dead or dormant.

Propagule survival was expressed as the ratio of propagules alive after treatment to the total treated. A completely randomized one-factor ANOVA model was used. Data for all variables were normally distributed. Analyses were made using GLM and UNIVARIATE of SAS (SAS Institute, Inc., 1982a,b).

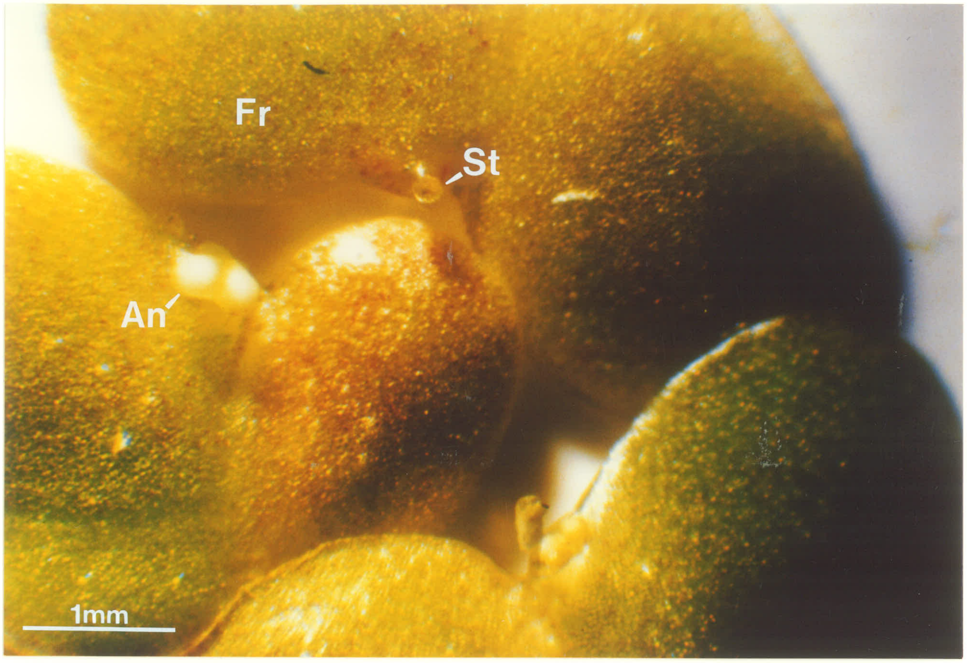
Phenological trends in two of the *Lemna* communities (Minor pond and Mixed ditch) were monitored in 1986 (June to October). In 1987, all sites were sampled for this purpose. Plants were sampled with a circular sieve (area=$45.6 cm^2$). Random samples were taken in Mixed ditch (n=12), Trisulca pond (n=12) and Minor pond (n=6) about every 21 days, starting June 24, 1986 Since phenological trends seemed different in the north - south and east - west arms of the Mixed ditch, sampling was stratified (6 samples taken in each arm of the ditch). Sampled material was placed in 9cm diameter plastic petri dishes and stored at 4°C until sorted.

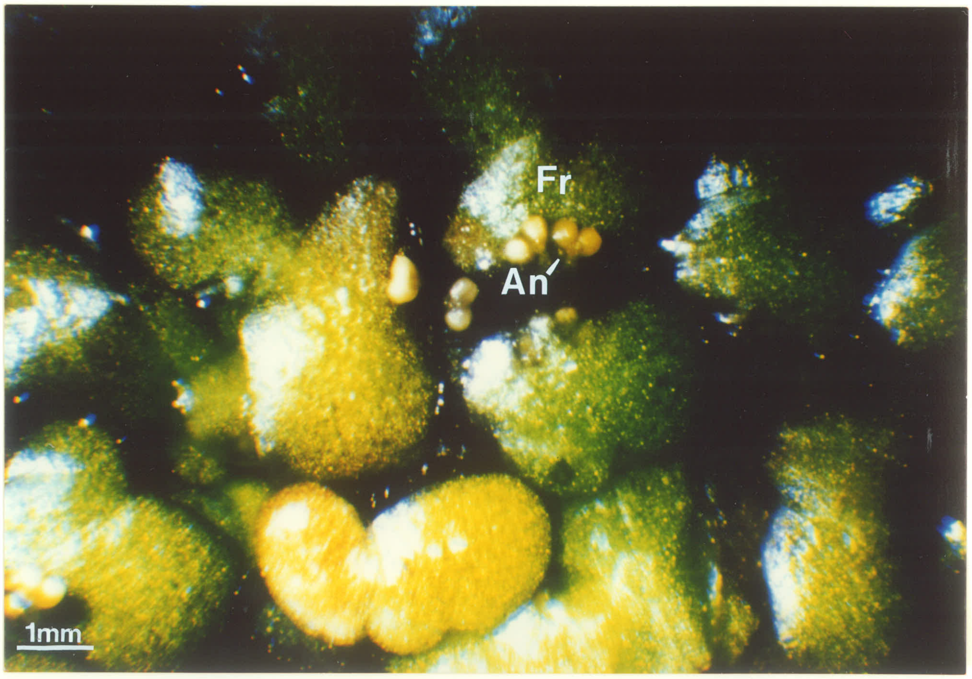
Samples were sorted by placing small amounts of *Lemna* in a white enamel pan (half filled with water), and separating fronds of each species by hand with a small triangular sieve (area=2cm^2) and forceps. Flower- bearing fronds were separated from vegetative ones in 1987. Fertile fractions were oven - dried at 110°C and desiccated over silica gel. A Cahn electrobalance used to measure dry weight to ±0.01mg. The number of mature fruits was recorded.

2.3 RESULTS AND DISCUSSION

Flowers of *L. minor* (Fig. 2.1a) and *L. trisulca* (Fig. 2.1b) occurred commonly in Delta Marsh. Casual searches at Morden, Manitoba and Buffalo Point, Manitoba also produced flowering *Lemna* plants. Jacobs (1947) suggests that, in Minnesota, *L. minor* flowers are not rare. Flowers of *L. minor* and *L. trisulca* were protogynous, as Hillman (1961) had suggested for Lemnaceae in general. While the morphology of flowering and vegetative fronds was similar in *L. minor*, flowering fronds in *L. trisulca* did not float beneath the surface as vegetative fronds did, but rather floated at the surface, develops a cuticle on part of their upper surface. This is similar to *Vallisneria* in which.male flowers detach from submerged plants and float on the surface (Faegri and Pijl, 1979). Frond tips curl down into the water and appear uncutinized. The surface exposed to air develops stomata (Den Hartog, 1964).

Figure 2.1: Flowering fronds of A)*Lemna minor* and B)*L. trisulca* (Fr=frond, St=stigma An=anther).


Fr
St
An
1mm


Fr
An
1mm

In both sections of Mixed ditch (1987), *L. trisulca* began flowering (Fig. 2.2a,b) on 7 May, peaked by about 1 June and finished flowering by 3 July *L. minor* flowering phenology lagged behind that of *L. trisulca* by about three weeks in the north - south arm; in the east - west arm, the onset time of flowering corresponded with that for *L. trisulca.*

In Minor pond a small flowering biomass of *L. minor* was recorded only on 3 July. (Fig. 2.3a). As Hicks (1932) and Den Hartog (1964) have noted, flowering rarely occurs under shaded conditions. The largest flowering biomass was recorded in Trisulca pond (Fig. 2.3b) where *L. trisulca* phenology was almost identical to that observed in Mixed ditch.

Kandeler (1985), in reviewing recent work on the physiological basis for flower induction in the Lemnaceae, concluded that although much work has been done, a consistent synthesis of information could not be made. Of the inducing factors he discusses, both photoperiod and nitrate assimilation might have ecological significance. He provided no information on *L. trisulca* flowering, but indicated that *L. minor* is a long - day plant. Tanaka *et al* (1986) showed that lowered nitrate availability or nitrate reductase inhibition (by enzyme inhibitors or by molybdenum deficiency) induced day neutral flowering in *L. paucicostata.* The similar flowering phenology of a given species in different sites suggests that a cue for flowering common to different sites may be involved. A photoperiodic response may be occurring and *L. trisulca* may respond to a somewhat shorter photoperiod than *L. minor.*

Figure 2.2: Seasonal patterns of *Lemna* flowering biomass in Mixed ditch: A) north - south arm, B) east - west arm. Biomass of flowering fronds of *L. minor* (——) and *L. trisulca* (— - —). Values are means ± SE.

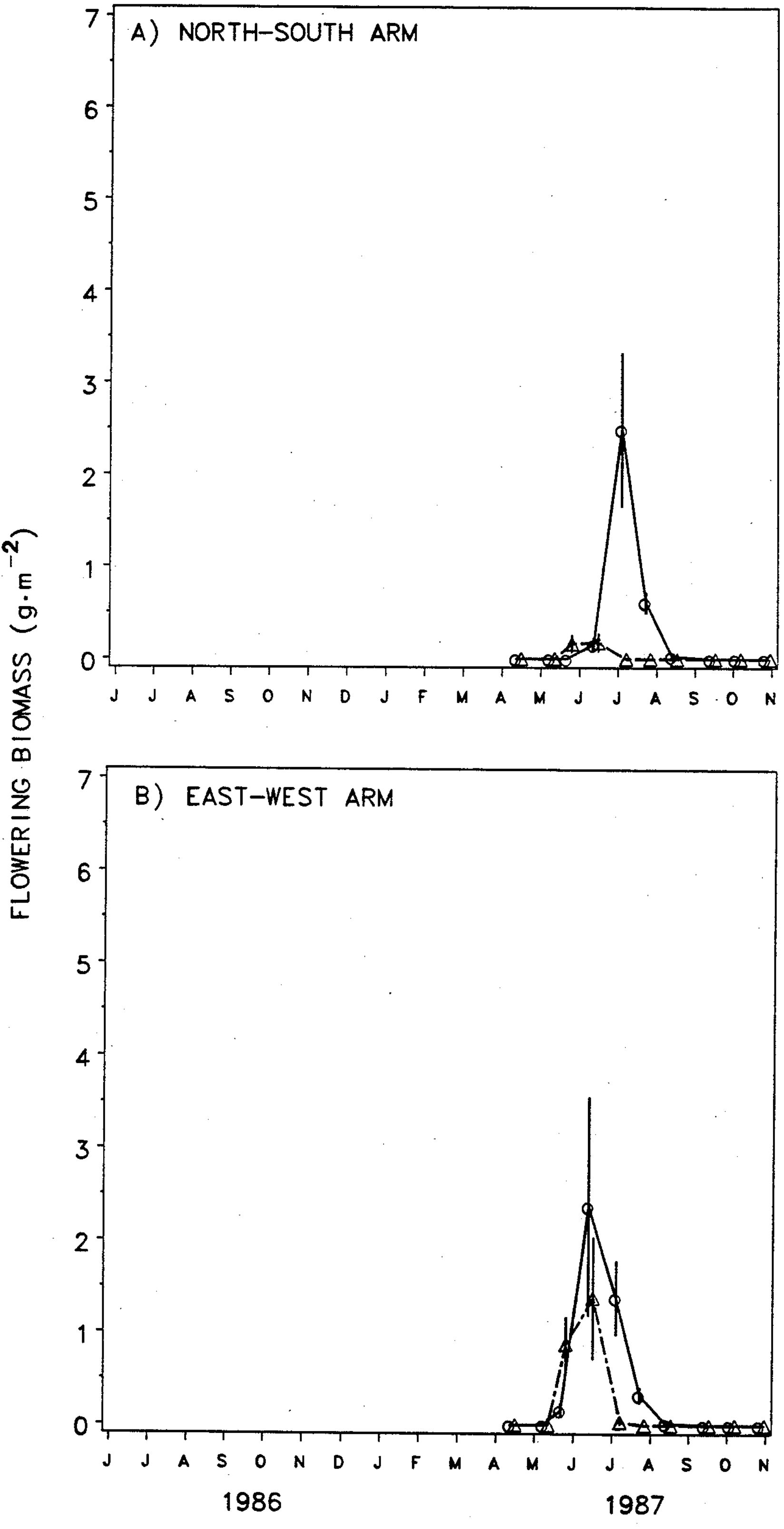
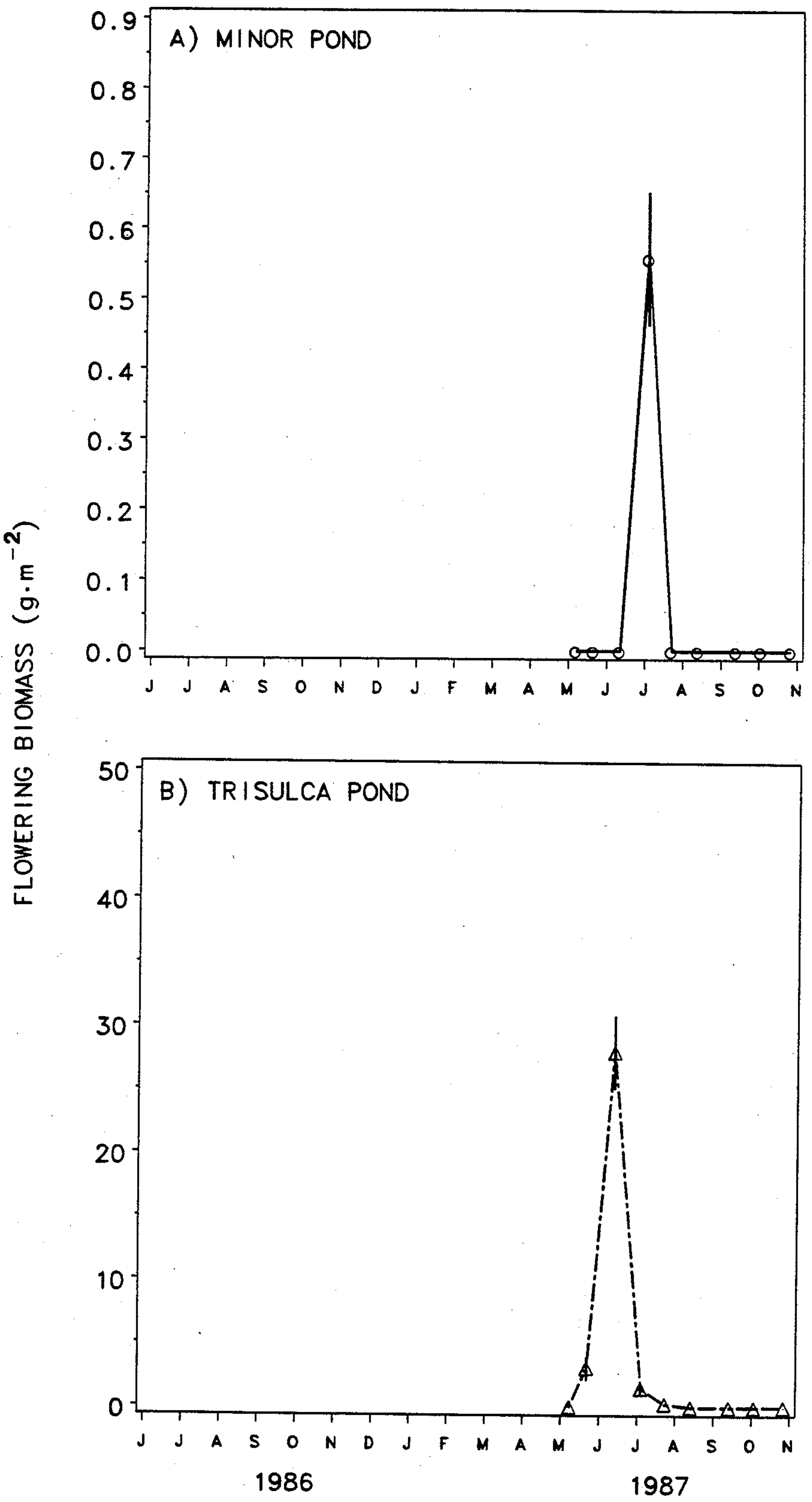

