

FINAL REPORT
RESTORATION AND RECOVERY OF MASON'S LILAEOPSIS:
PHASE II



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I. EXECUTIVE SUMMARY

Field reconnaissance of the littoral zones of the Sacramento-San Joaquin Delta, Suisun Marsh, and the Napa River Estuary, analyses of soil characteristics, and greenhouse experiments were initiated to investigate the geographic distribution, population threats from invasive aquatic weeds, and the ecophysiology of *Lilaeopsis masonii* Mathias & Constance (Mason's lilaeopsis). This report summarizes the results of this research, conducted from April 18, 1994 to December 31, 1995.

Documentation of the geographic distribution of *Lilaeopsis masonii* continues to expand with additional field reconnaissance, and the documented number of populations also continues to increase. As of October 3, 1995, 122 element occurrences are on record in the California Natural Diversity Data Base (NDDDB).

The aquatic weed, Water hyacinth (*Eichornia crassipes*), is now widespread throughout the Sacramento-San Joaquin Delta, with highest concentrations in sloughs of the central region. This taxon is an aggressive, widespread exotic weed that is a serious threat to *Lilaeopsis masonii* invading all but the western- and northern-most portions of its range. Water hyacinth appears to be limited by relatively higher salinity levels in the west portion of the Sacramento-San Joaquin Delta.

Results from greenhouse experiments suggested that the effects of salinity may depress biomass production in *Lilaeopsis masonii*. Competition experiments clearly demonstrated that *Lilaeopsis masonii* ramets from Pt. Edith and Brannan Island did not differ significantly from each other with respect to competitive ability. Effects of competition on *Lilaeopsis masonii* by *Scirpus cernuus* and *Hydrocotyle verticillata*, but not *Limosella subulata*, are significant, depressing the vegetative growth. However, effects of competition on *Lilaeopsis masonii* by *Limosella subulata* when grown together is not easily decipherable. When *Lilaeopsis* is grown with *Limosella* under fresh conditions, *Lilaeopsis* increases in biomass; it does not change significantly under 7 ppt salinity conditions. *Limosella* decreases under fresh conditions and produces consistently low biomass under 7 ppt salinity conditions. *Limosella* is more sensitive to high salinity values than is *Lilaeopsis*.

Greenhouse experiments have demonstrated that the effects of crude oil under fresh water conditions on the above ground persistence and total biomass of *Lilaeopsis masonii* are significantly detrimental at concentrations of 3.2 L/m² and greater. Under 7 ppt salinity conditions, ramet mortality is much higher at concentrations of 3.2 L/m² and greater than at fresh conditions.

Soil analyses conducted by an independent laboratory on eleven samples collected through the geographic range of *Lilaeopsis masonii* indicate primary (N/P/K) and secondary (Ca/Mg) nutrients range from moderate to fair high concentrations. Minor and trace nutrients range from low (e.g., Zn) to very high (e.g., Fe), but most are moderate to high. All pH's are fairly typical and most have fairly normal buffer indices. Most samples demonstrated very normal Ca:Mg ratios consistent with typical terrestrial soils. One sample (Venice Island), however, deviated from the others with respect to buffering capacity, H⁺ concentrations, and Ca:Mg ratios. Documented soil textures include loam, clay loam, clay, silty clay loam, sandy clay loam, loamy sand, and sand. Microbial levels in the eleven samples varied over several orders of magnitude.

Soils from Pt. Edith also were analyzed to assess the extent latent contamination from the 1987 Shell Oil spill. Results indicated that both soils oiled by the spill and adjacent soils not oiled contain measurable levels of petrochemical hydrocarbons, and should be considered contaminated. However, the oiled soil support contamination levels approximately ten times greater than the adjacent, non-oiled soil (2171 ppm TPH vs. 216.4 ppm TPH).

Many significant populations of *Lilaeopsis masonii* continue to be at risk, largely from human disturbance. Documented forms of disturbance include trampling from fishing and other human activities, riprap, loss of habitat due to development, erosion by wave and wake action, disintegration of pilings that support *Lilaeopsis* populations, and potentially by sea level rising and water quality degradation. The type locality of *L. masonii* presently remains overgrown by invasive species and may be threatened by loss of shoreline.

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II. INTRODUCTION, BACKGROUND, AND PROJECT OBJECTIVES

II.A. Introduction¹

As part of the legal settlement of the 1988 Shell Oil Refinery spill at Martinez, California, a cooperative agreement was entered involving the Shell Oil Spill Litigation Settlement Trustee Committee, San Francisco State University, and the California Department of Fish and Game. The purpose of the cooperative agreement was to begin the restoration and recovery of Mason's lilaeopsis (*Lilaeopsis masonii*; Apiaceae). Several populations of this state-listed rare plant were oiled during the 1988 spill from Shell Oil Company's Martinez refinery. As part of the settlement, various aspects of the geographic distribution, ecophysiology, and molecular genetics of Mason's lilaeopsis have been examined by the research team at San Francisco State University (SFSU), headed by Dr. Peggy Fiedler. This report outlines work accomplished by these researchers at San Francisco State University during Phase II of the restoration and recovery of *Lilaeopsis masonii*.

II.B. Background

II.B.1. History of the Spill

On April 23, 1988, the Shell Oil Company ("Shell") spilled approximately 432,264 gallons² into Peytonia Slough and the Carquinez Strait from its refinery in Martinez, California. The San Joaquin crude oil drained from a tank through a roof drain hose and flowed through an open levee storm drain into the adjacent slough. Shell Oil officials estimated that the tank was leaking for as long as 20 hours before the spill was discovered (EPA 1988). The spill occurred because of equipment failure and improper operation of the levee storm drain valve. Shell's failure to keep the valve closed was contrary to the written procedures that required the

¹We wish to thank Ms. Tish Sprague of the Shell Oil Spill Litigation Trust Committee, and Mr. Kevin Shaffer, Plant Conservation Program, California Department of Fish and Game, for the opportunity to conduct this research. We also would like to thank Lynn Christensen, Eileen Ecklund, Stephanie Kroll, Chris Rogers, Jake Sigg, Mike Vasey, and Mike Wood for assistance in the field.

²The total amount of oil spilled was never settled. Estimates varied, but it is agreed that greater than 400,000 gallons of crude oil were lost from the storage tank.

levee storm drains to be kept routinely closed (EPA 1988).

Crude oil polluted roughly eleven miles of shoreline, from Ryer and Roe Islands in the east to the Carquinez Bridge in the west (EPA 1988). Over 100 acres of wetlands in the 170 acre Shell Marsh were heavily coated with oil, and a layer of crude oil up to 18" thick covered more than a one-mile reach of Peytonia Slough. Heavy oil slicks were observed in the Carquinez Strait between the Carquinez Bridge and Peytonia Slough.

On April 26 and 27, 1988, David Showers, former Project Assistant, Endangered Plant Project, Non-Game Heritage Program, California Department of Fish and Game (CDFG), and warden Cynthia Pourroy, surveyed by boat the vicinity of the oil spill to assess damage potentially incurred by the native plant community and state-listed plant species. The area surveyed extended from the western end of the Martinez Regional Shoreline Park east to Point Edith and Seal Island, Contra Costa County. Showers (1988) reported that 20 percent of a population of *Lilaeopsis masonii* immediately west of Point Edith, extending approximately 30 m, was covered by oil, although most of the population to the east was unaffected. The population at the western-most tip of Seal Island also was oiled. Showers (1988) concluded that *L. masonii* was directly in the path of the oil slicks, and significant populations were covered by heavy crude oil. He recommended that a five-year monitoring program be initiated to assess the indirect consequences of the oil spill on the life history of *L. masonii*, specifically population vigor, reproductive success, latent plant mortality, and the ability of *L. masonii* to recover and reinvade damaged habitat (Showers 1988).

The enforcement actions against Shell Oil were oriented toward three goals: reimbursement of costs, institution of penalties, and natural resource restoration or mitigation. To achieve those goals, Shell Oil agreed to pay \$19,750,000.00 to the California Department of Justice. A portion of this settlement was allocated to the preservation of California plants, wildlife and fisheries, land acquisition, land improvements, and species propagation projects, including the

restoration and recovery of *Lilaeopsis masonii* and its freshwater tidal plant community.

IV.B.2. Natural History of *Lilaeopsis masonii*

IV.B.2.a. Taxonomy

Lilaeopsis masonii Mathias & Constance (Mathias & Constance 1977) is a member of the Apiaceae (Umbelliferae) (Figure 1). Thirteen species have been described for the genus, and all are characteristic of marshy or aquatic habitats in North and South America, and in Australasia (Affolter 1985). Two species, *L. occidentalis* and *L. masonii*, occur on the Pacific Coast, while two taxa, *L. carolinensis* and *L. chinensis*, are found along the Atlantic Coast of North America. *Lilaeopsis schaffneriana* is the fifth species found in North America, being restricted to southeastern Arizona, and central and northern Mexico. The genus has long been recognized as taxonomically difficult due to its vegetative simplicity and thus similarity among the various taxa. As a consequence of this morphology, *Lilaeopsis masonii* is easily overlooked in its native habitat.

IV.B.2.b. Autecology

Lilaeopsis masonii is a short, herbaceous perennial that spreads laterally by rhizomatous growth (Figures 2 and 3). Leaves form tufts borne along the horizontal rhizome or at the apex of vertical rhizome branches. Branches vary in length, from 1.5 to 7.5 (-15.0) cm long, and from (0.2-) 0.4 to 1.2 mm wide. They are terete, linear or filiform, and bear septa not easily seen unless held up to the light. Small white to greenish flowers occur in simple umbels, bearing 3-8 flowers per umbel. The flowering period is between April and October with the fruits maturing between June to October (Affolter 1985).

The geographic distribution of *Lilaeopsis masonii* is mainly within the Sacramento-San Joaquin Delta ("Delta") and Suisun Bay. Additional significant populations occur within the Napa River watershed and in Suisun Marsh. Although a voucher specimen for *Lilaeopsis masonii* from Tomales Bay (Marin County) is on deposit at the U.C. Berkeley Herbarium,

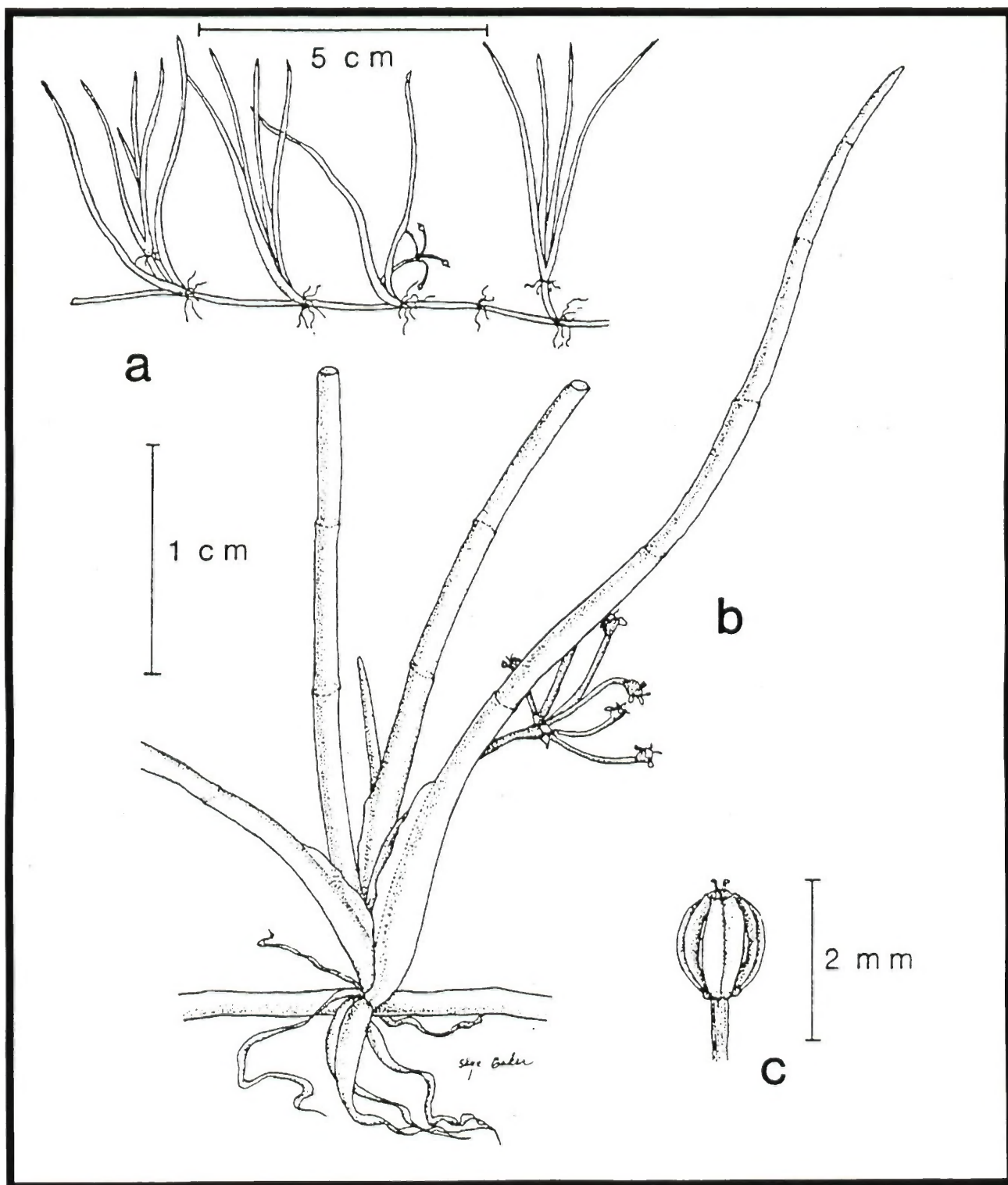


Figure 1. Drawing of *Lilaeopsis masonii* (Source: Affolter 1985).



