

POPULATION BIOLOGY AND GENETICS, HABITAT MODELING, AND PILOT RESTORATION FOR THREE CALFED AT-RISK PLANT SPECIES IN THE SACRAMENTO-SAN JOAQUIN DELTA AND SUISUN MARSH

Project Information

1. Proposal Title:

POPULATION BIOLOGY AND GENETICS, HABITAT MODELING, AND PILOT RESTORATION FOR THREE CALFED AT-RISK PLANT SPECIES IN THE SACRAMENTO-SAN JOAQUIN DELTA AND SUISUN MARSH

2. Proposal applicants:

ROBIN FALLSCHEER, California Department of Fish and Game
NIAL McCARTEN, ENVIRONMENTAL SCIENCE ASSOCIATES
LARRY RIGGS, BIOSPHERE GENETICS
RANDY ZEBELL, PRIVATE CONSULTANT
FRANK WERNETTE, CALIFORNIA DEPARTMENT OF FISH AND GAME
ROXANNE BITTMAN, CALIFORNIA DEPARTMENT OF FISH AND GAME

3. Corresponding Contact Person:

ROBIN FALLSCHEER
CALIFORNIA DEPARTMENT OF FISH AND GAME
CENTRAL VALLEY BAY-DELTA BRANCH 4001 N. WILSON WAY STOCKTON, CA
95205-2486
209 948-7163
rfallscheer@delta.dfg.ca.gov

4. Project Keywords:

At-risk species, plants
Genetic Conservation and Engineering
Native Plants

5. Type of project:

Implementation_Pilot

6. Does the project involve land acquisition, either in fee or through a conservation easement?

No

7. Topic Area:

8. Type of applicant:

State Agency

9. Location - GIS coordinates:

Latitude:

Longitude:

Datum:

Describe project location using information such as water bodies, river miles, road intersections, landmarks, and size in acres.

THE PROPOSED STUDY AREA INCLUDES CACHE, LINDSAY, AND BARKER SLOUGH WEST OF THE SACRAMENTO RIVER, JUST NORTH OF THE CITY OF RIO VISTA. IT INCLUDES THE SACRAMENTO RIVER SOUTH FROM RIO VISTA TO THE CONFLUENCE WITH THE SAN JOAQUIN RIVER; THEN WEST TO MARE ISLAND ON THE SOUTHERN NAPA RIVER. THE SOUTHEASTERN PART OF THE PROJECT AREA INCLUDES THE SAN JOAQUIN RIVER JUST WEST OF THE CITY OF STOCKTON, WEST ALONG THE RIVER TO THE CONFLUENCE WITH THE SACRAMENTO RIVER. THE SOUTHERN LIMIT INCLUDES OLD AND MIDDLE RIVERS. THE STUDIES INCLUDE SUISUN MARSH SOUTH OF SUISUN CITY, ALONG PEYTONA AND MONTEZUMA SLOUGH.

10. Location - Ecozone:

1.1 North Delta, 1.3 South Delta, 1.4 Central and West Delta, 2.1 Suisun Bay & Marsh, 2.2 Napa River, Code 15: Landscape

11. Location - County:

Contra Costa, Napa, Sacramento, San Joaquin, Solano, Yolo

12. Location - City:

Does your project fall within a city jurisdiction?

No

13. Location - Tribal Lands:

Does your project fall on or adjacent to tribal lands?

No

14. Location - Congressional District:

7th

15. **Location:**

California State Senate District Number: 4

California Assembly District Number: 8

16. **How many years of funding are you requesting?**

3 years

17. **Requested Funds:**

- a) Are your overhead rates different depending on whether funds are state or federal?

Yes

If yes, list the different overhead rates and total requested funds:

State Overhead Rate: 15%

Total State Funds: \$1,456,350

Federal Overhead Rate: 13.9%

Total Federal Funds: \$1,442,419

- b) Do you have cost share partners already identified?

No

- c) Do you have potential cost share partners?

No

- d) Are you specifically seeking non-federal cost share funds through this solicitation?

No

If the total non-federal cost share funds requested above does not match the total state funds requested in 17a, please explain the difference:

18. **Is this proposal for next-phase funding of an ongoing project funded by CALFED?**

No

Have you previously received funding from CALFED for other projects not listed above?

Yes

If yes, identify project number(s), title(s) and CALFED program.

1998-F23	SOUTH NAPA RIVER TIDAL SLOUGH RESTORATION	HABITAT RESTORATION
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19. **Is this proposal for next-phase funding of an ongoing project funded by CVPIA?**

No

Have you previously received funding from CVPIA for other projects not listed above?

No

20. **Is this proposal for next-phase funding of an ongoing project funded by an entity other than CALFED or CVPIA?**

No

Please list suggested reviewers for your proposal. (optional)

DIANE ELAM U.S. FISH AND WILDLIFE SERVICE 916/414-6600

BRENDA UNIVERSITY OF 530/752-4326 bgrewell@yolo.com
GREWELL CALIFORNIA, DAVIS

21. **Comments:**

Environmental Compliance Checklist

POPULATION BIOLOGY AND GENETICS, HABITAT MODELING, AND PILOT RESTORATION FOR THREE CALFED AT-RISK PLANT SPECIES IN THE SACRAMENTO-SAN JOAQUIN DELTA AND SUISUN MARSH

1. CEQA or NEPA Compliance

a) Will this project require compliance with CEQA?

Yes

b) Will this project require compliance with NEPA?

Yes

c) If neither CEQA or NEPA compliance is required, please explain why compliance is not required for the actions in this proposal.

2. If the project will require CEQA and/or NEPA compliance, identify the lead agency(ies). *If not applicable, put "None".*

CEQA Lead Agency: CA DEPARTMENT OF FISH AND GAME

NEPA Lead Agency (or co-lead:) US FISH AND WILDLIFE SERVICE

NEPA Co-Lead Agency (if applicable):

3. Please check which type of CEQA/NEPA documentation is anticipated.

CEQA

☒Categorical Exemption

-Negative Declaration or Mitigated Negative Declaration

-EIR

-none

NEPA

☒Categorical Exclusion

-Environmental Assessment/FONSI

-EIS

-none

If you anticipate relying on either the Categorical Exemption or Categorical Exclusion for this project, please specifically identify the exemption and/or exclusion that you believe covers this project.

THE PROJECT IS A SCIENTIFIC RESEARCH PROGRAM WITH A SMALL-SCALE PILOT RESTORATION COMPONENT. ONLY A SCIENTIFIC COLLECTING PERMIT IS REQUIRED.

4. CEQA/NEPA Process

a) Is the CEQA/NEPA process complete?

No

If the CEQA/NEPA process is not complete, please describe the dates for completing draft and/or final CEQA/NEPA documents.

DRAFT JOINT CEQA/NEPA: JANUARY 2003 FINAL JOINT CEQA/NEPA: MARCH 2003

b) If the CEQA/NEPA document has been completed, please list document name(s):

5. **Environmental Permitting and Approvals** (*If a permit is not required, leave both Required? and Obtained? check boxes blank.*)

LOCAL PERMITS AND APPROVALS

Conditional use permit

Variance

Subdivision Map Act

Grading Permit

General Plan Amendment

Specific Plan Approval

Rezone

Williamson Act Contract Cancellation

Other

STATE PERMITS AND APPROVALS

Scientific Collecting Permit Required

CESA Compliance: 2081

CESA Compliance: NCCP

1601/03

CWA 401 certification

Coastal Development Permit

Reclamation Board Approval

Notification of DPC or BCDC

Other

FEDERAL PERMITS AND APPROVALS

ESA Compliance Section 7 Consultation

ESA Compliance Section 10 Permit

Rivers and Harbors Act

CWA 404

Other

PERMISSION TO ACCESS PROPERTY

Permission to access city, county or other local agency land.

Agency Name:

Permission to access state land.

Agency Name: CA DEPARTMENT OF FISH AND GAME

Obtained

Permission to access federal land.

Agency Name:

Permission to access private land.

Landowner Name:

6. Comments.

Land Use Checklist

POPULATION BIOLOGY AND GENETICS, HABITAT MODELING, AND PILOT RESTORATION FOR THREE CALFED AT-RISK PLANT SPECIES IN THE SACRAMENTO-SAN JOAQUIN DELTA AND SUISUN MARSH

1. **Does the project involve land acquisition, either in fee or through a conservation easement?**

No

2. **Will the applicant require access across public or private property that the applicant does not own to accomplish the activities in the proposal?**

No

3. **Do the actions in the proposal involve physical changes in the land use?**

No

If you answered no to #3, explain what type of actions are involved in the proposal (i.e., research only, planning only).

RESEARCH AND MONITORING WITH SMALL-SCALE PLANTING OF NATIVE PLANTS.

4. **Comments.**

Conflict of Interest Checklist

POPULATION BIOLOGY AND GENETICS, HABITAT MODELING, AND PILOT RESTORATION FOR THREE CALFED AT-RISK PLANT SPECIES IN THE SACRAMENTO-SAN JOAQUIN DELTA AND SUISUN MARSH

Please list below the full names and organizations of all individuals in the following categories:

- Applicants listed in the proposal who wrote the proposal, will be performing the tasks listed in the proposal or who will benefit financially if the proposal is funded.
- Subcontractors listed in the proposal who will perform some tasks listed in the proposal and will benefit financially if the proposal is funded.
- Individuals not listed in the proposal who helped with proposal development, for example by reviewing drafts, or by providing critical suggestions or ideas contained within the proposal.

The information provided on this form will be used to select appropriate and unbiased reviewers for your proposal.

Applicant(s):

ROBIN FALLSCHEER, California Department of Fish and Game
NIALl McCARTEN, ENVIRONMENTAL SCIENCE ASSOCIATES
LARRY RIGGS, BIOSPHERE GENETICS
RANDY ZEBELL, PRIVATE CONSULTANT
FRANK WERNETTE, CALIFORNIA DEPARTMENT OF FISH AND GAME
ROXANNE BITTMAN, CALIFORNIA DEPARTMENT OF FISH AND GAME

Subcontractor(s):

Are specific subcontractors identified in this proposal? Yes

If yes, please list the name(s) and organization(s):

NIALl McCARTEN	ENVIRONMENTAL SCIENCE ASSOCIATES
LARRY RIGGS	BIOSPHERE GENETICS
RANDY ZEBELL	PRIVATE CONSULTANT
CHRIS ROGERS	ENVIRONMENTAL SCIENCE ASSOCIATES
MARK FOGIEL	ENVIRONMENTAL SCIENCE ASSOCIATES

Helped with proposal development:

Are there persons who helped with proposal development?

Yes

If yes, please list the name(s) and organization(s):

THOMAS TREXLER ENVIRONMENTAL SCIENCE ASSOCIATES

MICHAEL STEVENSON ENVIRONMENTAL SCIENCE ASSOCIATES

YOLANDA MOLETTE ENVIRONMENTAL SCIENCE ASSOCIATES

PETER BAYE U.S. FISH AND WILDLIFE SERVICE

Comments:

Budget Summary

POPULATION BIOLOGY AND GENETICS, HABITAT MODELING, AND PILOT RESTORATION FOR THREE CALFED AT-RISK PLANT SPECIES IN THE SACRAMENTO-SAN JOAQUIN DELTA AND SUISUN MARSH

Please provide a detailed budget for each year of requested funds, indicating on the form whether the indirect costs are based on the Federal overhead rate, State overhead rate, or are independent of fund source.

Independent of Fund Source

Year 1												
Task No.	Task Description	Direct Labor Hours	Salary (per year)	Benefits (per year)	Travel	Supplies & Expendables	Services or Consultants	Equipment	Other Direct Costs	Total Direct Costs	Indirect Costs	Total Cost
1	POPULATION						252,990			252990.0		252990.00
2	HABITAT EVALUATION AND MODEL						166,920			166920.0		166920.00
3	PILOT RESTORATION									0.0		0.00
		0	0.00	0.00	0.00	0.00	419910.00	0.00	0.00	419910.00	0.00	419910.00

Year 2												
Task No.	Task Description	Direct Labor Hours	Salary (per year)	Benefits (per year)	Travel	Supplies & Expendables	Services or Consultants	Equipment	Other Direct Costs	Total Direct Costs	Indirect Costs	Total Cost
1	POPULATION						248,991			248991.0		248991.00
2	HABITAT EVALUATION AND MODEL						85,260			85260.0		85260.00
3	PILOT RESTORATION						200,980			200980.0		200980.00
		0	0.00	0.00	0.00	0.00	535231.00	0.00	0.00	535231.00	0.00	535231.00

Year 3												
Task No.	Task Description	Direct Labor Hours	Salary (per year)	Benefits (per year)	Travel	Supplies & Expendables	Services or Consultants	Equipment	Other Direct Costs	Total Direct Costs	Indirect Costs	Total Cost
1	POPULATION						144,540			144540.0		144540.00
2	HABITAT EVALUATION AND MODEL									0.0		0.00
3	PILOT RESTORATION						166,710			166710.0		166710.00
		0	0.00	0.00	0.00	0.00	311250.00	0.00	0.00	311250.00	0.00	311250.00

Grand Total=1266391.00

Comments.