A) NORTH-SOUTH ARM
B) EAST-WEST ARM
FLOWERING BIOMASS ($g \cdot m^{-2}$)
0
1
2
3
4
5
6
7
J J A S O N D J F M A M J J A S O N
1986
1987

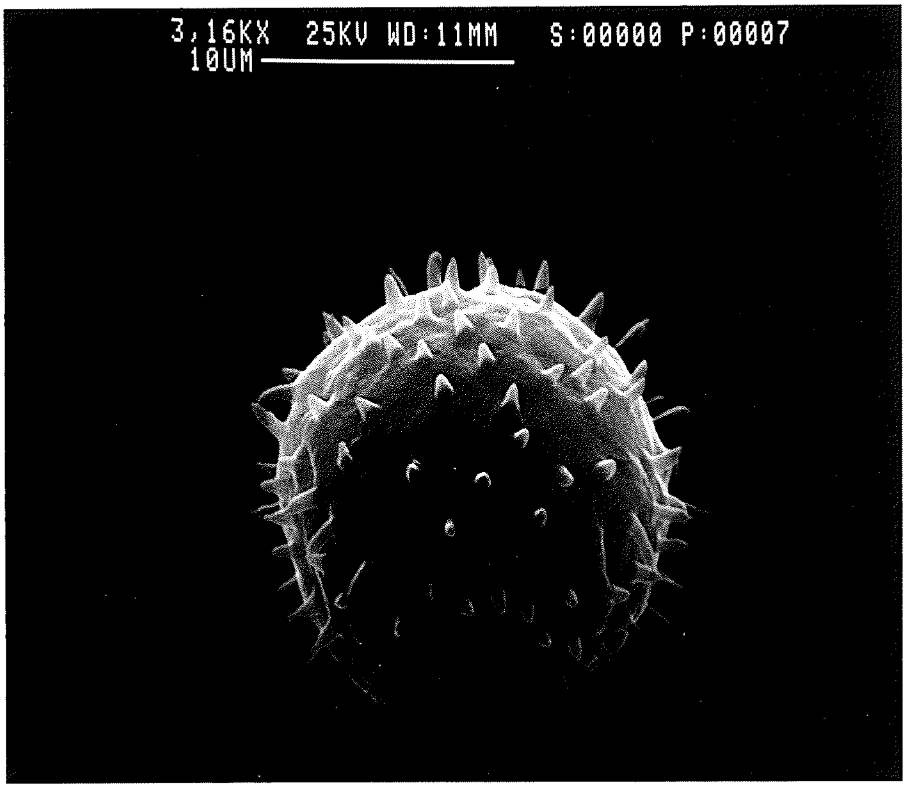
Figure 2.3: Seasonal patterns of *Lemna* flowering biomass in: A) Minor pond, B) Trisulca pond. Biomass of flowering fronds of *L. minor* (——) and *L. trisulca* (— - —). Values are means±SE.


A) MINOR POND
B) TRISULCA POND
FLOWERING BIOMASS (g·m⁻²)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
50
40
30
20
10
0
J J A S O N D J F M A M J J A S O N
1986
1987

Scanning electron microscope (SEM) pollen morphology was similar in both species (Fig. 2.4, grain from *L. minor* only). Grains are spherical, 15-20µm in diameter and covered with irregularly arranged conical spines ca. 1.5µm in length. SEM morphology illustrated in Martinsson (1984) for Swedish *L. minor* is similar except that spines in her material appear denser.

Suggested pollination mechanisms for *Lemna* include: anemophily , hydrophily and animal pollination (Arber, 1920). Wind pollination seems unlikely because the stigmatic area is small and pollen production low. Pollen capture by stigmas from water seems unlikely since stigmas are held above the surface. If plants are submerged and pollen is able to survive wetting, 'hyphydrophily' (pollination in water; Faegri and Pijl, 1979) may be possible. This seems to be an unlikely possibility, since *L. trisulca* does not remain submerged when it flowers. These plants may be pollinated by direct contact of anther and stigma in both *L. minor* (Lawalrée, 1961) and *L. trisulca* (Den Hartog, 1964). This is like the 'ephydrophily' (pollination on the water surface) of *Vallisneria* described in Faegri and Pijl (1979). Although this may work with *L. minor*, it seems more difficult to imagine in *L. trisulca* where flowers are often located in the right angle subtended by mother and daughter fronds. The presence of spiny pollen and the abundance of potential invertebrate pollen vectors occurring on *Lemna* mats (Insecta, Arachnida, Gastropoda and Hiridinea) suggested that animal pollination was also possible. The relative importance of ephydrophily vs. invertebrate pollination remains unclear.

Figure 2.4: Scanning electron micrograph of *L. minor* pollen grain.

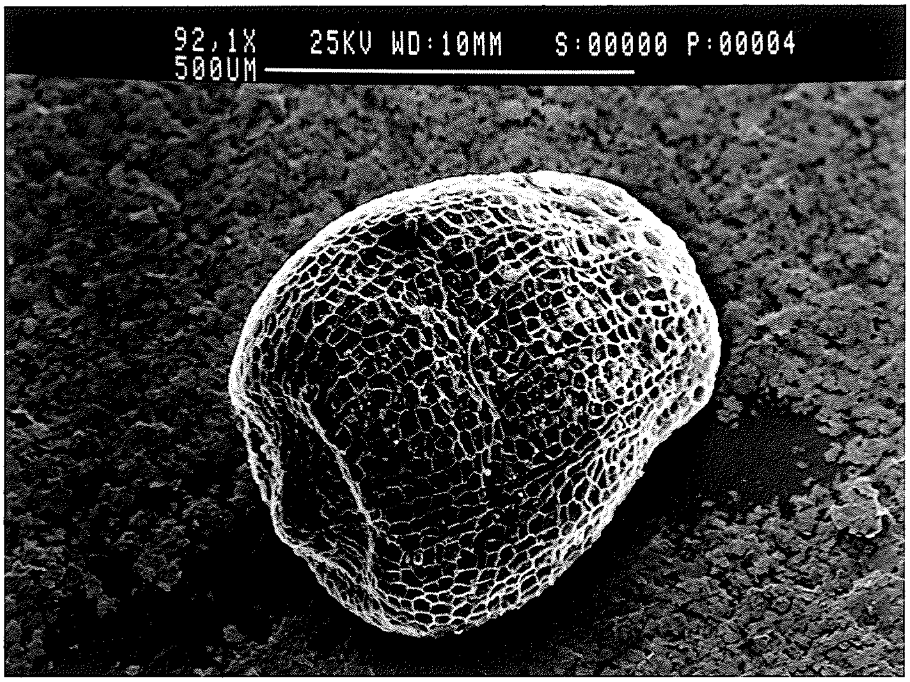

3,16KX 25KV WD:11MM S:00000 P:00007
10UM

Seeds of both species are contained in utricles (Smith, 1977) which remain green until the seed has matured. Seed (Fig. 2.5a,b) can be obtained by inspecting fresh plants or by sieving sediment. Seeds are 600-900μm long and are covered with a reticulate pattern of rectangular concave depressions (25 x 25μm) when dry. The *L. trisulca* seed was longitudinally ridged. Seed germinated well (> 95%) in distilled water under fluorescent light, but not in the dark or under incandescent light, suggesting that light is required for germination (dark dormancy). The observation of dark dormancy in *L. minor* and *L. trisulca* is not surprising given that similar responses have previously been found for seeds of *L. perpusilla* (Posner and Hillman,1962) and *L. gibba* (Rejmánková, 1976).

Seed-bearing fronds were only found in Mixed ditch (Fig. 2.6a,b), where the number of seeds produced suggests that sexual reproduction is an important part of *Lemna* life history. There is large variability in seed set between arms of the ditch, and between years in both species. *L. minor* seed density peaked in mid to late July. *L. trisulca* peaked before *L. minor* in 1986, but in 1987 it set little seed and peaked at about the same time. Comparison of peaks for flowering with those for mature seed density suggests that it may take from three to six weeks for seeds to mature.

The mechanism of dark dormancy involves phytochrome, with red light (R) stimulating and far-red light (FR) inhibiting germination (Smith,1982). Induction of dark dormancy in seeds of some plants, may result from the filtering of light reaching the seed. Chlorophyll in surrounding green tissue removes red light, and the developing seed receives light with a low R:FR ratio. Seeds exposed to low R:FR during development exhibit dark dormancy, while those developing in light with

Figure 2.5: Scanning electron micrograph of seeds: A) *Lemna minor* and B) *L. trisulca*.