Figure 2. Individual ramet of *Lilaeopsis masonii* at Brannan Island, California Natural Diversity Data Base population No. 23.



Figure 3. Extensive clonal colony of *Lilaeopsis masonii* along the shoreline of Montezuma Island.

additional surveys during Phase I of this research of the recorded site have not confirmed the Marin County location record. We believe that this population has been extirpated. Counties that have recorded populations of *Lilaeopsis masonii* include Contra Costa, Napa, Sacramento, San Joaquin and Solano.

Lilaeopsis masonii occupies the littoral zone in the freshwater and brackish tidal marshes of the Sacramento-San Joaquin Delta, Suisun Bay, Suisun Marsh, and the Napa River. As such, most populations of *L. masonii* are inundated twice daily by high tides, but are also exposed for a large portion of the day by low tides. However, because the timing of high and low tides vary daily, during some days *L. masonii* will spend a significant portion of the daylight period underwater when it would otherwise be photosynthetically active, while other times of the month just the opposite is true.

The habitat of *Lilaeopsis masonii* can be described as a tidally inundated, wave-cut beach or earthen levee. Any given population is therefore transient because erosion is constantly causing the substrate to slump into the adjacent water body. But because *Lilaeopsis masonii* appears to be so successful at spreading vegetatively by rhizomatous growth, it can tolerate these disturbances very well. Indeed, these natural episodes of erosion in its environment may be instrumental in protecting the diminutive plants of *Lilaeopsis masonii* from being overgrown and eventually replaced by larger plants that may require a more stable substrate. The largest and healthiest populations of *Lilaeopsis masonii* are found growing on the uninhabited islands in Suisun Bay where there is no riprap and little human activity. As might be expected, the smallest and most endangered populations occupy old pilings or sites with extensive riprap or significant foot traffic.

Lilaeopsis masonii is typically found growing on a clay or clay loam substrate, occasionally with significant amounts of silt or sand (see Results section IV.C. Soil Analyses and Table 3). Populations of this species also can be found on old pilings, shipping debris, or riprap. As

mentioned above, *Lilaeopsis masonii* reproduces primarily by vegetative lateral spread of its rhizomes; therefore, most populations are likely composed of clonal colonies (ramets). Given the broad tolerance of substrate texture, it is likely that substrate physical characteristics are not limiting the physical and geographic distribution of this rare plant. Small clonal tufts of *Lilaeopsis masonii* were observed floating in the Delta region. This may be a significant method by which sites are colonized.

Lilaeopsis masonii has been recorded growing on sites inundated by saline waters as low as 0.25 parts per thousand (ppt), and as high as 8.5 ppt (Golden and Fiedler 1991). Further field investigations of its distribution may show populations tolerating even higher salinities than presently recorded.

With regard to its protection status, *Lilaeopsis masonii* is listed currently as Rare by the state of California (California Department of Fish and Game 1996), and is a Category 2 candidate for federal listing by the U.S. Fish and Wildlife Service. It is also included on the California Native Plant Society's List 1B -- *i.e.*, plants that are rare, threatened, or endangered in California or elsewhere (Skinner and Pavlik 1994).

II.B.3. Research Completed in Phase I

Phase 1 of the Shell Oil Spill Settlement project for the recovery of *Lilaeopsis masonii* included field and laboratory research that resulted in the following findings.

1. The known distribution of this rare plant expanded greatly as additional sitings were made by various researchers (*e.g.*, Golden and Fiedler 1991, Golden 1992, Fiedler and Zebell 1993, Grewell unpublished) and consultants. More importantly, the number of populations of *Lilaeopsis masonii* also greatly increased.

2. Individual ramets of *Lilaeopsis masonii* grown under greenhouse conditions in a series of

water salinities revealed that:

- (a) ramets of *Lilaeopsis masonii* exhibited significantly decreased growth at 3 ppt, 6 ppt, 9 to 12 ppt, and 24 ppt salinity;
- (b) ramets of *Lilaeopsis masonii* exhibited significantly different responses to salinity regimes depending upon the collection locations of the ramets. Ramets from Harris Harbor and Pt. Edith (*i.e.*, relatively more saline locations) exhibited the highest weight increases across virtually all salinities investigated. Ramets from Collinsville (*i.e.*, relatively more fresh water) showed the lowest weight gains across virtually all salinities investigated. Ramets from Brannan Island displayed weight increases intermediate between these ranges;
- (c) ramets from Brannan Island gained the greatest weight of any ramet tested at a salinity above 0 ppt;
- (d) virtually no growth occurred for any ramet at 24 ppt; and
- (e) ramets grown from Pt. Edith did not appear to exhibit latent depressed growth as a result of the Shell Oil Refinery spill. Salinities in the experiment ranged from 0 ppt to 24 ppt, encompassing the water conditions naturally experienced throughout the range of this rare species.

3. Seed germination experiments of *Lilaeopsis masonii* in salt water revealed that:

- (a) water salinity and germination success of *Lilaeopsis masonii* seeds were negatively correlated. Percent germination decreased with increasing salinity;
- (b) time till germination (*i.e.*, radical emergence) increased as salinity increased. Thus, seeds took longer to germinate as salinity increased; and
- (c) seeds germinated in salinities as high as 12 ppt.

4. The molecular genetics of *Lilaeopsis masonii* were investigated using standard molecular genetic techniques. Base pair sequences from one internally transcribed spacer region (ITS2) of the nuclear ribosomal DNA were analyzed for 13 populations of *L. masonii* and one

population of *L. occidentalis*. Results revealed that:

- (a) it was possible to sequence the ITS2 section from *Lilaeopsis masonii* and *L. occidentalis* and match it to the similar region from carrot. The ITS2/25S boundary was thus identified;
- (b) no nucleotide variation was found either within *L. masonii* or between *L. masonii* and *L. occidentalis*; and
- (c) finding (b) suggests close phylogenetic affiliation between *L. masonii* and *L. occidentalis*.

II.C. Project Objectives for the Restoration and Recovery of *Lilaeopsis masonii*, Phase II

With specific regard to *Lilaeopsis masonii*, the primary objective of the legal settlement for the April 1988 oil spill at the Shell Oil Refinery at Martinez was to initiate research into its life history and the community dynamics of its freshwater tidal plant community for the implementation of the eventual recovery of the populations damaged by the spill. The project was initially divided into two phases. Phase I provided detailed background into the geographic distribution, ecophysiology, and molecular genetics. Phase II, the focus of this report, was initiated to continue additional field surveys, document aquatic weed threats, and to conduct soil analyses and greenhouse experiments on the effects of (a) competition on *Lilaeopsis masonii* by native members of the littoral community, and (2) crude oil on above- and below-ground growth and survival of *Lilaeopsis masonii*.

III. MATERIALS AND METHODS

III.A. Field Research

III.A.1 Distributional Surveys

It is generally agreed that the heightened salinity of San Pablo Bay limits the western-most occurrence of *Lilaeopsis masonii* to the mouth of the Napa River and the east end of the Carquinez Strait. It is less well known what limits *L. masonii* in the eastern, fresh water portion of its range. To better define this upstream limit of *L. masonii* we focused much of our survey efforts in this eastern zone .

Field surveys were conducted by car and boat. Boat surveys utilized a 10.5 foot Achilles inflatable raft with a 9.9 horse power Evinrude outboard motor. Fifteen field surveys (12 by boat and three by car) were conducted from July to October 1995 (Table 1). Information used to select final survey locations was in part obtained from personal communication with Ms. Brenda Grewell, Department of Water Resources, and Dr. Niall McCarten, Jones & Stokes.

Data collected during the field reconnaissance included the presence or absence of *L. masonii*, *Limosella subulata*, and *Eichornia crassipes*, as well as associated plant species. Casual vegetation data recorded included plant species associated with *L. masonii* within the littoral zone, as well as those plant species found behind the littoral zone as part of the near shore habitat. Plants were keyed in Hickman (1993), Mason (1969), or Munz (1973). Plant nomenclature follows Hickman (1993).

III.A.2. Estimation of Habitat Invasion by Water Hyacinth

Eichornia crassipes, Water hyacinth, is a member of the Pickerel-Weed family (Pontederiaceae), and originates from the American tropics. It is a free-floating, perennial herb typically bearing conspicuous inflated petioles that provide bouyancy for the individual. Lavender, bisexual flowers are produced on a spike or panicle inflorescence. Individual plants, however, multiply vegetatively, and are known to spread exceedingly rapidly. It is

considered by some to be the world's most noxious aquatic weed (Hickman 1993).

Table 1. Dates, locations and significant species found for 1995 *Lilaeopsis* field surveys.

Date	Boat/Car	Approximate location	Species found
7/26/95	Car	Brannan Island-SS and Hill Slough-SS	<i>L. masonii</i> , <i>E. crassipies</i>
8/8/95	Car	Collinsville-SS	<i>L. masonii</i> , <i>E. crassipies</i>
8/9/95	Boat	Steamboat, Miner, Cache-SS, and Sutter Sloughs	<i>L. masonii</i> *
8/10/95	Boat	Prospect Slough	<i>L. masonii</i> *
8/11/95	Boat	Petaluma River	None
8/23/95	Car	Vallejo Park-SS	<i>L. masonii</i>
8/25/95	Boat	Honker Cut, Venice Island-SS, Little Potato Slough, White Slough, and Disappointment Slough-SS	<i>L. masonii</i> *, <i>E. crassipies</i> , <i>L. subulata</i> ,**
9/5/95	Boat	Snodgrass Slough	<i>E. crassipies</i> , <i>L. subulata</i> **
9/6/95	Boat	Lost Slough	<i>E. crassipies</i> , <i>L. subulata</i> **
9/7/95	Boat	Prospect Slough-SS	<i>L. masonii</i> *
9/8/95	Boat	Pt. Edith-SS	<i>L. masonii</i>
9/22/95	Boat	Browns Island	<i>L. masonii</i> , <i>L. subulata</i>
10/2/95	Boat	Miner, Sutter, and Elk Slough	None
10/4/95	Boat	Honker Cut and Disappointment Slough	<i>E. crassipies</i>
10/5/95	Boat	Confluence of Mokelumne and Consumnes rivers	None

* = new locations

** = possibly new locations

SS = soil samples collected

Field surveys for Water hyacinth were conducted by car and boat. Boat surveys utilized a 10.5 foot Achilles inflatable raft with a 9.9 horse power Evinrude outboard motor. Fifteen field surveys were conducted from July to October 1995. Additional information about the present distribution of Water hyacinth in the Delta was obtained primarily from Ms. Valerie Van Weigh, California Department of Boating and Waterways, Sacramento, California.

III.A.3. Field Collection

III.A.3.a Soil Samples

Eleven soil samples were collected from sites distributed throughout the geographic range of *Lilaeopsis masonii*. Sites were chosen that include the major watersheds within the range of *L. masonii* (i.e., the Napa, Sacramento, and San Joaquin rivers and Suisun Marsh), and include sites known to exhibit the range of substrates upon which *L. masonii* grows, including the mostly sandy substrates of Brannan Island and the highly organic substrates of Venice Island. Soil also was collected from Point Edith both within and outside of areas covered by oil during the 1989 Shell oil spill. These samples were collected so as to assess the levels of petrochemical hydrocarbons remaining in the soils that support populations of *L. masonii*.

Soil samples consisted of three small subsamples collected at a single site within 30 m of each other within the root zone of *L. masonii* (i.e., top 5 - 8 cm). Subsamples were put into ziplock bags, kept on ice in the field, mixed into a single sample in the lab, and stored at 4°C until analyzed. Soil samples were analyzed by an independent laboratory, Environmental Technical Services, Petaluma, California, for texture, pH, organic content, macro- and micro-nutrients, microbial content, and petrochemical hydrocarbons for the Pt. Edith samples.

III.A.3.b. Material for Competition Experiments

Limosella subulata, *Scirpus cernuus*, and *Hydrocotyle verticillata* are native wetland species and all are indigenous to the San Francisco Estuary, Suisun and Delta ecosystems. These three taxa were included in the competition experiments because they are commonly associated with *L. masonii* in the littoral zone (Golden 1992) and are similar in size to *L. masonii*.

Limosella subulata Ives, Delta mudwort (Figure 4), is a diminutive, tufted perennial of the Figwort family (Scrophulariaceae) that bears linear, awl-like or subcylindrical leaves and a scapose inflorescence with a single, white flower. It resembles *Lilaeopsis masonii* superficially,

making identification of the two taxa difficult when they grow intermixed. *Hydrocotyle verticillata* (Figure 5) is a low-growing perennial of the Carrot family (Apiaceae) with a creeping rhizome that typically roots at the nodes. Leaves of *H. verticillata* are round-peltate and can be entire, shallowly or rarely deeply lobed. Small yellowish or greenish flowers are borne on a simple umbel, and the small fruit is elliptical or round with greatly compressed sides. *Scirpus cernuus* (Figure 6) is a small, densely-tufted annual bulrush in the Sedge family (Cyperaceae), with stems either erect or somewhat strongly arched. The inflorescence consists of a single spikelet with glabrous, greenish to pale or dark orange or red-brown floral bracts. The fruit is a tiny, sharply angled achene.