CA DEPARTMENT OF FISH AND GAME (DFG) STAFF WILL NOT BE REQUESTING HOURS OR EXPENSES AS PART OF THIS PROPOSALS BUDGET. DFG STAFF SALARIES ARE COVERED UNDER THE GOVERNORS BUDGET IN THE GENERAL FUND AS WELL AS

PROPOSITION 204 FUNDING. IN ADDITION, PROJECT WORK IN SUISUN MARSH WILL BE PAID FOR IN PART FROM CONTRACT FUNDS FROM THE CA DEPARTMENT OF WATER RESOURCES ONGOING BIOLOGICAL STUDIES. ALL SALARIES AND EXPENSES, OTHER THAN DFG OVERHEAD, WILL BE USED BY CONSULTING SCIENTISTS.

Budget Justification

POPULATION BIOLOGY AND GENETICS, HABITAT MODELING, AND PILOT RESTORATION FOR THREE CALFED AT-RISK PLANT SPECIES IN THE SACRAMENTO-SAN JOAQUIN DELTA AND SUISUN MARSH

Direct Labor Hours. Provide estimated hours proposed for each individual.

CA DEPARTMENT OF FISH AND GAME (DFG) LABOR IS NOT SPECIFIED BECAUSE NO BUDGET FOR STAFF IS REQUESTED.

Salary. Provide estimated rate of compensation proposed for each individual.

THERE IS NO SALARY REQUEST FOR DFG STAFF.

Benefits. Provide the overall benefit rate applicable to each category of employee proposed in the project.

NONE.

Travel. Provide purpose and estimate costs for all non-local travel.

NONE REQUESTED; CONSULTANT EXPENSE.

Supplies & Expendables. Indicate separately the amounts proposed for office, laboratory, computing, and field supplies.

DFG IS NOT REQUESTING FUNDS FOR SUPPLIES AND EXPENDABLES. SEE OTHER DIRECT COSTS FOR CONSULTANT.

Services or Consultants. Identify the specific tasks for which these services would be used. Estimate amount of time required and the hourly or daily rate.

CONSULTANT PARTICIPATION: LABOR AND SALARIES WE HAVE ON FILE A DETAILED SPREADSHEET PREPARED BY THE CONSULTANT SHOWING INDIVIDUALS, HOURS, AND RATES, PER TASK PER YEAR. CONSULTANT HOURS ARE GIVEN AS LABOR HOURS FOR ENVIRONMENTAL SCIENCE ASSOCIATES (ESA) AND LABOR DAYS FOR BIOSPHERE GENETICS INC. (BGI). CONSULTANTS WILL PARTICIPATE TO THE EXTENT IDENTIFIED BELOW: YEAR 1 YEAR 2 YEAR 3 TASK 1- 2256 HRS-ESA 1240 HRS-ESA 950 HRS-ESA 50.5 DAYS-BGI 64.5 DAYS-BGI 48 DAYS-BGI TASK 2- 1988 HRS-ESA 888 HRS-ESA TASK 3- 2384 HRS-ESA 1888 HRS-ESA CONSULTANTS BILLING RATES: PROJECT DIRECTOR AND SR. BOTANIST: \$100/HR; YEAR 1 \$110/HR; YEAR 2 \$115/HR; YEAR 3 STAFF BOTANISTS: \$85/HR AND \$70/HR ADMINISTRATIVE STAFF: \$60/HR LARRY RIGGS AND BGI STAFF: \$1,000/DAY RANDY ZEBELL WILL SUBCONTRACT TO ESA AT A RATE OF \$75/HR

Equipment. Identify non-expendable personal property having a useful life of more than one (1) year and an acquisition cost of more than \$5,000 per unit. If fabrication of equipment is proposed, list parts and materials required for each, and show costs separately from the other items.

NONE.

Project Management. Describe the specific costs associated with insuring accomplishment of a specific project, such as inspection of work in progress, validation of costs, report preparation, giving presentations, response to project specific questions and necessary costs directly associated with specific project oversight.

DFG PROJECT MANAGEMENT WILL NOT INCLUDE ANY DIRECT COSTS TO THE PROPOSAL: FRANK WERNETTE WILL MANAGE THE OVERALL PROPOSAL AND DFG STAFF, AND HE WILL ENSURE ALL CONSULTANTS ARE MEETING THEIR REQUIREMENTS UNDER THE SUBCONTRACTS. CONSULTANTS MANAGEMENT COSTS: NIAL McCARTEN (ESA) WILL USE 9 PERCENT, OR \$25,048, OF HIS BUDGET TO MANAGE THE OVERALL PROJECT TO MEET CORPORATE REQUIREMENTS, CONDUCT QUALITY CONTROL AND ASSURANCE, REVIEW ALL DELIVERABLES, AND COORDINATE WITH DFG STAFF.

Other Direct Costs. Provide any other direct costs not already covered.

DFG WILL NOT INCURE ANY DIRECT COSTS. CONSULTANTS DIRECT COSTS IN ADDITION TO SALARIES: TASK 1: BOAT RENTAL ESTIMATE=\$8,000 PER YEAR FOR 2 YEARS. BIOCHEMICAL SUPPLIES-REAGENTS AND MINOR EQUIPMENT (<\$750 FOR EQUIPMENT) = \$17,000. MICROSATELLITE LIBRARY DEVELOPMENT BY GENETIC IDENTIFICATION SERVICES = \$18,000. RAPDS PRIMERS FROM OPERON TECHNOLOGIES = \$5,000. OTHER TRAVEL COSTS: VEHICLE RENTAL=\$2,500, PLUS MILEAGE \$2,500. LABORATORY EQUIPMENT RENTAL (MICROSCOPE AND COMPUTING TIME)=\$500 PER YEAR FOR THREE YEARS. GLOBAL POSITIONING SYSTEM RENTAL=\$750. TASK 2: WATER AND SOIL ANALYSES FROM INDEPENDENT LABS=\$10,000. ECOLOGICAL AND WATER TESTING EQUIPMENT RENTAL=\$15,000. BOAT RENTAL=\$3,750 PER YEAR FOR TWO YEARS. OTHER TRAVEL COSTS: VEHICLE RENTAL=\$750 PLUS MILEAGE \$1500. TASK 3: WATER AND ECOLOGICAL MONITORING EQUIPMENT RENTAL=\$9,000. BOAT RENTAL=\$2,000 PER YEAR FOR 2 YEARS. OTHER TRAVEL COSTS: VEHICLE RENTAL=\$3,500 PLUS MILEAGE \$1,800.

Indirect Costs. Explain what is encompassed in the overhead rate (indirect costs). Overhead should include costs associated with general office requirements such as rent, phones, furniture, general office staff, etc., generally distributed by a predetermined percentage (or surcharge) of specific costs.

WE HAVE CALCULATED OVERHEAD COSTS FOR DFG ACQUISITION OF OUTSIDE FUNDING. THE OVERHEAD COSTS ARE BASED ON PERCENTAGES OF 15 PERCENT FOR STATE FUNDS AND 13.9 PERCENT FOR FEDERAL FUNDS. BECAUSE WE ARE NOT REQUESTING SALARIES OR EXPENSES FOR DFG OR ITS STAFF, WE WILL REQUEST THAT THE OVERHEAD CHARGE BE WAIVED. DFG OVERHEAD COSTS ARE STANDARD FOR STATE AGENCY ADMINISTRATIVE COSTS.

Executive Summary

POPULATION BIOLOGY AND GENETICS, HABITAT MODELING, AND PILOT RESTORATION FOR THREE CALFED AT-RISK PLANT SPECIES IN THE SACRAMENTO-SAN JOAQUIN DELTA AND SUISUN MARSH

THIS PROJECT WILL MEET SEVERAL IMPORTANT CALFED MILESTONES FOR THE MSCS, ERP IMPLEMENTATION PLAN GOALS AND OBJECTIVES, AND SCIENCE PROGRAM PRIORITIES. IT ALSO WILL MEET SEVERAL ERP REGIONAL PRIORITIES FOR THE DELTA AND EAST SIDE TRIBUTARIES AND BAY REGION, AND MULTIREGIONAL PRIORITIES FOR HABITATS, AT-RISK SPECIES, AND RESTORATION. WE PROPOSE TO STUDY THREE IMPORTANT TIDAL WETLAND AT-RISK PLANT SPECIES INCLUDING MASON'S LILAEOPSIS, DELTA MUDWORT, AND SUISUN THISTLE. OUR STUDIES WILL COLLECT DATA ON SPECIES POPULATION VIABILITY, LOCATION, GENETIC STRUCTURE AND DIVERSITY, AND LIFE-HISTORY CHARACTERISTICS FOR THE PURPOSE OF DEVELOPING A POPULATION MODEL. THESE DATA WILL BE USED TO DETERMINE PRESERVATION PRIORITIES AND PROVIDE INFORMATION ON POPULATION SOURCE GENETICS FOR RESTORATION AND LIFE-HISTORY CHARACTERISTICS THAT ARE IMPORTANT FOR POPULATION ESTABLISHMENT AND GROWTH. THE DATA COLLECTED FROM THE POPULATION SIZE AND VIABILITY WILL BE USED TO DEVELOP PERFORMANCE STANDARDS FOR RANKING POPULATION VIABILITY. WE ALSO WILL COLLECT DETAILED DATA TO DEVELOP A HABITAT MODEL BASED ON MICROHABITAT VARIABLES (INCLUDING TIDAL ELEVATION, TIDAL FLUX, SALINITY, TOTAL SUSPENDED SEDIMENTS, SURFACE AND SUBSURFACE LIGHT LEVELS, WATER AND SUBSTRATE NUTRIENTS, SUBSTRATE TEXTURE, AND PLANT ASSOCIATES) THAT ARE NEEDED TO DEVELOP RESTORATION STRATEGIES. WE WILL TEST OUR HABITAT MODEL BY DEVELOPING PILOT RESTORATION EXPERIMENTS. THESE EXPERIMENTS WILL BE MONITORED TO DETERMINE WHETHER THE AT-RISK SPECIES ARE ESTABLISHING AND GROWING SIMILAR TO NATURAL CONTROL POPULATIONS. THOSE EXPERIMENTS THAT SUCCEED WILL BE USED TO DEVELOP RESTORATION MODULES THAT CAN BE USED BY CALFED AND OTHER TIDAL RESTORATION EFFORTS.

Proposal

California Department of Fish and Game

**POPULATION BIOLOGY AND GENETICS, HABITAT MODELING, AND
PILOT RESTORATION FOR THREE CALFED AT-RISK PLANT SPECIES
IN THE SACRAMENTO-SAN JOAQUIN DELTA AND SUISUN MARSH**

ROBIN FALLSCHEER, California Department of Fish and Game

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FRANK WERNETTE, CALIFORNIA DEPARTMENT OF FISH AND GAME

ROXANNE BITTMAN, CALIFORNIA DEPARTMENT OF FISH AND GAME

POPULATION BIOLOGY AND GENETICS, HABITAT MODELING, AND PILOT HABITAT RESTORATION FOR THREE CALFED AT-RISK PLANT SPECIES IN THE SACRAMENTO-SAN JOAQUIN DELTA AND SUISUN MARSH.

A. Project Description

This project will build on existing studies, fill data gaps, and develop population and habitat models, and will develop and implement pilot restoration experiments for three CALFED At-risk plant species. The three species are Mason's lilaeopsis (*Lilaeopsis masonii*), Delta mudwort (*Limosella subulata*), and Suisun thistle (*Cirsium hydrophilum* ssp. *hydrophilum*). This project will develop testable models on these plant species' biological and habitat requirements. The population and habitat models will be directly tested to determine if the species follow the natural growth patterns. Successful experiments will be developed into restoration modules that can be included in future large-scale restoration projects in the Delta and Suisun Marsh.

1. Problem

The loss of high-quality tidal wetland habitat is considered the primary cause of rarity for Mason's lilaeopsis, Delta mudwort, and Suisun thistle. Most of the loss of tidal wetlands in the Delta and Suisun Marsh has been due to land use conversion and levee building. At least 90 percent of the former tidal wetlands have been lost. The CALFED Ecosystem Restoration Plan (ERP) and associated Implementation Plan and Multispecies Conservation Strategy (MSCS) have identified Mason's lilaeopsis, Delta mudwort, and Suisun thistle as species "At-risk" needing protection and restoration of habitat to assist in species recovery. Over the past 12 years, field investigations have identified the locations and distributional range of these species. Ecological studies by McCarten and Ornduff (1989, 1990, 1991, 1994), Golden and Fiedler (1991), Zebell and Fiedler (1993, 1996), and the California Department of Water Resources (DWR) (1994) identified a number of biological and physical parameters (i.e., salinity, light levels, biological competition, and tidal flux) that affect the distribution of these species. The extent these habitat variables control species distribution, or population establishment and viability, is not clearly understood.