92,1X 25KV WD:10MM S:00000 P:00004
500UM

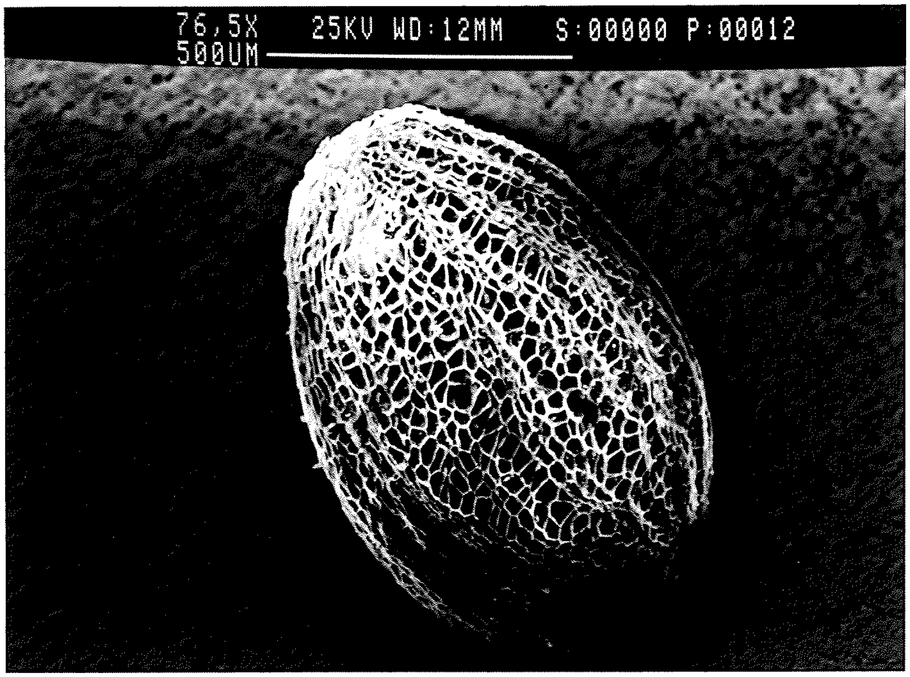
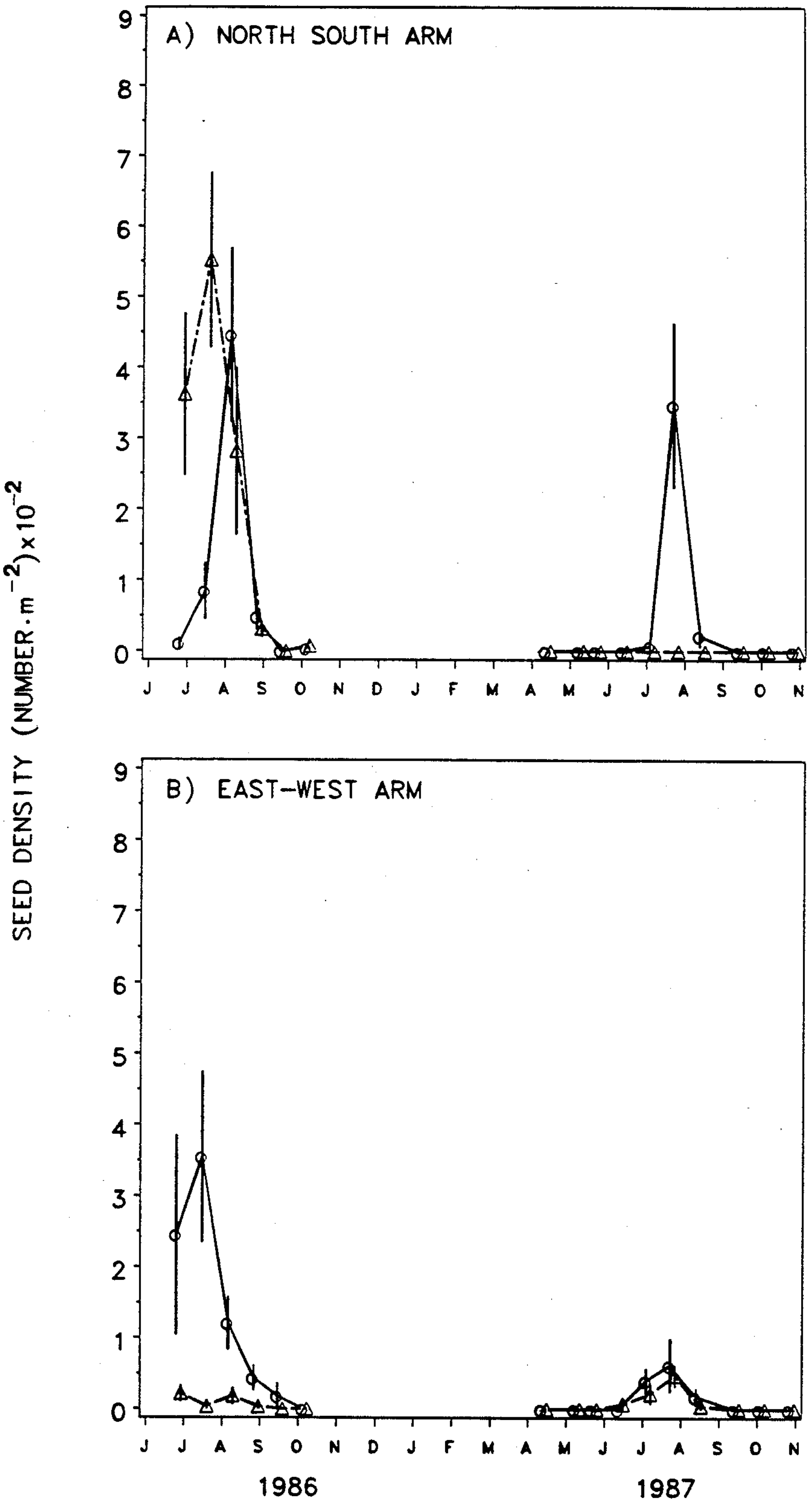

76,5X 25KV WD:12MM S:00000 P:00012
500UM

Figure 2.6: Seasonal patterns of *Lemna* seed number in Mixed ditch: A) north - south arm, B) east - west arm. Seed density for *L. minor* (——) and *L. trisulca* (— - —). Values are means±SE.


A) NORTH SOUTH ARM
B) EAST-WEST ARM
SEED DENSITY (NUMBER · m^{-2}) x 10^{-2}
0
1
2
3
4
5
6
7
8
9
J J A S O N D J F M A M J J A S O N
1986
1987

normal R:FR do not (Cresswell and Grime, 1981). *Lemna* seed behavior is consistent with this; seeds develop within a green utricle and often remain enclosed in it after fruit has fallen from the frond. Interestingly, seeds that become coated with green algae also fail to germinate, probably because algal chlorophyll attenuates red light.

Germination of seed in the dark or beneath dense mats of *Lemna* is probably disadvantageous. Seedlings that germinate in sediment are unlikely to survive because they do not develop an erect axis and are unable to grow out of the sediment. Seedlings that germinate beneath dense *Lemna* run a high risk of being outcompeted. A wavelength - dependent germination requirement would permit seeds to avoid both situations; in the dark or under a canopy of green plants, phytochrome would inhibit germination. Pons (1986) suggested that *Plantago major* seeds may not even germinate in early spring due to the low R:FR ratio of spring sunlight, and that the dark dormancy is responsible for the accumulation of seeds of some species in seedbanks.

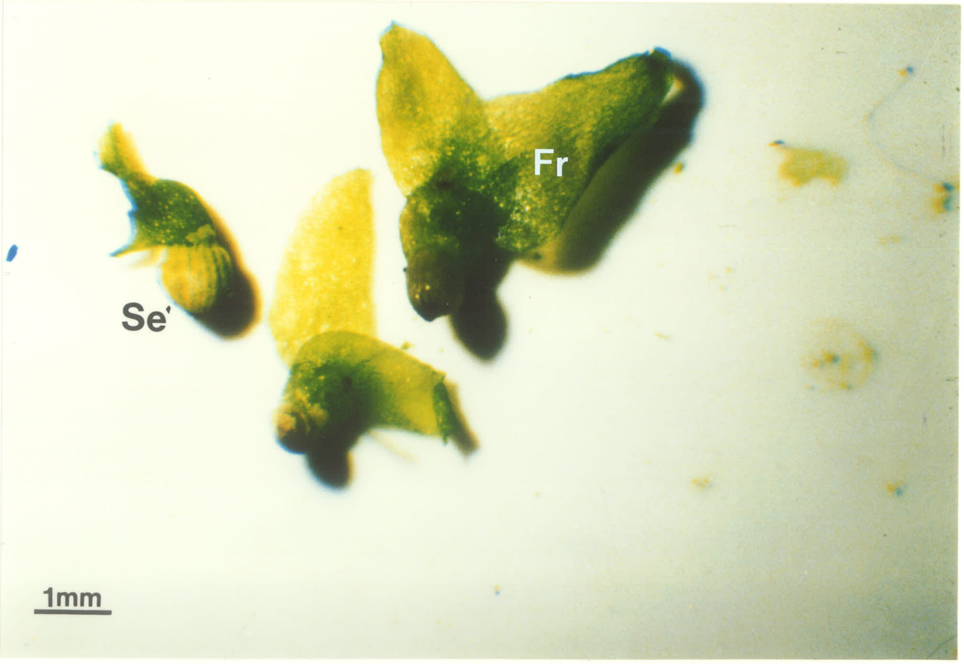
Large viable *Lemna* seedbanks exist; one Eckman grab sample from Minor pond, for example, contained 984 *L. minor* and 208 *L. trisulca* seeds. The fact that seed (in *L. gibba*) remains viable in 20°C water for up to 24 months (Rejmánková, 1976), and that *Lemna* seed has been present in Delta Marsh for at least 2,400 years (Sproule, 1972), suggest that seed and seedbanks are important in *Lemna* life history. Observations of seed germination behavior and accumulation in *Lemna* are consistent with Pons' (1986) mechanism for seed accumulation.

While the natural occurrence of *Lemna* seedlings was not observed during this study, laboratory observation do suggest a potential mechanism for seedling establishment. When sediment from beneath a *Lemna* mat was air dried in flasks at 32°C, crumbled by hand and rewetted with distilled water, most dry *Lemna* seeds floated to the surface (because their reticulate surface trapped air bubbles). Some rose and sank soon after. Others either germinated at the water surface, or germinated on top of the sediment and then floated to the surface. Some *L. minor* turions germinated, but most died. Analogous conditions would be produced in ponds where rain followed drought or by drawdown and reflooding of a marsh. Drought, which would tend to kill asexual propagules, followed by rewetting would favor seed germination and establishment. It would release seeds from sediment and place them in full sunlight. Seasonal recruitment from seed can be significant, as Rejmánková (1975b) noted for one *L. gibba* population in which 80% of spring recruitment was from seed. Rejmánková (1976) suggested that drought tolerance of seeds may be advantageous in ponds which tend to dry out, and cites Hegelmaier who observed germination of *Lemna* seed in a dried reservoir when it refilled.

Blodgett (1923) noted that when seed germinated, the young embryo swelled and pushed off the operculum of the seed coat. In this study, the lens-shaped plumule emerged first and appeared to have a cuticular upper surface in both species. The hydrophobic cuticle seemed to hold seedlings at the surface. *Lemna* seedlings (Fig. 2.7a,b) were easily identified to species once fronds developed.

Figure 2.7: Seedlings of a) *L. minor* and b) *L. trisulca* (Se=seed, Fr=frond).


Se
Fr
1mm


Fr
Se
1mm

Evidence for the long-term existence of successful sexual reproduction in addition to clonal growth in *Lemna* raises questions about the function of each means of propagation.

Results of the overwintering experiment (Table 2.1) indicated that only *L. trisulca* fronds suffered increased mortality at the shallowest depth in Minor pond ($p=0.04$, $F_{3,8}=4.5$). Seeds and *L. minor* turions appeared to tolerate the mild freezing that occurred at 75cm (Table 2.2). There were no treatment differences in Mixed ditch, probably because water did not freeze even at 75cm (Table 2.2). This may have been because the water level at this site rose soon after the experiment began and/or because water temperatures were somewhat warmer (Table 2.2) in Mixed ditch.

Other observations suggested advantages to the seed habit. Seeds survived and germinated after freezing at -20°C for 7 days, drying at 'room temperature' for 7 days or at 32°C for 12 hours. Jacobs (1947) suggested that turions of *Spirodela polyrrhiza* were more tolerant of freezing and high temperatures than normal fronds; turions survived for longer than three months at -4°C but died at -12°C in two days. Rejmánková (1976), suggested that *Lemna gibba* seeds can tolerate up to 17 months of desiccation at room temperature. Fronds of both species rapidly dry and lose viability at room temperature (Keddy, 1976; Wolek, 1981;) as do fronds and turions of *Spirodela polyrrhiza* (Jacobs, 1947). It thus appears that seeds have a higher tolerance of drought and freezing than fronds and turions. Turions seem somewhat more tolerant than fronds.

Clonal plants have potentially indefinite lifespans and are unlikely to die due to senescence (Harper, 1985). One could thus argue that for a *Lemna*

TABLE 2.1 - Survival of propagules (percent growing after treatment) which overwintered at levels above the sediment in 1986 - 87.

LEVEL (cm)	SITE			
	L. minor		L. trisulca	
	TURIONS	SEEDS	TURIONS	SEEDS
MIXED DITCH				
0	85	96	62	67
25	87	93	64	71
50	81	96	62	84
75	71	93	67	67
MINOR POND				
0	89	95	62	84
25	88	91	73	84
50	87	95	71	73
75	80	96	24	78

TABLE 2.2 - Site temperatures (°C) for overwintering experiment. Temperatures for levels above the sediment, the ice - snow interface (I/S), and air (overnight low) are listed.

LEVEL (cm)	DATE						
	7 DEC	28 DEC	2 JAN	24 JAN	8 MAR	28 MAR	11 APR
MIXED DITCH							
0	7.5	6.0	5.5	5.0	4.0	3.0	3.5
25	6.0	6.0	5.0	4.0	3.0	3.0	3.0
50	4.5	4.0	3.5	4.0	2.0	2.0	2.5
75	4.2	3.5	3.0	2.0	2.0	1.5	2.0
I/S	-3.0	-1.5	-3.5	-6.0	0.0*	.	5.0*
MINOR POND							
0	4.0	3.5	3.5	2.5	2.0	2.0	2.0
25#	.	.	.	.	.	.	.
50	3.0	3.0	2.5	2.0	2.0	2.0	2.0
75	-0.5	-1.0	-1.0	-2.0	0.5	0.0	1.0
I/S	-5.0	-1.0	-3.0	-11.0	-3.0	0.0	6.0*
AIR	24.5	-8.5	-13.0	-39.0	-3.5	-21.0	-5.0

* Liquid water present

#Malfunctioning thermistor

clone, drought and freezing events are highly probable on an evolutionary time-scale and make extirpation likely. This suggests that *Lemna* has been and is exposed to strong selection pressure for drought tolerance. Delta Marsh has undergone a number of drawdown events in the past (Walker, 1965) and *Lemna* was likely exposed to drought. Since low water levels in temperate climates may increase the risk that the bottom of a pond freezes, adaptation to freezing should also be expected.