Plant material of *L. subulata*, *S. cernuus*, and *H. verticillata* used in this competition experiment was collected from Brannan Island on July 13, 1994. Plant material of *L. masonii* used in this experiment was collected from Brannan Island and Pt. Edith on September 10 and September 12, 1992, respectively, and had subsequently been maintained in the SFSU greenhouse.

III.B. Laboratory Greenhouse Studies

III.B.1. Initial Competition Experiment

We chose to conduct competition experiments on four commonly or occasionally occurring taxa within the littoral zone of the Delta and Suisun Marsh. These are *Lilaeopsis masonii*, *Limosella subulata*, *Scirpus cernuus*, and *Hydrocotyle verticillata*. *Lilaeopsis masonii* and *Limosella subulata* are considered rare species, and *Scirpus cernuus* and *H. verticillata* were included because they are both common, and commonly associated with *L. masonii* in the littoral zone (Golden 1992).

III.B.1.a. Experimental Design

Three treatment factors were assessed: (1) species associations; (2) collection location of *L. masonii*, (Brannan Island and Point Edith); and, (3) salinity (0 ppt and 7 ppt). Three

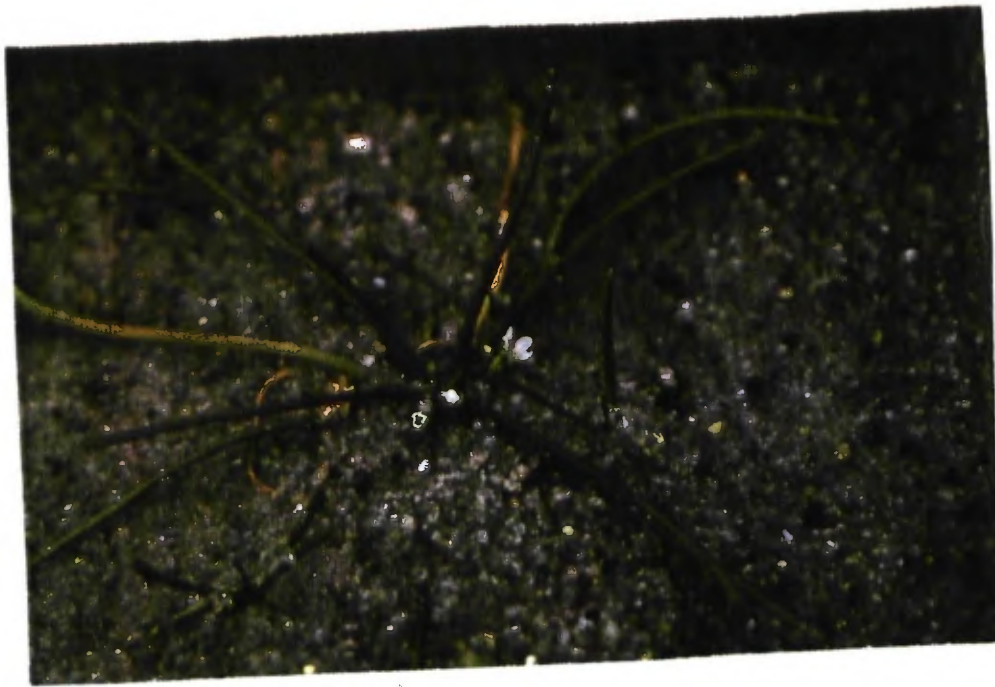


Figure 4. Greenhouse material of *Limosella subulata* used in the competition experiments.

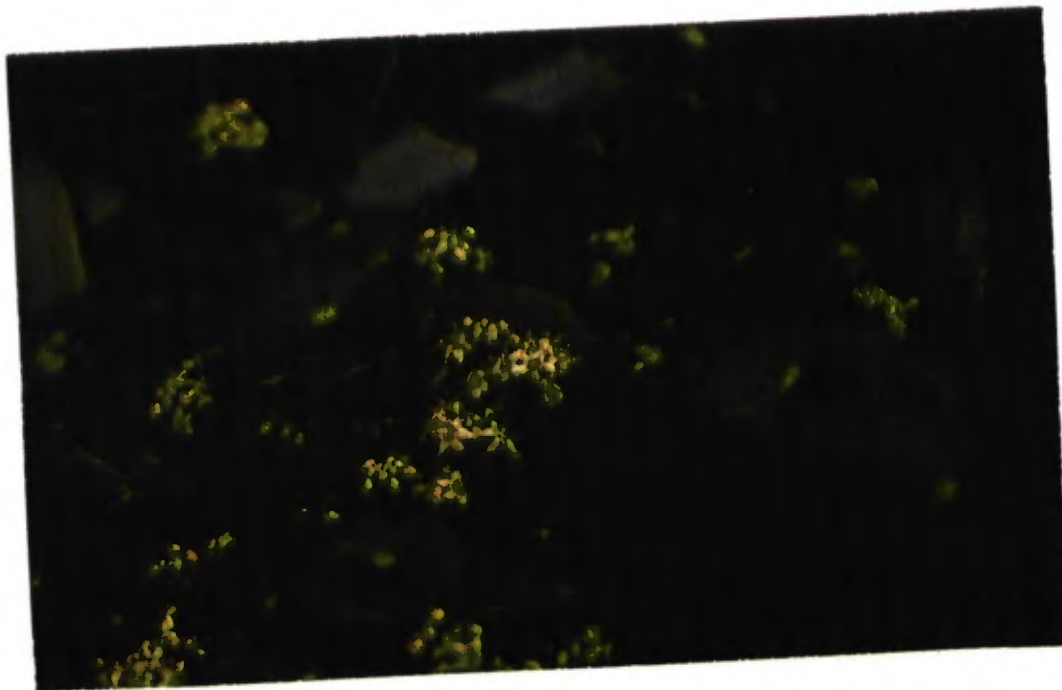


Figure 5. Greenhouse material of *Hydrocotyle verticillata* used in the competition experiments.

different species assemblages also were compared: (1) *L. masonii* and *L. subulata*; (2) *L. masonii*, *L. subulata*, and *S. cernuus*; and, (3) *L. masonii*, *L. subulata*, *S. cernuus*, and *H. verticillata*. Four individual ramets of each species were planted per flat (10 cm x 17 cm) with five replicate flats per treatment condition. All flats were planted with the same species pattern depicted below. Only those species making up a particular assemblage were included in any experimental flat. Planting positions of species not comprising the assemblage were left absent.

<i>L. masonii</i>	<i>S. cernuus</i>	<i>H. verticillata</i>	<i>L. subulata</i>
<i>L. subulata</i>	<i>H. verticillata</i>	<i>L. masonii</i>	<i>H. verticillata</i>
<i>S. cernuus</i>	<i>L. subulata</i>	<i>S. cernuus</i>	<i>L. masonii</i>
<i>H. verticillata</i>	<i>L. masonii</i>	<i>L. subulata</i>	<i>S. cernuus</i>

This planting pattern stratified each flat into four distinct regions: corners, interior, and sides (two types, narrow and wide; the flats are not square). Within each region, species were randomly positioned so that the ramets were not directly adjacent to a conspecific neighbor. This species pattern randomly distributed ramets throughout each flat within each distinct flat region while minimizing within species interactions.

Treatment effects were quantified as grams dry weight biomass relative to controls. Controls consisted of flats containing a single species planted in the same pattern, density, and replication as the other experimental flats. Initial dry weight conditions for each species were established by drying and weighing five randomly selected subsets of four ramets each immediately prior to planting.

Total number of flats in this experiment was 110 ((number of species combinations [3; *Lilaeopsis*, *Limosella*, *Scirpus*] x collection locations [2; Point Edith and Brannan Island] x salinities [2; 0 and 7 ppt] x replicates [5; sample size of treatments]) + (controls [5; sample size of monotypic flats] x salinities [2; 0 and 7 ppt] x replicates [5; sample size of controls]) = 110)). Total number of plants included was 920 (320 *L. masonii* [160 each from Brannan Island and Pt. Edith], 280 *L. subulata*, 200 *S. cernuus*, and 120 *H. verticillata*).

III.B.1.b. Experimental Run

The competition experiment was established on October 6 and October 7, 1994, in the greenhouse at SFSU (Figure 7). Target conditions were achieved November 1, 1994 and the experiment continued until January 9, 1995, for a total of 70 days. During the experiment plants were watered as needed with tap water until saturated, and every three weeks with a nutrient solution consisting of 1/4 strength Hoagland's solution. Flats subjected to the salinity treatment had 7 parts per thousand (ppt) "Instant Ocean" added to the watering solution. Salinity was increased gradually to 7 ppt over the first three weeks to minimize osmotic shock. Every three to four weeks flats were randomly rearranged to minimize effects of subtle variation in sunlight and temperature across the greenhouse bench. Flats were sprayed every few weeks with "Safer Soap" to prevent plant pest damage from aphids, white flies, and mealy bugs.

The competition experiment was disassembled January 9 through January 11, 1995. Control plants were cleaned, divided into roots (*i.e.*, tissue at and below the point of attachment of the rhizome/stolon) and shoots (*i.e.*, tissue above the point of attachment of the rhizome/stolon), and pooled by flat into a paper bag. Each bag was dried and weighed. Remaining plants were similarly cleaned but left whole, then pooled by flat and species into a paper bag, dried, and weighed. Data were entered into Microsoft Excell, analyzed using Biostat 1 version 2.0, and summarized in graphical form using DeltaGraph Pro version 3.1.

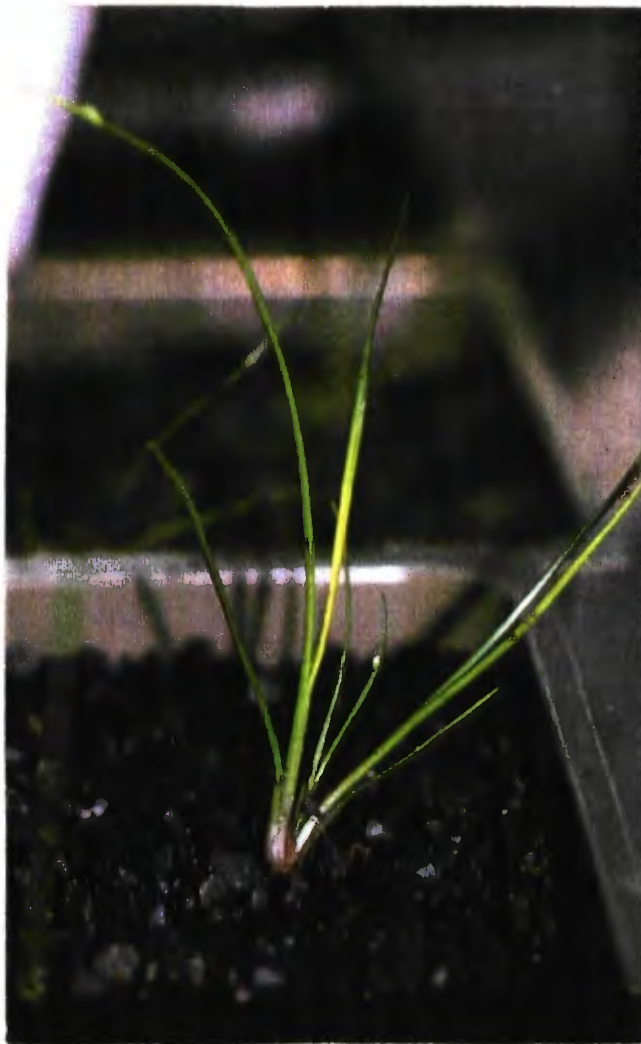


Figure 6. Greenhouse material of *Scirpus cernuus* used in the competition experiments.

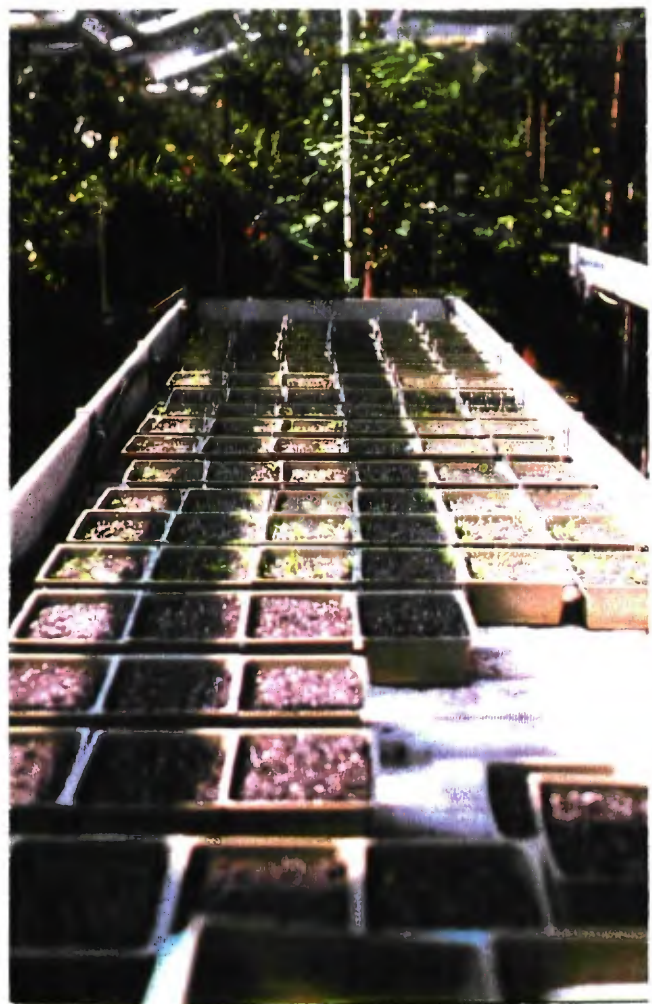


Figure 7. Competition experiment for *Lilaeopsis masonii* at the greenhouses at San Francisco State University.