Focus on Species of Concern

Mason's lilaeopsis and Delta mudwort. Mason's lilaeopsis (Figure 1) is a member of the Apiaceae (carrot family). It is a California endemic plant listed as "Rare" by the California Department of Fish and Game (DFG). It is a small (3-5 cm wide) herbaceous perennial that forms rosettes and vegetatively reproduces by sending out tillers and subsequently forms new rosettes (Figure 1). Delta mudwort is a member of the Scrophulariaceae (snapdragon family). This species is extremely rare with a habitat and growth pattern much like Mason's lilaeopsis; however, the rosettes are generally smaller (1-3 cm wide). The California Natural Diversity Database (CNDDB) tracks these species using ArcOracle GIS database and mapping. Individuals voluntarily submit the information gathered on rare plants, animals, and natural communities to CNDDB. In many cases, the data for Mason's lilaeopsis and Delta mudwort in the CNDDB is over 5 years old (or older). Most of the data lacks voucher specimens. Recently, Robin Fallscheer (DFG botanist) determined that herbarium species of these two species had been misidentified. Further, field observations following the high water and flooding period of 1997 and 1998 determined that numerous known populations of these species had been lost due to the loss of in-channel islands and erosion of levee banks. Many of the remaining populations had become reduced in size and their viability is uncertain. Very little is known about the population structure, genetic diversity, life-history characteristics, and quantitative habitat parameters for these two species.

Suisun thistle. Suisun thistle is a member of the Asteraceae (sunflower family). It is a California endemic plant that is federally listed as "Endangered" and considered to be the rarest plant in Suisun Marsh (DWR 1994). Very little is known about this species' population structure, genetic diversity, life history, and habitat parameters. This species has an extremely limited distribution (DWR 1994, Peter

Baye U.S. Fish and Wildlife Service [USFWS] pers. com.) and a primary means to recovery will be through creating habitat and establishing new populations (Peter Baye USFWS pers. com.). This species is associated with first order tidal channels and tidal mosquito control channels (DWR 1994). Habitat modification in Suisun Marsh has led to the loss of first order channels and disturbance to the habitat has caused the invasion on non-native invasive plants, including pepperweed (*Lepidium latifolium*), that invade the habitat of Suisun thistle further affecting the At-risk species. Field observations suggest this species also may be hybridizing with other species of thistle (Horenstein 1987). Hybridization has been shown to increase the risk of plant extinction (Wolf et al. 2001). This is particularly true if habitat exists or is created that causes hybridizing species to overlap to a greater degree than occurs naturally. Suisun thistle is a biennial to short-lived perennial herbaceous plant and its unusual life history and habitat characteristics may present a special challenge to recovery efforts.

Goals and Objectives of the Study

The goal of this study is to develop models of plant population biology, life history, and habitats of these species that can be used in future preservation, management, and restoration to aid in the recovery of these three species. The specific objectives of this study include: 1) determine the current species' distribution and population viability, 2) determine the genetic diversity and its distribution/allocation among and within populations, 3) identify life-history traits that may affect the species' long-term survivorship, 4) identify tidal wetland habitat parameters that will lead to the development of a functional habitat model, and 5) develop a series of pilot restoration experiments based on the habitat model to determine if we accurately understand the species' habitat requirements. A goal of the habitat model and pilot restoration project is to develop a restoration module that can be included in larger tidal restoration projects to ensure that habitat for these species is included in the design.

2. Justification

CALFED has identified the need for ecosystem restoration to benefit the plant and animal species through the restoration of their habitats and return to the ecological processes that allow these species to become self-maintaining. Large-scale tidal restoration projects will benefit the ecosystem, but such efforts currently lack information necessary to ensure that a diversity of habitat conditions are produced that will result in higher biodiversity. Our proposed project will conduct population and habitat studies, develop models, and conduct pilot restoration experiments that will provide critical information that will benefit other CALFED restoration projects (Figure 2). Both Mason's lilaeopsis and Delta mudwort naturally occur at low tidal elevations on tidal marsh banks, high mudflats, and tidal channels. However, natural tidal marsh banks and channels have become rare habitats, and these two rare plants now live tenuously on the highly erodible mineral soils of man-made levees and remnants of in-channel islands that are the result of dredge cuts. Habitat and population studies will provide key information for these species (Figure 3).

Importance of genetics. The proposed DNA marker studies are an important step toward developing information on both the evolutionary ecology of the system to be restored and the possible impacts of alternative restoration measures (Figure 3). We will obtain important baseline information on the genetic architecture of each species to: 1) describe within and among population variation using molecular markers effective in measuring variation at individual, population, and geographic levels for each species; and 2) determine the relative importance of vegetative and sexual reproduction in existing populations. Findings will be applicable to four questions that arise repeatedly in restoration work: 1) how to preserve or restore individual population assemblages, 2) how to preserve and maintain genetic diversity/variation within populations (the genetic contribution to population viability), 3) how to preserve and manage for population identity or distinctness (in the context of evolved and evolving population differentiation in a species), and 4) how to avoid domestication selection and other management effects that may be

associated with propagation and restoration activities. Results of this part of the study will provide preliminary recommendations for design and implementation of the trial restoration effort (Task 3) and will lay important groundwork for defining the preservation and restoration practices for the species of interest that may be formalized in the future.

3. Approach

The project is divided into three primary tasks. Each task is designed to answer specific questions (Table 1) related to developing the population and habitat models, developing the conservation and habitat restoration strategy, and ultimately implementing a recovery program. The first two tasks require field data gathering, analysis, and the development of models on the population demography and habitat ecology. Each of these first two tasks is independent and will be conducted separately. The third task is dependent on the information gathered in the first two tasks and particularly the task relating to the habitat mode. In what follows, approaches applicable to all three species will be described under procedural headings; those specific to one or two species will be described under taxonomic headings.

Task 1 - Population Biology and Genetics

This task includes three subtasks each of which gathers data on the three At-risk species that will be used to develop a model of population structure and dynamics.

Subtask A - Population Description and Distribution

Mason's lilaeopsis and Delta mudwort. Field investigations will be conducted by boat and vehicle at low tides throughout the known distribution areas of these species in the Sacramento-San Joaquin Delta and Suisun Marsh. Some effort will be made to locate new populations that could extend the geographic range of these species in the Delta. The populations will be mapped and sites digitally identified using a global positioning system (GPS). Measurements of the population sizes will be taken as an area because it is unlikely we can differentiate individual clones or plants in the field. Photographs will be taken of each population to document the condition of the site. The CNDDDB ranks populations into four categories based on potential population viability. This study, in coordination with CNDDDB botanists, will develop a standard measurement method for ranking the condition and viability of these populations so it can be used in future surveys. Under permit from the DFG Endangered Species Program, we will make voucher specimens so that future researchers can be assured we correctly identified the species. In some instances, vouchering may not be feasible due to the small size of the populations. Voucher specimens, when available, will be deposited at the UC Jepson herbarium in Berkeley and/or at the UC Tucker herbarium in Davis.

Suisun Thistle. Suisun thistle is located only in Suisun Marsh in the upper elevational zones of tidal brackish marsh (DWR 1994). We will use the recently developed GIS vegetation map for Suisun Marsh developed by DFG and DWR (1999-2000) to determine potential locations of Suisun thistle using a spatial analysis based on the same vegetation types where the species currently occurs. We will then conduct field surveys of potential locations. Additional field surveys will be conducted based on information from experts on our team. We recognize that extensive field investigations have already been conducted (DWR 1994). These field investigations also will be useful to determine potential locations for the pilot restoration experiments (Task 3). All populations will be mapped and digitally determined using GPS. Photographs will be taken to document populations. Voucher specimens of plants from each new population discovered will be collected under permit from USFWS and DFG. Voucher specimens will be deposited at the UC Jepson herbarium in Berkeley and/or at the UC Tucker herbarium in Davis. We have been informed that only one known population may remain due to a recent fire that may have destroyed the second population (DWR 1994). We will establish permanent markers to locate ends of transects that will be placed to position quadrats for counting plants. We will use the existing data to ensure that the previous data can be used in evaluating population variation. Individual plants will be counted in each

quadrat along the transects. Populations of this species will be monitored during the three-year study to measure changes in population size (see life-history subtask below).

Subtask B - Population Genetics

Success in restoration efforts requires an effective integration of habitat, life history, and genetics information in the context of specific circumstances on the ground. To date, there is no genetic information pertinent to restoration design and implementation on any of the three species focused on in this proposal. One previous genetic study of Mason's lilaeopsis (Zebell and Fiedler 1993), using randomly amplified polymorphic DNA markers (RAPD), could not differentiate this species from another species (Western lilaeopsis). Other members of the carrot family have been studied using allozyme (protein) and DNA markers (RAPD, ISSR, and SSR). We will use molecular markers appropriate to addressing two key questions about the genetics of Mason's lilaeopsis relating to design of restoration initiatives: 1) how is genetic variation in this species distributed within-and-among populations, and 2) how is vegetative vs. sexual reproduction manifested in individual populations.

At this time, we are not aware of any genetic information available for the Delta mudwort. The essential questions important for restoration of this species are the same as those outlined for Mason's lilaeopsis.

Mason's lilaeopsis will be sampled throughout the geographic range of the species (Figure 4). At least 19 geographic sites will be sampled. Each site will include addition individual genet/ramet samples. The geographic sampling will include samples from more saline areas such as the Lower Napa River, Suisun Marsh, and Browns Island. Some of these sites were previously studied by Zebell and Fiedler (1993, 1996), and we will apply their data on salinity tolerance to help determine the presence of genetic ecotypes. Samples made from the higher salinity locations will be contrasted with samples from tidal freshwater areas in the west and east Delta (Figure 4). We will specifically include relatively isolated populations at the ends of sloughs such as Barker Slough in Solano County. Delta mudwort will be sampled throughout the geographic range of the species to delineate genetic architecture. Fewer sample sites will be studied due to the limited range of this species relative to Mason's lilaeopsis. We propose to sample five ramets/individual plants from each of the five geographic locations. When possible, Delta mudwort and Mason's lilaeopsis will be sampled at the same sites for analytical data and logistical reasons. The methods proposed for the molecular genetics of Delta mudwort will follow the same procedures identified for Mason's lilaeopsis.

There is no genetic information currently available for Suisun thistle. It has been suggested that this species also may hybridize with other species of the genus *Cirsium* (Horenstein 1987, Grewell in DWR 1994). Hybridization with the non-native species (common thistle) is believed to be a potential threat. However, these species differ in chromosome number (*C. hydrophilum* $2n=32$, *C. vulgare* $2n=68$). Hybridization of Suisun thistle with other species may not produce fertile hybrids, but will reduce reproductive output. We will evaluate hybridization in two ways: pollen stainability (see life history below) and molecular genetics. The hybridization study will include sampling non-native species occurring in the vicinity. We also will take a sample of the closely related variety Mt. Tamalpais thistle (*C. hydrophilum* ssp. *vaseyi*) that occurs in serpentine seeps on Mt. Tamalpais in Marin County. The molecular genetic study will sample 50 plants per population (based on two populations, if only one population is found to remain, we will sample 100 plants). The sampling will include first year plants and mature flowering plants. The steps to collect plant material and identify the best molecular marker method are the same as those given under Mason's lilaeopsis.

All of the sample handling, DNA extraction and purification, DNA amplification, and DNA fractionation techniques proposed for use in this study have been used extensively in published and unpublished work by many laboratories. The basis for selecting methods applied to each species discussed above and additional details are provided in Attachment A. Scientists associated with the laboratory managed by

Dr. Riggs have direct experience with all of these techniques. We will have access to special expertise required to address any problems that may arise through a permanent panel of scientific and technical advisors available to Dr. Riggs, as well as through several of the scientific reviewers named for this study.

Subtask C – Life-History Characteristics

Mason's lilaeopsis and Delta mudwort. Both Mason's lilaeopsis and Delta mudwort reproduce vegetatively using an extensive type of clonal morphology (Silander 1985). Typically, clonal plant populations are based on the development and loss of ramets and rarely through seed germination and the recruitment of genets (Cook 1985). Multiyear observations of populations of Mason's lilaeopsis found ramets to expand seasonally from April through August (McCarten and Ornduff 1994). A more thorough and systematic study of population structure for Mason's lilaeopsis and Delta mudwort is proposed and will include: 1) determining the extent of seasonal ramet growth measured by tiller length and the number of ramets produced, and 2) determining the extent of ramet connectivity in single populations. Populations will be measured using 0.5 meter square quadrats, each quadrat with 25 5x5 cm grid cells, which overlay the center of a population. A total of 10 populations will be studied. One hypothesis is that growth rates, including the formation of ramets and length of tillers, vary between populations depending on whether the soils are clay with high organic content or silts or sands with low organic content. We will study five populations from each of the two sets of substrate conditions. Our study will monitor the 10 populations over three years. We will include populations in Barker Slough and Suisun Marsh (both contain clay, high organic substrates) and levees along Brannan Island State Recreation Area (silt-sand, low organic content substrate). The extent of ramet connectivity will be measured by carefully washing off substrate with a squirt bottle to expose the tillers that are within 1-2 cm below the surface. Close-up photographs of the network of ramets and tillers will document the specific patterns and connections. Substrate will be replaced over the tillers after the photos have been taken. The photos will be developed and digitized and a mapping of the tiller-ramet network will be done on computer.