An advantage of clonal growth in *Lemna* results from the large and diffuse nature of its clones. Because a clone is composed of separate modules (Harper, 1977), the probability that it may become extinct is lower than if it were integrated. In fact if clone fragments are completely independent, $P_E=\Pi P_{ei}$ where P_E is the probability that a clone will become extinct and P_{ei} are equal to the separate probabilities that each fragment may become extinct (Cook, 1985). Clone fragmentation appears adaptive in habitats where local extinction risk is patchy.

Sexual reproduction carries a 'meiotic cost' (Silander, 1985) for a clone since a parent clone can only pass on 50% of its genome to sexually produced offspring. This reproductive mode also carries physiologically costs because it diverts resources from growth (Sohn and Policansky, 1977). In *L. trisulca*, for example, frond production appeared to be slowed in flowering and fruiting colonies. Since maturation of seed apparently toke about three to six weeks, allocation of resources to sexual reproduction could have considerably reduced the size of a clone and the number of asexual propagules it could have produced. For sexuality to have been retained, sexually produced seeds must be advantageous in a way that asexual propagules are not.

The primary advantage of sexuality lies in the production of genetic variation (Silander, 1985). Genetic variability may increase the chance that novel genotypes will survive in the new environment following the period of adverse conditions. This in turn means that genes of a clone may have a better chance of being perpetuated. If environmental change is likely, it makes sense for a clone to reproduce sexually in an attempt to ensure that at least some of its genes are perpetuated. Indeed, Law and Lewis (1983) suggested that species which live inside others (inhabitants) in mutualistic symbioses tend to have reduced sexuality compared with exhabitants. They speculate that because exhabitants ameliorate the environment for inhabitants, genetic variation is less advantageous to the latter, and there is in turn less selection pressure for sexuality. Such new gene combinations should be 'protected' from adverse environmental changes. In *Lemna*, high drought and freezing tolerance of seeds may reflect past selection pressure (at the sexual phase of the life-cycle) for protection of seed from these conditions.

When their environment is relatively stable it seems appropriate for *Lemna* clones to perpetuate and enlarge themselves by investing in relatively low-cost asexual propagules. Seeds may be produced on an ongoing basis as insurance, since adverse conditions occur unpredictably. By using both strategies, a *Lemna* clone may be able to: a) take advantage of locally favorable conditions through asexual propagation, and b) preserve some of its genes during probable adverse conditions, like drought, and in the changed conditions which follow this perturbation.

The argument just outlined contains the hidden assumption that sex is the only mechanism for producing genetic variation in *Lemna*. Significant amounts of genetic variation have been observed in populations of obligate or nearly obligate asexual organisms such as *Daphnia* (Hebert *et al.*, 1988) or a variety of clonal plants (Ellstrand and Roose, 1987). As Harper (1977) suggests and Dawkins (1983) justifies, clonal propagation from multicellular meristems, like those of *Lemna*, does not constitute reproduction. A mutation which occurs in a given meristematic cell may produce a chimera, but is unlikely to give rise to a frond line of a new genotype. It seems unlikely that evolutionarily relevant variation could occur through asexual propagation as it occurs in *Lemna*. Parthenogenetic *Daphnia* and agamospermous plants (e.g. *Taraxacum*) differ in that the asexual phase of their life - cycle passes through a single cell phase. If a mutant allele is present in this cell, the whole embryo contains this mutation. Such an allele enters the germ line and is evolutionarily relevant.

The dispersal ability of propagules is also important, primarily in diffusing extinction risk. Since fronds of Lemnaceae have limited tolerance to drying, both Keddy (1976) and Wolek (1981) argue that there is limited potential for dispersal of *L. trisulca* and *L. minor* on the bodies of animals (e.g. muskrat and waterfowl). Jacobs (1947) and Malek (1981) suggested that turions only tolerate mild drying. It seems likely that asexual propagules have relatively poor dispersal potential. Seeds tolerant of drying would be highly dispersible and might confer a lowered risk of extinction on genes of a clone and in addition permit the clone to 'explore' new habitats.

Clearly, further research is required before we fully understand the sexual life histories of *L. minor* and *L. trisulca*. We need to determine how widespread sexual reproduction in *Lemna* is and whether or not the degree of sexuality varies between populations (i.e. is sexuality less prevalent in areas subject to less drought and freezing?). A detailed investigation of the factors inducing flowering in nature is required; possibly, multivariate methods could be used to determine which factor(s) are most important. A detailed study of the mechanism of pollination is lacking; because flowering is easily induced in *L. perpusilla* (Posner and Hillman,1962), it could be used for this purpose. More information is required on the conditions necessary for successful seed germination and seedling establishment in nature. A study conducted to determine the actual cost of sexuality and the potential for self pollination within clones would be illuminating.

Chapter III

III. INTERACTION AND COMPETITION

3.1 INTRODUCTION

In Manitoba, communities of free floating aquatic plants may include the following species of Lemnaceae: *L. minor* L. (includes *L. turionifera* Landolt), *Lemna trisulca* L., *Spirodela polyrrhiza* (L.) Schleid., *Wolffia columbiana* Karsten, as well as the hepatics *Riccia fluitans* L. and *Ricciocarpus natans* (L.) Corda. Manitoba communities may be monospecific or may include several of the above species, but associations of the first two species are most commonly encountered. Landolt and Wildi (1977) also indicated that associations of *L. minor* and *L. trisulca* are common. This association in Manitoba covers a range from virtually monospecific stands of one species to monospecific stands of the other. Den Hartog (1964) describes similar situations in Holland, where *L. trisulca* dominated dune ponds, while *L. minor* and *L. trisulca* occurred together in ponds of the more fertile Polderland.

Competition may, in part, explain these patterns of association. A number of studies have examined competition in Lemnaceae (Clatworthy and Harper, 1962; Rejmánková, 1975a; Docauer, 1983). Docauer studied competition for nutrients between floating species. Only Keddy (1976) considered the submerged *L. trisulca*. He concentrated on competition for light between *L. minor* and *L. trisulca*, and predicted that *L. minor* should outcompete *L. trisulca* in situations where the two coexist.

Tilman (1980, 1982) outlines a theory which relates competition and species diversity to rates of supply and consumption of resources, where a resource is "any substance or factor which can lead to increased growth rates as its availability in the environment is increased, and which is consumed by an organism." In its simplest form, the model is used to predict the outcome of competition for one resource under steady state conditions, in which birth and death rates, and supply and consumption rates are equal. At equilibrium, the species which has the lowest requirement for a given resource will displace those with higher requirements. The approach can be extended to model competition for two resources.

Tilman has applied his resource competition models to phytoplankton competition (Tilman, 1981a) and to the study of succession in old - fields (Tilman, 1986a; 1987). He has extended application of his model to consider environmental factors like temperature (Tilman, 1981b). He suggests that variance in resource supply, herbivory and non - equilibrium solutions could increase the number of species that the model predicts to coexist (Tilman, 1982). Indeed, non - steady state resource supply does appear to enhance coexistence (Sommer, 1985; Gaedeke and Sommer (1986).

This chapter presents results of field experiments designed to investigate interaction between *L. minor* and *L. trisulca,* and attempts to explain their interaction in terms of resource competition. It also documents seasonal dominance changes within a *Lemna* community in which *L. minor* and *L. trisulca* occur in comparable densities. Phenological patterns are compared with those observed in two communities dominated by one species. Trends are interpreted in the light of life-history, species interaction and extrinsic factors.

3.2 MATERIALS AND METHODS

3.2.1 Site description and environmental measurements

In addition to sites described in Chapter 2, this study includes:

Minor pond2, which like Minor pond, is dominated by *L. minor*. This site is located at the University of Manitoba Field Station, Delta Marsh. It is ca. 3m x 5m, with a maximum depth of 0.8m. Sediment is sand and organic material. This site is heavily shaded in summer by *Acer negundo*.

Dissolved nutrients enter water and are consumed concurrently. When a water sample is taken, the measured concentration of a given nutrient in the sample is related to the difference between supply and consumption rates. If both rates are equal, the measured concentration will be zero. For this reason, water nutrient analysis does not provide a reliable indication of nutrient availability. Conductivity gives an rough indication of overall water nutrient status.

Water was sampled periodically in 1986. Conductivity was measured with a YSI model 33 S-C-T meter and pH with a Fisher pH meter. Data were analyzed using ANOVA (SAS Institute, Inc., 1982a,b).

Photosynthetically active radiation (P.A.R., units = $E{\cdot}m^{-2}{\cdot}day^{-1}$) reaching sites was measured with a Licor quantum sensor and Licor solar integrator in July and August by placing the sensor at various locations within sites. Data were expressed as a percentage of the 24h integration recorded from an unshaded sensor. Light data were non-normally distributed and could not be transformed; statistical tests were not performed.

3.2.2 Competition experiments

Three different types of competition experiments were conducted in natural *Lemna* habitats to study competitive interaction in *L. minor* and *L. trisulca* Replacement series experiments, in which the total density of plants was kept constant while the proportion of the two species was varied (De Wit, 1960), were used in 1986 to assess the potential for coexistence of *L. minor* and *L. trisulca*. An addition series experiment, in which both density and proportions were varied (Harper, 1977), was conducted to examine intra- and interspecific competition. To assess the effect of introduction of species to a community at a large numerical disadvantage, 'introduction' experiments were conducted.

Competition experiments were conducted in floating rafts. Each raft was constructed by joining 5cm lengths of clear acrylic tube (2.5cm I.D., 3mm wall) together with methylene dichloride solvent to form a five by six cell matrix. Matrices were suspended from 3mm diameter acrylic rods attached to a Styrofoam™ float. A cone of fiberglass window screen was placed beneath each apparatus to exclude frogs, fish and most *L. minor* turions. Covers of aluminum window screen (1986) or 3mm clear acrylic sheet bordered by window screen (1987) were used to exclude animals, reduce the impact of raindrops, and limit the input of *Lemna* fronds during rain while permitting air flow. *Lemna* was collected from Mixed ditch and placed in a white enamel pan of marsh water. Healthy fronds were then removed to containers of strained marsh water with feather forceps. Fronds were transferred to raft cells in the field with a small paintbrush. Treatments were assigned randomly to cells in rafts and involved placing various numbers of fronds (M-*L. minor* , T-*L. trisulca*) in cells at the beginning of the experiment.

Replacement were conducted in 1986 in Mixed ditch and Minor pond. Two sets of experiments were conducted with six replicates of 48M:0T, 36M:12T, 24M:24T, 12M:36T and 0M:48T, the first from 2 June to 16 June and the second from 27 June to 25 July.

The addition series experiment was conducted in Mixed ditch from 10 July to 24 August, 1987. Five replicates of 50M+50M, 50M, 50M+50T, 50T+50T and 50T were used.

Introduction experiments were conducted in Mixed ditch, Minor pond and Trisulca pond with five replicates of 100M, 2M, 2T+100M, 2M+100T, 100T, 2T from 16 July to 13 August, 1987.

At the end of competition experiments, rafts were removed and fronds counted. Data for replacement series experiments were analyzed using De Wit (1960) ratio diagrams. Addition and introduction experiment data, were expressed as a 'frond ratio' (output / input) where output is the number of fronds taken out at the end of an experiment and input is the initial number of fronds. For treatments 50M+50M and 50T+50T of the addition series experiment, half the final density was used in the frond ratio. Data were analyzed using single factor ANOVA, with a separate ANOVA being run for each species in an experiment. Residuals were checked for normality and non-normal data were transformed (a log transformation was effective in these cases). Multiple comparisonswere made using the Scheffé multiple comparison procedure. Significant differences were assessed using transformed data and back - transformed means used in summary tables. Analyses were made using GLM and UNIVARIATE of SAS (SAS Institute, Inc., 1982a,b).

3.2.3 Transplant experiment

An experiment was conducted in which *Lemna* from Mixed ditch was transplanted to cylinders in all three sites and to shaded cylinders in Mixed ditch. *Lemna* was enclosed in clear acrylic cylinders (7cm I.D, 3mm wall), supported in 15cm x 15cm x 2cm Styrofoam™. These were tethered to stakes. Cylinders bounded an area of 38.6cm^2. They were covered below with black fiberglass window screen held on with aquarium grade silicon rubber. Clear acrylic (9cm x 9cm x 3mm) rain shields were held above each cylinder on lengths of acrylic rod (3mm diam.) by sections of Tygon™ tubing (length-1cm, I.D.-3mm). Shields in the shaded treatment were of translucent white acrylic (Acrylite™ 015-2) with a layer of black window screen attached. These excluded 90% of incident photosynthetically active radiation (P.A.R.). Sections of poultry wire (15cm high) and of window screen (5cm high) were attached to the perimeter of floats to discourage muskrats and to limit input of *Lemna* during heavy rain.

Disks of the *Lemna* mat were collected with a sieve (area-45.6cm^2) within a 1m^2 area of Mixed ditch and placed in petri dishes; this area was chosen because in contained almost equal biomass of each species. Disks were cut in half, and cylinders filled with half-disks. Five randomly -

selected half - disks were sorted and the biomass of each species determined as described in Chapter 2.