III.B.2. Second Competition Experiment

III.B.2.a. Experimental Design

Because of the high mortality of *L. subulata* during the initial competition experiment, a second, smaller competition experiment was rerun with *L. subulata* and *L. masonii* (Pt. Edith source material). The design of this second experiment was similar to the first with the same plant density, planting pattern, replication, and controls, with a similar nutrient solution and/or 7 ppt "Instant Ocean" watering regime, and gradual salinity increase for minimizing osmotic shock. The following changes were made, however, with the intent of increasing *L. subulata* survival: (1) use of a more coarse and evenly grained sand as the planting medium; (2) spraying with two fungicides prior to planting as well as during the first few weeks after planting; and (3) 30% shade cloth erected around the entire experimental area.

Treatment effects were similarly quantified using grams dry weight biomass relative to controls. Initial dry weight conditions were established by drying and weighing plant material from five additional flats upon achieving the target experimental conditions of 7 ppt salinity. The total number of flats in this experiment was 25 ((salinities [2; 0 and 7 ppt] x replicates [5; sample size of treatments]) + (*L. subulata* controls [10; sample size of controls]) + (flats for initial conditions [5; sample size of treatments]) = 25)). Total number of plants was 160 (100 *L. subulata* and 60 Pt. Edith *L. masonii*).

III.B.2.b. Second Experimental Run

This competition experiment was established in conjunction with the crude oil experiment described below. Material was planted February 14, 1995 with 7 ppt salinity conditions being achieved by March 9, 1995. No mortality of *L. subulata* occurred prior to the establishment of 7 ppt salinity. Individuals of *L. subulata* in the fresh water treatment exhibited vigorous growth. Plants were watered every few days with water and/or 7 ppt "Instant Ocean." Every two weeks a nutrient solution (1/4 strength Hoagland's solution) was added to the watering mix. Every three to four weeks flats were randomly rearranged to minimize effects of subtle

variation in sunlight and temperature across the greenhouse bench.

The experiment was disassembled June 6 and 7 1996 in the greenhouse at SFSU after 88 days. Plants were cleaned, divided into roots (*i.e.*, tissue at and below the point of attachment of the rhizome/stolon) and shoots (*i.e.*, tissue above the point of attachment of the rhizome/stolon), pooled by species and by flat into paper bags. Each bag was dried and weighed. Data were entered into Microsoft Excell, analyzed using Biostat 1 version 2.0, and summarized in graphical form using DeltaGraph Pro version 3.1.

III.B.3. Crude Oil Experiments

An experiment was established in the SFSU greenhouse to assess the toxicity of crude oil on the vegetative growth of *L. masonii*. Approximately four liters of Alaskan North Slope crude oil were obtained from the California Department of Fish and Game for this experiment. Plant material used in the oil experiment was propagated from material collected at Pt. Edith on September 12, 1992, and subsequently maintained in the SFSU greenhouse.

III.B.3.a. Experimental Design

The crude oil experiment was designed to be similar to the competition experiment to facilitate meaningful comparisons. The same density of plant material (*i.e.*, four ramets of Pt. Edith *L. masonii*) was planted in the same size flat (10 cm x 17 cm), in the same spatial pattern, with the same replication (five flats each treatment dosage) and controls, with the same watering regime of a nutrient solution (1/4 strength Hoagland's) and/or 7 ppt "Instant Ocean", and a similar gradual increase of salinity to minimize osmotic shock. The following changes were made so that experimental controls also would apply to the second competition experiment described above: (1) use of a more coarse and evenly grained sand; (2) spraying with two different fungicides prior to planting as well as during the first few weeks after planting; and (3) 30% shade cloth erected around the entire experimental area.

Two treatment factors were assessed: dosage of crude oil and salinity (0 ppt and 7 ppt). Treatment effects were quantified using grams dry weight biomass relative to controls. Initial dry weight conditions were established by drying and weighing plant material from five extra flats upon achieving the target experimental conditions of 7 ppt salinity and oil application.

To obtain a more detailed assessment of vegetative response to crude oil application, percent plant cover for each flat was recorded. A transparent clip board on which were drawn 12 four cm squares was placed above each flat and the percent vegetative cover of live leaves was recorded for each square. Percent cover was recorded weekly for the first month and bi-monthly for the duration of the experiment. Mortality also was recorded.

Crude oil dosages were chosen to provide a wide range of application. Grant (1993) applied 0.2 L/m² of crude oil, a dosage he considered small, in his experiments with grey mangrove seedlings. DaLaune (1979) applied from 1 to 32 L/m² oil in his crude oil experiments involving Cordgrass (*Spartina alterniflora*). We chose to test 0.0, 0.2, 0.8, 3.2, and 12.8 L/m² of oil dosages to encompass the range of applications of crude oil tested in the literature and in anticipation of finding a significant effect. These dosages corresponded to 0, 4, 16, 64, and 256 ml of crude oil per flat. The oil was then applied to each flat in a total volume of 300 ml with the non-oil volume being made up with water. This was done to provide a sufficient application volume so that the oil would pool, at least temporarily, on the sand surface and cover completely the leaf surfaces, even at low oil dosages.

Prior to application, the crude oil was aged following the method of Grant (1993). Aging consisted of floating the crude oil on salt water (33 ppt "Instant Ocean") for 24 hours under a vented hood. Aging the oil prior to application is thought to more closely mimic an actual oil spill since most spills travel over open water prior to reaching land. While traveling over open water the oil vents off most of its volatile components.

The experimental flats were placed on 1/4 inch dowels over a plastic lined greenhouse bench (Figure 8). Plastic sheeting was used to contain the oil and thus prevent it from draining outside of the experimental area. Dowels elevated the experimental flats off the surface of the plastic so that liquid draining from adjacent flats would not contaminate others. Excess drained solution was syphoned off the plastic surface with a three foot piece of rubber tubing and stored in plastic tubs as hazardous waste. Total number of flats in this experiment was 55 (crude oil dosages [5; 0.0, 0.2, 0.8, 3.2, and 12.8 L/m²] x salinities [2; 0 and 7 ppt] x replicates [5; sample size of treatments]) + (flats for initial conditions) [5; sample size of controls]) = 55). Total number of ramets was 220.

III.B.3.b. Experimental Run

We established the above experiment in conjunction with the second competition experiment described in Section III.B.2.a. Material was planted February 14, 1995, with 7 ppt salinity conditions being achieved March 9, 1995. Aged crude oil was applied March 10, 1995. In the lower dosage applications, oil drained through the sand relatively quickly, generally within a few hours. In the higher dosage applications, oil remained pooled on the surface of the sand for up to five days. In all oil dosages, leaves of *L. masonii* were completely covered by the oil/water mixture. Plants were watered every few days with water and/or 7 ppt "Instant Ocean." Every two weeks a nutrient solution (1/4 strength Hoagland's solution) was added to the watering mix. Every three to four weeks flats were randomly rearranged to minimize effects of subtle variation in sunlight and temperature across the greenhouse bench. Percent cover and mortality were recorded weekly for the first month and bi-monthly for the duration of the experiment.

The experiment was disassembled June 6 and 7 1996 in the greenhouse at SFSU after 88 days. Plants were cleaned, divided into roots (i.e., tissue at and below the point of attachment of the rhizome/stolon) and shoots (i.e., tissue above the point of attachment of the rhizome/stolon), pooled by flat into a paper bag. Each bag was dried and weighed. Data

were entered into Microsoft Excell, analyzed using Biostat 1 version 2.0, and summarized in graphical form using DeltaGraph Pro version 3.1.

IV. RESULTS AND DISCUSSION

IV.A. Geographic Distribution

The number of known locations of *Lilaeopsis masonii* continues to expand as additional efforts are made to determine the geographic extent of *Lilaeopsis masonii* and other rare plants of the Sacramento-San Joaquin Delta, Suisun Marsh, and San Francisco Estuary. As of October 3, 1995, 122 element occurrences are on record in the California Natural Diversity Data Base (CNDDB) (Plant Conservation Program, California Department of Fish and Game).

As part of this project we conducted 14 boat surveys in an attempt to find additional locations in previously unrecorded regions of the Bay Area. The Petaluma River was surveyed from its mouth to just below the city of Petaluma on 2 August 1993. There were scattered areas of apparently suitable habitat, but no plants were found. A survey of the lower reach of the Napa River on 27 September 1993 revealed one new location near the Napa/Solano County border. An additional survey on 12 October 1992 revealed two new locations, one each in Disappointment and Whiskey Sloughs in San Joaquin County south of Terminous. Table 2 lists the new locations recorded during this phase of research.

IV.A.1. Summary of Findings

- (1) *Lilaeopsis masonii* is widely distributed throughout the Sacramento-San Joaquin Delta, and in the lower reach of the Napa River. It may properly be considered one of the most widespread of all California state-listed rare species.
- (2) It is found on a wide variety of substrates and salinities, and varies considerably in population size.
- (3) No populations have been located west of the Napa River system.

IV.B. Geographic Distribution of *Eichhornia crassipes* and Impact to Mason's *Lilaeopsis*

One goal of this project was to document the range of geographic encroachment of Water hyacinth (*Eichhornia crassipes*) into *Lilaeopsis masonii* habitat in order to assess the threat *E.*

Table 2. Locations of new populations of *Lilaeopsis masonii* found during field surveys conducted for Phase II.

<u>General Location</u>	<u>USGS Quadrangle/ County</u>	<u>Dates Surveyed</u>	<u>Approximate Population Number/Size/Cover</u>
Prospect Slough	Rio Vista & Liberty Island/Solano	8/9/95; 8/10/95; 9/7/95	22 small scattered populations found on interior islands and slough banks ranging in size from one to 50 ft ² each up to 100% vegetative
Disappointment Slough	Terminous/ San Joaquin	8/25/95	One small population approximately 25 ft ² in size and 25% vegetative cover on Tower Island near mouth of Honker Cut
Ward Cut	Terminous/ San Joaquin	8/25/95	One small population approximately 25 ft ² in size and 25% vegetative cover on small island across from Tinsley Island
White Slough	Terminous/ San Joaquin	8/25/95	One small population approximately 10 ft ² in size and 60 percent vegetative cover found on interior islands

crassipes possesses to long-term viability of *L. masonii* populations. We found large floating mats of Water hyacinth primarily in the Central Delta. For example, large populations of Water hyacinth line the shores of Venice Island (Figure 9), and individual clumps of both *Lilaeopsis* and *Eichornia* can be found together, suggesting light and other resource competition.

In addition to our field investigations, and as mentioned previously, we also obtained background information on the current and historic distribution of *E. crassipes* in California, primarily from the California Department of Boating and Waterways, but also from personnel at the USDA Aquatic Weed Lab in Davis, California; the California Department of Agriculture's Weed Herbarium. All agency offices are located in Sacramento, California.

Eichornia crassipes has a long history of establishment in California and continues to spread throughout the state. *Eichornia crassipes* was first recorded in California in 1904 from Yolo

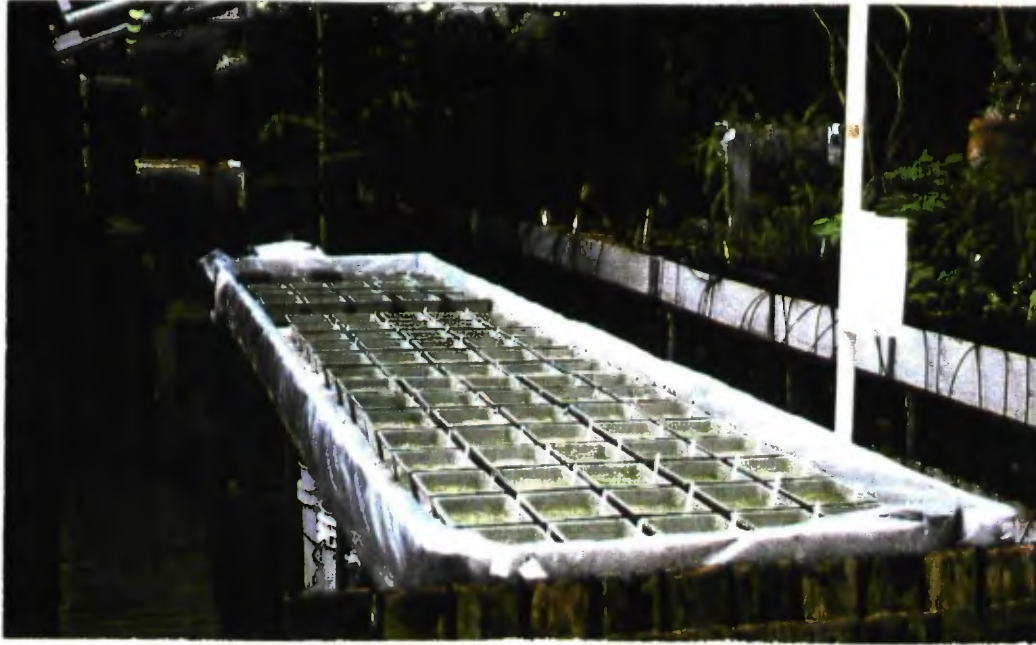


Figure 8. Crude oil experiment for *Lilaeopsis masonii* at the greenhouses at San Francisco State University.



Figure 9. Water hyacinth (*Eichornia crassipes*) lining the shores of the San Joaquin River at Venice Island.