Sexual reproduction on these two species is not well understood. Both species produce minute flowers that form during summer. Seed production and the fate of seed and mode of dispersal are unknown, although we currently assume the seeds may float. The molecular genetics studies will contribute to understanding the level of genetic diversity that should be maintained through sexual reproduction. We will investigate seed production and mode of transport in two simple ways: 1) determine the amount of seed production by collecting seed from 50 flowers of each species and conducting a germination study for seed viability (it is assumed that these flowers are self-fertilizing and any pollen transfer occurs by water), and 2) the length of time the seeds can float will be measured by collecting an additional 100 seeds and placing them in an aquarium with a water pump to replicate natural water movement. The floating experiment will be conducted for a period of 60 days. The miniscule size of the seed prevents accurate field studies to track dispersal.

Suisun thistle. An understanding of the life-history characteristics of this species will help develop a population model and strategy for species recovery. Three life-history traits will be measured including population age structure, reproduction (self-fertilization vs. outcrossing), and the potential for hybridization. Population structure will be studied to determine three components: 1) number and proportion of plants in each age category (first year, second year, and third year), 2) distribution of age categories within the habitat since they may differ in the center versus the edges, and 3) number and proportion of flowers on plants and their distribution throughout the habitat. We will determine outcrossing rates by excluding potential pollinators from developing flower heads. We will bag a single flowering head on plants with multiple inflorescences in order to compare the amount of seed set on individual plants. Ten plants will be sampled. On each plant, one flowering head will be used as a control and another flowering head will be bagged to prevent external pollination. The number of fertile seed sets will be used to determine the level of outcrossing. Pollen stainability (see below) will be used to

evaluate the accuracy of the outcrossing study since hybridization could influence the level of fertile seed produced. A preliminary pollinator study will be conducted to determine what species are pollinating and which are native vs. non-native pollinators.

We will evaluate the question of hybridization by using molecular genetics (see previous subtask) and through the measurement of reduced fertility. We assume that hybridization will result in lower pollen fertility than non-hybrids because other thistles in the vicinity have different chromosome numbers (Keil and Turner 1993). Pollen taken from 25 flowering heads will be stained using Acetocarmine in glycerine jelly (Radford et al. 1974). At least 100 pollen grains from each flowering head will be scored for stainability.

Task 2 - Habitat Structure and Ecological Processes

The habitat measurements are divided into three components: 1) tidal hydrology, salinity, and sedimentation; 2) substrate texture, soil moisture, stability, and nutrient composition; and 3) biological factors including potential plant competition.

Mason's *lilaeopsis* and Delta mudwort

Tidal factors. Tidal flux and elevation have been used to define the habitat for these two species as occurring in the low tidal elevation or littoral zone (McCarten and Ornduff 1994, DWR 1994, Zebell and Fiedler 1993, 1996). We will establish two study areas, Suisun Marsh (a brackish marsh) and Barker Slough (a freshwater marsh). The duration of exposure of the plants below and above water has several significant consequences including availability of light and carbon below water, and the limitation of moisture when exposed above water for an extended period. We propose to measure the specific diurnal and seasonal tidal flux for populations occurring in our study areas to establish the range of tidal exposure. LiCor quantum light meters will be used to measure the amount of photosynthetically active radiation (PAR) the plants receive in the field. Erosion and sedimentation will be measured to determine whether the plants are restricted to eroding banks as has been suggested (Grewell in DWR 1994, Peter Baye USFWS pers. com.). As previously noted, the affects of salinity on Mason's *lilaeopsis* have been extensively studied, and field measurements in Suisun Marsh have identified potential salinity thresholds in the field (DWR 1994). We will measure salinity in the water of the populations to determine potential ranges throughout the year because seasonal timing has a significant influence on the salinity levels and the stage of plant development (Houle et al. 2001, Noe and Zedler 2000).

Substrate factors. Soil substrate at these plants' sites has been found to contain a high percentage of clay with high organic matter content or low percent clay with high percent sandy or silt substrate with low organic matter content (McCarten unpublished data). Nutrients may not be a limiting factor, but the texture of the substrate does affect the resistance to tidal and wave action. We will measure soil characteristics including texture (sand, silt, clay), organic content, pH, and basic nutrients (N, P, K). The substrate studies will include the Suisun Marsh and Barker Slough, and will be extended to at least 10 other sites in the Delta to get a broader sampling of soil conditions since our hypothesis is that soil stability may be a factor in the suitability of habitat for these species.

Biological factors. Competition from other wetland plants has been suggested and tested to some extent (Zebell and Fiedler 1996). These previous studies have determined that a diverse group of plants co-occur with Mason's *lilaeopsis* and Delta mudwort. The exact relationship and interaction between the At-risk species and other tidal plants is not clear. Zebell and Fiedler (1996) concluded that the ecological relationships between Mason's *lilaeopsis* and other low elevation intertidal plants could play an important role in the distribution of this species. They recommended that a plant community analysis be conducted to measure potential correlations with plant associates. In addition, McCarten and Ornduff (1994) suggested that shading in dense tule marshes could be a limiting factor. A species association and community study will be conducted to correlate potential positive and negative relationships with

Mason's lilaeopsis and Delta mudwort. Sites will be selected to incorporate the complete range of associated plant species. We will collect data on five sites from Suisun Marsh and five sites from Barker Slough. We will include 10 sites from other geographic areas including Old and Middle Rivers in the South Delta, Brannan Island, Napa River, and Frank's Tract in the central and north Delta. We will perform an ordination on the species data using canonical correlation analysis (Johngman, et al. 1995) using the software program "CANOCO," (ter Braak 1987-1992). This will determine potential species relationships and establish if specific environmental gradients are co-occurring. Also, we will incorporate water and substrate variables measured for the 20 study sites to correlate potential species interactions with environmental variables.

Suisun Thistle

Tidal factors. A significant amount of existing data exists for this species regarding the effects of tidal fluctuations and salinity due to the extensive work by Brenda Grewell (DWR 1994). It was observed that this species occurs at higher tidal elevations along first degree tidal channels, and that decreases in salinity may contribute to an increase in this species population size (DWR 1994, Grewell pers.com.). We propose to make additional measurements on tidal flux and salinity to confirm how these parameters may define habitat limitations. Our measurements will specifically address whether seasonal salinity levels at the location of the existing population and historic sites differs from locations elsewhere in Suisun Marsh. Seasonal salinity levels may directly affect early development stages of the Suisun thistle as has been noted with other saltmarsh species (Houle et al. 2001). We will establish field equipment at the known and historic population sites to measure the tidal flux, salinity, conductivity, and sedimentation or erosion rates.

Substrate factors. Substrate for Suisun thistle is currently known to contain a high percentage of clay and organic matter, often called peat soils, (Peter Baye USFW pers com.). Recovery of this species by establishing new populations first requires determination as to whether the species has an ecological requirement for these soils. The organic soils have higher water holding capacity and may prevent permanent wilting point (PWP) during low tides, while sandier soils could cause draught conditions. Soils from populations will be analyzed for texture (sand, silt, clay), organic content, pH, and basic nutrients (N, P, K). In addition, the soils will be measured for PWP and field capacity to determine the soil water holding capacity. Soil moisture probes will be placed in 10 locations within Suisun thistle populations to measure diurnal fluctuations in soil moisture during one growing season. Soil moisture probes also will be placed in five sites occurring at identical tidal elevation zones that have sand or silt soils with low organic content.

Biological factors. The effect of plant associates on Suisun thistle is not known, and it may be susceptible to hybridization. Hybridization could have eliminated Suisun thistle from some locations where the plant was in proximity to other common species of thistle (Task 1). There is a strong correlation with the occurrence of plant species and their distance from tidal channels (Sanderson et al. 1999). Changes in habitat conditions could affect the plant associates which could have a negative affect on Suisun thistle. One species of particular concern is pepperweed (*Lepidium latifolium*). Pepperweed is recognized in the CALFED Implementation Plan as a particularly noxious non-native invasive plant species. Grewell (pers. com.) indicated that pepperweed in Suisun Marsh may be having a significant negative effect on Suisun thistle populations. Within the square meter quadrats along the permanent transects (see Task 1) we will quantitatively measure plant associates for numbers of plants or percent cover if individuals cannot be identified. We will perform canonical correlation analysis on the species data using CANOCO (ter Braak 1987-1992). The ordination analysis will measure correlations between Suisun thistle and other species associates as well as the habitat variables.

Task 3 - Pilot Restoration Project

The best method to evaluate the species population and habitat models is to create the habitat conditions and then measure whether the species are growing and reproducing following the population model predictions. We propose to conduct a series of experimental pilot restorations at multiple sites for each of the three At-risk plant species: Mason's lilaeopsis, Delta mudwort, and Suisun thistle. All proposed pilot restoration experiments will be sited on permanently protected lands such as DFG reserves.

Mason's lilaeopsis

Ten pilot restoration experiments will be conducted for this species. The sites for these restoration experiments will include Barker Slough, Calhoun Cut (both part of the DFG Calhoun Cut Reserve), and Suisun Marsh. Due to the small size of the plant ramets (3-4 cm diameter) the habitat creation sites will be approximately 1 x 0.5 meter in area. The restoration area will be measured spatially by using a grid composed of 5 x 5 cm grid cells. The small area will allow a minimum of disturbance to the tidal banks and existing wetlands and provide adequate space to measure the growth rate of the plants. Specific methodology for the experimental restoration efforts will be determined as part of the pilot restoration plan following completion of Tasks 1 and 2.

Analytical Methods. Two methods will be used to measure performance of the pilot restoration: 1) an increase in the number of ramets and aerial growth by tillering, and 2) a statistical comparison between the pilot restoration sites with natural unmanipulated "control" populations growing nearby. Statistical analyses will include an analysis of variance (ANOVA) using SYSTAT ver. 10. We also will employ the method used by McCarten and Ornduff (1989) to measure the success of translocated plants of Mason's lilaeopsis relative to control populations.

Control populations will need to be evaluated using cover values since the number of ramets may be large even over a small area. In that case, a proportional comparison using percent cover values in each grid cell will be measured in the pilot and control populations. The goal will be to determine whether the pilot populations are growing and seasonally fluctuating similar to control populations.

Delta Mudwort

Ten pilot restoration experiments will be conducted for this species. The sites for these restoration experiments will include Barker Slough, Calhoun Cut (both part of the DFG Calhoun Cut Reserve), and Suisun Marsh. Due to the small size of the plant ramets (3-4 cm diameter) the habitat creation sites will be approximately 1 x 0.5 meter in area. The restoration area will be measured spatially by using a grid composed of 5 x 5 cm grid cells. The small area will allow a minimum of disturbance to the tidal banks and existing wetlands and provide adequate space to measure the growth rate of the plants. Specific methodology for the experimental restoration efforts will be determined as part of the pilot restoration plan following completion of Tasks 1 and 2.

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Suisun Thistle

The limiting habitat component for Suisun thistle is believed to be the availability of first order and possibly second order tidal channels (DWR 1994). Most of these finer channel systems were lost when Suisun Marsh was historically diked. In addition, the loss of organic peat soils due to oxidation and subsequent subsidence led to the use of mineral soils to fill the diked wetlands. Therefore, organic-peat soils are much less common and it may be necessary to aid Suisun thistle recovery by establishing populations on mineral soil substrates. The proposed pilot tidal restoration experiments will be conducted in high elevation tidal marsh areas. One set of experiments will be established on existing organic-peat soils and the second set of experiments will be conducted on mineral soils. The restoration experiment will involve creating three first order tidal channels (approximately 0.5 to 1 meter wide and 5 meters long; specific size of channel will be determined from the habitat studies). A set of three tidal channels will be created in each of the substrate types (i.e., organic peat and mineral soils). The existing populations and habitats will be monitored as control sites. Digging the channels and linking them to larger channels will create the first order channels.

The restoration experiments will include seeding and planting Suisun thistle along the created tidal channels. Seed will be planted in specified plots along the channel and away from the channel equivalent (i.e., the distance of the plants in their natural population from the channel). Plants will be germinated in pots in the greenhouse to establish first year rosettes. These plants will be translocated to the created tidal channels after the first season during the dormant period. The number of plants germinated will be dependent on the number of seed that can be safely harvested from the existing population. It is DFG policy that not more than 5 percent of the seed from an endangered plant population can be collected for studies. The restoration project for Suisun thistle will include an evaluation of an eradication program for pepperweed. We will identify experimental methods to test the efficacy of implementing an eradication plan if it is determined that peppergrass eradication can be used to enhance the existing habitat quality for Suisun thistle.

We will monitor each of the six pilot restoration experiment sites three times each year during the two-year period of the pilot restoration. Field monitoring will count the number of seeds germinated and size of seedlings. Both seedlings and first-year rosette plants will be measured for rosette leaf number, length, and width. Plants surviving to flowering will be measured for the number of heads and flowers per head. The plant data from the experiment sites will be quantitatively compared with similar data collected at the control population. An ANOVA test will be used to statistically compare experimental populations with controls.