The experiment began May 15, 1987 but was restarted June 29, 1987 because of muskrat damage to replicates in Mixed ditch. The following cylinders remained by August 25 (number of replicates in parentheses): Mixed ditch unshaded (15), Mixed ditch shaded (12), Minor ditch (10) and Minor ditch2 (10). Data for Trisulca pond were discarded because larvae of the moth *Synclita obliteralis* (Wlk.) (Pyralidae) apparently removed most or all *Lemna* from cylinders two weeks prior to harvest. The final biomass of each the species in each treatment was determined in the same manner as initial biomass, and data for each species analyzed separately using ANOVA (see above). Paired biomass differences were also determined (*L. minor* - *L. trisulca* on a per - cylinder basis).

3.2.4 Mat sampling

Mat sampling methods are as outlined in Chapter 2 with the exception that vegetative fractions were weighed with a Mettler PE360 electronic balance to ±1mg. Since phenological trends in the two arms of mixed ditch were different, results were presented separately for each arm. Data were presented as biomass per unit area, and as the paired biomass difference (on a per sample basis). A t-test was performed on paired differences for each date and arm to determine whether differences were significantly different from zero.

3.3 RESULTS

3.3.1 Environmental measurements

Environmental data indicated that there was a decreasing gradient of conductivity ($p<0.0001$, $F_{3,19}=60.73$) from Minor pond and Mixed ditch to Minor pond2 to Trisulca pond (Table 3.1). The fact that Trisulca pond was located in marginal farmland, had sediments low in organic material and had clear water of low conductivity, suggested that this site was the most oligotrophic (i.e. had the lowest nutrient supply rate). Trisulca pond pH was higher than in other sites ($p<0.0001$, $F_{3,16}=19.25$) (Table 3.1). A decreasing gradient of light appeared to exist from Mixed ditch to Trisulca pond to Minor pond2 (Table 3.2).

3.3.2 Competition experiments

For replacement series experiments conducted in Mixed ditch (1986), ratio diagrams predicted codominance of the two species in the first experiment and *L. minor* dominance in the second (Fig. 3.1). Ratio diagrams for replacement series experiments run in Minor pond suggest that interaction of the two species favors dominance of *L. minor* (Fig. 3.2). *L. minor* dominance appeared slightly greater in the later experiment. For both species, the pure stand density (Table 3.3) was significantly larger (ANOVA for: *L. minor* $p<0.0001$, $F_{1,10}=542.18$; *L. trisulca* $p=0.0003$, $F_{1,10}=36.26$) for

TABLE 3.1 - Mean water pH, and specific conductivity (µS) in *Lemna* sites*

SITE	pH	n	Specific Conductivity	n
MIXED DITCH	7.4a	7	1600.1a	8
MINOR POND	7.6a	7	1597.0a	8
MINOR POND2	7.7a	2	979.6b	2
TRISULCA POND	8.3b	4	353.3c	5

* Sites with different letters are significantly different (Scheffé multiple comparison, $p<0.05$).

TABLE 3.2 - Amount of shading (percentage of full sunlight) in *Lemna* sites.

SITE	n	RANGE	MEAN±SD	C.V.*
MIXED DITCH	12	55.0-93.6	79.6±8.9	11.2
TRISULCA POND	5	50.3-69.1	58.7±5.2	8.9
MINOR POND	9	2.3-47.4	24.6±20.9	85.1
MINOR POND2	6	1.2-10.0	5.4±3.3	60.9

* C.V. is the coefficient of variation.

Figure 3.1: Ratio diagrams for replacement series experiments in Mixed ditch, a) 2 June to 16 June and b) 27 June to 25 July. Plotted lines are linear regressions with 95% confidence bands for the mean. Diagonal reference line joins equal input and output ratios. M=*L. minor*, T=*L. trisulca.*

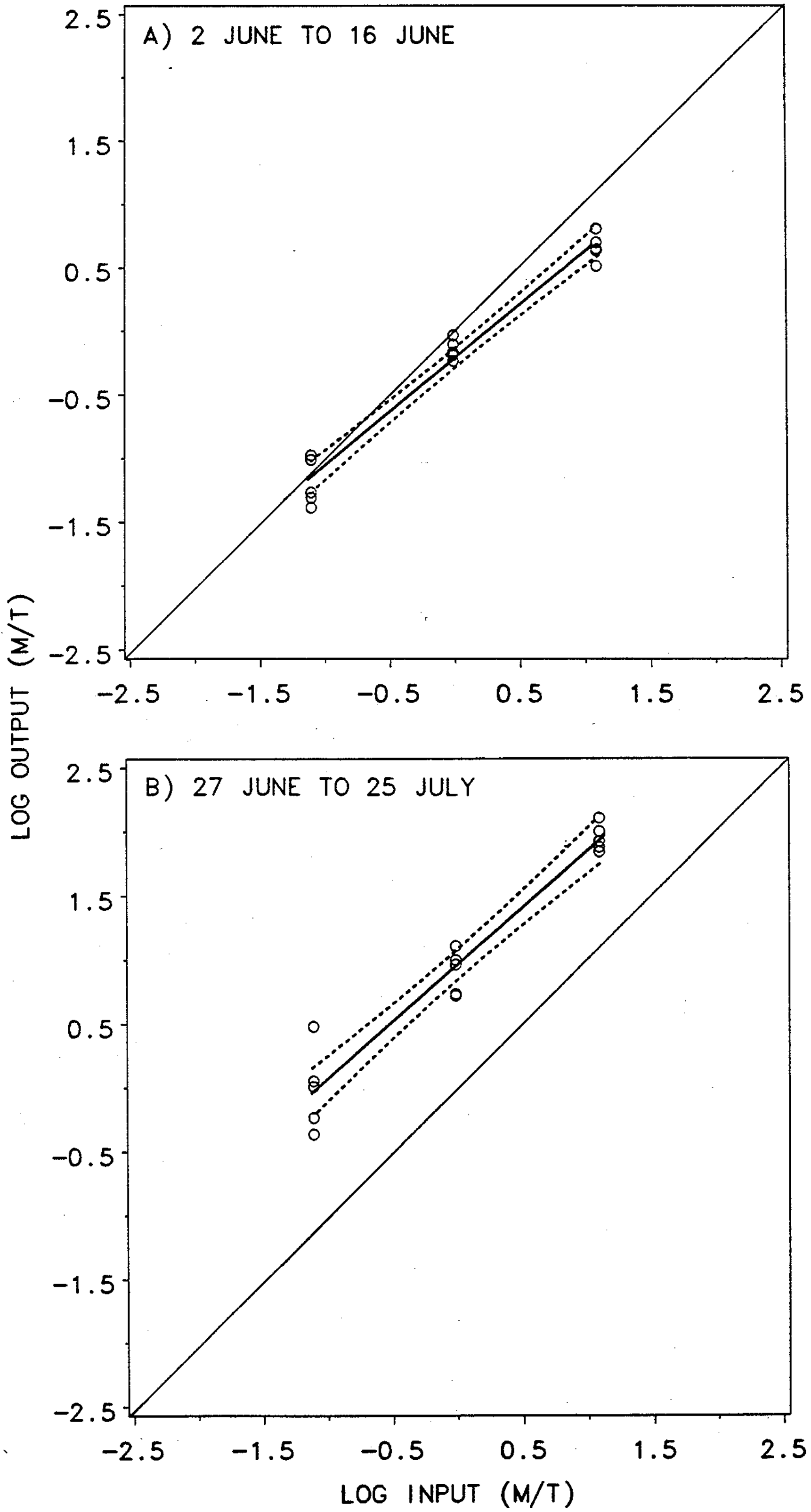

A) 2 JUNE TO 16 JUNE
B) 27 JUNE TO 25 JULY
LOG OUTPUT (M/T)
LOG INPUT (M/T)
2.5
1.5
0.5
−0.5
−1.5
−2.5
−2.5
−1.5
−0.5
0.5
1.5
2.5

Figure 3.2: Ratio diagrams for replacement series experiments in Minor pond, a) 2 June to 16 June and b) 27 June to 25 July. Plotted lines are linear regressions with 95% confidence bands for the mean. Diagonal reference line joins equal input and output ratios. M=*L. minor*, T=*L. trisulca.*

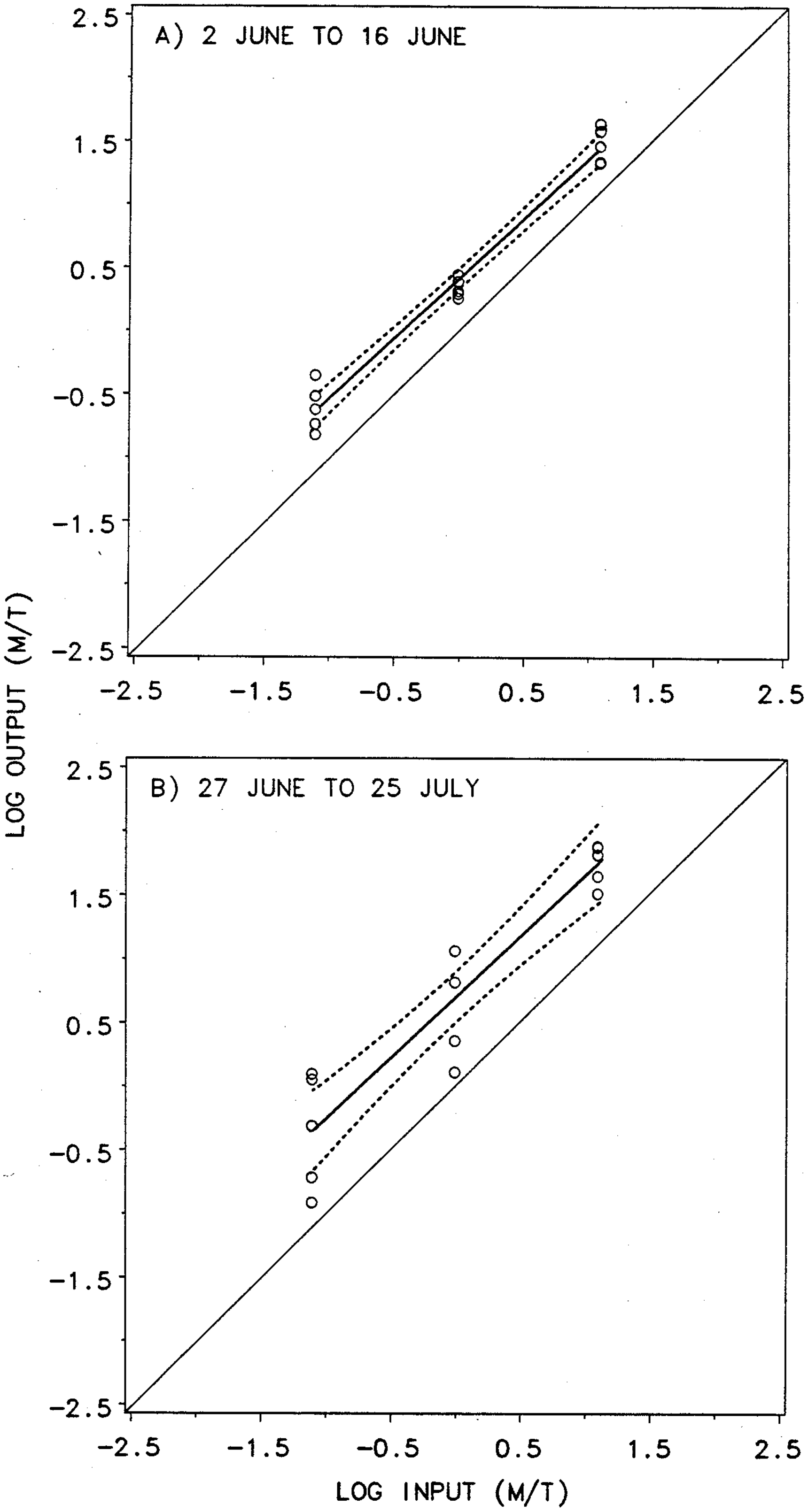

A) 2 JUNE TO 16 JUNE
B) 27 JUNE TO 25 JULY
LOG OUTPUT (M/T)
LOG INPUT (M/T)
2.5
1.5
0.5
−0.5
−1.5
−2.5
−2.5
−1.5
−0.5
0.5
1.5
2.5

TABLE 3.3 - Pure stand densities (mean fronds per cell in 48T and 48M treatments) in replacement series experiments*

EXPERIMENT[†]	SITE			
	MIXED DITCH		MINOR POND	
	L. minor	*L. trisulca*	*L. minor*	*L. trisulca*
2 JUNE-16 JUNE	80a	103a	142a	101a
27 JUNE-25 JULY	275b	156b	198b	111a

* M and T are the number of fronds of *L. minor* and *L. trisulca*, respectively, at the beginning of the experiment. Treatment means (compared between dates) with different letters are significantly different ($p<0.05$).

[†] Dates of initiation and competition of experiments.

the longer July experiment conducted in Mixed ditch. In Minor pond, the pure stand density of *L. minor* was higher in the second experiment ($p<0.0163$, $F_{1,10}=9.19$), but *L. trisulca* density was not ($p=0.2973$, $F_{1,10}=1.24$).