County. The first attempt at eradicating *E. crassipes* in California dates from 1947 when the herbicide 2,4-D mixed with diesel fuel was applied to plants in Rock Slough. In 1982, California Senate Bill 1344 was signed into law appropriating money and designating the Department of Boating and Waterways as the lead agency to implement and develop short and long range programs for the control of *E. crassipes*. Mechanical harvesting, herbicides, and biological controls (i.e., two species of weevil) have been used in California. Since 1982, 4,800 acres have been sprayed with herbicides throughout California to eradicate *E. crassipes*.

The 1994 Summary of Operations Water hyacinth Chemical Control Program report (CDBW 1994) from the California Department of Boating and Waterways divided the spraying operation into four geographic areas: West, North, Central, and South Sacramento-San Joaquin Delta. In 1994, 2,742 acres throughout the Delta were treated with herbicides to control *E. crassipes*. In the West Delta only 48 acres were treated. Growth of *Eichornia crassipes* is inhibited by the increased salinity in this area, however. Most of the spraying (2,071 acres) occurred in the Central Delta -- a region that includes high quality *Lilaeopsis masonii* habitat.

The 1995 Summary from the California Department of Boating and Waterways (CDBW 1995) reported that because 1995 was an extremely wet year, high water levels were sustained until late June. In addition, the water remained cool such that vegetative growth of Water hyacinth remained slow through June. Eradication efforts concentrating in the Central Delta began April 13, 1995, and ended December 10, 1995. Little spraying occurred in the Tuolumne and Upper San Joaquin rivers. Specifically, 1,246 acreages were treated in the Central Delta in 1995, 127 acres in the North Delta, 160 acres in the West Delta, 44 acres in the San Joaquin River area, and 17 acres in the Tuolumne River (CDBW 1995). The primary herbicide used as Weedar 64^R, with a limited amount of Rodeo^R applied as well.

The 1995 CDBW summary also reported further spreading of Water hyacinth throughout the Delta. It was reported that because of the higher freshwater content of the water in the West Delta, the hyacinth spread to Grizzly Island in Solano County. California Department of Fish and Game (CDFG) personnel notified the CDBW Water hyacinth team of this problem, and in November of 1995, four sites were treated with herbicide.

IV.B.1. Summary of Findings

- (1) *Eichornia crassipes* is widespread throughout the Sacramento-San Joaquin Delta, with high concentrations in sloughs of the central region.
- (2) The Water hyacinth eradication program has been in place for 10 years beginning in Merced County, primarily on the Merced River and to a lesser degree, the San Joaquin River and its tributaries.
- (3) We have observed that populations of *Lilaeopsis masonii* within the San Joaquin River system are negatively impacted by *Eichornia crassipes*, most likely through: (a) competition for light; (b) physical obstruction of the shoreline habitat, preventing colonization; and (c) physical disturbance by individuals and/or clumps of individuals of *Eichornia crassipes* on existing populations of *Lilaeopsis masonii* and their habitat, as *Eichornia crassipes* is forced onto the shoreline by waves and boat wakes.
- (4) While undocumented, *Lilaeopsis masonii* populations may be negatively impacted by the application of herbicides targeted for *Eichornia crassipes* either by direct contact with herbicide due to wind drift or by smothering from dead vegetative *Eichornia* mats.
- (5) Personnel from the CDBW Chemical Control Program are eager to work with other state personnel to minimize the adverse effects of herbicide spraying on *Lilaeopsis masonii*.

IV.C Soil Analyses

Soil analyses conducted by an independent laboratory, ETS, on eleven samples collected throughout the geographic range of *Lilaeopsis masonii* indicated primary (N/P/K) and secondary (Ca/Mg) nutrients range from moderate to fairly high concentrations (Table 3).

Greatest variability among samples documented for macronutrients was found in levels of potassium (K). Minor and trace nutrients range from low levels (*e.g.*, zinc [Zn]) to very high levels (*e.g.*, iron [Fe]), but most nutrient levels are moderate to high. All soil pH's are fairly typical (range of 6.1 at Pt. Edith to 7.2 at Brannan Island east), and most have fairly normal buffer indices (Table 3). Salinities range from levels indicating normal terrestrial soils (*e.g.*, 0.11 mmhos/cm at Disappointment Slough; Table 3) to moderately saline soils. Samples from Pt. Edith are roughly 20% marine salinity, and the soil sample from Vallejo Park is approximately 36%, indicating the greatest marine influence. This is consistent with soil from Vallejo Park being the western-most sample collected. Soil from Hill Slough was intermediate in salinity among all samples.

Most samples demonstrated very normal Ca:Mg ratios consistent with typical terrestrial soils (Table 3). One sample (Venice Island), however, deviated from others with respect to Ca:Mg ratios as well as buffering capacity and H⁺ concentrations. Documented soil textures included a very broad range of loam, clay loam, clay, silty clay loam, sandy clay loam, loamy sand, and sand. Thus, soil texture values indicated hydraulic conditions ranging from typical sporadic runoff to conditions of slack water or water with more or less continuous (or periodic) moderate velocity. Of all the variables assessed, only the very high iron content values showed any consistency among samples.

All soils were tested for microbial populations, and counts for heterotrophic bacterial varied significantly among samples (Table 4). Various, mostly independent variables control microbial populations, including soil texture (primarily clay content), salinity, temperature, periods of inundation and insolation, oxygen tension, OM (organic matter) content, vegetation cover, and soil nutrient levels. However, something must be known about each variable for each soil sample to interpret the microbial results. Because a detailed soil analyses was beyond this scope of work, a thorough explanation of the variability among samples is not possible. Thus while there appears to be no correlation between heterotrophic

YOUR SAMPLE ID	AREA &/or TYPE of SAMPLE	PERCENT ORGANIC MATTER	TOTAL N TKN ppm	NITRATE N ppm	AMMONIA N ppm	PHOSPHOROUS P ppm	POTASSIUM K ppm	MAGNESIUM Mg ppm	CALCIUM Ca ppm	SULFATE-S SO4-S ppm	ZINC Zn ppm	MANGANESE Mn ppm	COPPER Cu ppm	IRON Fe ppm
D1/C (#1)	Sediment	32.1	---	9	73	19	114	939	3345	20	0.9	7.7	1.6	255.9
D2/C (#2)	Sediment	11.8	---	2	88	25	121	872	3333	29	5.1	30.6	5.8	267.8
D3/C (#3)	Sediment	1.1	---	1	22	25	43	190	568	29	1.0	39.6	1.6	130.2
D4/C (#4)	Sediment	0.5	---	2	20	5	30	140	427	2	0.5	1.7	0.5	18.6
D5/C (#5)	Sediment	6.5	---	1	70	19	789	1303	693	170	5.1	110.5	5.2	160.8
D6/C (#6)	Sediment	8.8	---	2	38	45	353	1462	2128	304	5.0	106.0	5.4	239.8

YOUR SAMPLE ID	AREA &/or TYPE of SAMPLE	SODIUM Na ppm	BORON B ppm	SOIL pH -log[H+]	ALKALINITY EXC CARB (Qual.)	SOLUBLE SALTS mmhos/cm	Ca/Mg ratio	%K (1-7%)	ACTUAL %Mg (15-25%)	PERCENT %Ca (60-75%)	%Na (0-5%)	CEC %H/Al (0-15%)	TOTAL CEC (Summation)
D1/C (#1)	Sediment	89	0.9	6.5	VL	0.40	3.56	0.8	21.7	46.3	1.1	30.1	36.1
D2/C (#2)	Sediment	89	1.0	6.4	VL	0.11	3.82	1.0	24.5	56.2	1.3	16.9	29.6
D3/C (#3)	Sediment	22	0.6	6.2	VL	0.21	2.99	1.9	27.2	48.7	1.6	20.6	5.8
D4/C (#4)	Sediment	10	0.3	7.2	VL	0.07	3.05	2.2	34.1	62.4	1.3	0.0	3.4
D5/C (#5)	Sediment	4030	2.4	6.6	L	9.67	0.53	5.4	29.0	9.3	46.9	9.4	37.4
D6/C (#6)	Sediment	744	3.4	6.5	L	1.47	1.46	3.0	40.0	34.9	10.6	11.5	30.5

SAMPLE ID	SOIL TEXTURE	USDA CLASSIFICATION
D1/C (#1)	{Sandy Mud}	{Sandy Clay Loam}
D2/C (#2)	Sandy Mud	Clay Loam
D3/C (#3)	Silty Sand	Loamy Sand
D4/C (#4)	Sand	Sand
D5/C (#5)	Mud	Silty Clay
D6/C (#6)	Sandy Mud	Clay

Table 3. Elemental and texture characteristics of eleven soils that support populations of *Lilaeopsis masonii* throughout its geographic range. Sample #1= Venice Island; Sample #2 = Disappointment Slough; Sample #3 = Brannan Island, East; Sample #4 = Brannan Island, West; Sample #5 = Vallejo Park; and Sample #6 = Hill Slough. Laboratory analyses were performed by ETS, Petaluma, California.

YOUR SAMPLE ID	AREA &/or TYPE of SAMPLE	PERCENT ORGANIC MATTER	TOTAL N TKN ppm	NITRATE N ppm	AMMONIA N ppm	PHOSPHOROUS P ppm	POTASSIUM K ppm	MAGNESIUM Mg ppm	CALCIUM Ca ppm	SULFATE-S SO4-S ppm	ZINC Zn ppm	MANGANESE Mn ppm	COPPER Cu ppm	IRON Fe ppm
D7/C (#7)	Sediment	6.1	---	18	40	7	129	608	1537	35	2.1	6.2	25.9	102.3
D8/C (#8)	Sediment	3.4	---	14	38	5	91	666	1857	22	1.4	4.2	4.5	102.3
D9/C (#9)	Sediment	5.9	---	5	88	5	157	1068	2679	21	0.8	5.3	6.8	111.3
D10/C (#10)	Sediment	15.5	---	3	60	20	324	1102	835	278	5.1	22.4	7.2	311.3
D11/C (#11)	Sediment	8.5	---	19	57	21	681	1178	816	171	4.0	40.2	9.6	150.4
YOUR SAMPLE ID	AREA &/or TYPE of SAMPLE	SODIUM Na ppm	BORON B ppm	SOIL pH -log[H ⁺]	BUFFER INDEX -log[H ⁺]	ALKALINITY EXC CARB (Qual.)	SOLUBLE SALTS mmhos/cm	Ca/Mg ratio	%K (1-7%)	ACTUAL %Mg (15-25%)	PERCENT %Ca (60-75%)	%Na (0-5%)	TOTAL CEC (0-15%)	TOTAL CEC (Summation)
D7/C (#7)	Sediment	113	0.8	7.4	7.1	VL	0.54	2.53	2.2	33.7	51.0	3.3	9.8	15.1
D8/C (#8)	Sediment	45	0.6	6.6	7.0	VL	0.39	2.79	1.3	32.1	53.7	1.1	11.8	17.3
D9/C (#9)	Sediment	65	0.9	7.0	7.0	VL	0.38	2.51	1.6	35.5	53.4	1.1	8.4	25.1
D10/C (#10)	Sediment	2100	2.0	6.1	6.8	VL	2.45	0.76	3.2	34.9	15.9	34.7	11.4	26.3
D11/C (#11)	Sediment	2050	2.2	6.9	7.0	L	3.92	0.69	6.6	37.2	15.5	33.7	7.0	26.4
SAMPLE ID		SOIL TEXTURE							USDA CLASSIFICATION					
D7/C (#7)	TEXT ANAL ->	47.6 % Sand	32.6 % Silt	19.8 % Clay	---	Sandy Mud	---	Loam						
D8/C (#8)	TEXT ANAL ->	42.0 % Sand	26.6 % Silt	31.4 % Clay	---	Sandy Mud	---	Clay Loam						
D9/C (#9)	TEXT ANAL ->	19.0 % Sand	31.6 % Silt	49.4 % Clay	---	Sandy Mud	---	Clay						
D10/C (#10)	TEXT ANAL ->	19.4 % Sand	49.0 % Silt	31.6 % Clay	---	Sandy Mud	---	Silty Clay Loam						
D11/C (#11)	TEXT ANAL ->	19.4 % Sand	45.0 % Silt	26.4 % Clay	---	Sandy Mud	---	Silty Clay Loam						

Table 3 (Continued). Elemental and texture characteristics of eleven soils that support populations of *Lilaeopsis masonii* through its geographic range. Sample #7 = Collinsville; Sample #8 = Cache Slough; Sample #9 = Prospect Slough; Sample #10 = Pt. Edith, West (oiled); Sample #11 = Point Edith, East (non-oiled). Laboratory analyses were performed by ETS, Petaluma, California.

count and soil texture, other site factors must be involved.

Soils from Pt. Edith also were analyzed to assess the extant, latent contamination from the Shell Oil spill in 1987. Results indicated that both soils oiled by the spill (sample #10) and adjacent soils not oiled (sample #11) contain significant levels petrochemical hydrocabons, and should be considered contaminated. However, the oiled soil supports contamination levels approximately ten times greater than the adjacent, non-oiled soil (2171 ppm total TPH [petrochemical hydrocarbons] vs. 216.4 ppm TPH).

Table 4. Spread plate count for microbial poulations for eleven soils that support populations of *Lilaeopsis masonii* throughout its geographic range. Sample #1= Venice Island; Sample #2 = Disappointment Slough; Sample #3 = Brannan Island, East; Sample #4 = Brannan Island, West; Sample #5 = Vallejo Park; and Sample #6 = Hill Slough. Laboratory analyses were performed by ETS, Petaluma, California.