4. Feasibility

Several components of our proposal make this an extremely feasible project in terms of scheduling, travel logistics, accessibility to plant populations, and ability to conduct and evaluate technical approaches. The most important component is the team of scientists that are participating. We have botanists, ecologists, molecular geneticists, water quality experts, and resource agency staff that are recognized as regional experts on these three species, have extensive knowledge of the locations of all the populations of the plants, and extensive knowledge of the Delta and Suisun Marsh. The scientists also include individuals that are recognized in the study of molecular genetics of rare species and have specific knowledge on the molecular genetics of Mason's lilaeopsis. We have direct access to study sites and plant populations because they are on public lands and are protected and managed by DFG, DWR, and California Department of Parks and Recreation (DPR). All of these agencies participate in the protection of At-risk species and support this project.

5. Performance Measures

Our project includes a team of scientific and peer reviewers. We specifically include budget to cover peer review time and expenses. Paying for peer review is not considered a conflict of interest because it is common to pay for review services, and we will retain qualified individuals if we compensate them appropriately. The performance measures for Tasks 1 and 2 include scientific and peer review of administrative draft reports by recognized scientists and experts. The proposed reviewers for Task 1 are: Dr. Diane Elam (USFWS) to review population biology and genetics for all three species; Dr. Bruce Baldwin (Jepson Herbarium, University of California at Berkeley) to review Suisun thistle population biology and genetics; and Dr. Carla D'Antonio (Department of Integrative Biology, University of California at Berkeley) to review habitat model. The proposed reviewers for Task 2 are: Dr. John Callaway (University of San Francisco) and Brenda Grewell (University of California at Davis) to review habitat model.

Progress on the genetics component of this study is easily quantifiable in terms of numbers of samples processed through each project stage: sample collection, accession, and DNA extraction and purification. For analytic activities, a useful progress parameter is the number of markers resolved via PCR, fractionation, data recording, and analysis. Targets will be established relative to the work plan at the beginning of the study, and progress toward those targets will be identified in each quarterly report.

The Task 3 pilot restoration will include a detailed design plan and description of methods for statistical analysis of the data. A monitoring plan will be developed that will measure species At-risk and the physical and biological variables that may affect the outcome of the experimental pilot restoration. We will have the pilot restoration and monitoring plans reviewed prior to implementation. We propose to have Dr. John Callaway (Department of Environmental Studies, University of San Francisco) review these plans, and Dr. Kevin Rice (University of California at Davis) review the monitoring plan.

6. Data Handling and Storage

Field data will be entered into computer databases (Microsoft Access), word processing (Microsoft Word), and spreadsheets (Microsoft Excel). Mapping information and GPS data will be entered into ArcInfo/ArcView GIS databases and new maps will be produced. The data will be printed out and staff will check the accuracy (QA/QC) of the data transferred to computer with the original collected data. Copies of all QA/QC data will be stored at the DFG Bay-Delta Program office. Field survey forms and maps identifying the location of rare plant populations will be filled out and copies provided to the CNDDDB.

Genetic data acquired from laboratory analyses will be maintained in databases associated with the program Phoretix 1D (Phorestix International Ltd., Birmingham, UK). Data is exportable via Excel files to database systems maintained by Environmental Science Associates and DFG. Reports will be provided in one or more of the following forms as preferred: hard copy; Microsoft Word, Microsoft Excel, PDF, and/or HTML files on CD-ROM; e-mail attachment; or Web-accessible files.

7. Expected Products/Outcomes

8. Work Schedule

The results of the genetics component of this study will provide a baseline description of the genetic architecture of each species and begin to measure the relative importance of clonal propagation in Mason's lilaeopsis and Delta mudwort. The genetic information obtained will provide a foundation for preservation and restoration practices for these species and help identify any further research needed to address questions about the relative importance of microevolutionary processes (selection, drift, gene flow, and recombination) and their relationship to species' distribution and life history. Data will support

the definition of specific alternative hypotheses explaining the patterns observed, thereby identifying productive avenues for future inquiry.

B. Applicability to CALFED ERP and Science Program Goals and Implementation Plan and CVPIA Priorities

1. ERP, Science Program, and CVPIA Priorities

CALFED MSCS Milestones

Delta and East Side Tributaries. Restore tidal slough habitat to benefit Delta mudwort; restore tidal emergent wetland to benefit Mason's lilaeopsis and Delta mudwort.

Suisun Marsh and Bay Habitat. Restore tidal marsh habitat for Suisun thistle. Identify at least three protected and managed sites for introduction of at least three additional populations of Suisun thistle, increase overall population size. Restore suitable, occupied slough edge habitat for Delta mudwort in the Suisun Bay and Marsh and in the Napa River; species to benefit are Mason's lilaeopsis and Delta mudwort.

ERP Strategic Goals and Objectives

Goal 1: Endangered and other At-risk species and native biotic habitats.

Objective 1: Recovery and self-sustaining populations for Suisun thistle and Mason's lilaeopsis.

Objective 2: Contribute to recovery of Delta mudwort.

Goal 2: Ecosystem processes and biotic communities.

Goal 4: Habitats - protect, restore functional habitat types, including Delta and Suisun Marsh.

Science Program Goals

Build population models for At-risk species, and conduct adaptive management experiments.

ERP Implementation Plan Priorities

Study Needs for At Risk Species: Suisun thistle, Mason's lilaeopsis and Delta mudwort.

Multi-Regional Priority 6: Ensure recovery of At-risk species by developing conceptual understanding and models that cross multiple regions.

Delta and East Side Region

Priority 1: Restore habitat corridors including develop, test, assess techniques to restore tidal wetlands.

2. Relationship to Other Ecosystem Restoration Projects

This project will benefit from previous and on-going projects in Suisun Marsh including restoration of soft bird's beak (CALFED 1999-N05). CALFED proposed and on-going restoration planning projects involving tidal restoration will be able to use the restoration modules.

3. Requests for Next Phase Funding

Not applicable.

4. Previous Recipients of CALFED Program or CVPIA Funding

Not applicable.

5. System-Wide Ecosystem Benefits

The studies and model developed for the low-elevation tidal habitats will benefit restoration and other projects throughout the tidal freshwater and brackish water regions of the Delta and Bay Region. It will identify the locations of important At-risk plant populations and develop a strategy for protection and restoration priorities in those regions.

6. Additional Information for Proposals Containing Land Acquisition

Not applicable.

C. Qualifications

The project team is composed of botanists, geneticists, and tidal wetland ecologist that have extensive experience on the CALFED At-risk plant species and their habitats proposed in this study.

Robin Fallscheer is a botanist with DFG's Central Valley Bay-Delta Branch in Stockton. She received both her B.A. and M.S. in Biological Sciences at California State University, Chico. As a member of the CALFED Ecosystem Restoration Unit of the DFG Central Valley Bay-Delta Branch, she is intimately involved in the CALFED restoration efforts in the Sacramento-San Joaquin Delta. Ms. Fallscheer will be the project coordinator for DFG on this project.

Roxanne Bittman is a senior biologist (Botany) with DFG's CNDDDB. She has been with the CNDDDB since 1986. She received her B.S. in Environmental Biology from UC Santa Barbara and an M.S. in Botany from Ohio State University. She has over 17 years of experience in plant conservation including surveys, monitoring, management planning and database work. Habitats of expertise include tidal wetlands, vernal pools, grasslands, desert vegetation, and high elevation wetlands. She participated in the development of the original CALFED Multispecies Conservation Strategy and in the development of the Milestones. Ms. Bittman will participate in the collection and evaluation of population data for the three At-risk plant species.

Niall McCarten is senior biologist with Environmental Science Associates and Research Associate with the Section of Plant Biology at UC Davis, and the UC Jepson Herbarium at UC Berkeley. He received his B.A. in botany at UC Santa Barbara, M.A. in Ecology and Systematics at San Francisco State University, and Ph.D. in botany at UC Berkeley. He is a nationally recognized botanist and plant ecologist with peer-reviewed papers and conference presentations on rare and endangered plants, wetlands ecology and monitoring. His area of research is on the ecology and population biology of aquatic and wetland plants and plant adaptations to edaphic factors such as those found on serpentine and gabbro soils. He has conducted research on rare plants for federal and California natural resources agencies, including studies on Mason's lilaeopsis, Delta mudwort, and Suisun marsh aster. He is a recognized expert on the Sacramento-San Joaquin Delta system and has conducted studies on the rare plants and wetlands, and conducted detailed habitat assessments and mapping. He was one of the few non-public agency scientists asked to participate in the development of the original CALFED ERP plan, and participated in the development of the CALFED Natural Community Conservation Plan (NCCP). He was chairman of conservation and vice-president of the California Botanical Society.

Larry Riggs is a population geneticist and evolutionary biologist who has been working at the interface between research and application for the past 20 years. He received his A.B. from Dartmouth College. As a graduate student at the University of Colorado, Boulder, he participated in the first NEPA-mandated environmental impact studies with a team of plant ecologists working throughout western Colorado before moving to the University of California, Berkeley, where he received his Ph.D. in Zoology. He taught at the University of California, Santa Barbara, and worked with USFWS and the National Council on Gene Resources' California Gene Resources Program before consolidating his independent consulting activities under the name of Genetic Resource Consultants. He was a co-founder of Biosphere Genetics,

Inc. in 1991 and has been a principal investigator on projects applying genetic information and a variety of molecular marker techniques to conservation, restoration, and resource management for the past 10 years. He currently serves as the company's president and CEO.

Randy Zebell is a regionally recognized botanist and independent consultant. He received his B.A. and M.A. in Biology and Ecology and Systematics from San Francisco State University. He was the primary researcher for a multi-year study on Mason's lilaeopsis that evaluated the effects of potential oil spills on this rare plant. As part of that study he conducted extensive field investigations and population sampling. He also conducted preliminary molecular genetic studies on Mason's lilaeopsis using Randomly Amplified Polymorphic DNA Sequences (RAPDs).

Chris Rogers is a plant ecologist with Environmental Science Associates. He received his B.A. from San Francisco State University. He is considered to be a regional expert on wetland plant ecology and tidal wetland restoration. He has participated in several projects involving Mason's lilaeopsis, Delta mudwort and Suisun thistle. Specifically, he conducted three years of field monitoring of natural and translocated populations of Mason's lilaeopsis in Barker Slough as part of an experimental project to relocate plants. He has extensive experience in the Sacramento-San Joaquin Delta, Suisun Marsh, and the San Francisco Bay Region.

Yolanda Molette is a botanist and plant ecologist with Environmental Science Associates and has extensive experience with identification and ecology of California flora. She received her B.A. in Biology (emphasis botany) and M.A. in Conservation Biology from San Francisco State University. Her experience ranges from conducting rare plant surveys, vegetation mapping, and ecological monitoring and interpretation to preparing habitat restoration plans, erosion control plans and experience with non-native invasive plant species. Ms. Molette also analyzes impacts to biological resources, and performs habitat assessments for various types of projects and environmental compliance monitoring for construction projects.

Frank Wernette is an environmental program manager at the Central Valley Bay-Delta Branch of DFG. He received his B.S. in Wildlife Management from California State University, Humboldt. As the supervisor of the Water Project Planning and Ecosystem Restoration Program, he is currently responsible for the evaluation of proposed State Water Project water storage and conveyance projects throughout the State with an emphasis on the Sacramento-San Joaquin Delta. He oversees analysis of fish and wildlife impacts associated with water project development. Currently, he is assisting CALFED in developing the comprehensive ERP Plan. He acts as DFG's technical lead in assessing fish and wildlife impacts of the Delta Wetlands Project and developing appropriate mitigation measures. Previously (1980-1990), he was the DFG representative on the Suisun Marsh Technical Committee. He performed wildlife habitat monitoring studies in the Suisun Marsh and provided wildlife input to the committee for planning and implementing the Suisun Marsh Plan of Protection. He provided technical wildlife input to the principal DFG negotiators working on the Suisun Marsh Preservation Agreement. From 1975-1980, he was the Region 2 Wildlife Biologist, with a primary focus on wildlife and habitat issues in the Sacramento-San Joaquin Delta.

D. Cost

1. Budget

The estimated cost of this project over a three-year period is \$1,266,391.

2. Cost-Sharing

Not applicable.

E. Local Involvement

Due to the large multiregional geographic range covered by this project, we will have numerous types of local involvement. Our studies will include State parks owned and managed by DPR, including Brannan Island, Delta Meadows, Frank's Tract, and local DFG and DWR preserves including Calhoun Cut DFG preserve and Suisun Marsh. The Suisun Marsh studies will be coordinated with the Suisun Marsh Planning Committee, and the developing Suisun Marsh Charter Program. We will involve the DFG and DWR Delta Levees and In-channel Islands programs. There are proposed levee restoration projects on U.S. Army Corps of Engineers levees and we will coordinate with those projects. There are private Reclamation Districts throughout our study area and we will coordinate and involve those districts.

F. Compliance with Standard Terms and Conditions

This proposal will comply with the standard terms and conditions for State and Federal contracts as described in Attachments D and E of the Proposal Solicitation Package.