In the addition series experiment conducted in Mixed ditch, both *L. minor* ($p=0.003$, $F_{2,12}=8.77$) and *L. trisulca* ($p<0.0001$, $F_{2,12}=21.28$,) showed significant treatment differences (Table 3.4). Both were inhibited in the presence of other members of the same species. Final *L. minor* density was not significantly depressed by *L. trisulca*, but *L. trisulca* was depressed to the same degree by *L. minor* as it was by individuals of its own species.

In the introduction experiment (Mixed ditch), *L. minor* was not significantly depressed by *L. trisulca* ($p>0.2$, $F_{1,8}=1.95$) but *L. trisulca* was by *L. minor* ($p=0.0004$, $F_{1,6}=50.85$) (Table 3.5). A very low value and a zero value were removed from the *L. trisulca* analysis since they inflated variance. These low values were probably due to gastropod (*Limnaea* sp., *Helisoma* sp.) grazing.

In the introduction experiment (Minor pond), neither *L. minor* ($p>0.45$, $F_{1,8}=0.62$) nor *L. trisulca* ($p>0.51$, $F_{1,8}=0.51$) appeared significantly affected by the presence of 100 fronds of the other (Table 3.5). Green algae were abundant in all cells which began with two *L. trisulca* fronds, but only the lowest density *L. minor* cell had noticeable amounts of green algae.

In the introduction experiment (Trisulca pond), *L. minor* was depressed by *L. trisulca* ($p=0.0126$, $F_{1,8}=14.44$) and vice versa ($p<0.0001$, $F_{1,8}=82.53$)(Table 3.5). One very low and two zero values were excluded from

TABLE 3.4 - Mean frond ratio (Output/Input) in addition experiment*

TREATMENT	*L. minor*	*L. trisulca*
50M	13.6a	-
50M+50T	11.1ab	-
50M+50M	7.7b	-
50T	-	10.4a
50T+50M	-	7.3b
50T+50T	-	5.5b

* M and T are the number of fronds of *L. minor* and *L. trisulca*, respectively, at the beginning of an experiment. Treatments with different letters are significantly different (Scheffé multiple comparison, $p<0.05$).

TABLE 3.5 - Mean frond ratio (Output / Input) in introduction experiments*

TREATMENT	SITE					
	MIXED DITCH		MINOR POND		TRISULCA POND	
	L. minor	*L. trisulca*	*L. minor*	*L. trisulca*	*L. minor*	*L. trisulca*
2M	54.3a	-	63.2a	-	75.3a	-
2M+100T	28.0a	-	56.0a	-	11.6b	-
2T	-	23.5a	-	3.1a	-	37.2a
2T+100M	-	6.9b	-	2.8a	-	2.7b

* M and T are the number of fronds of *L. minor* and *L. trisulca*, respectively, at the beginning of an experiment. Treatments with different letters are significantly different ($p<0.05$).

the *L. minor* analysis. These values inflated variance and were probably due to *Synclita* grazing (see also: methods, transplant experiment).

3.3.3 Transplant experiment

In the transplant experiment, significant differences in biomass were found between treatments for *L. minor* ($p<0.0001$, $F_{3,43}=42.61$,) and *L. trisulca* ($p=0.0128$, $F_{3,43}=4.05$). The Scheffé comparison showed that *L. minor* biomass was significantly higher in the unshaded Mixed ditch (control) than in other treatments, was half as high as the control under 90% shade in Mixed ditch, and was significantly lower than the control in Minor pond and Minor pond2. *L. minor*biomass increased from initial values in the first two treatments and declined in Minor pond and Minor pond2. *L. trisulca* biomass was similar in shaded Mixed ditch, Minor pond and Minor pond2. Biomass in the control was significantly higher than in the shaded Mixed ditch. *L. trisulca* biomass decreased in all treatments from the amount initially added to cells (Table 3.6). Actual mean mat biomass (measured August 13) was 117.6 $g \cdot m^{-2}$ ± 31.5SD for *L. minor* and 48.2 $g \cdot m^{-2}$ ± 29.6SD for *L. trisulca* in Mixed ditch , and 49.1 $g \cdot m^{-2}$ ± 25.8SD for *L. minor* in Minor pond. Paired differences indicate *L. minor* dominance in control and shaded treatments, and slight *L. trisulca* dominance in the others.

3.3.4 Mat sampling

Data from samples of the *Lemna* mat in the Mixed ditch indicated that *L. trisulca* tended to dominate in spring and fall, *L. minor* in summer. This pattern was repeated each year. The east - west arm (Fig. 3.3a) appeared to

TABLE 3.6 - Mean dry weight per quadrat (g·m^{-2}) in transplant experiment*

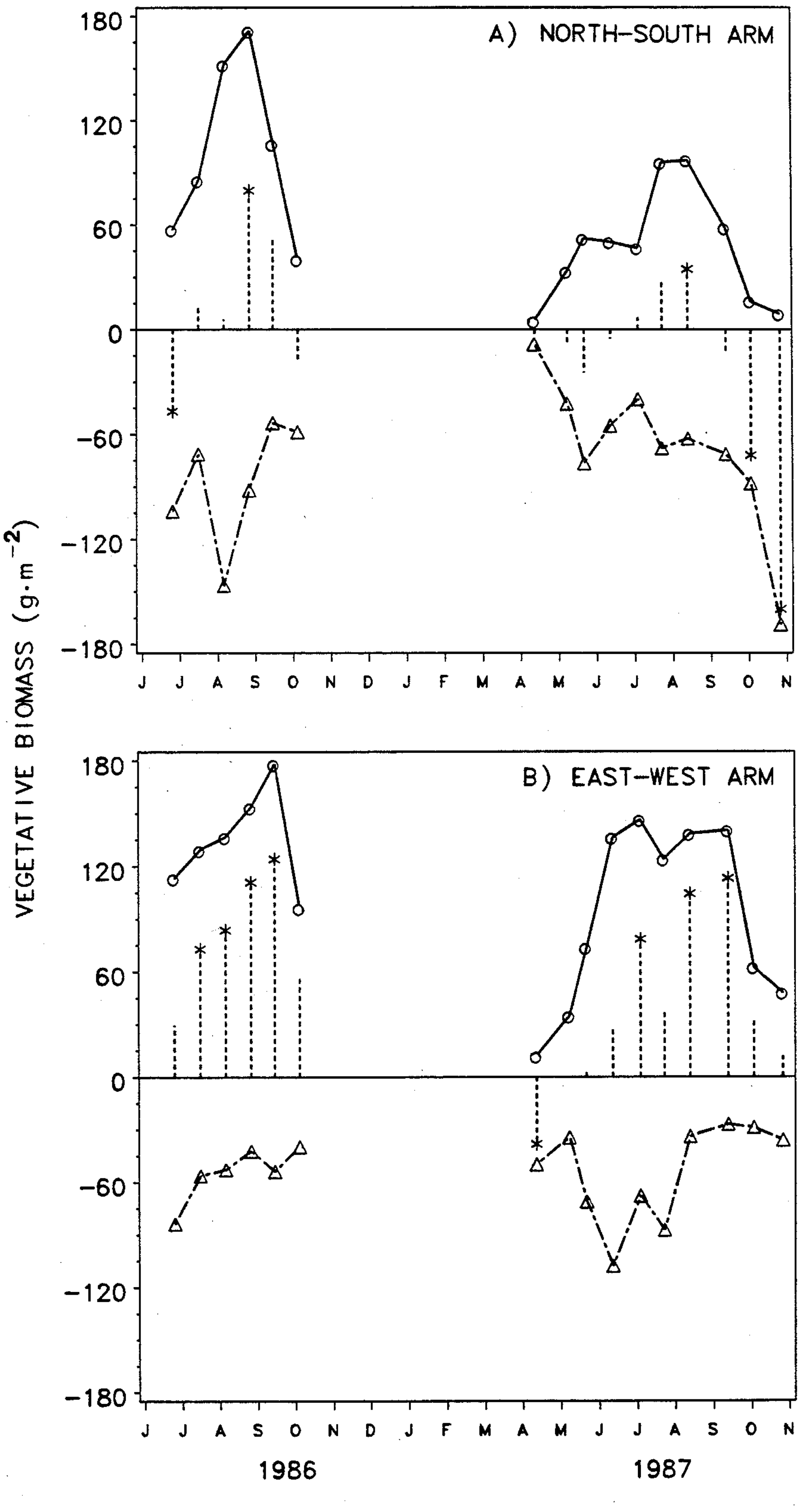
TREATMENT	*L. minor*	*L. trisulca*	Paired Difference
INITIAL[†]	58.7	54.0	.
CONTROL	157.6a	54.1a	109.2
90% SHADE	77.7b	34.4b	48.2
MINOR POND	36.3c	50.9ab	-12.5#
MINOR POND2	31.1c	49.5ab	-12.7#

* Treatments with different letters are significantly different (Scheffé multiple comparison, $p < 0.05$).

\# Negative values indicate greater *L. trisulca* biomass.

[†] Estimated biomass of *Lemna* transplanted at the beginning of the experiment

Figure 3.3: Seasonal patterns of *Lemna* dominance in Mixed ditch: a) North-South arm, b) East-West arm. Paired biomass difference (*L. minor* - *L. trisulca*, $p<0.05$ (*)); *L. minor* dominates above the the zero line, *L. trisulca* below. Mean biomass of *L. minor* (———) is represented by the positive upper scale, of *L. trisulca* (— - —) by the lower negative scale.


A) NORTH-SOUTH ARM
B) EAST-WEST ARM
VEGETATIVE BIOMASS (g·m⁻²)
180
120
60
0
-60
-120
-180
J J A S O N D J F M A M J J A S O N
1986
1987

be dominated more by *L. minor* than the north - south arm (Fig. 3.3b). Both species increased in biomass in spring, but *L. trisulca* stopped increasing first in summer. *L. trisulca* increased as *L. minor* senesced in late summer to fall.

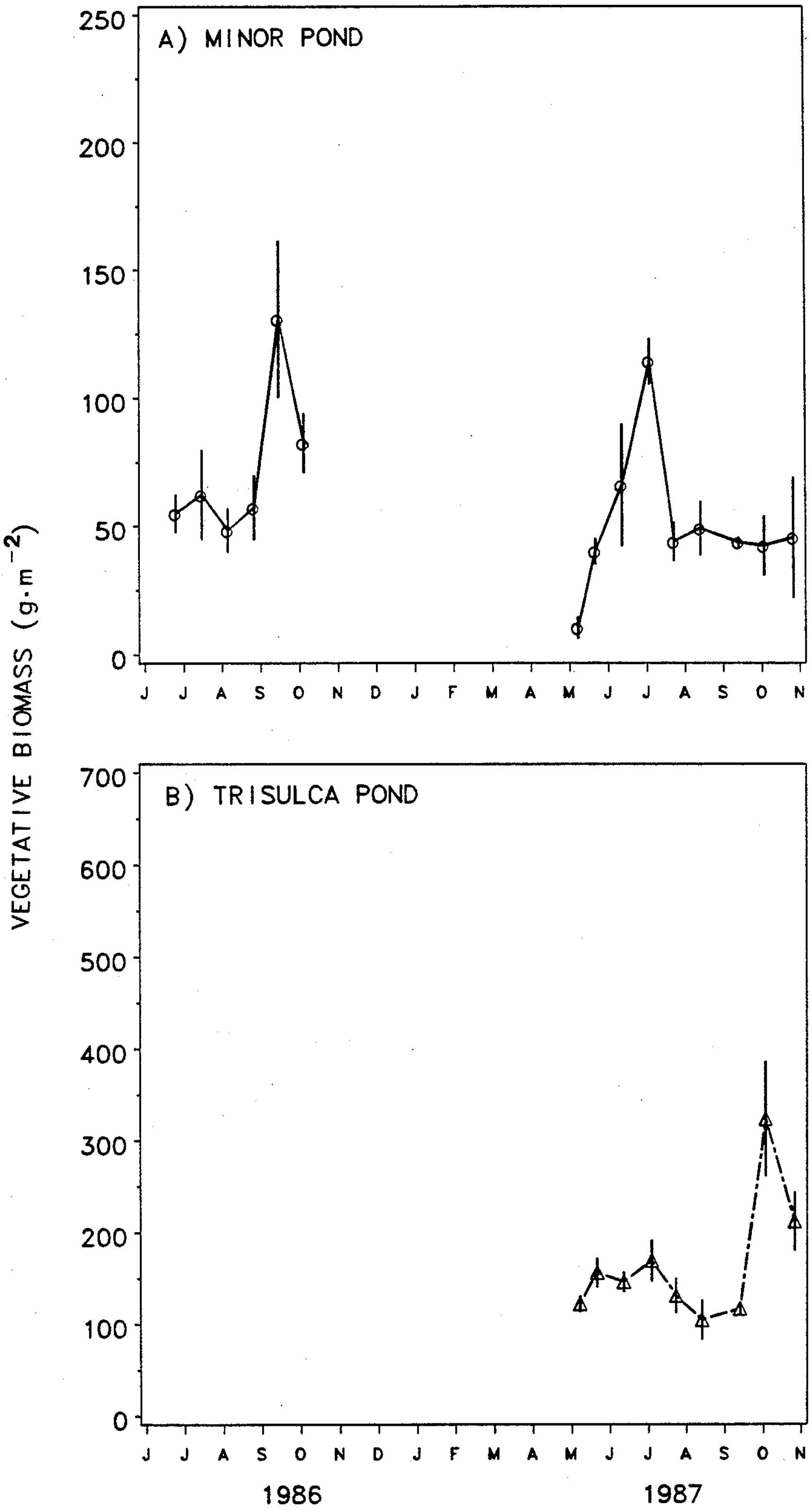
Minor pond and Trisulca pond (Fig. 3.4) do not show biomass peaks as distinct as those observed in Mixed ditch and are essentially monodominant. The existence of *L. trisulca* in Minor pond is questionable. *L. minor* exists in Trisulca pond, but at densities too low to be shown on the figure (<0.1% of mat biomass). Wind drift of *L. trisulca* contributed to the high variability in data for Trisulca pond.