<u>Sample Number</u>	<u>Heterotrophic Count</u>
1	1.45 x 10 ⁹ cfu/gm
2	3.39 x 10 ⁶ cfu/gm
3	1.89 x 10 ⁷ cfu/gm
4	8.60 x 10 ⁷ cfu/gm
5	4.45 x 10 ⁷ cfu/gm
6	1.66 x 10 ⁷ cfu/gm
7	9.62 x 10 ⁷ cfu/gm
8	1.09 x 10 ⁹ cfu/gm
9	8.31 x 10 ⁷ cfu/gm
10	7.68 x 10 ⁷ cfu/gm
11	7.63 x 10 ⁸ cfu/gm

IV.C.1. Summary of Findings

(1) Soil analyses conducted on eleven samples collected throughout the geographic range of *Lilaeopsis masonii* indicated primary and secondary nutrients range from moderate to fairly high concentrations. Greatest variability for macronutrients among samples was found in levels of potassium.

- (2) Minor and trace nutrients range from low to very high levels, but most are moderate to high.
- (3) pH's are fairly typical and most have fairly normal buffer indices.
- (4) Salinities range from levels indicating normal terrestrial soils to moderately saline soils.
- (5) Most samples demonstrated very normal Ca:Mg ratios consistent with typical terrestrial soils.
- (6) The soil sample from Venice Island deviated from others with respect to Ca:Mg ratios, buffering capacity, and H⁺ concentrations.
- (7) Soil textures included a very broad range of loam, clay loam, clay, silty clay loam, sandy clay loam, loamy sand, and sand.
- (8) Counts for heterotrophic bacteria varied significantly among samples. No correlation between heterotrophic count and soil texture was found.
- (8) Soils from Pt. Edith are contaminated with petrochemical hydrocabons. The oiled soil supports contamination levels approximately ten times greater than the adjacent, non-oiled soil (2,171 ppm total TPH [petrochemical hydrocarbons] vs. 216.4 ppm TPH).

IV.D. Laboratory Greenhouse Studies

IV.D.1 First competition experiment

Three of the four species, *Lilaeopsis masonii*, *Hydrocotyle verticillata*, and *Scirpus cernuus*, grew substantially and flowered during the course of the experiment. Only *Limosella subulata* fared poorly under these experimental conditions. Of the 280 individuals of *L. subulata* included in this experiment, 211 died prior to completion. Because of this high mortality, we were unable to include the *L. subulata* data in further analysis. The high mortality of *L. subulata* may have been caused by a fungal pathogen because ramets wilted at the sand surface with the roots dying away. This condition is suggestive of "damping off", a common cause of mortality in greenhouses by a cosmopolitan fungal pathogen (*Phytophthora* spp.).

Despite this high mortality, an interesting feature of *Limosella subulata* autecology was revealed. Approximately 850 seedlings sprouted from capsules present on the ramets, in spite of the deaths of individual *L. subulata* ramets. All seedlings were removed during the course of the experiment because we did not want seedling presence and establishment to influence the experiment. The only other species to produce apparently viable seedlings during the course of the experiment was *Scirpus cernuus*. Four seedlings were recorded.

Data analyses indicated that *Lilaeopsis masonii*, *Scirpus cernuus*, and *Hydrocotyle verticillata* grow more vigorously in fresh water than in 7 ppt "Instant Ocean" (Figure 10; Table 5). Growth of *H. verticillata* is decreased by more than 50 percent by 7 ppt salinity, whereas *S. cernuus* growth decreased less than 20 percent, and *L. masonii* growth by about 25 percent. Both *Scirpus cernuus* and *Hydrocotyle verticillata* depress growth of *L. masonii*. An ANOVA suggests that salinity and competition are significant factors, but that location of source *L. masonii* material (Brannan Island vs. Pt. Edith) is not.

IV.D.1.a. Summary of Findings

- (1) The effects of salinity (0 vs. 7 ppt) are significant on the growth of *Lilaeopsis masonii* and its common littoral zone associates.
- (2) The effects of the location of source *L. masonii* material are not ($p = 0.0978$).
- (3) The effects of competition on *L. masonii* are significant ($p < 0.0001$).
- (4) Growth response of *L. masonii* to *Scirpus* and to *Scirpus* and *Hydrocotyle* treatments is not significantly different from each other, but is significantly different from the response of *L. masonii* grown by itself.

Figure 10. First competition experiment. Brannan Island and Pt. Edith *Lilaeopsis masonii* growth responses to two competitors, *Scirpus cernuus* and *Hydrocotyle verticillata*. Data are gdw for each treatment and species at end of experiment to control. n=5.

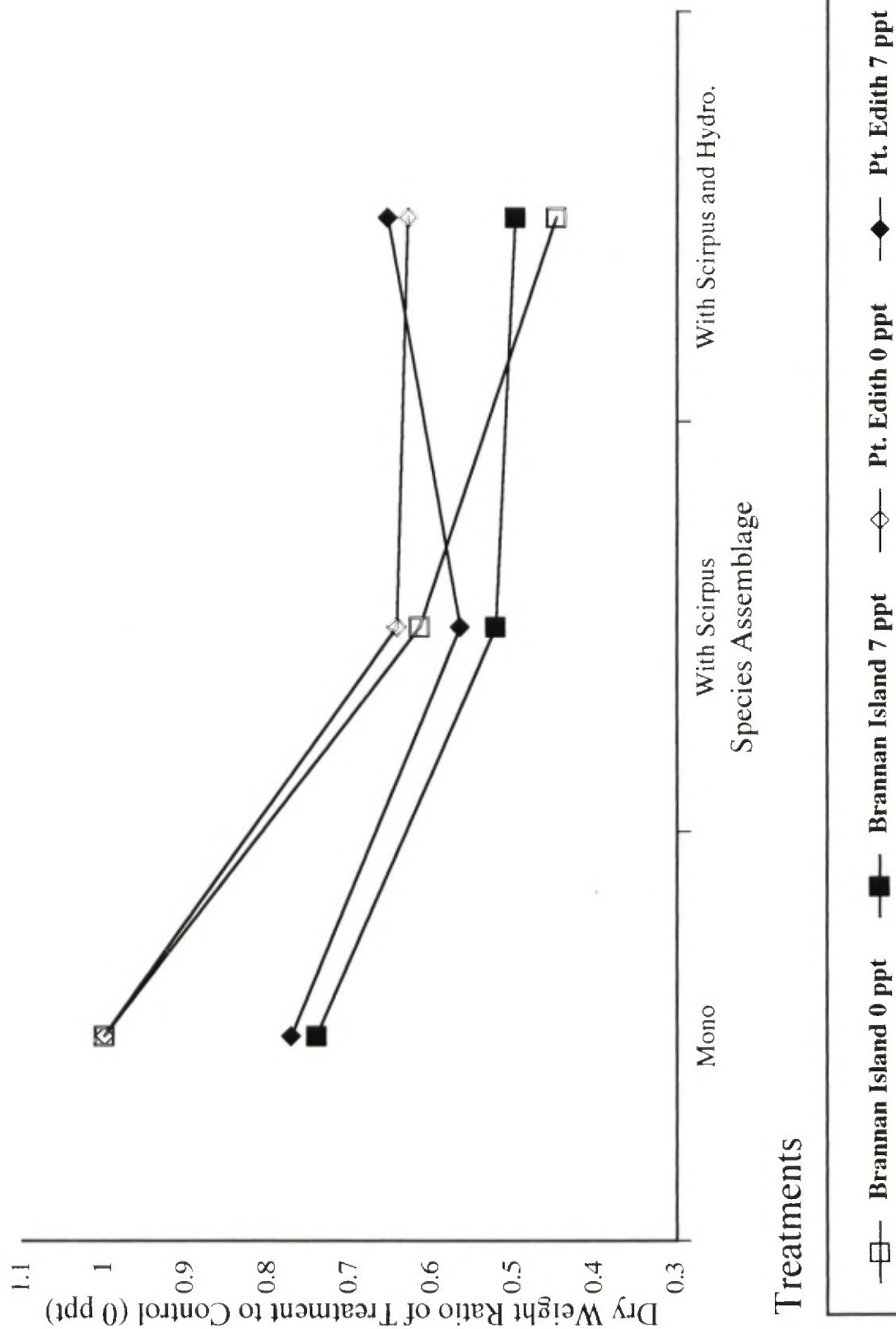


Table 5. *Lilaeopsis masonii*: First competition experiment. Average grams dry weight per treatment. Standard deviations within parenthesis. N = 5.

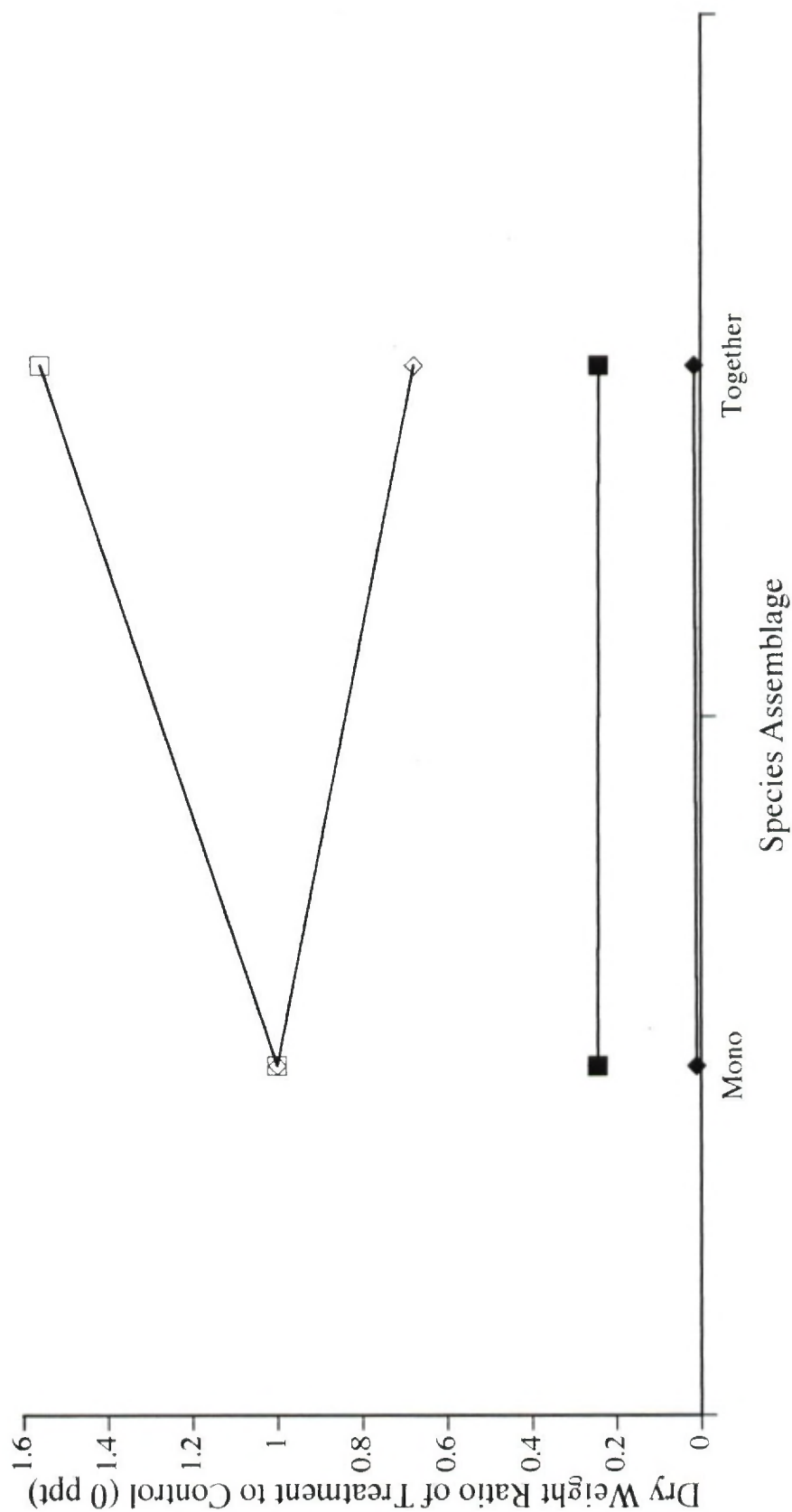
<u>TREATMENT</u>	<u>MONOTYPIC</u>	<u>W/SCIRPUS</u>	<u>W/ SCIRPUS & HYDROCOTYLE</u>
Brannan Island			
0 ppt	1.760 (0.253)	1.082 (0.237)	0.788 (0.183)
7 ppt	1.304 (0.240)	0.918 (0.087)	0.918 (0.087)
Point Edith			
0 ppt	1.472 (0.057)	0.946 (0.241)	0.926 (0.161)
7 ppt	1.136 (0.135)	0.832 (0.175)	0.962 (0.088)

IV.D.2. Second competition experiment

The effects of competition on *Lilaeopsis masonii* by *Limosella subulata* when grown together is not easily decipherable. *Limosella subulata* grew vigorously during this experiment, and as expected, decreased growth under competitive conditions at 0 ppt salinity conditions. However, when *Lilaeopsis* was grown with *Limosella* under 0 ppt salinity conditions, *Lilaeopsis masonii* increased in biomass. When the two species were grown together under 7 ppt salinity conditions, neither *Lilaeopsis* nor *Limosella* grew or declined in biomass. Thus, there was very low growth of either species at the 7 ppt salinity conditions. This may have been due to the shade cloth (and thus lower light levels), or to the different experimental set-up (different sand as growth medium). The notably low growth of *Limosella subulata* is indicative of its lower tolerance of brackish or saline water conditions. Results of this experiment under 7 ppt salinity conditions can be seen in Figure 11 and Table 6.