G. Literature Cited

- Cook, R.E. 1985. Growth and Development of Clonal Plant Populations. In Population Biology and Evolution of Clonal Organisms. J. Jackson, L. Buss, and R.E. Cook eds. Yale University Press, New Haven.
- Department of Water Resources. 1994. Summary of Sensitive Plant and Wildlife Resources in Suisun Marsh During Water Years 1984-1994. Technical report of the Environmental Services Office, Sacramento. 107 pp.
- Golden, M. and P. Fiedler. 1991. Characterization of the Habitat for *Lilaeopsis masonii* (Umbelliferae): A California State-listed Rare Plant Species. Technical Report prepared for the California Department of Fish and Game, Sacramento, CA. 72. pp.
- Horenstein, J. 1987. Natural Diversity Database field survey form for *Cirsium hydrophilum* ssp. *hydrophilum* and associated notes in files at the California Department of Fish and Game.
- Houle, G., L. Morel, C. Reynolds, and J. Siegel. 2001. The effect of salinity on different development stages of an endemic annual plant, *Aster laurentianus* (Asteraceae). Amer. Journ. Botany 88:62-67.
- Johnsman, R., C.J.F. ter Braak, and J. Van Tongeren. 1995. Data Analysis in Community and Landscape Ecology. Cambridge University Press, Cambridge, UK. 299 pp.
- Keil, D. and C. Turner. 1993. The Genus *Cirsium*. In the New Jepson Manual. University of California Press, Berkeley, CA.
- McCarten, N.F. and R. Ornduff. 1989. Study of Sensitive Plant Species in the Littoral Zone of Brannan Island State Recreation Area. Technical report prepared for the California Department of Parks and Recreation, Inland Region, Lodi, CA. 20 pp.
- McCarten, N.F. and R. Ornduff. 1990. Study of Sensitive Plant Species Occurring in the Delta Meadows State Park. Report prepared for the California Department of Parks and Recreation, Inland Region, Lodi, CA. 16 pp.
- McCarten, N.F. and R. Ornduff. 1991. Monitoring of Transplanted Mason's *Lilaeopsis* Populations in Barker Slough, Solano County: 1989-1990. Technical report prepared for the California Department of Water Resources, Sacramento, CA.
- McCarten, N.F. and R. Ornduff. 1994. Monitoring of Transplanted Mason's *Lilaeopsis* Populations in Barker Slough, Solano County: 1989-1993. Technical report prepared for the California Department of Water Resources, Sacramento, CA. Contract #B58893.

- Noe, G. and J. Zedler. 2000. Differential effects of four abiotic factors on the germination of salt marsh annuals. *Amer. Journ. Botany* 87:1679-1692.
- Radford, A., W. Dickison, J. Massey, and C.R. Bell. 1974. *Vascular Plant Systematics*. Harpor and Rowe Publishers, New York.
- Sanderson, E.S. Ustin, and T. Foin. 1999. The Influence of tidal channels on the distribution of salt marsh plant species in Petaluma marsh, CA. Technical paper- Center for Spatial Technologies and Remote Sensing (CSTSARS), University of California at Davis.
- Silander, J. 1985. Microevolution in clonal plants. In *Population Biology and Evolution of Clonal Organisms*. J. Jackson, L. Buss, and R.E. Cook eds. Yale University Press, New Haven.
- ter Braak, C.J.F. 1987-1992. CANOCO- a FORTRAN program for canonical community ordination. Microcomputer Power, Ithaca, New York.
- Wolf, D., N. Takebayshi, and L. Rieseberg. 2001. Predicting the risk of extinction through hybridization. *Conservation Biology* 15:1039-1053.
- Zebell, R. and P. Fiedler. 1993. Restoration and Recovery of Mason's *Lilaeopsis* Phase I. Technical report prepared for the Shell Oil Spill Settlement Trustee Committee and California Department of Fish and Game, Sacramento, CA. 47 pp.
- Zebell, R. and P. Fiedler. 1996. Restoration and Recovery of Mason's *Lilaeopsis* Phase II. Technical report prepared for the Shell Oil Spill Settlement Trustee Committee and California Department of Fish and Game, Sacramento, CA. 50 pp.

Attachment A

Basis for Selection of Molecular Methods Appropriate for Proposed Genetics Activity

Available Molecular Methods

Since the polymerase chain reaction (PCR™, Roche Molecular Systems) was introduced over a decade ago, an impressive toolbox of molecular methods has arisen enabling alternative approaches to genetic work proposed in this study. Marker systems appropriate to description of within-and-among population variation include: 1) microsatellites, also known as simple sequence repeats (SSR) or simple tandem repeats (STR); 2) inter-simple sequence repeats (ISSR); 3) randomly amplified polymorphic DNA (RAPD); and 4) Amplified Fragment Length Polymorphism (AFLP®, Keygene Inc.). Each of these methods has been used for both of the two principal aims we propose as illustrated by numerous examples in the literature.

RAPD or AFLP are the “fingerprinting” methods used by many working in crop plant genetics to identify clones (Sydes and Peakall 1998) and distinguish closely related varieties, cultivars or populations (Chalmers et al. 1994, Rossetto et al. 1995, Sharma et al. 1996, Tohme et al. 1996, Del Rio et al. 1997b, Hongtradul et al. 1997, Muluvi et al. 1999, Nebauer et al. 1999, Kiers et al. 2000, Lowe et al. 2000). However, microsatellites are the current standard for individual identification in human forensics and are preferred by many population geneticists working on plants (Thomas et al. 1994, Yang et al. 1994, Akagi et al. 1997, Green et al. 2001, Bonnin et al. 2001, Perrsson and Gustavsson 2001). ISSR techniques (very similar to RAPD except for the use of primers for SSR sequences) are beginning to be more widely applied and have been demonstrated to have utility in work on species in the Asteraceae and Scrophulariaceae (Wolfe et al. 1998b, Lutz and Wolfe 2000), as well as the Apiaceae (Briard et al. 2001). A number of studies have compared the relative utility of different marker classes for various purposes (Lin et al. 1996, Powell et al. 1996, Yang et al. 1996, Fang et al. 1997, Milbourne et al. 1997, Nagaoka and Ogihara 1997, Olufowote et al. 1997, Parsons et al. 1997, Russell et al. 1997, Pejic et al. 1998, Wolfe and Liston 1998a, Virk et al. 2000). Generalizations applicable to previously unstudied species are tentative, but at least one study finds ISSR to be at least as informative as AFLP (an average of 24 markers per primer) with less effort and cost. Some useful information is returned by most techniques; even sequence data from nuclear and mitochondrial regions have proven useful in prioritizing conservation decisions (Zoller et al. 1999), although in many taxa most such regions that have been studied exhibit too little variability to be useful in distinguishing entities below the sub species level (e.g., Zebell and Fiedler, 1993).

A handful of studies relevant to the plant families of interest to this proposal should receive special note. ISSR, microsatellites, and RAPDs have been compared for varietal identification of carrot (family Apiaceae) genotypes (Briard et al. 2001). Studies of the rare plant, *Cirsium pitcheri* (family Asteraceae), found geographic differences among populations of using using RAPD markers (Bell et al. 2000). Work with ISSR on members of the Asteraceae and Scrophulariaceae is mentioned by Wolf and Liston (1998). In work on the Scrophulariaceae, there are reasons to question whether markers obtained with the primers used in these studies would be homologous in taxa not known to be closely related (Datwyler et al. 2000, Olmstead et al. 2001); however marker homology is not critical to the population genetic focus taken in this proposal.

Selection of Methods Appropriate to Species and Key Questions

Although the published literature provides some basis for choice of marker system, tradeoffs between difficulty of application and information return cannot be evaluated in advance of preliminary work or previously unstudied species. An appropriate first step in this study will be to screen samples obtained

from the field in Year 1 using two or three of the available methods in each case. Year 1 results will determine which method/marker system is most consistently and efficiently implemented with each species. As sampling proceeds in years 2 and 3, laboratory work will proceed with the method or methods most effective at generating data addressing the aims specified in body of the proposal (see Justification section). Other aspects of the method used in the molecular genetics work proposed are widely used in conservation biology and other specialty areas and are summarized below.

Field Sampling and Sample Handling. Field sampling methods include selecting populations in the field during the course of fieldwork performed under Task 1. One to several leaves providing an equivalent of 3-5 cm² of leaf tissue will be collected from each sampled plant or the equivalent of one ramet rosette. About 1 cm² is used in an individual DNA extraction. The balance is needed to provide backup material for optimization work and repeat analyses. Material remaining at the end of the study will be made available for archiving in conjunction with voucher material at the herbaria named above. Leaves will be inserted in special labeled envelopes, which will be placed in a plant press at the end of each field session for transport and initial storage. After a suitable period of drying, envelopes will be removed from the press and stored in a desiccating environment. Material collected in this way can be extracted for DNA at any time without need for ultracold freezer storage or time-sensitive handling. Year 1 fresh material will be collected from a subset of sampled individuals and extracted for DNA soon after collection in order to perform a comparison with dry leaf extracted DNA verifying this approach.

DNA Extraction and Purification. Unless we find recently published work demonstrating effective DNA extraction using simpler methods from each of the three species, we will use a CTAB/organic solvent method as has been repeatedly demonstrated to be most reliable with plant tissue material. A number of comparisons of DNA extraction techniques have been published but none pertain directly to the species under study here. One study on domesticated carrot evaluated seven alternative extraction methods using tissue from flowers, fresh leaves, lyophilized leaves, calli, and tap roots (Boiteux et al. 1999). Criteria included: 1) DNA yield, 2) DNA purity, 3) DNA cleavage with HindIII, 4) DNA integrity, and 5) DNA suitability for amplification in a random amplified polymorphic DNA (RAPD) system. Our own work with RAPD markers has indicated the importance of identifying an effective combination of procedures for reliable resolution of particular markers. An assessment of methods most likely to produce satisfactory results for each species and targeted class of markers will be performed after preliminary sampling is done during Year 1.

Primer Selection and Acquisition. Candidate primers for screening of RAPD and ISSR markers will be identified from available sets of random primers with reference to prior studies on species from the families Apiaceae, Asteraceae, and Scrophulariaceae. Prior information for related species may increase the chances of resolving informative variation for the species of interest to this proposal. Otherwise, it may be necessary to screen 50-100 primers on a representative subset of population samples to identify markers useful to this study.

RAPD primers identified for use in this study will be ordered from the University of British Columbia Forest Biotechnology Laboratory or a commercial supplier. Synthesis of ISSR primers and other primer sequences used in the study will be ordered from Operon Technologies Inc (Alameda, CA).

The emphasis in the proposed genetics work is on obtaining maximally informative data for Mason's *lilaeopsis*. RAPD and ISSR marker data are relatively easy and inexpensive to obtain for species not previously studied. Such data provide a good overview on the distribution of genetic variation within and among populations and are effective at recognizing clones in sampled material. However, good estimates of population structure and gene flow parameters important to heuristic and application-oriented modeling of population genetics require co-dominant marker data, as provided by variation at microsatellite loci. For Mason's *lilaeopsis*, we will obtain representative material (leaf tissue) from several populations and

outsource development of a genomic library enriched for variable microsatellite sequences. The vendor probably most appropriate to this task is Genetic Identification Services, Inc. Genetic Identification Services, Inc., guarantees production of at least 12 variable microsatellite sequences in libraries they develop.

Preliminary Sample Screening. For Mason's lilaeopsis, we will screen preliminary sample extracts for variation using existing panels of primers for RAPD, ISSR, and possibly AFLP (beginning with primers used successfully in published work on carrot), and for microsatellite markers (primers developed for domesticated carrot). Apparently, there are no primers available to amplify microsatellite regions in any member of the Scrophulariaceae family. Work on Delta mudwort will begin by screening a selection of RAPD and ISSR primers. For Suisun thistle, we will screen a selection of microsatellite primers developed for sunflower (Paniego et al. 1999) in addition to testing RAPD and ISSR primers.

Optimization of PCR-based Methods. Molecular genetics work with species (and sometimes even populations) and primers not previously studied in combination often requires optimization of PCR methods to obtain markers that can be fractionated and scored reliably. One round of PCR optimization will occur in conjunction with primer screening (template quantity and reaction components indicated by the literature to affect amplification). If informative markers are indicated for particular primers but are not well or consistently expressed, a second round of optimization may be useful before beginning population survey runs.

Fractionation and Result Recording. PCR products will be fractionated using either slab gels, capillary electrophoresis (CE), or fragment sizing methods for D-HPLC. In year 1, marker screening and methods optimization work will rely primarily on slab gel techniques. For RAPD and ISSR markers, agarose gels stained with ethidium bromide will be digitally photographed on a transilluminator. For SSR and AFLP, fractionation will be done on polyacrylamide gels stained with silver stain and either photographed with a digital camera (Kodak 120) on a transilluminator or read using an FMBIO II system (Miraibio, Inc./Hitachi Genetics, Alameda, CA). CE and D-HPLC equipment that may be used in years 2 and 3 to generate equivalent data via fluorescence event recording.

Data Handling and Analysis. Data generated by slab gel analysis will be obtained via digital imagery of transilluminated gels. The program Phoretix 1D provides a full suite of tools for reading, editing and verifying marker data. Similar capabilities are available in software associated with CE and D-HPLC equipment, which may be used in years 2 and 3. Data converted to marker identity and presence/absence by one or more of these methods will then subject to analysis by standard methods used in population genetic and phylogeographic analyses.