3.4 DISCUSSION

In this discussion, I outline characteristics of *L. minor* and *L. trisulca* which are relevant when considering resource competition for light and nutrients; roles of mortality factors and differences in maximum growth rates of the species are also considered. A model involving these factors is developed and experimental results are interpreted in light of the model.

L. minor is superior to *L. trisulca* in its shading ability because it floats at the surface and is shaped like an oblate spheroid. Niklas and Kerchner (1984) indicated that this shape coupled with horizontal orientation very efficiently intercepts light from any angle above horizontal. Dale and Gillespie (1976)

Figure 3.4: Seasonal patterns of *Lemna* dominance in: A) Minor pond, B) Trisulca pond. Biomass of *L. minor* (———) and *L. trisulca* (— - —). Values are means±SE.


A) MINOR POND
B) TRISULCA POND
VEGETATIVE BIOMASS (g·m^{-2})
250
200
150
100
50
0
700
600
500
400
300
200
100
0
J J A S O N D J F M A M J J A S O N
1986
1987

found light transmittance through a *L. minor* mat to be 2 to 6%. Thick mats like those found in Mixed ditch often had 0% transmittance.

L. trisulca on the other hand seems better suited for efficient nutrient uptake. Unlike *L. minor*, fronds of *L. trisulca* are submerged, thin and elongate and have a large surface area for potential nutrient uptake.

Ford and Diggle (1981) and Weiner and Thomas (1986) suggest that competition for light is often one-sided (one-way competition). Light is supplied directionally from its source and can be intercepted. Plants which intercept light are 'canopy plants' while those they shade are 'understory plants'. Forest understory plants often have life history characteristics which permit them to tolerate heavy shade, to persist for long periods (Hubbel and Foster, 1986) and to grow when gaps occur in the canopy. Schemske's (1977) work suggests that some understory plants use phenological avoidance of shading to exist beneath deciduous canopies, completing their life cycle in spring before canopies close.

Shaded understory plants have higher photosynthetic efficiency (Boardman, 1977), so one might conclude that they are superior competitors for light. However, this becomes irrelevant if a competing canopy plant intercepts all of the light and thus makes it unavailable to an efficient shade plant.

The growth habits of *L. minor* and *L. trisulca* place the former in the canopy and the latter in the understory. *L. minor* is physically able to intercept light before submerged *L. trisulca* is able to use it. Observations made in the laboratory and field suggest that both species are able to persist in low light or even darkness for long periods. In winter, these plants are exposed to low light when fronds are on or in sediment, often under ice deeply covered with snow.

Mortality rates vary depending on the plant species involved and the types of mortality sources active in given habitats at given times (Harper, 1977). If canopy species suffer high mortality, differential mortality (e.g. from herbivory) might favor understory plants when they are being suppressed by canopy species. For example, *Trifolium* excludes *Dactylis* by shading it. With slug grazing, *Dactylis* can coexist with *Trifolium* because slugs prefer *Trifolium* (Cottam, 1986).

Different *Lemna* habitats had different dominant herbivores. The most common were gastropods (*Lymnaea* and *Helisoma*), duckweed weevils (*Tanysphyrus* sp.), and the moth *Synclita*. In Minor pond, gastropods and *Tanysphyrus* were moderately abundant. In Mixed ditch, gastropods were abundant, *Tanysphyrus* moderately abundant and *Synclita* rare. In Trisulca pond, *Synclita* was abundant, while gastropods and *Tanysphyrus* were rare.

Gastropods spent days beneath and within the *Lemna* mat, but appeared on the surface at night in large numbers (100's per square metre) in Mixed ditch. They fed on both species of *Lemna*. *Synclita* oviposits beneath the leaves of floating aquatic plants like *Lemna* (Monroe, 1972). Larvae made cases from both species and appeared to feed on both. *Tanysphyrus* adults chew circular holes in floating fronds of both species, while larvae are leaf miners (Scotland, 1934; 1940). It appeared that both *Lemna* species would suffer low mortality from grazing in Minor pond and high mortality in Mixed ditch. In Trisulca pond, mortality may be biased against *L. minor* if its floating habit makes it preferable for *Synclita* oviposition.

Differences in maximum growth rates of the two species may be important. For example, Tilman (1986b) showed that differences in maximum growth rate had important effects on equilibrium resource competition models of succession. Some plants are slow growers even under optimal growing conditions. Crawley (1986) indicated that slow growth is characteristic of plants adapted to habitats with low nutrient availability. *L. trisulca* has such a low maximum growth rate (Landolt, 1957).

The evidence just outlined can be used to develop a hypothetical resource competition model to help predict competitive outcomes of interaction between these *Lemna* species, and to explain differences in their relative abundance in different habitats. Tilman (1982) suggests that when two plants compete for two resources and coexist, each should be better at consuming the resource which limits the growth of the other. It seems plausible that *L. minor* is a superior competitor for light and is most limited by nutrients, while *L. trisulca* is a superior competitor for nutrients (e.g. phosphate) and is limited by light. However, plants which intercept light (e.g. *L. minor*) effectively inhibit potential reciprocal inhibition by those they shade (e.g. *L. trisulca*) because shaded plants cannot grow rapidly and are unable to effectively limit canopy plants by competing with them for nutrients. Thus, Tilman's (1980, 1982) resource competition model for two resources cannot be applied here because one-way competition for light does not permit *L. trisulca* to compete for light with *L. minor*.

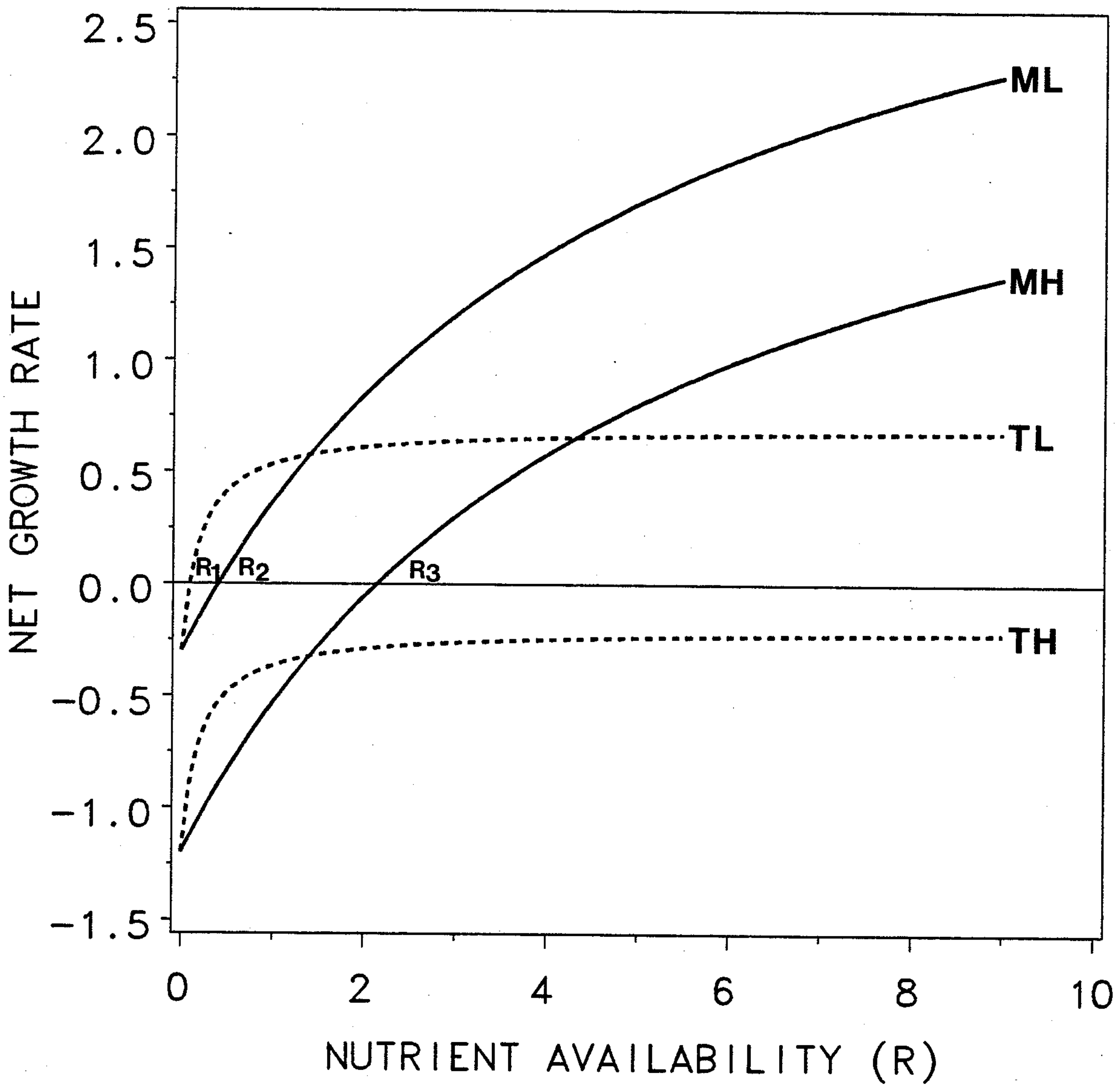
Tilman's one resource model, however, is helpful when considering competition for nutrients. Figure 3.5 illustrates an hypothetical model of the relationship between net growth rate (NGR) and nutrient availability (R), incorporating unequal maximum growth rates and differential mortality (high and low). The Monod equation, NGR = (R•Gmax / (R + K)) - m, models net growth, where Gmax is maximum growth rate, K a constant representing nutrient uptake efficiency at low nutrient availabilities, and m is mortality rate. Gmax = 4.0 and K = 5.0 for *L. trisulca*, while values are 1.0 and 0.2 respectively for *L. minor*. The high mortality rate is 1.2 while low mortality rate is 0.3. These values are arbitrary, and were chosen to reflect three assumptions of the model: 1) that *L. trisulca* is most efficient at growing with low nutrient availability, 2) that *L. trisulca* has the lower maximum growth rate (K for *L. trisulca* is low) and 3) that differential mortality may occur.

The species requiring the least nutrient to survive can reduce nutrient availability below the level necessary to support the other. The species with the lowest equilibrium requirement (R_i) for nutrients wins. Figure 3.5 suggests that if *L. trisulca* mortality is high, *L. minor* wins. If *L. trisulca* mortality is low it wins.

But, these plants also compete for light. If one - way competition for light is considered in addition to this model of nutrient competition, a numberofpossiblescenariosaresuggested.

In a relatively unshaded, eutrophic site (e.g. Mixed ditch), with low initial *L. minor* density, either species could eventually dominate. If *L. minor* mortality is low, it could gradually become dense and shade

Figure 3.5: Hypothetical net growth curves for *L. minor* (———M) and *L. trisulca* (------T). ML and TL are curves for low mortality, MH and TH for high mortality. **R_1**, **R_2** and **R_3** represent equilibrium nutrient availabilities (points where net growth rate is zero).


ML
MH
TL
TH
R1
R2
R3
NET GROWTH RATE
NUTRIENT AVAILABILITY (R)
2.5
2.0
1.5
1.0
0.5
0.0
−0.5
−1.0
−1.5
0
2
4
6
8
10

L. trisulca. Until this happens, however, *L. trisulca* could limit nutrient availability enough to slow the progression toward *L. minor* dominance. If *L. trisulca* were dense and *L. minor* suffered high mortality *L. trisulca* might be able to dominate.

With high initial *L. minor* density, *L. minor* attenuates most incident light and *L. trisulca* is light limited. *L. trisulca*'s slow growth prevents it from limiting *L. minor* by competing with it for nutrients.

In a shaded eutrophic site (e.g. Minor pond), light limitation prevents *L. trisulca* from becoming dense enough to limit nutrient availability. Only high *L. minor* mortality would prevent it from eventually shading *L. trisulca* completely.

In a relatively unshaded, oligotrophic site (e.g. Trisulca pond), with low initial density, *L. minor* attenuates little incident light. *L. trisulca* receives light and can limit growth of *L. minor* by competing with it for nutrients. With low nutrient supply and low *L. trisulca* mortality, *L. trisulca* outcompetes *L. minor* for nutrients and holds it at low density. With high *L. trisulca* mortality and low *L. minor* mortality, *L. minor* could exist alone in low numbers. If an oligotrophic site were shaded, both species might survive in low numbers, but extirpation of both seems likely.