Interpretation of the results of this experiment is problematic. Increasing growth of *Lilaeopsis masonii* under competitive conditions (i.e., resource limitation) is not likely under natural settings. Because of the high variance in growth responses, these competition experiments should be repeated with a larger sample size at different planting densities, both in the

Figure 11. Second competition experiment. Point Edith *Lilaeopsis masonii* and *Limosella subulata* grown separately and together. Data are the proportion of each treatment to the respective species grams dry weight (gdw) at 0 ppt. N = 5.



Treatments

—□— Pt. Edith 0 ppt —■— Pt. Edith 7 ppt —◇— Limosella 0 ppt —◆— Limosella 7 ppt

Table 6. *Lilaeopsis masonii*: Second competition experiment. Average grams dry weight per treatment. Standard deviations within parenthesis. N = 5.

<u>SPECIES</u>	<u>TREATMENT</u>	<u>MONOTYPIC</u>	<u>TOGETHER</u>
<i>Lilaeopsis masonii</i>	0 ppt	0.592 (0.371)	0.922 (0.142)
	7 ppt	0.144 (0.052)	0.918 (0.096)
<i>Limosella subulata</i>	0 ppt	0.628 (0.174)	0.425 (0.376)
	7 ppt	0.0064 (0.011)	0.001 (0.021)

greenhouse and under field conditions.

One interesting aspect of the biology of *Limosella subulata* was revealed during this second competition experiment. *Limosella* produced an abundance of seeds and seedlings throughout the experimental run preferentially in the 0 ppt salinity conditions treatment, suggesting that: (1) sexual reproduction is an important mode of reproduction for this perennial herb, and (2) salinity greatly limits seed germination. This result is in striking contrast to *Lilaeopsis masonii*, which has produced few if any viable seedlings throughout both study phases of the restoration and recovery, and whose seeds demonstrated an ability to germinate in salinity conditions up to 12 ppt (Fiedler and Zebell 1993). Table 7 illustrates the seedling establishment of *Limosella subulata* in the second competition experiment.

IV.D.1.a. Summary of Findings

(1) *Lilaeopsis masonii* increased in biomass when grown under competitive conditions with *Limosella subulata* at 0 ppt salinity conditions. *Limosella subulata* decreased under the same

Table 7. Number of seedlings pulled of *Limosella subulata* during second competition experiment by date and treatment (0 ppt vs 7 ppt).

Date	0 ppt	7 ppt	Grand Total
3/24/95	1	5	6
4/7/95	56	0	56
4/10/95	80	0	80
4/21/95	34	0	34
5/2/95	195	0	195
5/10/95	267	0	267
5/15/95	560	0	560
5/19/95	198	0	198
5/24/95	95	0	95
6/2/95	71	0	71
6/5/95	31	0	31
Total	1,588	5	1,593

conditions.

(2) *Lilaeopsis masonii* and *Limosella subulata* did not change significantly in biomass when grown under competitive conditions at 7 ppt salinity conditions.

(3) *Limosella subulata* produced an exceptional number of seedlings during the experiment, suggesting that sexual reproduction is an important if not primary mode of reproduction for this species. Germination is inhibited under 7 ppt salinity conditions.

IV.E. Crude Oil Contamination Experiment

Most of the leaves present when the oil was applied died, but all individuals of *Lilaeopsis masonii* in the flats across all treatments grew significantly following the application of oil.

Total ramet mortality of *Lilaeopsis masonii* was insignificant (3/200) under all five dosages of oil under 0 ppt salinity conditions, but more substantial (22/200 ramets) under 7 ppt salinity conditions (Table 8). By the completion of the experiment, a total of 25 of the 200 ramets of *L. masonii* died, with the greatest mortality occurring in the combined highest dosages of crude oil and 7 ppt salinity.

Table 8. *Lilaeopsis masonii* crude oil experiment. Total ramet mortality by treatment. N = 200.

<u>Treatment</u>	<u>0 ppt</u>	<u>7 ppt</u>
A. No crude oil	0	0
B. 0.2 L/m ² crude oil	0	1
C. 0.8 L/m ² crude oil	1	0
D. 3.2 L/m ² crude oil	2	12
E. 12.8 L/m ² crude oil	3	9

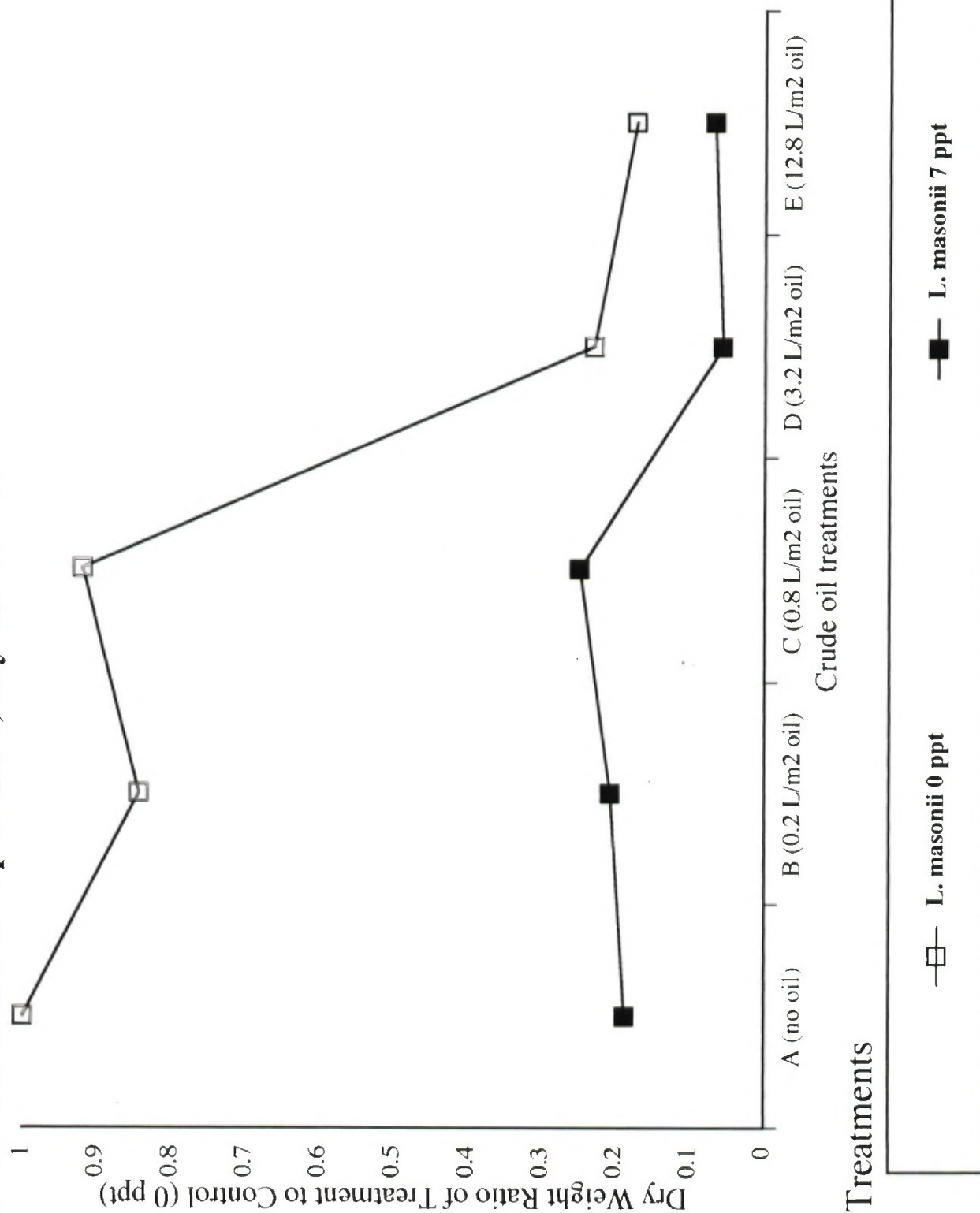
The effects of crude oil on the growth of *Lilaeopsis masonii* also were evaluated by examining the production of biomass (root/shoot ratio grams dry weight [gdw]) and by cover values. As measured by grams dry weight, results were not significant (Table 9), although as expected, growth was significantly slower under the 7 ppt salinity conditions (Figure 12). In addition, the interaction between crude oil and salinity was not significant ($p = 0.271$).

As evaluated by cover statistics, the effect of salinity on the growth of *Lilaeopsis masonii* was highly significant ($p = 1 \times 10^{-6}$), as was the effect of crude oil ($p = 1.2 \times 10^{-4}$) (Table 10, Figures 13 and 14). Treatments D and E (*i.e.*, higher dosages of 3.2 L/m² crude oil and 12.8

Treatment	0 ppt				7 ppt			
	Root	Shoot	Total	Root/Shoot	Root	Shoot	Total	Root/Shoot
A (no crude oil)	0.353 (0.203)	0.239 (0.168)	0.592 (0.371)	1.597 (0.294)	0.096 (0.040)	0.048 (0.016)	0.144 (0.052)	2.063 (0.671)
B (0.2 L/m2 crude oil)	0.318 (0.113)	0.224 (0.089)	0.542 (0.198)	1.463 (0.207)	0.140 (0.027)	0.075 (0.026)	0.215 (0.049)	1.974 (0.414)
C (0.8 L/m2 crude oil)	0.331 (0.168)	0.236 (0.104)	0.566 (0.271)	1.373 (0.122)	0.114 (0.041)	0.108 (0.021)	0.222 (0.058)	1.041 (0.250)
D (3.2 L/m2 crude oil)	0.098 (0.025)	0.075 (0.022)	0.174 (0.036)	1.441 (0.737)	0.035 (0.036)	0.023 (0.028)	0.059 (0.062)	1.541 (1.507)
E (12.8 L/m2 crude oil)	0.085 (0.039)	0.054 (0.027)	0.139 (0.065)	1.650 (0.553)	0.042 (0.022)	0.053 (0.044)	0.095 (0.065)	1.092 (0.496)

Table 9. *Lilaeopsis masonii* crude oil experiment. Average grams dry weight (gdw) of ramet roots and shoots at the end of the experiment. Standard deviations in parentheses. N = 5 for root and shoot values; N = 10 for totals and ratios.

Figure 12. *Lilaeopsis masonii* crude oil experiment. Pt. Edith *Lilaeopsis masonii* growth response to five levels of crude oil application. Data are grams dry weight (gdw) for each treatment at end of experiment, day 88. N = 5.



Treatment	Days since experiment began															
	8		15		22		29		43		56		71		88	
	0 ppt	7 ppt	0 ppt	7 ppt	0 ppt	7 ppt	0 ppt	7 ppt	0 ppt	7 ppt	0 ppt	7 ppt	0 ppt	7 ppt	0 ppt	7 ppt
(no crude oil)	4.48 (1.13)	2.90 (0.84)	3.63 (0.70)	2.18 (0.38)	3.57 (0.93)	1.98 (0.80)	4.23 (0.97)	1.92 (0.66)	3.58 (0.90)	2.20 (0.75)	7.32 (3.55)	2.90 (1.08)	12.88 (8.16)	2.43 (0.97)	21.00 (14.32)	3.95 (1.50)
(0.2 L/m ² crude oil)	4.33 (0.75)	2.92 (0.91)	5.22 (1.22)	2.93 (0.80)	4.88 (1.47)	1.87 (0.68)	5.84 (2.61)	1.87 (0.49)	6.02 (2.59)	1.70 (0.61)	9.03 (2.42)	3.35 (1.02)	12.62 (4.29)	3.57 (1.74)	17.72 (7.18)	4.37 (1.74)
(0.8 L/m ² crude oil)	3.70 (0.84)	2.27 (0.68)	4.77 (1.36)	2.80 (0.43)	4.35 (1.32)	2.48 (0.88)	4.52 (1.60)	2.27 (1.10)	5.78 (2.16)	1.93 (0.30)	8.45 (4.83)	3.03 (0.82)	14.93 (6.59)	3.63 (1.10)	19.32 (7.23)	5.23 (1.17)
(3.2 L/m ² crude oil)	2.77 (0.86)	1.24 (0.66)	2.80 (0.45)	1.21 (0.54)	1.93 (0.64)	0.73 (0.50)	2.20 (0.77)	0.68 (0.60)	2.68 (0.71)	0.76 (0.72)	2.70 (0.55)	0.77 (0.61)	3.22 (1.42)	1.15 (1.28)	4.83 (2.63)	1.18 (1.52)
(12.8 L/m ² crude oil)	1.80 (1.06)	1.76 (0.60)	1.43 (0.61)	1.35 (0.68)	1.43 (0.80)	0.82 (0.61)	1.97 (0.91)	0.94 (0.63)	2.27 (1.10)	0.97 (0.72)	2.83 (0.93)	1.28 (0.79)	2.37 (0.86)	1.28 (0.80)	3.63 (2.06)	1.42 (1.20)

Table 10. *Lilaeopsis masonii* crude oil experiment. Average percent cover values. Standard deviations in parentheses. N = 5.

L/m² crude oil, respectively) were significantly different from Treatments A, B, and C (*i.e.*, lower dosages of 0.0 L/m² crude oil, 0.2 L/m² crude oil, and 0.8 L/m² crude oil, respectively). The higher dosages of Treatments D and E were not significantly different from each other, nor were the lower dosages of Treatments A, B, and C (Figures 13 and 14). These results suggest a threshold effect, in that dosages of 3.2 L/m² or greater of crude oil significantly decrease the growth of *Lilaeopsis masonii*.