References Cited in Attachment A

- Akagi H., Y. Yokozeki, A. Inagaki, and T. Fujimura. (1997). Highly polymorphic microsatellites of rice consist of AT repeats, and a classification of closely related cultivars with these microsatellite loci. *Theoretical and Applied Genetics*, 94: 61-67.
- Bell, T.J., M. Bowles, J. McBride, and K. Havens. 2000. Viability analysis of a restored population of the federal threatened Pitcher's Thistle (*Cirsium pitcheri*) in Illinois. Paper presented at: Botany 2000! Oregon Convention Center, 6-10 August, 2000 Portland, OR Abstract in: *American Journal of Botany* 87(6).
- Boiteux, L.S., M.E.N. Fonseca, and P.W. Simon. 1999. Effects of plant tissue and DNA purification method on randomly amplified polymorphic DNA-based genetic fingerprinting analysis in carrot. *J. Amer. Soc. Hort. Sci.* 124(1):32-38.

- Bonnin, I., J. Ronfort, F. Wozniak, and I. Olivieri. 2001. Spatial effects and rare outcrossing events in *Medicago truncatula* (Fabaceae). *Molecular Ecology* 10(6):1371-1383.
- Briard, M., V. LeClerc, A.E. Mauset, and A. Veret. 2001. A comparative study on the use of ISSR, microsatellites and RAPD markers for varietal identification of carrot genotypes. IN: Dore, C., F. Doba, and C. Baril (eds.). *International Symposium on Molecular Markers for Characterizing Genotypes and Identifying Cultivars in Horticulture*. ISHS Acta Hort. 546:
- Chalmers K.J., A.C. Newton, R. Waugh, J. Wilson, and W. Powell. 1994. Evaluation of the extent of genetic variation in mahoganies (Meliaceae) using RAPD markers. *Theoretical and Applied Genetics*, 89: 504-508.
- Datwyler, S.L. and A.D. Wolfe. 2000. Assessment of ISSR band homology by southern hybridization with implications for data analysis and microsatellite development. Paper presented at: Botany 2000! Oregon Convention Center, 6-10 August, 2000 Portland, OR (Abstract).
- Del Rio A.H., J.B. Bamberg, and Z. Huaman. 1997a. Assessing changes in the genetic diversity of potato gene banks. 1. Effects of seed increase. *Theoretical and Applied Genetics*, 95: 191-198.
- Del Rio, A.H., J.B. Bamberg, Z. Huaman, A. Salas, and S.E. Vega. 1997b. Assessing changes in the genetic diversity of potato gene banks. 2. *In situ vs ex situ*. *Theoretical and Applied Genetics*, 95: 199-204.
- Fang, D.Q., M.L. Roose, R.R. Krueger, and C.T. Federici. 1997. Fingerprinting trifoliate orange germplasm accessions with isozymes, RFLPs, and inter-simple sequence repeat markers. *Theoretical and Applied Genetics*, 95: 211-219.
- Green, J.M., J.H.A. Barker, E.J.P. Marshall, R.J. Froud-Williams, N.C.B. Peters, G.M. Arnold, K. Dawson, and A. Karp. 2001. Microsatellite analysis of the inbreeding grass weed Barren Brome (*Anisantha sterilis*) reveals genetic diversity at the within- and between-farm scales. *Molecular Ecology* 10(4):1035-1045.
- Hongtrakul, V., G.M. Huestis, and S.J. Knapp. 1997. Amplified fragment length polymorphisms as a tool for DNA fingerprinting sunflower germplasm: genetic diversity among oilseed inbred lines. *Theoretical and Applied Genetics*, 95: 400-407.
- Kiers. A.M., T.H.M. Mes, R. van der Meijden, and K. Bachmann. 2000. A search for diagnostic AFLP markers in *Cichorium* species with emphasis on endive and chicory cultivar groups. *Genome*, 43: 470-476.
- Lin, J.J., J. Kuo, M. Jin, D.A. Saunders, H.S. Beard, M.H. MacDonald, W. Kenworthy, G.N. Ude, and B.F. Matthews. 1996. Identification of molecular markers in soybean comparing RFLP, RAPD and AFLP DNA mapping techniques. *Plant Molecular Biology Reporter*, 14: 156-169.
- Lowe. A.J., A.C.M. Gillies, J. Wilson, and I.K. Dawson. 2000. Conservation genetics of bush mango from central/west Africa: implications from random amplified polymorphic DNA analysis. *Molecular Ecology*, 9: 831-841.
- Lutz, A.W. and A.D. Wolfe. 2000. Population genetics of the endemic species, *Penstemon caryi* Pennel. Paper presented at: Botany 2000! Oregon Convention Center, 6-10 August, 2000 Portland, OR (Abstract).
- Milbourne D., R. Meyer, J.E. Bradshaw, E. Baird, N. Bonar, J. Provan, W. Powell, and R. Waugh. 1997. Comparison of PCR-based marker systems for the analysis of genetic relationships in cultivated potato. *Molecular Breeding*, 3: 127-136.

- Muluvi, G.M., J.I. Sprent, N. Soranzo, J. Provan, D. Odee, G. Folkard, J.W. McNicol, and W. Powell. 1999. Amplified fragment length polymorphism (AFLP) analysis of genetic variation in *Moringa oleifera* Lam. *Molecular Ecology*, 8: 463-470.
- Nagaoka, T., and Y. Ogihara. 1997. Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. *Theoretical and Applied Genetics*, 94: 597-602.
- Nebauer, S.G., L. del Castillo-Agudo, and J. Segura. 1999. RAPD variation within and among natural populations of outcrossing willow-leaved foxglove (*Digitalis obscura* L.). *Theoretical and Applied Genetics*, 98: 985-994.
- Olmstead, R.G., C.W. dePamphilis, A.D. Wolfe, N.D. Young, W.J. Elisons, and P.A. Reeves. 2001. Disintegration of the Scrophulariaceae. *Amer. J. Bot.* 88: 348–361.
- Olufowote, J.O., Y. Xu, X. Chen, W.D. Park, H.M. Beachell, R.H. Dilday, M. Goto, and S.R. McCouch. 1997. Comparative evaluation of within-cultivar variation of rice (*Oryza sativa* L.) using microsatellite and RFLP markers. *Genome*, 40: 370-378.
- Panigo, N., M. Nunoz, M. Echaid, L. Fernandez, P. Faccio, R. Zandeomeni, E. Suarez, and E. Hopp. 1999. Microsatellite development for sunflower. Paper presented at: Plant and Animal Genome VII Conference, San Diego, CA. January 17-21, 1999. (Abstract).
- Parsons, B.J., H.J. Newbury, M.T. Jackson and B.V. Ford-Lloyd. 1997. Contrasting genetic diversity relationships are revealed in rice (*Oryza sativa* L.) using different marker types. *Molecular Breeding*, 3: 115-125.
- Perrsson, H.A. and B.A. Gustavsson. 2001. The extent of clonality and genetic diversity in lingonberry (*Vaccinium vitis-idaea* L.) revealed by RAPDs and leaf-shape analysis. *Molecular Ecology* 10(6):1385-1397.
- Pejic, I., P. Ajmone-Marsan, M. Morgante, V. Kozumplick, P. Castiglioni, G. Taramino, and M. Motto. 1998. Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs, and AFLPs. *Theoretical and Applied Genetics*, 97: 1248-1255.
- Powell, W., M. Morgante, C. Andre, M. Hanafey, J. Vogel, S. Tingey, and A. Rafalski. 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding*, 2: 225-238.
- Rossetto, M., P.K. Weaver, and K.W. Dixon. 1995. Use of RAPD analysis in devising conservation strategies for the rare and endangered *Grevillea scapigera* (Proteaceae). *Molecular Ecology*, 4: 321-329.
- Rossetto, M., M. Shepherd, F. Harriss, R. Henry, S. Lee, and G. Cordeiro. 1999. Cross species amplification of microsatellite loci – A valuable tool for genetic studies in plants. Paper presented at: Plant & Animal Genome VII Conference, San Diego, CA, January 17-21, 1999. (Abstract).
- Russell, J.R., J.D. Fuller, M. Macaulay, B.G. Hatz, A. Jahoor, W. Powell, and R. Waugh. 1997. Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. *Theoretical and Applied Genetics*, 95: 714-722.
- Sharma, S.K., M.R. Knox, and T.H.N. Ellis. 1996. AFLP analysis of the diversity and phylogeny of *Lens* and its comparison with RAPD analysis. *Theoretical and Applied Genetics*, 93: 751-758.
- Sydes, M.A., and R. Peakall. 1998. Extensive clonality in the endangered shrub *Haloragodendron lucasii* (Haloragaceae) revealed by allozymes and RAPDs. *Molecular Ecology*, 7: 87-93.

- Thomas, M.R., P. Cain, and N.S. Scott. 1994. DNA typing of grapevines: A universal methodology and database for describing cultivars and evaluating genetic relatedness. *Plant Molecular Biology*, 25: 939-949.
- Tohme, J., D.O. Gonzalez, S. Beebe, and M.C. Duque. 1996. AFLP analysis of gene pools of a wild bean core collection. *Crop Science*, 36: 1375-1384.
- Virk, P.S., J.Zhu, H.J. Newbury, G.J. Bryan, M.T. Jackson, and B.V. Ford-Lloyd. 2000. Effectiveness of different classes of molecular marker for classifying and revealing variation in rice (*Oryza sativa*) germplasm. *Euphytica*, 112: 275-284.
- Wolfe, A.D., and A. Liston. 1998a. Contributions of PCR-based methods to plant systematics and evolutionary biology. in: *Plant Molecular Systematics II* D. E. Soltis, P. S. Soltis and J. J. Doyle. pp. 43-86. Boston, Kluwer.
- Wolfe, A.D., Q.-Y. Xiang and S.R. Kephart. 1998b. Assessing hybridization in natural populations of *Penstemon* (Scrophulariaceae) using hypervariable inter simple sequence repeat markers. *Molecular Ecology* 7:1107-1125.
- Yang, G.P., M.A. Saghai Maroof, C.G. Xu, Q. Zhang, and R.M. Biyashev. 1994. Comparative analysis of microsatellite DNA polymorphism in landraces and cultivars of rice. *Molecular and General Genetics*, 245: 187-194.
- Yang, W., A.C. de Oliveira, I. Godwin, K. Schertz, and J.L. Bennetzen. 1996. Comparison of DNA marker technologies in characterizing plant genome diversity: variability in Chinese sorghums. *Crop Science*, 36: 1669-1676.
- Zebell, R. and P. Fiedler. 1993. Restoration and Recovery of Mason's *Lilaeopsis* Phase I. Technical report prepared for the Shell Oil Spill Settlement Trustee Committee and California Department of Fish and Game, Sacramento, CA. 47 pp.
- Zebell, R. and P. Fiedler. 1996. Restoration and Recovery of Mason's *Lilaeopsis* Phase II. Technical report prepared for the Shell Oil Spill Settlement Trustee Committee and California Department of Fish and Game, Sacramento, CA. 50 pp.
- Zoller, S., F. Lutzoni, and C. Scheidegger. 1999. Genetic variation within and among populations of the threatened lichen *Lobaria pulmonaria* in Switzerland and implications for its conservation. *Molecular Ecology*, 8: 2049-2059.

Table 1. List of Tasks/Subtasks and Key Questions


Tasks/Subtasks	Key Questions	Relevance to Species
Task 1 - Population Biology and Genetics	Key: ML= Mason's lilaeopsis, DM=Delta mudwort, ST=Suisun thistle	
<i>Subtask A – Population Description and Distribution</i>		
Population description and distribution	What are the current distribution, population size, and structure?	ML, DM, ST
Population viability and habitat quality	What is the viability of individual populations and the potential to protect or restore the habitat?	ML, DM, ST
<i>Subtask B – Population Genetics</i>		
Species and population genetics	How is genetic diversity distributed within and among populations throughout species range?	ML, DM, ST
	Is population differentiation related to salinity tolerance indicated by genetic marker data?	ML, DM
	How is vegetative vs. sexual reproduction reflected in genetic population structure?	ML, DM
	Is this species at higher risk from extinction due to hybridization?	ST
<i>Subtask C – Life History Characteristics</i>		
Species life history	What life-history traits limit the species' dispersal and distribution?	ML, DM, ST
	What life-history traits do we need to understand to help recover the species?	ML, DM, ST
Task 2 - Habitat Structure and Ecological Processes		
	What tidal elevations, water quality, sedimentation, soils, and biological parameters define the habitat constraints for these species?	ML, DM, ST
	What unique suite of ecological processes effects the distribution of each species?	ML, DM, ST
Task 3 - Pilot Restoration Project	Based on the development of population and habitat models, can we restore habitat and aid recovery of the species?	ML, DM, ST
	Will species growth patterns and life-history characteristics function the same in created habitats as in natural ones?	ML, DM, ST

Table 2. Task Products, Outcomes, and Work Schedule

Tasks	Products, Outcomes and Schedule
Task 1 - Population and Biology Genetics	
Field Studies and Report: Distribution of populations of Mason's lilaeopsis and Delta mudwort.	Field studies August through September 2002. Report produced December 2002.
Distribution of populations of Suisun thistle	Field studies August through September 2002. Report produced December 2002.
Life-history and population genetics of Mason's lilaeopsis and Delta mudwort.	Field studies August 2002 through September 2003. Preliminary report on life history December 2003, final report December 2004. Genetics report November 2004.
Life history and population genetics of Suisun thistle.	Field studies August 2002 through August 2003. Preliminary report on life history December 2003, final report December 2004. Genetics report November 2004.
Task 2 - Habitat Structure and Ecological Processes	
Biological and physical parameters and habitat model for Mason's lilaeopsis and Delta mudwort.	Field studies August 2002 through July 2003. Report produced October 2003.
Biological and physical parameters and habitat model for Suisun thistle.	Field studies August 2002 through July 2003. Report produced October 2003.
Task 3 - Pilot Restoration Project	
Restoration design and monitoring plan for Mason's lilaeopsis and Delta mudwort.	Draft report for review December 2003. Final report produced February 2004.
Restoration design and monitoring plan for Suisun thistle.	Draft report for review September 2003. Final report produced November 2003.
Implement restoration plan for Mason's lilaeopsis and Delta mudwort.	March-April 2004.
Implement restoration plan for Suisun thistle.	December-January 2004.
Report: Annual monitoring of Mason's lilaeopsis and Delta mudwort.	First season results November 2004, Second season results October 2005.
Report: Annual monitoring of Suisun thistle.	First season results November 2004, Second season results October 2005.