The predictions of the light - nutrient resource competition model outlined above are useful in interpreting experimental results. Interpretations are conservative because substitutive (replacement series) and additive experiments have recently received criticism (Connolly, 1987), especially since they are usually conducted at a single total density. Law and Watkinson (1987) and Connolly (1987) have recently outlined better methods for quantifying degrees of inter- and intraspecific interaction between two species. But as Law and Watkinson (1987) indicate, substitutive and additive experiments still provide useful information about the trajectory of competitive interaction. Substitutive experiments can be improved by using a total density which results in a final yields approaching maximum yields of the species in monoculture (Aarssen, 1985). Given this, results from the experiments conducted can only give a partial indication of the interactive relationship of *L. minor* and *L. trisulca.* Since *Lemna* tends to fragment as it grows, density increases as experiments run. This may partially alleviate problem of inadequate density in the *Lemna* system because plants can attain the maximum density for the experimental conditions if the experiment is allowed to run long enough.

Mixed ditch replacement series experiments indicated that when experiments were run for a longer period of time, final densities of each species increased. This increase may have resulted in the shift from a prediction (by the ratio diagrams) of codominance in the first experiment to one of *L. minor* dominance in the second. At high density, *L. minor* would attenuate most of the light and inhibit reciprocal competition by *L. trisulca.* This observation seems to correspond well with patterns of seasonal dominance in the two species. In spring when *L. minor* is at low density both species grow and codominate (Fig. 3.3); later, when *L. minor* is dense, *L. trisulca* declines and there is a shift toward dominance by *L. minor.* The increase in *L. minor* density in the Minor pond experiment could similarly explain the slightly stronger *L. minor* dominance prediction of the second replacement series experiment.

Results of the addition series experiment indicated that strong intraspecific competition can occur between plants of both species. Interspecific competition limited *L. trisulca* as much as intraspecific competition, while *L. minor* seemed unaffected by interspecific competition. *L. minor* probably limited the light available to *L. trisulca* sufficiently to curtail its growth and inhibit reciprocal competition by it for nutrients.

In the Mixed ditch (nutrients high, light high) introduction experiment, *L. trisulca* was probably depressed because *L. minor* reduced light availability. One hundred *L. trisulca* were probably unable to suppress *L. minor* because the two *L. minor* grew rapidly and daughters soon shaded *L. trisulca.* This suggests that it is unlikely that *L. trisulca* could invade the Mixed ditch if the site were dominated by *L. minor.*

In the Minor pond (nutrients high, light low) introduction experiment, *L. trisulca* was probably shaded by *L. minor* enough to prevent its growth in the 2T+100M treatment, while in the control (2T), algal competition for nutrients and/or light may explain the lack of a significant difference. Rejmánková (1975b), has reported that green algae strongly inhibited growth of *L. gibba*. It is not surprising that 100 *L. trisulca* had little effect on *L. minor,* since *L. minor* rapidly covered the water surface in experimental cells. In such a heavily shaded site, this would reduce light availability to *L. trisulca* to very low levels and prevent it from competing with *L. minor* for nutrients. The eutrophic nature of this site would make it even less likely that *L. trisulca* could significantly limit nutrients. *L. trisulca* appears unlikely to be able to invade such a site.

In Trisulca pond (nutrients low, light moderate to high) introduction experiment, nutrient levels were probably low enough to slow growth of *L. minor*, allowing *L. trisulca* to grow and further limit *L. minor* growth through its nutrient consumption. *L. minor* when present in high density shaded *L. trisulca* and slowed its growth. Mortality biased toward *L. minor* would enhance the trend toward *L. trisulca* dominance. It seems likely that either species could invade such a site.

Explanation of transplant experiment results is somewhat more complex. Controls contained biomass of each species similar to those found in the surrounding mat. This suggests that enclosures had little effect on species composition. Shading treatment in Mixed ditch caused biomass declines in both species such that species composition in this treatment began to approach that of the naturally shaded sites. In the Mixed ditch, high mortality in both species, combined with eutrophy, would favor *L. minor*, the superior competitor for light.

The similarity in biomass for both species in Minor pond and Minor pond2 is not surprising since these sites have similar environmental conditions (high conductivity, low light). The decrease in biomass observed for both species in these sites indicates that mortality occurred and that light availability was probably insufficient to support the initial biomass. Biomass of *L. minor* in Minor pond cylinders was quite similar to actual *Lemna* biomass in that site. An unexpectedly large biomass of *L. trisulca* persisted in these shaded sites despite the high biomass of *L. minor*. This suggests that *L. trisulca* mortality was relatively low in these sites. *L. trisulca* 's ability to persist in low light where when mortality is low could explain its high biomass. Possibly persistence is facilitated by heterotrophic metabolism.

Lemna has specific systems for the uptake of organic compounds like sugars (Datko and Mudd, 1985). Interestingly, *L. trisulca* is unable to grow in purely inorganic media (Bowker *et al*, 1980). Even so, *L. trisulca* would probably require periods of reduced shading for long - term persistence to be possible.

Experimental results and resource competition theory permitted predictions which were in agreement with actual dominance patterns observed in the study sites. Species that competed well in experiments conducted in Minor pond and Trisulca pond dominated in these sites. In Mixed ditch, *L. trisulca* dominated in spring when *L. minor* density was low and gradually declined as *L. minor* attained dominance in summer. In fall when *L. minor* formed turions and its biomass declined in the mat, *L. trisulca* again dominated. If it were not for the periods of low *L. minor* density induced by its life-history response to seasonal change, it is likely that *L. trisulca* could not codominate with *L. minor* in Mixed ditch.

Factors not included in the model, but which would further permit *L. trisulca* to coexist with *L. minor* are mat disturbance and patchy mortality. In this study swimming muskrat and Blue-winged Teal parted the cover of *L. minor*, exposing *L. trisulca* to full sunlight. In some cases this included >50% of the mat. Rains tended to close the canopy of *L. minor*. Wind tended to thin the mat on the windward side, and to thin it on the lea side of ponds. Patchy mortality occurred, as the pattern of loss of *Lemna* from experimental cells indicated. The resource competition model assumes uniform mortality. Patchy mortality could create a mosaic of dominance patterns within a site, some of which could favor *L. trisulca*.

Temperature affects growth rate. In Trisulca pond, where three species occurred, they appeared at different times. *L. trisulca* was present in May, while *L. minor* and *Spirodela polyrrhiza* did not appear before June 29. *Spirodela polyrrhiza* has a more southerly distribution than *L. trisulca* (Landolt, 1984), possibly because it does not grow below 14°C (Landolt, 1957), and the minimum temperature for germination of its turions is 15°C (Jacobs 1947). This may explain its late appearance in Trisulca pond. The minimum temperature for *L. minor* growth is somewhat higher than for *L. trisulca* (Landolt, 1957). Thus, *L. trisulca* may be able to grow early in spring before *L. minor* turions germinate and grow in Mixed ditch. It has also been suggested (S. Gurney, pers. comm.) that the presence of a *L. trisulca* mat may shade turions and slow their germination in spring.

The model described here applies well to the *L. gibba* / *L. minor* competition described by Rejmánková (1975a). Gibbous form *L. gibba* has a higher maximum growth rate than *L. minor* and is able to overtop and shade it. In Czechoslovakia, *L. gibba* dominates in eutrophic waters while *L. minor* dominance is restricted to relatively oligotrophic sites. Landolt (1975) suggests that *L. turionifera* (*L. minor* in part) growth is less inhibited in oligotrophic water than that of *L. gibba* Chambers and Kalff (1987) and Chambers (1987) have also suggested that in submerged aquatic macrophytes short (understory) species are superior competitors for nutrients while taller (canopy) species are better competitors for light. Short species dominated communities of submerged aquatics with low nutrient availabilities, while tall species dominated with high nutrient availability. These patterns are remarkably similar to the pattern observed in *L. minor* / *L. trisulca* communities in Manitoba.

Floating *Lemna* spp. are also able to shade submerged macrophytes and limit their photosynthesis (McLay, 1974; Jacobs, 1947). The high pH caused by photosynthesis of submerged macrophytes apparently inhibits growth of *L. perpusilla* (McLay, 1974). Plausibly, inhibition results from the limitation of nutrient availability caused by the high pH. Since macrophytes are able to obtain some nutrients from sediment (Huebert and Gorham, 1983) this phenomenon may permit them to limit *Lemna* by lowering nutrient availability in the water.

An interesting link can be made from this system to that of forest trees. Horn (1975) predicted that plants normally found in shaded habitats (<25% of full sunlight) should have a single layered leaf architecture, while plants normally found in full sun should have a diffuse multilayered architecture. The reason for this is that photosynthesis tends to be light saturated at about 500 $\mu E \cdot m^{-2} \cdot sec^{-1}$ P.A.R (25% full sun). *L. gibba* can actually be photoinhibited at 650 $\mu E \cdot m^{-2} \cdot sec^{-1}$ (Ögren and Hällgren, 1984). Self-shading architecture should permit more efficient use of light in full sun while monolayers should be optimal in shade. *L. minor* forms monolayers in shaded sites and *L. trisulca* (in sunny oligotrophic sites) has a highly branched architecture. Observation indeed suggests that *L. minor* is frequently an understory plant found growing in the shade of trees or macrophytes, while *L. trisulca* dominates in sunny oligotrophic habitats. *L. trisulca* is also able to modify its chloroplast orientation in response to light intensity, orienting them in a plane perpendicular to the direction of the light source at low light levels, and parallel to it at saturating intensities. In this way, the plant may be able to photosynthesize efficiently over a wide range of light intensity while avoiding photochemical damage (Seitz, 1982).

It is possible that *L. minor* and *L. trisulca* the type of architecture each has in response to past selection pressures which favored growth of *L. minor* in shaded eutrophic sites like forest pools, and growth of *L. trisulca* in sunny oligotrophic pools.

Further research should be aimed at determining the response of each species to a range of light and nutrient levels. One-way light competition should be examined experimentally. The degree of herbivory (and other mortality factors) should be quantified if possible. Factorial lab experiments which include treatments of varying light intensity, nutrient supply and mortality could be conducted; these would give us a much clearer understanding of resource competition between *L. minor* and *L. trisulca*

Chapter IV

SUMMARY

In an unshaded eutrophic ditch at Delta Marsh, both *Lemna minor* L. and *L. trisulca* L. flowered abundantly in 1986 and 1987. *L. trisulca* began flowering by 7 May, peaked about 1 June and finished flowering by 3 July. The phenology of *L. minor* lagged behind that of *L. trisulca* by about three weeks. In a shaded site, *L. minor* flowered, but had little flowering biomass. The largest flowering biomass recorded was for *L. trisulca* in an unshaded oligotrophic farm dugout. Phenology in the latter sites was similar to that observed in the unshaded marsh ditch.

Pollen grains and seeds of both species were examined with scanning electron microscopy. Although pollen morphology of the two was similar, *L. trisulca* seed differed from that of *L. minor* in having a longitudinally ribbed surface. Pollination may be by contact (ephydrophily) or invertebrates.

Seed - bearing fronds of both species were found in the unshaded marsh ditch, but not in the other sites. Seed set varied between years and within the ditch. Peak seed densities occurred 3-6 weeks after biomass of flowering plants peaked, indicating a 3-6 week seed development time. Seed of both species exhibited dark dormancy, and tolerated drying and freezing. A large *Lemna* seedbank was present at Delta Marsh, but seedlings were not observed in nature even though greater than 95% of seed extracted from sediment was viable. In the laboratory, seeds floated after being dried, and germinated at the water surface. This suggested that drought, followed by rain might liberate seed from sediment and permit it to germinate.

It is suggested that the retention sexuality and tolerance of seed to drought and freezing may function as insurance, allowing some of a clone's genes to survive periods of environmental change.

Replacement series experiments predicted codominance of *L. minor* and *L. trisulca* in an unshaded marsh ditch when run for two weeks, but predicted *L. minor* dominance when run for three weeks. Similar experiments conducted in a shaded eutrophic site predicted *L. minor* dominance.

Addition series experiments showed that intra- and interspecific competition occurred between species in the unshaded ditch. Intraspecific competition in *L. minor* was greater than interspecific competition. In *L. trisulca* both types of competition were of equal magnitude.

'Introduction' experiments demonstrated that the effectiveness of a large density of one species in inhibiting a small density of the other depended on where the experiment was conducted. In the unshaded ditch, only *L. minor* could suppress *L. trisulca*. In the shaded site, *L. minor* and green algae inhibited *L. trisulca* and in the oligotrophic site, each was able to inhibit the other.

A transplant experiment was conducted. Portions of the *Lemna* mat from a marsh ditch were transplanted to unshaded enclosures and shaded enclosures in the ditch, and to enclosures in two shaded eutrophic sites. Species composition in the shaded treatment approached that of enclosures in the two shaded sites. *L. trisulca* persisted in the shaded sites and shaded treatment.

Vegetative biomass trends in the marsh ditch indicated spring and fall dominance of *L. trisulca* and summer dominance of *L. minor*. The degree of dominance varied between two arms of the ditch. The shaded site was dominated by *L. minor* , while the oligotrophic site was dominated by *L. trisulca*.

A model was developed to explain dominance patterns (in time and space) by considering resource competition for light and nutrients, different maximum growth rates of the two species and differential mortality. Coexistence of *L. trisulca* with *L. minor* in the unshaded marsh ditch could not be predicted by the model. Consideration of seasonal life history responses of the species helped explain coexistence.

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