IV.E.1. Summary of Findings

- (1) The effect of salinity (0 vs. 7 ppt) on the growth of *Lilaeopsis masonii* is significant ($p \leq 1 \times 10^{-6}$).
- (2) The effect of crude oil on the growth of *Lilaeopsis masonii* is significant ($p \leq 1.2 \times 10^{-4}$).
- (3) As measured by grams dry weight, the effect of salinity is not significant ($p = 0.835$).
- (4) As measured by grams dry weight, the effect of oil is not significant ($p = 0.213$).
- (5) As measured by grams dry weight, the interaction between salinity and oil is not significant ($p = 0.271$).
- (6) As measured by cover values, dosage levels of 3.2 L/m² and 12.8 L/m² crude oil are significantly different from the three lower dosage treatment levels (0.0, 0.2, and 0.8 L/m²).
- (7) As measured by cover values, dosage levels 3.2 L/m² and 12.8 L/m² crude oil are not significantly different from each other.
- (8) As measured by cover values, dosage levels 0.0, 0.2, and 0.8 L/m² crude oil are not significantly different from each other;
- (9) As measured by cover values, the interaction between salinity and oil is significant ($p = 0.0179$).

Figure 13. *Lilaeopsis masonii* crude oil experiment. Pt. Edith *Lilaeopsis masonii* growth response to 0 ppt salinity and five levels of crude oil application as measured by average percent cover. N = 5.

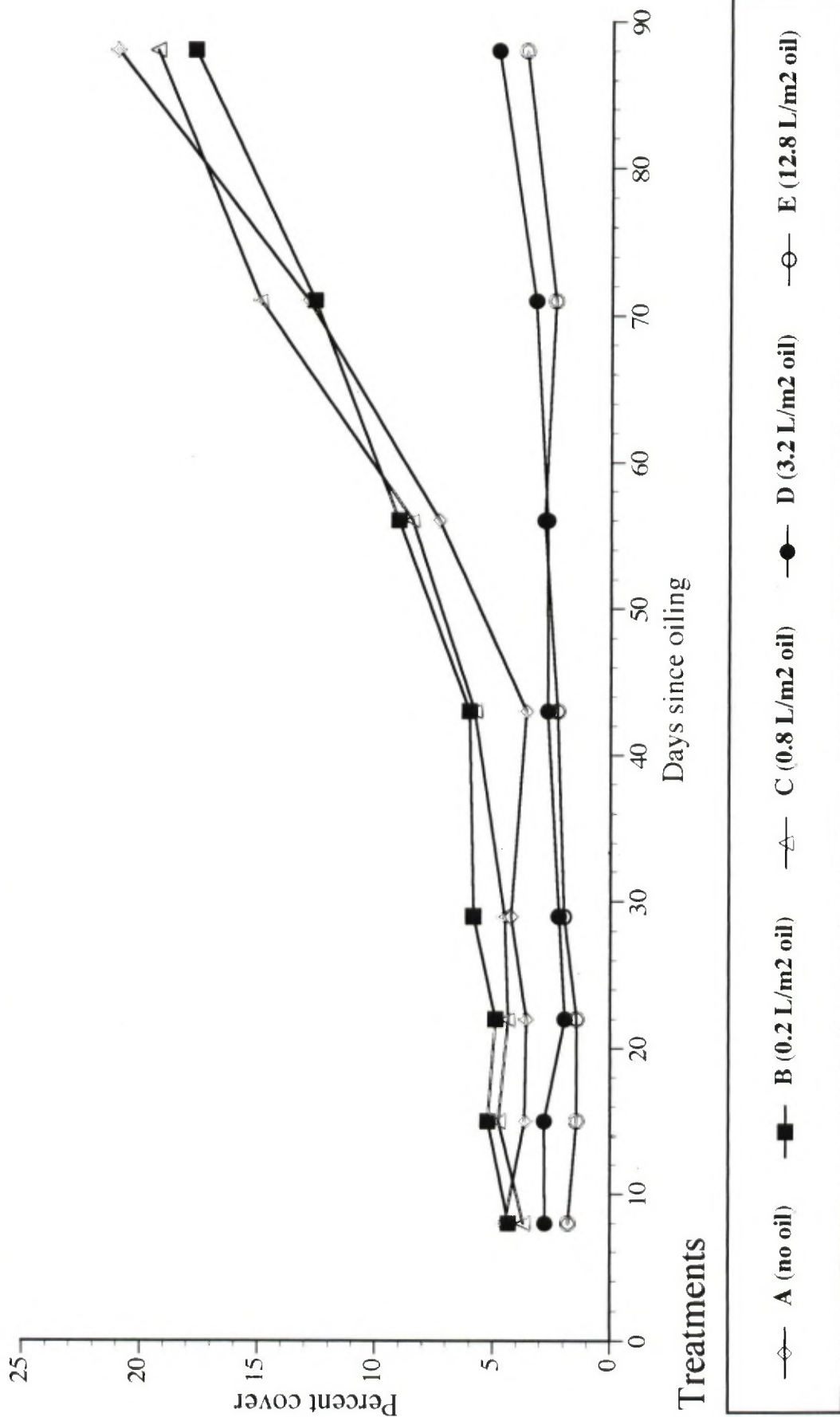
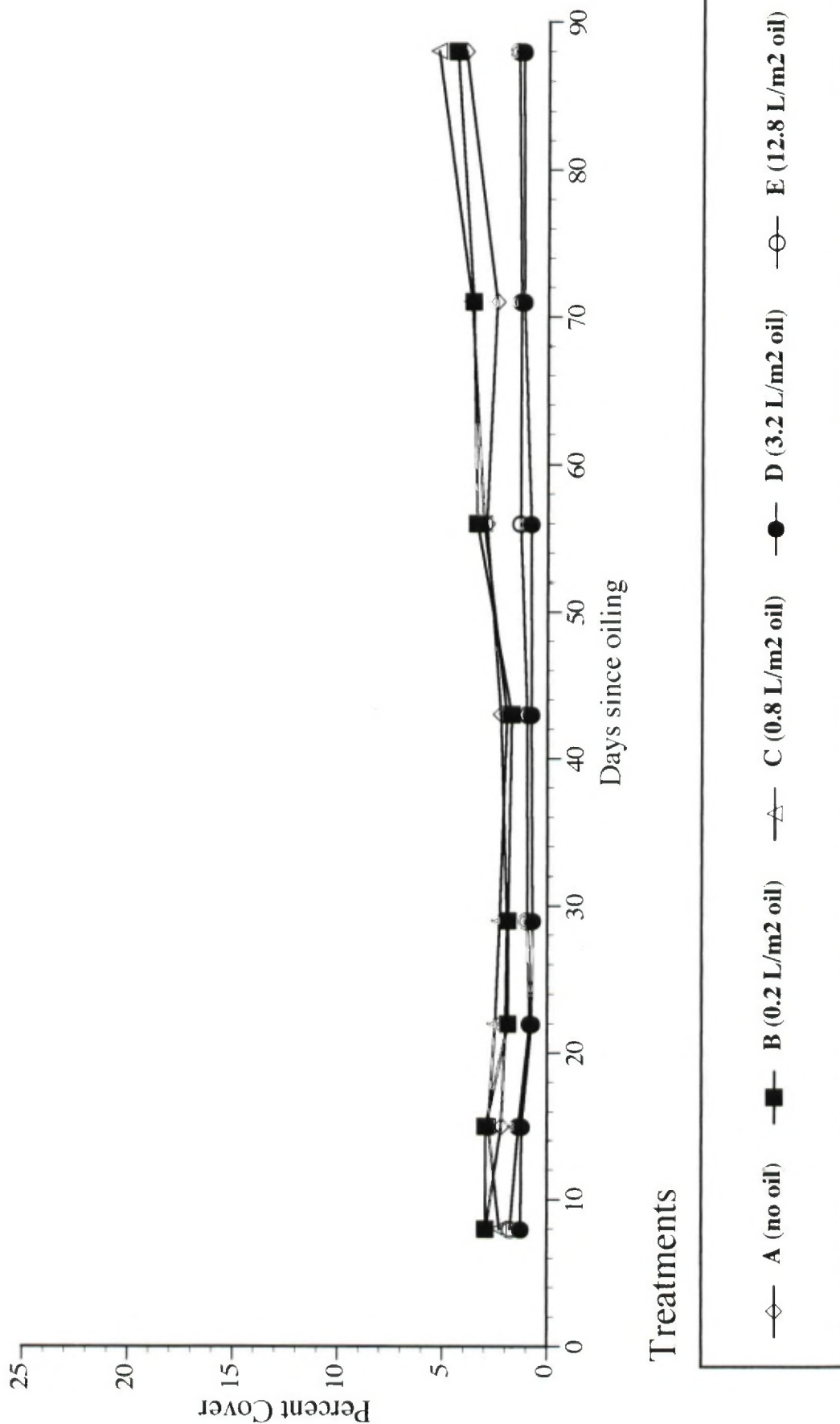


Figure 14. *Lilaeopsis masonii* crude oil experiment. Pt. Edith *Lilaeopsis masonii* growth response to 7 ppt salinity and five levels of crude oil application as measured by average percent cover. N = 5.



V. CONCLUSIONS

V.A. Relationship of *Lilaeopsis masonii* to *L. occidentalis*

The morphological distinctiveness of *Lilaeopsis masonii* from *L. occidentalis* is still in question, despite several years of intensive field and laboratory research on the taxon in Phase I, and more casual observations in Phase II of this study. Greenhouse observations of these two taxa indicate a continuum in leaf and ramet size, with no absolute morphological disjunction. Field observations during Phase II at Mare Island in the western most portion of the range of *Lilaeopsis masonii*, have verified this morphological continuum within a single *Lilaeopsis* patch. Genetic studies completed in Phase I did not provide conclusive corroborating evidence for the morphological or taxonomic distinctiveness of *L. masonii* due primarily to the overall low level of genetic variability found within the genomic region investigated. It is possible that additional genetic studies could be initiated in the future that ultimately may clarify its taxonomic status.

V.B. Ecophysiology of *Lilaeopsis masonii*

Overall, *Lilaeopsis masonii* demonstrated remarkably similar growth responses across a broad range of water salinities, but consistently shows depressed growth and reproduction in high salinity levels. Therefore, our results in both phases of this research have demonstrated that increasing salinity will depress ramet growth and seedling germination and establishment of *Lilaeopsis masonii*. The extent of this response may be either ecotypic or genetically determined.

V.C. Competitive Ability of *Lilaeopsis masonii*

When grown in combination with common littoral associates, *Lilaeopsis masonii* is compromised. While the results from the two common species, *Scirpus cernuus* and *Hydrocotyle verticillata* are straightforward, those from the experiments with *Limosella subulata* are less so. This is possibly the result of the experimental design and implementation and less likely the reflection of the natural history of the *Lilaeopsis* or *Limosella*. We suspect that the four littoral species all influence biomass production of their associates in a way that is dictated by the external features of each site, e.g., salinity, substrate characteristics, time of year, and inundation regime. In particular, *Limosella* may be

light limited by the potentially significantly greater stature of some *Lilaeopsis* ramets, leading to competitive dominance by *Lilaeopsis*. The reverse for these two species is not true. Thus, deciphering the interactions of competitive ability of each species will require additional extensive field and laboratory experiments.

V.D. Restoration and Recovery of *Lilaeopsis masonii*

Because *Lilaeopsis masonii* is one of the most widespread state-listed species as evidenced by the large number of populations not documented, it does not appear to require additional protection at the present time. We urge that this evaluation, however, should be tempered by our results described herein and in 1993 (Fiedler and Zebell 1993), and our observations described below.

Growth and sexual reproduction have been demonstrated to be depressed at high levels of salinity, and this likely account for the geographic limitation of *Lilaeopsis* west of the Napa River. Should levels of salinity in the San Francisco Estuary and Sacramento-San Joaquin Delta increase significantly due to water diversion, the viability and geographic integrity of this species likely will be compromised.

Effects of crude oil on the above ground growth of *Lilaeopsis* has been demonstrated to be significantly detrimental at levels above 0 ppt salinity. Soils around Pt. Edith retain significant levels of petrochemical hydrocarbons, presumably from the 1987 Shell Oil spill. However, the adjacency of Pt. Edith to the Martinez refinery suggests additional chronic low-level loading of hydrocarbons in the adjacent wetlands. Should another oil spill occur within the San Francisco Estuary, then at minimum, the shoots of *Lilaeopsis* will die back. That ramet death and local extirpation of *Lilaeopsis* populations is the inevitable result of an oil spill was not determined during this research.

Invasion of exotic species, particularly the Water hyacinth (*Eichornia crassipes*), is a serious threat to the health and viability of populations of *Lilaeopsis*, particularly in the central Delta. Increasing encroachment into the geographic range of *Lilaeopsis* by *Eichornia* should be carefully monitored.

In summary, *Lilaeopsis masonii* is a rare species found within a narrow and precarious habitat in the Sacramento-San Joaquin Delta, Suisun Marsh, and San Francisco Estuary of California. The species is susceptible to habitat degradation by the effects of boat wakes, oil spills, and trampling at accessible locations. It is susceptible to habitat loss by the effects of boat wakes, sea level rise, and by the natural slumping of the littoral zone. *Lilaeopsis masonii* is susceptible to the negative effects of competition by both common and native plant associates, and importantly by the invasion of the exotic species, *Eichornia crassipes*. Therefore, a large number of populations may be required for the rare plant species *Lilaeopsis masonii* to persist in the narrow and precarious habitat to which it is restricted.

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