Figure 1
Mason's *Lilaeopsis*

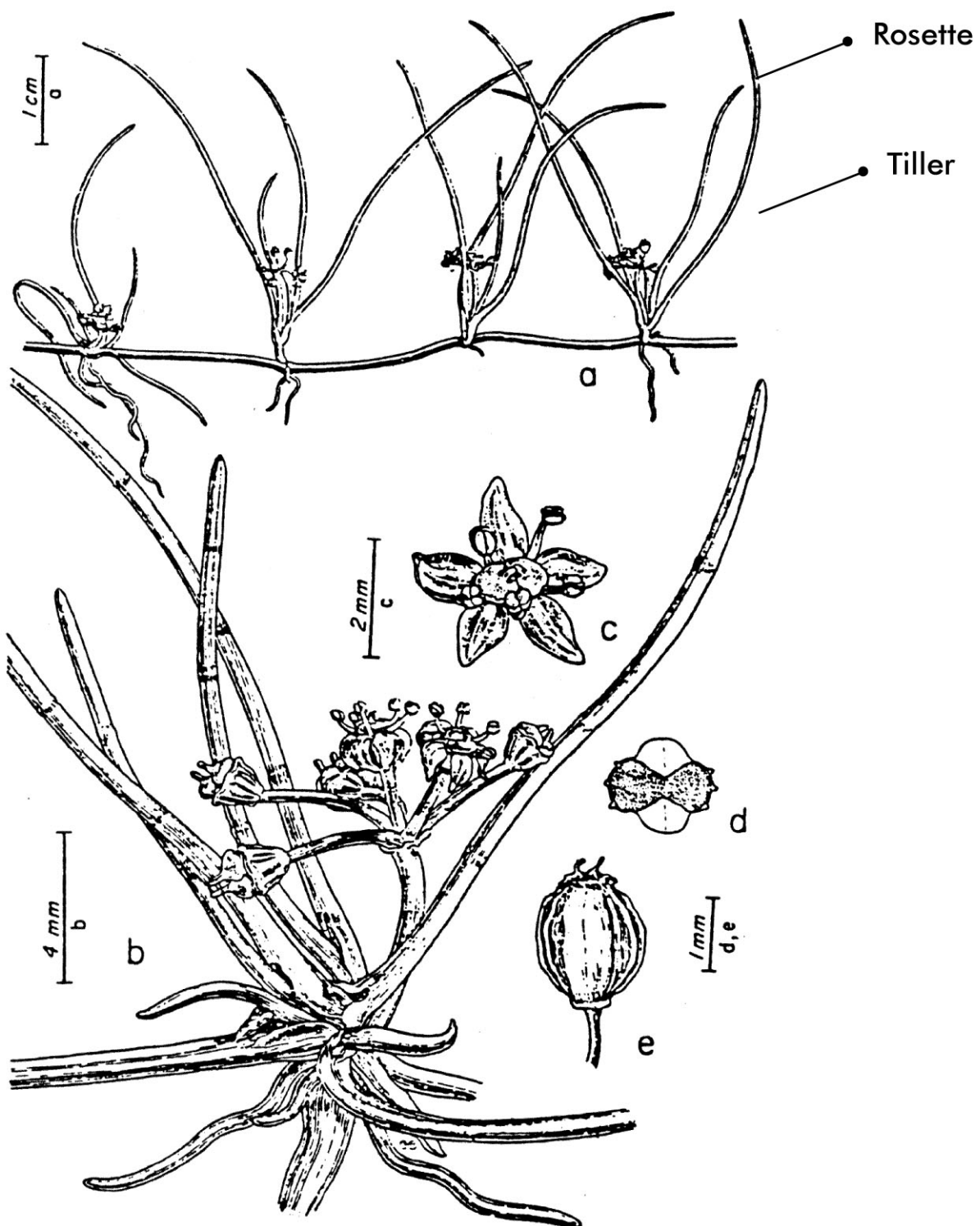


Figure 2
Conceptual Model
Within CALFED Adaptive Management Program

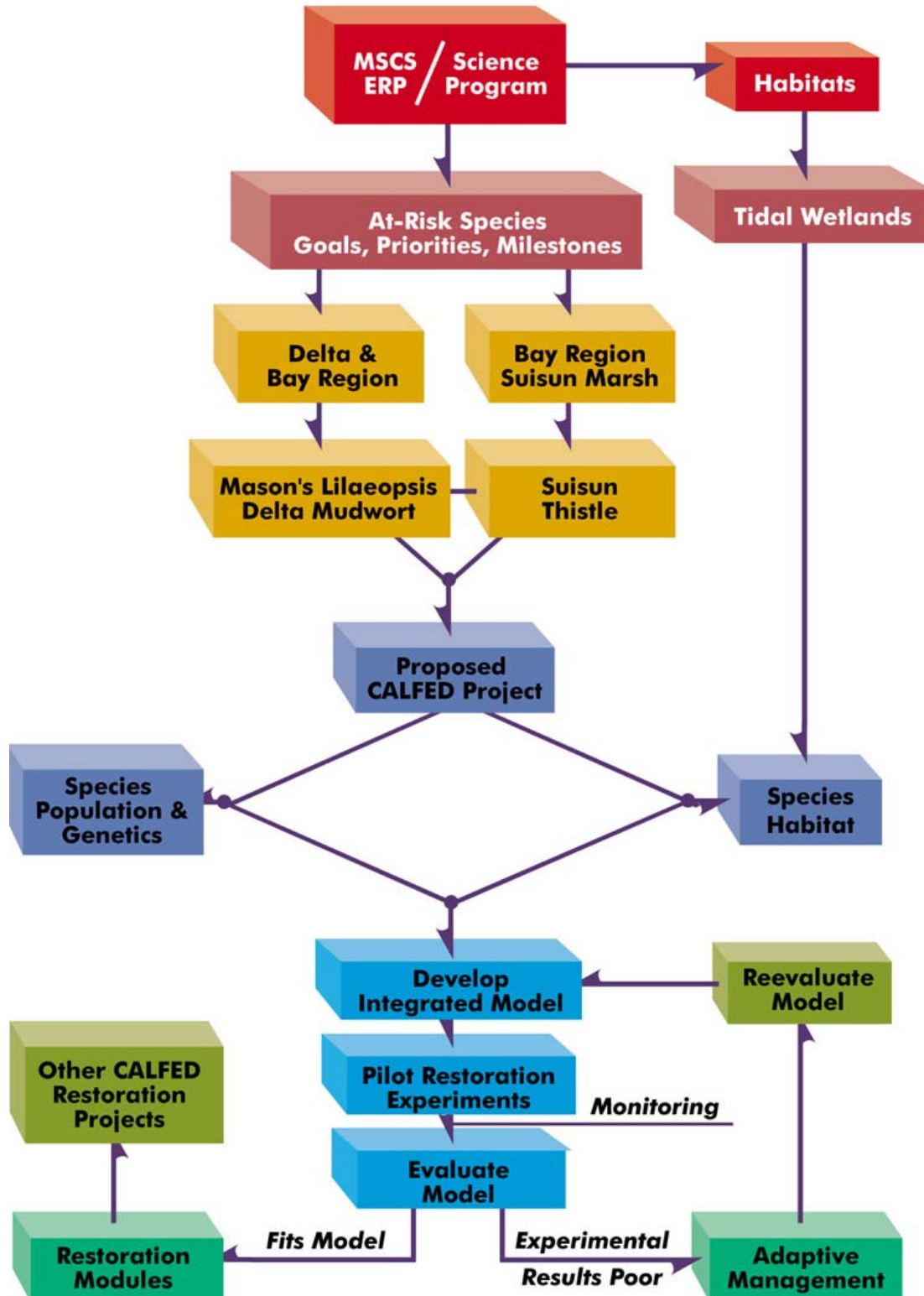


Figure 3
Conceptual Model
for Mason's Lilaeopsis and Delta Mudwort

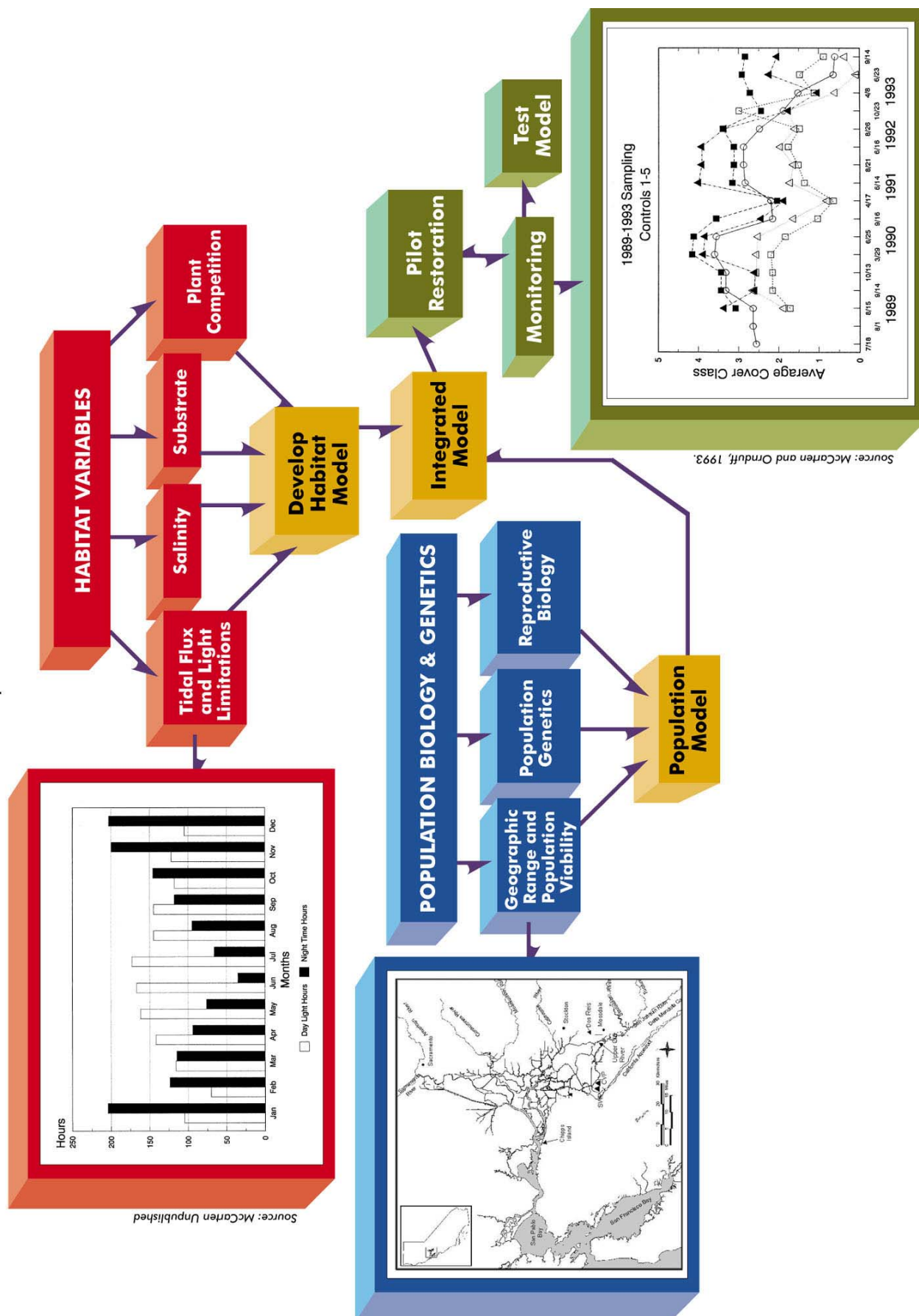


Figure 4
Sample Site Locations and Population Range for Mason's *Lilaeopsis*

